BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-013192
Article Type:	Research
Date Submitted by the Author:	25-Jun-2016
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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Global health, Paediatrics
Keywords:	Nutrition < TROPICAL MEDICINE, Community child health < PAEDIATRICS, Geographical mapping < TROPICAL MEDICINE

Impact of iron fortification on the geo-spatial patterns of infection risk among young children in rural Ghana

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Keywords
Spatial analysis, infection, child health, GIS, nutrition

Word count: 5,714 (including tables and references)

Abstract

Objectives: Patterns of infection among children with varying levels of iron status in a malaria endemic area may vary spatially in ways requiring integrated infection and iron deficiency control programs. The objective of this secondary analysis was to determine the geo-spatial factors associated with malaria and non-malaria infection status among young Ghanaian children at the end of a 5-month iron intervention trial.

Design: Cluster-randomised controlled trial

Setting: Rural Ghana

Participants: 1943 children (6-35 months of age) with geocoded compounds.

Interventions: Point-of-use fortification with micronutrient powders containing vitamins and minerals with or without iron.

Primary and secondary outcome measures: Generalized linear geostatistical models with a Matern spatial correlation function were used to analyse four infection response variables, defined using different combinations of inflammation (C-reactive protein, CRP >5 mg/L) and malaria parasitaemia. Analyses were also stratified by treatment group to assess the independent effects of the iron intervention.

Results: The by-group and combined-group analyses both showed that baseline infection status was the most consistent predictor of endline infection risk, particularly when infection was defined using parasitaemia. In the No-iron group, age above 24 months and weight-for-length z-score at baseline were associated with high CRP at endline. Higher asset score was associated with a 12% decreased odds of endline infection, defined as CRP >5 mg/L and/or parasitaemia (OR 0.88, 95% CrI 0.78, 0.98), regardless of group. Maps of the predicted risk and spatial random effects showed a defined low-risk area around the District centre, regardless of how infection was defined.

Conclusions: In a clinical trial setting of iron fortification, where all children receive bed nets and access to malaria treatment, there may be predictable geographical variation in the risk of infection with distinct high- and low-risk areas, particularly around municipal centres.

Trial registration: clinicaltrials.gov, NCT01001871.

Strengths and limitations of this study

- The geostatistical analyses conducted in this study are the first of their kind to use model-based geostatistics with Bayesian inference and integrated nested Laplace approximations (INLA) to explore the spatial variation and associated risk factors of malaria and non-malaria infection risk among children in rural Ghana after a 5-month randomized iron intervention trial.
- These analyses also provide input into the potential utility of geographical indicators, particularly for assessing infection risk potential, which could help guide the implementation of iron interventions in areas where infectious diseases are prevalent.
- Since satellite-derived spatial data often vary at a higher level than the individual (e.g. village or region), the use of these data in statistical models with individual-level outcomes may increase the risk of a change of support problem.
- The use of C-reactive protein (CRP) as an indicator of non-malaria infection may have led to the underestimation of this outcome, since CRP is an acute phase protein that only rises in accordance with the first 48 hours of the inflammatory response.
- Straight-line distance, rather than distance by road, was used to estimate proximity to a health facility or the district centre, and thus may not have fully accounted for travel impedance.

Background

The leading causes of death in children less than 5 years of age are infection-related, and child mortality rates are highest in low- and middle-income countries (LMICs).[1] Malnutrition is also a large contributor to mortality (45% of all deaths),[1] including micronutrient deficiencies. The most common nutritional disorder worldwide is iron deficiency,[2] which is estimated to account for 2.2 million disability-adjusted life years lost per year among children less than five years of age.[3] While iron deficient children are more vulