Good Food for Children Study

Report on study findings and policy implications for Cambodia

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Abstract

Background

In Cambodia, 62% of children under-five years are anaemic and 44% are stunted. In efficacy trials in Cambodia and elsewhere, micronutrient powders (Sprinkles) have significantly reduced anaemia. Whether this can be achieved by delivery through existing government health services is uncertain.

Methods

We did a cluster-randomised trial in one health district evaluating the effectiveness of daily Sprinkles mixed with complementary foods in infants 6 to 11 months of age, alongside infant and young child feeding (IYCF) education. We followed a sub-sample of 1350 randomly selected children at 6 monthly intervals to 24 months of age, to establish whether any observed effects on anaemia, deficiencies of iron, vitamin A, zinc and growth were sustained.

Findings

Home fortification with micronutrient Sprinkles along with IYCF education reduced any anaemia at 12 months compared to IYCF education alone by 24% (Haemoglobin (Hb)<110 g/L, Incidence Rate Ratio (IRR) 0·76, 95% Confidence Interval (CI) 0·64 to 0·89, p=0·001) and moderate anaemia (Hb<100 g/L) by 57% (IRR 0·43, 95% CI 0·33 to 0·56, p<0.001). Iron depletion was reduced by 57% (IRR) 0·43, 95% CI 0·32 to 0·58, p<0.001 and both iron deficiency anaemia (IDA) and non-IDA were reduced for moderate anaemia at 12 months. We found a significant increase in mean serum zinc concentration (0·44 μmol/L, 95% CI 0·04 to 0·83, p=0·028) but no difference in the prevalence of zinc and vitamin A deficiencies, or growth at any time point. Compared to national surveys, we found a slowing of stunting and underweight.

Interpretation

Sprinkles micronutrient powder, delivered through government health services, reduced anaemia, and increased mean serum zinc concentration in Cambodian infants. These effects were in addition to IYCF education alone, but did not persist beyond the intervention period. Therefore it may be best to continue Sprinkles until 24 months of age.

Funding

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1 BACKGROUND

Indicators of child survival are improving globally and in Cambodia[1]. Nevertheless, 62% of Cambodian children under-five years are anaemic[2] and 44% are stunted. Rates of anaemia in preschool children in Cambodia are among the highest in the Asia-Pacific region, especially among infants aged 6 to 23 months (81%)[2]. Those aged 6 to 23 months are at highest risk for anaemia[2] and micronutrient deficiencies, which together may lead to impairments in growth and immune function, cognitive and learning difficulties, and increased mortality[3, 4]. The aetiology of anaemia is multi-factorial including iron deficiency, other micronutrient deficiencies, infections and genetic haemoglobin (Hb) disorders. The latter are found in 30 to 70% of Cambodians[5, 6].

Micronutrient deficiencies during early childhood prompted the development of in-home fortification with multi-micronutrient powders, termed Sprinkles. In efficacy trials in Cambodia[7] and elsewhere[8], Sprinkles significantly reduced anaemia in infants and young children when mixed with complementary foods. However, whether reduction in anaemia and micronutrient deficiencies can be achieved by delivering Sprinkles through existing government health services is uncertain. Therefore, we conducted a cluster-randomised controlled trial in Cambodia to evaluate the effectiveness of daily Sprinkles mixed with home-based complementary foods in infants from age 6 to 11 months, alongside infant and young child feeding (IYCF) education. We evaluated the effect on anaemia, deficiencies of iron, vitamin A, and zinc, and on wasting, underweight and stunting. We followed the children to aged 24 months to establish whether any observed effects were sustained.

2 METHODS

Study setting and design
All children born between August 2007 and January 2008 in Svay Rieng Operational Health District, Cambodia were eligible to participate. Enrolment was between March and August 2008. The study was a cluster-randomised trial with health centre (HC) catchment area as the unit of randomization. All 20 HC catchment areas of the health district were included.

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, 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 sub-sample selected for more intensive follow-up at enrolment.

Procedures
Health centres (clusters) were randomised by permuted block design to intervention (IYCF education plus Sprinkles; 10 clusters) and control (IYCF education alone; 10 clusters). IYCF and Sprinkles education was provided to caregivers by trained Government village health workers called village health support groups (VHSG's) in verbal, written and pictorial form, together with cooking demonstrations, based on Cambodia IYCF Policy recommendations. Infants in the intervention arm received daily Sprinkles in single dose sachets (Table 1), delivered monthly to their homes by VHSG's. Sprinkles were mixed with the infant's meal immediately before serving. Adherence was assessed monthly by counting the number of unused sachets from each household. VHSG's were monitored by HC staff through monthly meetings and spot checks. HC staff were monitored by district, provincial, national and study staff overseeing the study. Immunizations, bi-annual vitamin A capsules and mebendazole tablets (for deworming) were provided to all children according to Cambodia Ministry of Health guidelines.

A sub-sample of 675 children in each arm was randomly selected within month blocks. Caregivers and children attended their local HC on one of five mornings per month over the enrolment period. Data on socio-demographic status, antenatal and postnatal practices were recorded via questionnaires and anthropometry and blood samples were collected. The sub-sample children were followed at 6 monthly intervals corresponding to age 6, 12, 18 and 24 months. On each occasion, a blood sample, information on feeding practices and anthropometry were collected. All sub-sample children were invited to attend each round (Figure 1).

Non-fasting venous samples were taken using International Zinc Collaborative Group (IZiNCG) procedures[9]. Blood samples were drawn into an EDTA-containing tube for complete blood count (CBC) and a trace-element (TE) free tube (Beckton Dickinson, Frankton Lakes, NJ, USA) at least 30 minutes after applying topical anaesthetic

(Ametop [TM] gel (tetracaine 4%, Smith & Nephew). All blood samples were refrigerated immediately after collection. CBC's were performed using an automated haematology analyser (Sysmex Corporation, Japan) at the National Institute of Public Health Laboratory, Cambodia. Serum aliquots were frozen in TE-free polyethylene vials at -20°C, and later at -70°C prior to shipment to the University of Otago for zinc, and DBS-Tech, Willstaett, Germany for serum ferritin, retinol binding protein (RBP), transferrin receptor (sTfR), C-reactive protein (CRP), and α-1-glycoprotein (AGP) analysis.

Serum zinc was analysed using flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 800; Perkin Elmer Corp., Norwlak, CT, USA). Serial replicates of a pooled serum sample and a certified reference material (CRM) (Bovine Serum Reference Material No. 1598; National Institute of Standards and Technology) were used to check the precision and accuracy of the method. The inter-assay coefficient of variation (CV) for zinc (as percent) was 5·8% (n=278) and the value for the CRM was 13·19 (SD 0·76 μmol/L (CV 5·8%, n= 31) compared with the certified value of 13·46 (95% CI, 0·38 to 13·84) μmol/L. Serum ferritin, RBP, sTfR, CRP and AGP were analysed using a sandwich ELISA technique[10]. Acute and chronic inflammation was assessed by serum CRP>5mg/L and AGP>1g/L, respectively[11].

Screening for genetic Hb disorders was performed at aged 18 months, using the SEBIA MINICAP analyzer and HEMOGLOBIN (E) program, designed for the separation of normal haemoglobins (A, A_2 and F) and detection of major haemoglobin variants, including HbE, HbH and HB Constant Spring. MINICAP results were controlled using Sebia Normal and Pathological Haemoglobin A_2 controls. In addition, IC α THAL Strip test was performed for the determination of α thalassaemia.

Anthropometry was conducted using standardized procedures and calibrated equipment. Recumbent length was measured using a portable length measuring board (Shorr Board, Shorr Productions), weight using an electronic scale (UNIscale, UNICEF, Item No. 0141015), and head circumference and mid-upper-arm circumference (MUAC) using a non-stretch retractable circumference tape (Chasmors CTM08 Circumference Measure, Chasmors Ltd, London, UK). Monthly data consistency analyses were performed. Z-scores for weight-for-age (WAZ), weight-for-length (WLZ) and length-for-age (LAZ) were calculated using WHO international growth reference data (WHO, 2006)

Sample Size

We estimated 3600 eligible children were in the study area. The sub-sample size of 1350, was calculated based on a cluster-randomised study design, allowing for a 20% drop-out and an intraclass correlation coefficient (ICC) of 0·15. It was calculated to determine a significant difference for the primary outcome of the prevalence of anaemia and taking into consideration the secondary outcomes of vitamin A deficiency, haemoglobin concentration, serum zinc, serum ferritin and z-scores of stunting and wasting with a power of 90% and 5% level of significance.

Data management and statistical analysis

Data entry was blinded. Interviews and anthropometric data were double entered into an SPSS Version 11·5 database and checked for consistency. CBC and biochemical data were entered at the respective laboratories. Statistical analyses were performed using Stata V11 (StatCorp, College Station, TX, USA). All means are reported ± standard deviation (SD). Differences between groups are reported with 95% confidence intervals (CI). Analysis was by randomised group, but no imputation has been used for missing data. Mixed models were used for the analysis with health centre as a random effect. All models adjust for baseline (6 month) values.
This trial is registered, number ACTRN12608000069358

Role of the funding source

This study was funded from multiple sources. No sponsor had any role in collection, analysis and interpretation of the data, or the report writing. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

3 RESULTS & INTERPRETATION

A total of 3112 infants were listed and invited to participate in the study; 1350 children were recruited to the sub-sample, 650 in the intervention arm (Sprinkles + IYCF education and 650 in the active control arm (IYCF education alone)(see 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). 93% of eligible children used Sprinkles and the median number of Sprinkles sachets consumed per month per child was 23·8 (range 0 to 30).

3.1 Anaemia response

Using the WHO cut off for children 6-59 months, rate of any anaemia (Hb<110 g/L) at 12 months was 294/441 (66·7%) in the intervention group compared with 410/484 (84·7%) in the control group (Risk difference 20·6%, 95% CI 9·4 to 30·2, p=0·001), meaning that there was a 20·6% decrease in anaemia in the intervention group (Sprinkles + IYCF) compared to the control groups (IYCF alone). At later follow ups (age 12,18 and 24 months) there were no significant differences in any anaemia (Hb<110g/L) for intervention versus control with 64·0% vs. 72·3% (Risk difference 10·0%, 95% CI -2·9 to 20·6, p=0·120) at 18 months and 49·4% vs 56·7% (Risk difference 8·9%, 95% CI -1·2 to 17·2, p=0·082) at 24 months. Note, there were differences seen with a decrease in anaemia of 10·0% at 18 months and 8·9% at 24 months for intervention compared to control, but these differences were not statistically significant.

For moderate anaemia[12] (Hb<100 g/L) the prevalence at 12 months was 21·5% in the intervention group compared with 47·3% in the control group (Risk difference 27·1%, 95% CI 21·0 to 31·8, p<0.001) (Table 3), meaning that there was a 27·1% decrease in moderate anaemia at endline (children aged 12 months) in the intervention compared to the control group. At later follow ups there were no significant differences in moderate anaemia for intervention versus control (the same as we found if we used any anaemia (Hb<110g/L).

The overall mean (SD) baseline haemoglobin was 100·3 ± 9·1 g/L and did not differ between groups. Mean haemoglobin levels increased from baseline to 12 months in the intervention group compared to controls (mean difference 6·1 g/L, 95% CI 4·7 to 7·4, p<0·001 (Table 3). At 18 and 24 months, mean haemoglobin differences were 2·2 g/L (95% CI 0·8 to 2·2, p=0.003) and 1·1 g/L (95% CI -0·1 to 2·2, p=0·076) respectively. So, we found significant differences in mean haemoglobin at endline (6·1 g/L)(12 months) and a small but statistically significant difference at first follow-up (2·2 g/L)(18 months) but not at the 2nd follow-up (24 months).

For children who were anaemic at baseline (Hb<110 g/L), rate of recovery from anaemia at 12 months was 94/330 (28·5%) in the intervention group compared with 27/350 (7·7%) in controls (IRR 0·84, 95% CI 0·73 to 0·96, p=0·013). So we found that

28·5% of children who were anaemic at baseline had become non-anaemic at endline in the Sprinkles + IYCF group compared with only 7·7% of children in the IYCF group. This still means that 71·5% of children anaemic at baseline did not recover from their anaemia due to Sprinkles. At later follow-ups, there were no significant differences between the groups.

Genetic haemoglobin disorders impacted mean Hb and prevalence of anaemia at 12, 18 and 24 months. Sprinkles intervention had a similar proportional effect in decreasing anaemia whether the child had a Hb disorder or not at 12 months (20·9% vs. 16·8%, p= 0·460). Anaemia prevalence in children with a Hb disorder was higher at 12, 18 and 24 months. This means that even if the child had a genetic Hb disorder (thalassemia or haemoglobinopathy), they still responded to Sprinkles, although they ended up more anaemic than those without a disorder

3.2 Anaemia Interpretation

Our effectiveness study showed a 24% risk reduction in any anaemia (Hb<110 g/L) and a 57% risk reduction in moderate anaemia (Hb<100 g/L) at 12 months. Risk reduction is derived from the IRR or Incidence Rate Ratio (a measure of the effect of children who received Sprinkles compared to those who did not). Hence, for any anaemia (Hb<110g/L) the IRR is 0·76 (which when taken away from 1 i.e. 1-0·76=0·24) yields the 24% reduction in risk of anaemia. If we use moderate anaemia (Hb<100g/L) we find an IRR of 0·43, which means a 57% reduction in the risk of moderate anaemia (1-0·43=0·57). The prevalence of anaemia was significantly lower in both groups at aged 18 and 24 months, consistent with the age-related pattern seen in the Demographic and Health Surveys (DHS) in Cambodia[2] and elsewhere[13] (Figure 2).

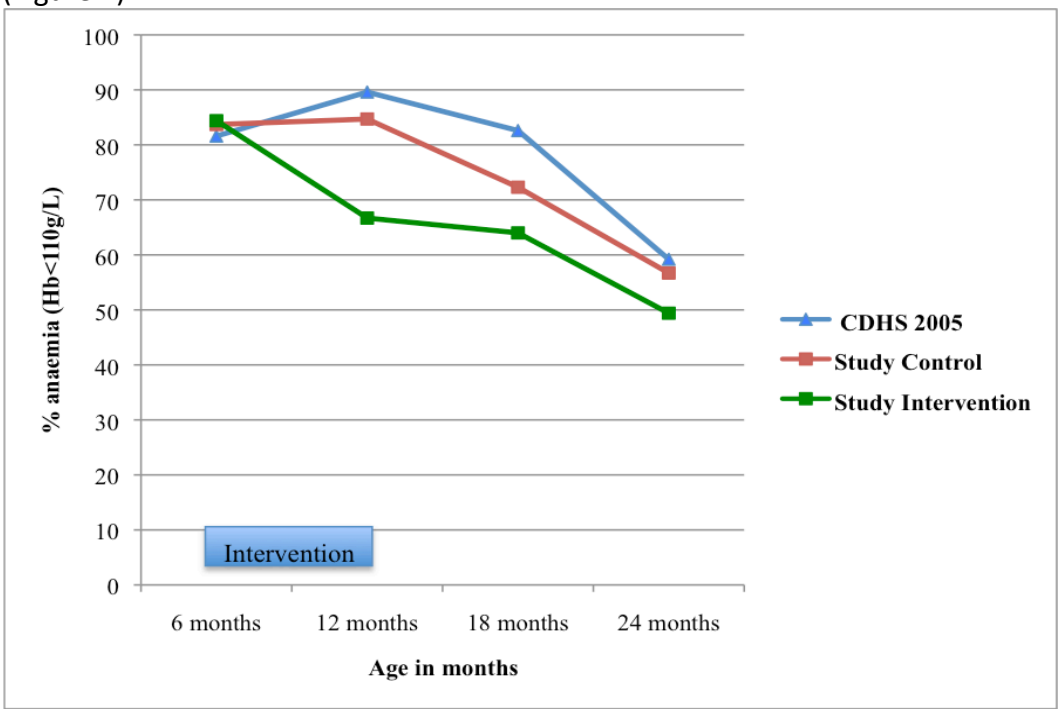


Figure 1: Anaemia by age: Comparison with Cambodia Demographic & Health Survey (CDHS) 2005

For example, examination of the CDHS 2000 and 2005 anaemia results for children 6-59 months by age, reveals a similar pattern whereby the prevalence of anaemia increases slightly up to 12 months of age and then steadily decreases. Results for CDHS 2000 and 2005, and then DHS reports from other developing countries in Asia, Africa and South America all show the same pattern. Unfortunately, this pattern is not so marked in many reports because all the ages are grouped together e.g. 6-59 months or 6-23 months, preventing this age-related pattern (month by month) from being seen. One reason why anaemia might be so high from 6-12 months, is that probably the cut off of 110 g/L for anaemia is probably too high. Even in WHO documents where they look at specific ages they state the -2 SD limit for children age 6 months should be Hb 95 g/L (from Assessing the iron status of populations: Report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, Geneva, Switzerland, 6–8 April 2004. – 2nd ed.)

TABLE 1
The mean and lower standard deviation (–2 SD) of normal haemoglobin concentrations (g/l) in a
Caucasian population

Age range	Mean	-2 SD
3–6 months	115	95
0.5–2 years	120	110
2–6 years	125	115

Adapted, from Hoffman (11), with permission of the publisher.

Other studies have also shown that the normal physiological haemoglobin level for infants may be lower than the current WHO cut offs (from Domellof et al, J Nutr. 132: 3680-3686, 2002):

TABLE 4

Suggested 2 SD cut-off values for iron status variables at 4, 6 and 9 mo of age, based on iron-replete, breast-fed infants[1]

	4 mo	6 mo	9 mo
Hb, *g/L*	<105	<105	<100
MCV, *fL*[2]	<73	<71	<71
ZPP, *μmol/mol heme*	>75	>75	>90
Ferritin, *μg/L*	<20	<9	<5
TfR, mg/L	>11	>11	>11

[1] Hb, hemoglobin; MCV, erythrocyte mean cell volume; ZPP, zinc protoporphyrin; TfR, soluble transferrin receptors.
[2] Based on Swedish infants.

So, the most accurate Hb cutoff level for estimating anaemia infants is not entirely clear, which is why we have reported both *any* anaemia (Hb <110g/L) which is the current WHO recommended cut off, as well as a lower cutoff for *moderate* anaemia (Hb<100g/L). The latter has actually been used in most Sprinkles study reports or publications, including the meta-analysis systematic review (Dewey et al, 2009).

Examination of the Sprinkles studies that reported following up children to see how long the effect of Sprinkles lasted reveals that other follow-up studies have been restricted to those children whose anaemia were successfully treated[14-16], i.e. only the children who responded to Sprinkles and recovered from anaemia. This means that only about half the children in those studies were then followed up and no information has been given on what has happened to the other 50% of the children who did not respond adequately: did they need Sprinkles for longer to recover? Did they have other problems causing their anaemia? This information is not given. Therefore, the conclusion that you only need to give Sprinkles for 2 or 4 months only applies to those who actually responded to Sprinkles and recovered from anaemia. In a real life situation, we need to take into consideration all children, and work out what is the best policy that would give the best outcomes for all the children. Most studies have ignored the characteristic age-related improvements in haemoglobin. Hence, sometimes they report an improvement in children when they are at an older age, without taking into consideration that the anaemia would be probably be improving anyway. In one Sprinkles study where they compared daily Sprinkles for 2 months and flexible Sprinkles over 4 months, the group who received Sprinkles for 4 months were reported as doing the best. However, there are two factors that were not considered in this study: a) the children were 2 months older and so not directly comparable and b) the usual decrease in anaemia with increasing age was ignored.

In our study, Sprinkles were effective in reducing anaemia in children both with genetic Hb disorders and without. This is the same as was found in the CESVI trial (but they did not publish those genetic Hb disorder results). It is also the same finding as that found in the GTZ Foodlet trial (although they gave 3 RDA (Recommended daily allowance) twice a week as a foodlet (large micronutrient tablet which was crushed and eaten or mix with food) so it was not directly comparable with our study.

3.3 Anaemia conclusions:

Sprinkles given daily for 6 months from age 6-11 months saw a reduction in risk of any anaemia of 24% and a reduction in risk of moderate anaemia of 57% at the end of intervention. This effect did not last through to 18 months or 24 months, although due to the age-related decrease in anaemia, the children were less anaemic at 18 and 24 months.

The policy question then becomes: How long do we need to give Sprinkles for to reduce anaemia to cover the critical growth period of 6-24 months? In the CESVI trial, they gave daily Sprinkles for 12 months, from age 6-17 months, and at endline (age 18 months) they had an anaemia rate (for any anaemia, Hb<110g/L) of 38.5% for MMN and 37.5% for Fe/Fol compared with 71% for placebo. So, although they saw a marked decrease (from around 80% with any anaemia at baseline), they still had nearly 40% of children anaemic after 12 months of daily Sprinkles. This compares with the GFC study for children at 18 months (with only 6 months of daily Sprinkles given from age 6-12 months) where 64% of children were still anaemic at

18 months (72·3% for IYCF alone).

If we look at moderate anaemia (Hb<100g/L), the CESVI trial had a moderate anaemia rate at 18 months (after giving daily Sprinkles from age 6-17 months) of 13·8% for MMN and 7·8% for Fe/Fol compared with 32·3% for placebo. This compared with the findings of the GFC study for moderate anaemia at 18 months of 25·8% for Sprinkles + IYCF and 27·5% for IYCF alone.
The GFC study shows that at 24 months (after 6 months of intervention) the rate of any anaemia (Hb<110g/L) was 49·4% for Sprinkles + IYCF versus 56·7% for IYCF alone which is still at a level considered to be a severe public health problem; or for moderate anaemia 12·4% for Sprinkles + IYCF versus 14·9% for IYCF alone (mild public health problem).
Using the current WHO Hb cutoff for anaemia, it seems clear that the intervention (Sprinkles) should continue until the children are aged 24 months if we want to see a reduction in anaemia in this population. Even using the lower Hb cutoff for moderate anaemia (Hb<100g/L), the population would still have anaemia at a level classified as having a mild public health problem (*Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System, WHO 2011*).

Table 4

Classification of public health significance of anaemia in populations on the basis of prevalence estimated from blood levels of haemoglobin

Category of public health significance	Prevalence of anaemia (%)
Severe	40 or higher
Moderate	20.0 – 39.9
Mild	5.0 – 19.9
Normal	4.9 or lower

Regarding flexible versus daily dosing, there is actually only one study that reports on flexible dosing but it has the same limitations as the ones described above (4 months versus 2 months, different ages etc). While there is no firm evidence to support flexible compared with daily dosing, pragmatically (for cost and possibly adherence reasons) it may be reasonable to adopt flexible dosing regimens such as proposed for Cambodia (15 doses per month) but covering the whole age range from 6-23 months.
Other countries (e.g. Mongolia) are trying various flexible regimens, and until more evidence is available, the best regimen will remain uncertain. This uncertainty has been highlighted by WHO in the recent WHO Guideline: Use of multiple micronutrient powders for home fortification of foods consumed by infants and children 6–23 months of age, WHO 2011. The suggested scheme is as follows (but please note that in the full document they note the limitations in evidence for flexible dosing and for the exact composition, saying further research is needed for

the determination of the most appropriate dose of zinc and other vitamins and minerals to be included in multiple micronutrient powders and the effects of these micronutrients on indicators of nutritional status other than iron deficiency and anaemia (e.g. improvement of iodine status, prevention of vitamin A deficiency, prevention of zinc deficiency) and on important functional outcomes including growth and motor and cognitive skills.

Table 1

Suggested scheme for home fortification with multiple micronutrient powders of foods consumed by infants and children 6–23 months

Composition per sachet[a]	• Iron: 12.5 mg of elemental iron, preferably as encapsulated ferrous fumarate[b] • Vitamin A: 300 μg of retinol • Zinc: 5 mg of elemental zinc, preferably as zinc gluconate
Frequency	One sachet per day
Duration and time interval between periods of intervention	At minimum, for a period of 2 months, followed by a period of 3–4 months off supplementation, so that use of the micronutrient powders is started every 6 months
Target group	Infants and children 6–23 months of age, starting at the same time as weaning foods are introduced into the diet
Settings	Populations where the prevalence of anaemia in children under 2 years or under 5 years of age is 20% or higher

[a] The recommendation for the composition of the powder is based on the doses and nutrients included in the systematic review (*13*). In addition to iron, vitamin A and zinc, multiple micronutrient powders may contain other vitamins and minerals at currently recommended nutrient intake (RNI) doses for the target population (*14*).
[b] 12.5 mg of elemental iron equals 37.5 mg of ferrous fumarate, 62.5 mg of ferrous sulfate heptahydrate or 105 mg of ferrous gluconate.

3.4 Zinc response

The overall mean baseline serum zinc concentration was 9·7 ± 2·1 μmol/L and did not differ between groups (Table 4a). (Note: normal serum levels are said to be >9·9 μmol/L, although it should be noted that this cutoff set by IZiNCG was based on very limited data for infants and young children). There was a significant difference in mean (SD) serum zinc in the intervention group compared with controls at 12 months (10·1 ± 2·4 vs. 9·6 ± 1·7 μmol/L; mean difference of 0·44 μmol/L, 95% CI 0·04 to 0·83, p=0·028), but no difference at 18 months or 24 months. After adjusting for baseline and infection by excluding children with AGP>1g/L and CRP>5mg/L, there was no significant difference in the prevalence of zinc deficiency (Zn<9·9 μmol/L) for the intervention group compared with the controls at 12 months (54·4% vs. 61·9%, p=0·396), or at later follow-ups (Table 4b).

3.5 Zinc Interpretation

We found a significant but small increase in serum zinc concentration due to Sprinkles at 12 months (Table 4a). This is the first Sprinkles study to show an improvement in serum zinc. Moreover, although not statistically significant, there was an 8% reduced risk of zinc deficiency at 12 months. Although we used a higher zinc level (as gluconate) in our Sprinkles compared to that used earlier (10mg vs. 4·5mg)[17], we did not see a decrease in zinc deficiency and in fact, the prevalence of low serum zinc concentrations in the intervention group at 12 months was still well above the level (i.e. >20%) indicative of population zinc deficiency[9]. Such a modest response in serum zinc despite the high prevalence of zinc deficiency and stunting (which is associated with a risk of zinc deficiency and can be used as a proxy measure for zinc deficiency in a population) among the infants at baseline, is disappointing (Table 4b and 4c). However, our finding is consistent with earlier reports for zinc fortificants in cereal-based porridges compared to liquid supplements[23]. Poor zinc absorption arising from either high phytate cereal-based porridges fortified with zinc, or interference by iron fortificants (causing decreased absorption of zinc) have been implicated[9], although in our study, the fortified complementary foods were rice-based with low phytate content[24], and an iron to zinc ratio (1·25:1) proven not to adversely affect absorption of zinc fortificants[25]. We failed to show a lasting improvement in mean serum zinc or zinc deficiency at follow up. This was not unexpected, as plasma zinc declines rapidly following withdrawal of zinc supplementation[26]. It is unclear, why the increased amount of zinc in our Sprinkles did not see a larger improvement in serum zinc, and a significant decrease in the prevalence of zinc deficiency. Perhaps the zinc fortificants in the Sprinkles was not absorbed properly, because theoretically the content of zinc in the Sprinkles should have been an adequate dose. Other studies have found the same and no one has a clear answer yet.

3.6 Zinc Conclusions:

We saw a small but significant increase in serum zinc in the Sprinkles + IYCF group compared to IYCF alone at endline. I would recommend continuing with the 10mg Zinc gluconate while waiting for further evidence from other studies for the reason why the serum zinc concentrations were not increased more (poor absorption may not be the reason). Because at least half the children were zinc deficient based on the cutoffs currently set by IZiNCG for children of this age, our findings do suggest that zinc deficiency is a major public health problem among this age group that needs to be addressed.

3.7 Retinol binding protein response (Vitamin A Deficiency)

The overall mean baseline retinol binding protein (RBP) was 1·07 ± 0·24 μmol/L and did not differ between groups (Table 4a) (Note: normal RBP is >0·7 μmol/L). The prevalence of vitamin A deficiency (RBP<0·70 μmol/L), again after adjusting for

infection (i.e. AGP>1g/L and CRP>5mg/L) was low (<3·5% at any time point), with no significant difference between groups at any time point (Table 4b).

3.8 Vitamin A Deficiency Interpretation

The overall prevalence of vitamin A deficiency was very low at any time-point (<3·5%), with our RBP levels corresponding to the serum retinol concentrations within the normal range when they are homeostatistically controlled[27]. This means that concentrations of serum retinol that reflect normal Vitamin A levels in the body (i.e. not deficient or only mildly deficient) are controlled by a feedback mechanism that keeps the retinol at the correct level for the body to use. It is only if the body is very vitamin A deficient that the serum retinol level would be very low. So, our study did not show a significant reduction in vitamin A deficiency due to Sprinkles (Table 4b). In contrast, a national micronutrient survey in 2000 showed a relatively high (22%) prevalence of vitamin A deficiency[28]. Cambodia has a national vitamin A supplementation program with improved coverage in recent years which may account for this discrepancy[2, 29]. It looks like the Vitamin A Supplementation programme in Cambodia has been very successful in decreasing Vitamin A deficiency, as both groups of our children should have received Vitamin A through the normal system. (Very unfortunately, we did not record this unless it was on the Yellow Card (very few); mother's were not asked if their child had received Vitamin A in the previous 6 months – this was an oversight). Children in our study may have also eaten Vitamin A rich foods to contribute to this low level. It would take another national micronutrient survey to document if this is only occurring in our study area, or is nation wide.

3.9 Vitamin A Deficiency Conclusions:

Vitamin A deficiency as measured by serum retinol binding protein (RBP) in our study was surprisingly low at all age points. There were very small significant differences seen in mean RBP levels between intervention and control, but no difference in the prevalence of Vitamin A deficiency between groups at any time point. Hence, the national Vitamin A Supplementation programme in Cambodia should be continued together with giving Sprinkles containing a daily dose of Vitamin A.

3.10 Iron response

The overall mean ferritin was 40·3 ± 28·0 μg/L at baseline with no difference between groups. Iron deficiency, as measured by ferritin <12μg/L in the absence of infection (i.e. AGP>1g/L and CRP>5mg/L) was 10·1% at baseline and did not differ between groups (Table 4b). There was a significant difference in the prevalence of iron deficiency for the intervention group vs. controls at 12 months (66/349 (18·9%) vs. 165/386 (42·7%), p<0·001), and 18 months (75/346 (21·7%) vs. 124/360 (34·4%), p=0·005) but not at 24 months (26/286 (9·1%) vs. 36/308 (11·7%), p=0·647).

The risk of iron deficiency anaemia (IDA) as measured by Hb<100g/L and ferritin<12μg/L in the absence of infection was reduced for the intervention group at 12 months (IRR 0·30, 95% CI 0·18 to 0·52, p<0·001) and 18 months (IRR 0·45, 95% CI 0·02 to 0·23, p=0·018) but not at 24 months (IRR 0·70, 95% CI 0·29 to 1·72, p=0·44). The risk of non-IDA was also reduced at 12 months (IRR 0·50, 95% CI 0·33 to 0·76, p=0·001) but not at 18 months (IRR 1·11, 95% CI 0·67 to 1·83, p=0·672) or 24 months (IRR 0·76, 95% CI 0·43 to 1·34, p=0·341) (Figure 3).

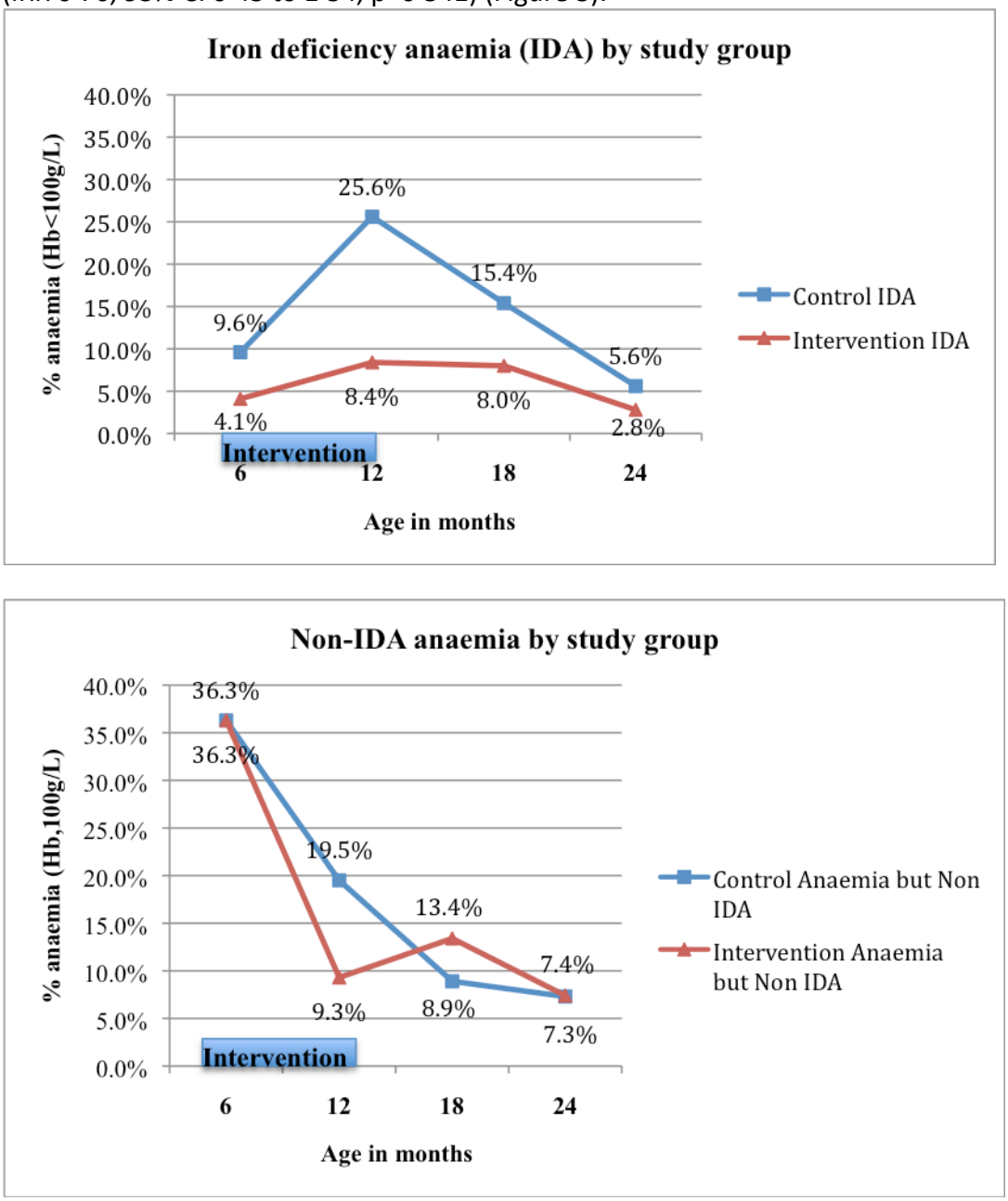


Figure 2: Iron deficiency anaemia (IDA) and non-IDA anaemia (Hb<100g/L) by study group

3.11 Iron Response Interpretation

There was a low prevalence of iron deficiency at 6 months of age (baseline, Table 4b), based on low serum ferritin values adjusted for infection. However, the prevalence of iron deficiency in both groups had increased by the age of 12 months. This age-related increase has been reported in other Sprinkles studies, in Cambodia[7] and elsewhere[17], and is attributed to increased iron requirements of the growing infant, and insufficient available iron from non-milk foods[18]. Importantly, the rise was significantly less in the intervention group compared to the controls, confirming a treatment effect of Sprinkles on serum ferritin with a 57% reduction in risk of iron deficiency (IRR 0·43, 95% CI 0·32 to 0·58, p<0·001). While much of the impact on anaemia at 12 months was due to a slowing in the rise of iron deficiency, this is the first Sprinkles study to show an effect on non-IDA for moderate anaemia (Figure 3). Of note, iron deficiency remained significantly lower at 18 months but not at 24 months in the intervention group, whereas the effect on non-IDA was only observed at 12 months and not at 18 or 24 months. Although there was a statistically significant difference (increase) in mean haemoglobin levels for IDA and non-IDA children for the intervention group compared to the control at endline (12 months) and first follow-up (18 months), the effect on non-IDA was not seen when we defined any anaemia based on Hb<110g/L, as the mean Hb was much lower than that cut-off point.

We used serum ferritin to detect iron deficiency in our population, as recommended by WHO[19]. Serum transferrin receptor, a biomarker known to be elevated in iron deficiency and unaffected by inflammation or infection[20], was elevated in the children with certain genetic haemoglobin disorders, consistent with reports elsewhere[21]. STfR is of limited use as a biomarker of tissue iron levels in our population[22]. In contrast, there was no significant difference in baseline serum ferritin according to genetic haemoglobin disorder (data not shown). We had hoped that by using sTfR, which is not affected by infection (unlike ferritin which is strongly affected by infection and therefore can give inaccurate results if you do not take infection into account) we would be able to clearly estimate the prevalence of iron deficiency. Unfortunately, for children with haemoglobinopathies or thalassemias, the sTfR results are not accurate and so, because 50% of our study children had Hb disorders, it was not possible to use sTfR.

3.12 Iron Response Conclusions:

Sprinkles had a large effect in reducing iron deficiency in our study that persisted through to age 18 months, but not through to 24 months. Most of the anaemia reduction was due to a reduction in iron deficiency. However, we also saw a smaller but significant reduction in moderate anaemia (Hb<100 g/L) not associated with iron deficiency, although only at 12 months (not lasting through to 18 or 24 months of age).

Serum transferrin receptor (sTfR) is not a useful marker for estimating the prevalence of iron deficiency in a population with a high proportion of genetic Hb disorders such as Cambodia.

3.13 Notes on adjusting for infection

Ferritin is an acute phase reactant (or acute phase protein) with the level of serum ferritin increasing (positive reactant) if the person has an infection. Because we use ferritin as a measure of iron deficiency, if the person has an infection at the time of measuring ferritin, it may be high due to infection even though the person is actually iron deficient, resulting in a misleading diagnosis. Indeed, iron deficiency may still be present with serum ferritin values up to 60-100μg/L. This is important for populations with expected high rates of infection (e.g. children in developing countries who are exposed to many pathogens) as, if you do not adjust for infection, your prevalence of iron deficiency may be lower than expected.

Retinol binding protein (for measuring Vitamin A status) and serum zinc are also acute phase reactants, but they both decrease (negative reactant) in the presence of infection. If we do not adjust for infection when measuring serum retinol and zinc, the prevalence of vitamin A and zinc deficiency would be misleadingly high i.e. it would appear that more of the population have vitamin A or zinc deficiency due to low serum retinol binding protein and low serum zinc levels than is the case. Instead, the high prevalence is due to the infection and does not reflect true deficiency states.

We used both CRP (C-reactive protein) and AGP (alpha-1 glycoprotein) to measure infection. CRP is the best measure for acute infection (it starts rising about 4 hours after an infection begins but then falls again after about 48 hours) and AGP is better to measure post-acute/recent infection as it begins to rise after about 6 hours, reaches its peak (highest point) at about 3 days and then decreases, although it still remains at a higher level than normal for the next 3-4 days. Therefore, by using both measures we could see which children were affected by acute infection and those recovering from infection (convalescent state). The WHO recommendation is to use at least one infection marker to interpret iron deficiency when using ferritin (either CRP or AGP [or another marker called ACT that not many people use]. Most studies only measure one marker, but other scientific literature (including WHO) recommend that measurement of both using both CRP [or ACT] and AGP, may help to interpret the changes as it will give a much better idea if the person has an acute infection or is recovering from an infection (i.e. is in a convalescent state), both of which may affect measures of iron deficiency (i.e. ferritin), vitamin A (i.e. retinol binding protein) and zinc (i.e. serum zinc). Consequently, in our study, we excluded children who had either a raised CRP or AGP (or both) from the analysis in order to give a more accurate or true estimate of the prevalence of iron, vitamin A and zinc deficiency. In some studies, they have a much higher proportion of subjects with an elevated CRP and/or AGP, so there are some scientists that use an 'adjustment' factor rather than excluding the subjects from the analysis. We needed to exclude

less than 10% of children in our study, and so still had adequate numbers of children to carry out the proper statistical tests.

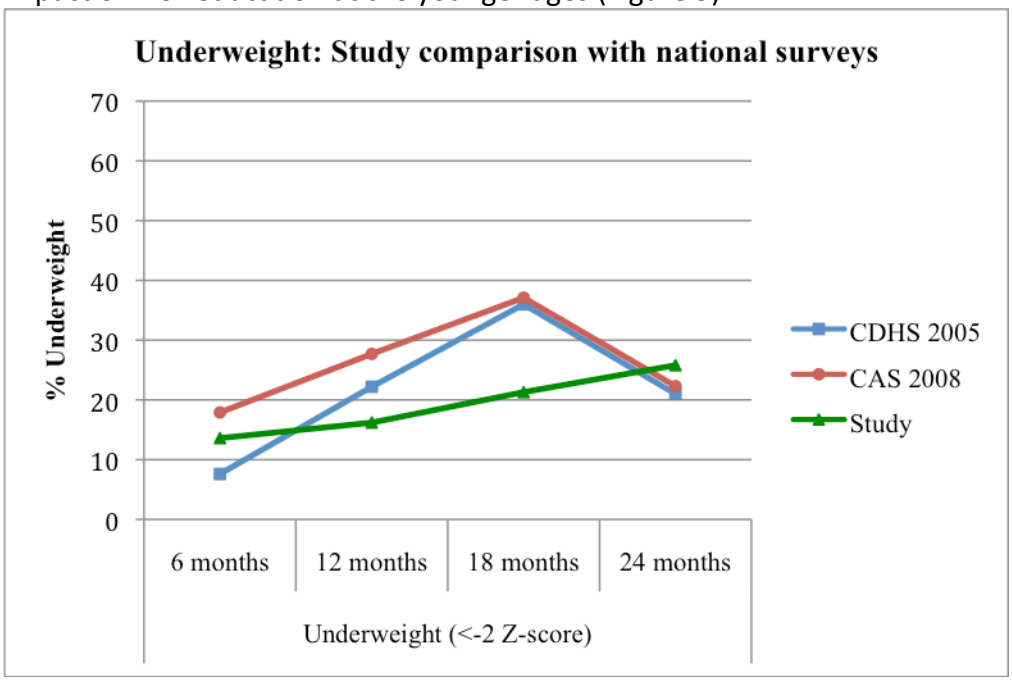
We also measured serum transferrin receptor (sTfR) which is a measure of tissue iron deficiency (sTfR levels are increased in iron deficiency) and is not affected by infection. This is also recommended as a possible measure of iron deficiency by WHO. However, research has shown that for people with a genetic haemoglobin disorder (haemoglobinopathy or thalassemia), the sTfR level may also increase due to an increase in erythropoetic activity (more blood cells being made because the abnormal blood cells do not have the normal length of life). Therefore, for populations with a high prevalence of genetic Hb disorders, sTfR is not an accurate test to use (it would give a much high prevalence of iron deficiency than there actually is). In our study, around 50% of the children had a genetic Hb disorder, so we could not use the sTfR test as too many children were affected.

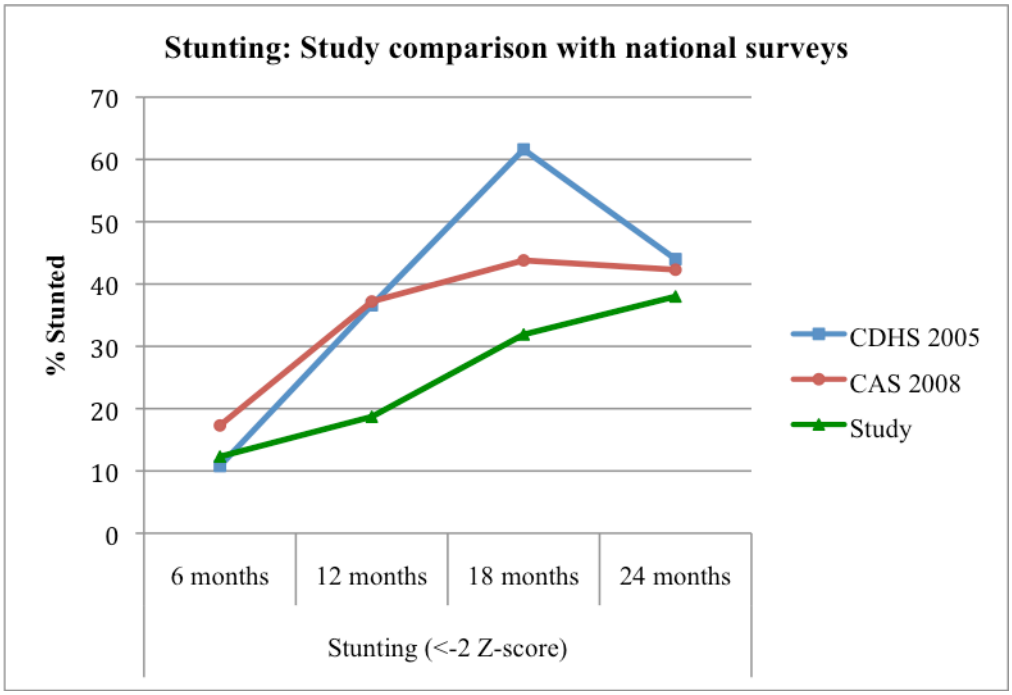
3.14 Anthropometry

There was no difference between intervention and control groups for any of the anthropometric indicators at any time point (Table 4c). However, when compared with national data, at 12 and 18 months there was a significant decrease in underweight ($p<0{\cdot}001$) and stunting ($p<0{\cdot}001$) (Figure 4).

3.15 Anthropometry Interpretation

The lack of positive growth response due to Sprinkles was disappointing. However, the trends to improved growth (slowing of stunting and underweight to 18 months of age), in both groups compared to national surveys[2, 29] suggests there was an impact of IYCF education at the younger ages (Figure 5).





CDHS: Cambodia Demographic & Health Survey, 2005
CAS: Cambodia Anthropometric Survey, 2008
Figure 3: Underweight and stunting comparisons with study (total).

3.16 Anthropometry/Growth Conclusions:

The comparison with national data suggests that there was an impact of IYCF education at the younger ages (up to 18 months) although there were no differences at 24 months. However, it must be noted that this finding was based on national data rather than Svay Rieng specific data as there were not enough children to perform the analysis on this subgroup alone.

More effort needs to go on IYCF education for the whole period (6-23 months and beyond) in order to reduce stunting and underweight in Cambodia. As noted from the qualitative TIPs survey on the IYCF guidelines, both the amount and quality of complementary food given to children at the various ages needs to be monitored and improved to ensure they consume adequate intakes of macro and micro-nutrients.

4 FINAL CONCLUSIONS

We have shown that home fortification with micronutrient Sprinkles along with IYCF education in rural Cambodia reduces anaemia and iron depletion compared to IYCF education alone. We achieved high adherence rates, confirming the acceptability of micronutrient powders found in other studies[8] and have shown that the program can be implemented through existing Government health structures.

Efficacy trials in Cambodia and elsewhere, have shown that Sprinkles are as efficacious as iron drops in reducing anaemia, with fewer side effects and better acceptance[8]. In a meta-analysis, anaemia risk (Hb<100g/L) was halved, although the effects on plasma zinc and retinol binding protein (a measure of vitamin A levels) were mixed[8].

This study provides clear evidence supporting the roll out of Sprinkles as a micronutrient intervention in Cambodia and similar settings. It also provides indirect evidence that Sprinkles interventions should be accompanied by IYCF education.

Because the observed reduction in anaemia was not sustained beyond the intervention period, a critical question is the optimal duration of Sprinkles implementation. Research indicates that the first two years of life is the period of greatest vulnerability[30], 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 until 24 months of age. It seems reasonable that such a policy be adopted immediately and monitored to confirm ongoing benefits of Sprinkles in children. It should also be possible, with widespread implementation of Sprinkles, to monitor the effect on growth and major infectious diseases in different settings.

Sprinkles	Study Composition
Fe (microencapsulated ferrous fumarate)	12·5mg
Zinc (gluconate)	10 mg
Vitamin A (retinol acetate)	300 μg
Iodine	90 μg
Vitamin B1	0·5 mg
Vitamin B2	0·5 mg
Vitamin B6	0·5 mg
Vitamin B12	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

Table 1: Nutrient composition of sprinkles

	Control (n = 582)	Intervention (n = 578)
Sex		
Female	275 (51·2%)	269 (50·4%)
Age (months)	6·16 ± 0·37	6·13 ± 0·34
Number of household members	5·35 ± 1·80	5·45 ± 1·88
Number of children under 5 year in household	1·30 ± 0·50	1·33 ± 0·53
Literate	43·4%	41·4%
Number of months pregnant at first ANC visit	5·26 ± 12·44	5·68 ± 13·08
Total number of ANC visits	5·84 ± 13·07	5·54 ± 11·76
Number of iron tablets consumed during pregnancy	72·42 ± 34·36	70·57 ± 39·05
Took Mebendazole during pregnancy	323 (62·2%)	282 (53·7%)
Still breastfeeding at baseline (age 6-7months)	512 (95·5%)	510 (96·0%)
How many times breastfed during previous 24 hours	10·30 ± 5·65	10·42 ± 5·51
Birth weight	3·03 ± 0·52	3·09 ± 0·60
Electricity (mains)	15·0%	17·4%
Television	64·4%	61·0%
Motorcycle/Moped/Scooter	56·1%	50·2%
Ownership of agricultural land	89·4%	89·3%
Markers of infection, iron & zinc status (mean ± SD)		
AGP (α-1 glycoprotein)	0·77 ± 0·22	0·77 ± 0·21
CRP (C-reactive protein)	2·96 ± 3·90	3·00 ± 4·08
sTfR (serum transferrin receptor)	9·26 ± 3·10	9·58 ± 3·69
RBP (retinol binding protein)	1·07 ± 0·23	1·08 ± 0·25
Ferritin	39·5 ± 27.2	41·1 ± 28·9
Zinc	9·7 ± 2·0	9·7 ± 2·1
Genetic Haemoglobin Disorders (%)	51·6%	49·4%
Anthropometry		
Weight (kg)	7·21 ± 1·97	7·15 ± 0·88
Length (cm)	65·51 ± 3·48	65·63 ± 3·04
Head Circumference (cm)	41·71 ± 1·83	41·68 ± 1·83
Mid Upper Arm Circumference (cm)	13·77 ± 1·17	13·79 ± 1·12
WAZ (Underweight <-2 Z-score)	14·7%	12·5%
LAZ (Stunting <-2 Z-score)	11·3%	13·3%
WLZ (Wasting <-2 Z-score)	5·1%	4·4%
BMI-for-age (<-2 Z-score)	6·8%	5·5%
Head-circumference-for-age (<-2 Z-score)	17·7%	16·3%
Mid-upper-arm-circumference (<-2 Z-score)	4.2%	4.4%

Data are number (%); mean ± SD; or n/N (%)

Table 2: Baseline household and participant characteristics

	Control (n/N (%))	Intervention (n/N (%))	Difference* (95% (CI))	IRR	p value**	ICC
Anaemia (Hb < 110 g/L)						
Baseline (6 months)	411/491 (83·7%)	410/486 (84·4%)	----	----	----	----
Endline (12 months)	410/484 (84·7%)	294/441 (66·7%)	20·6% (9·4 to 30·2)	0·76	0·001	0·000
Follow-up (18 months)	287/397 (72·3%)	231/361 (64·0%)	10·0% (-2·9 to 20·6)	0·86	0·120	0·000
Follow-up (24 months)	259/457 (56·7%)	219/443 (49·4%)	8·9% (-1·2 to 17·2)	0·84	0·082	0·000
Anaemia (Hb < 100 g/L)						
Baseline (6 months)	238/491 (48·5%)	218/486 (44·9%)	----	----	----	----
Endline (12 months)	229/484 (47·3%)	95/441 (21·5%)	27·1% (21·0 to 31·8)	0·43	<0·001	0.000
Follow-up (18 months)	109/397 (27·5%)	93/361 (25·8%)	3·3% (-5·4 to 9·7)	0·88	0·415	0.000
Follow-up (24 months)	68/457 (14·9%)	55/443 (12·4%)	-0·9% (-11·3 to 9·4)	0·88	0·515	0.000
*Difference is the IRR (Incidence Rate Ratio) for intervention vs. control, adjusting for anaemia at baseline						
Mean Haemoglobin (g/L)	**(mean ± SD)**	**(mean ± SD)**				
Baseline (6 months)	99·8 ± 9·3	100·8 ± 8·9	----		----	----
Endline (12 months)	100·0 ± 10·0	105·8 ± 8·8	6·06 (4·7 to 7·4)		< 0·001	0.016
Follow-up 1 (18 months)	104·2 ± 9.9	105·8 ± 9·0	2·20 (0·8 to 3·7)		0·003	0.014
Follow-up 2 (24 months)	107·9 ± 8·8	109·0 ± 8·8	1·05 (-0·1 to 2·2)		0·076	0.002
*Mean Difference Between Intervention Groups (with 95% Confidence Intervals) adjusting for cluster design						

** p value for Comparison Between Intervention Groups, adjusting for Baseline (6 months) value

Table 3: Primary Outcomes: Anaemia and Mean Haemoglobin

	Control (mean ± SD)	Intervention (mean ± SD)	Difference* (95% CI)	p value**
Zinc (µmol/L)				
Baseline (6 months)	9·7 ± 2·0	9·7 ± 2·1	----	----
Endline (12 months)	9·6 ± 1·7	10·1 ± 2·4	0·44 (0·04 to 0·83)	0·028
Follow-up (18 months)	9·7 ± 2·3	9·9 ± 1·8	0·09 (-0·22 to 0·41)	0·560
Follow-up (24 months)	9·7 ± 1·6	9·6 ± 1·9	-0·02 (-0·39 to 0·34)	0·893
Retinol Binding Protein (µmol/L)				
Baseline (6 months)	1·1 ± 0·2	1·1 ± 0·2	----	----
Endline (12 months)	1·1 ± 0·2	1·2 ± 0·3	0·07 (0·03 to 0·11)	< 0·001
Follow-up (18 months)	1·2 ± 0·2	1·2 ± 0·3	0·05 (0·01 to 0·09)	0·022
Follow-up (24 months)	1·2 ± 0·3	1·2 ± 0·3	0·01 (-0·10 to 0·07)	0·724
Ferritin (µg/L)				
Baseline (6 months)	39·5 ± 27·2	41·1 ± 28·9	----	----
Endline (12 months)	19·1 ± 17·3	29·0 ± 21·2	10·4 (6·3 to 14·5)	< 0·001
Follow-up 1 (18 months)	21·0 ±14·8	25·8 ±16·7	5·5 (1·4 to 9·5)	0·008
Follow-up 2 (24 months)	33·1 ± 17·7	33·7 ± 17·2	0·3 (-3·3 to 4·0)	0·854

* Mean Difference Between Interventions Groups (with 95% Confidence Intervals), adjusting for infection by excluding cases with AGP>1g/L and CRP>5mg/L)

**p value for Comparison Between Intervention Groups, adjusting for Baseline (6 months) value

Table 4a: Secondary Outcomes

	Control (n/N (%))	Intervention (n/N (%))	Difference (95% CI)	IRR* (95% CI)	p value**
Iron depletion (ferritin < 12mg/l)					
Baseline (6months)	47/381 (12·3%)	35/380 (9·2%)		----	----
Endline (12months)	165/386 (42·7%)	66/349 (18·9%)	24·3% (17·7 to 29·1)	0·43 (0·32 to 0·58)	< 0·001
Follow-up (18 months)	124/360 (34·4%)	75/346 (21·7%)	12·4% (4·4 to 18·2)	0·63 (0·47 to 0·87)	0·005
Follow-up (24 months)	36/308 (11·7%)	26/286 (9·1%)	1·3% (-5·9 to 5·7)	0·88 (0·52 to 1·50)	0·647
Zinc deficiency (Zn<9.9µmol/L)					
Baseline (6months)	223/370 (60·3%)	217/370 (58·7%)		----	----
Endline (12months)	237/383 (61·9%)	186/342 (54·4%)	5·2% (-7·6 to 15·7)	0·92 (0·7 to 1·1)	0·396
Follow-up (18 months)	212/359 (59·1%)	187/337 (55·5%)	4·0% (-8·9 to 14·5)	0·93 (0·8 to 1·2)	0·513
Follow-up (24 months)	179/313 (57·2%)	167/290 (57·6%)	1·6% (-13·1 to 13·2)	0·99 (0·8 to 1·2)	0·945
Vitamin A deficiency (RBP <0.7µmol/L)					
Baseline (6months)	8/384 (2·1%)	4/380 (1·1%)		----	----
Endline (12months)	5/388 (1·3%)	2/349 (0·6%)	0·3% (-4·7 to 1·1)	0·77 (0·12 to 4·6)	0·773
Follow-up 1 (18months)	4/363 (1·1%)	5/346 (1·4%)	-0·4% (-5·7 to 0·8)	1·38 (0·31 to 6·16)	0·616
Follow-up 2 (24months)	9/314 (2·9%)	10/291 (3·4%)	-0·7% (-8·7 to 1·8)	1·24 (0·39 to 4·03)	0·695

* Mean Difference Between Interventions Groups (with 95% Confidence Intervals)

** p-value for Comparison Between Intervention Groups, adjusting for Baseline (6 months) value and infection (excluding cases with AGP>1g/L or CRP>5mg/L)

Table 4b: Secondary Outcomes

Anthropometry	Control (n/N (%))	Intervention (n/N (%))	Difference* (95% CI)	p value**
Weight-for-age (Underweight <-2 Z-scores)				
Baseline (6 months)	78/529 (14·7%)	66/528 (12·5%)	----	----
Endline (12 months)	79/498 (15·9%)	83/504 (16·5%)	-2·4% (-6·1 to 1·2)	0·190
Follow-up 1 (18months)	100/492 (20·3%)	107/481 (22·2%)	-2·8% (-7·4 to 1·8)	0·234
Follow-up 2 (24months)	139/499 (27·9%)	116/490 (23·7%)	3·2% (-2·7 to 9·1)	0·283
Length-for-age (Stunting <-2 Z-scores)				
Baseline (6 months)	60/530 (11·3%)	70/528 (13·3%)	----	----
Endline (12 months)	95/498 (19·1%)	92/504 (18·3%)	3·5% (-0·7 to 7·8)	0·105
Follow-up 1 (18 months)	146/492 (29·7%)	164/481 (34·1%)	-2·2% (-7·6 to 3·3)	0·437
Follow-up 2 (24 months)	196/499 (39·3%)	180/490 (36·7%)	0.7% (-5·9 to 7·4)	0·829
Weight-for-length (Wasting <-2 Z-scores)				
Baseline (6 months)	27/528 (5·1%)	23/526 (4·4%)	----	----
Endline (12 months)	38/498 (7·6%)	36/504 (7·1%)	-0·3% (-3·5 to 2·9)	0·842
Follow-up 1 (18 months)	39/492 (7·9%)	30/481 (6·2%)	1·4% (-2·2 to 5·1)	0·434
Follow-up 2 (24 months)	24/499 (4·8%)	28/490 (5·7%)	-0·2% (-3·2 to 2·6)	0·850

* Mean Difference Between Intervention Groups (with 95% Confidence Intervals)

** p value for Comparison Between Intervention Groups, adjusting for Baseline (6 months) value

Table 4c: Secondary Outcomes

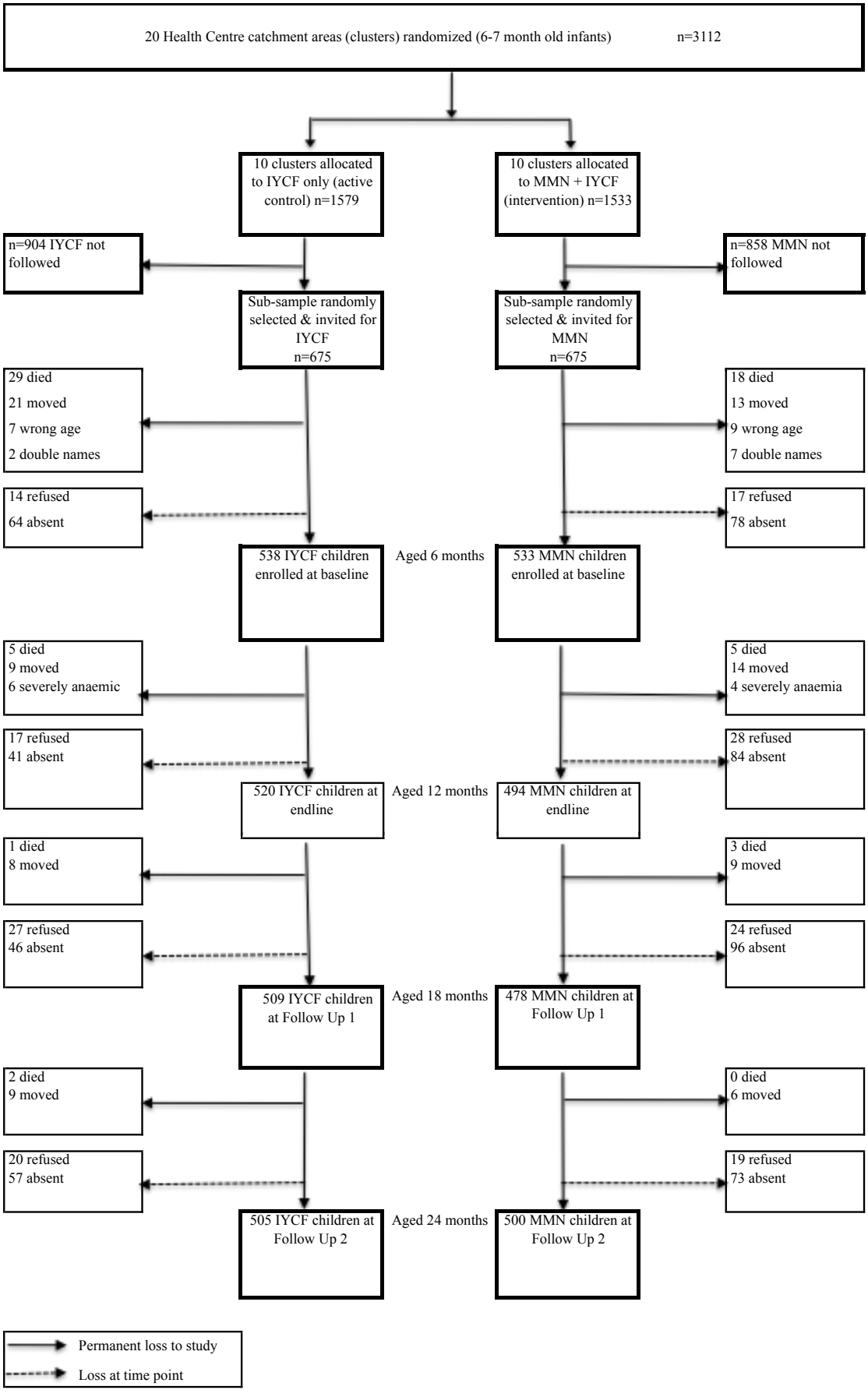


Figure 4: Sub-sample profile

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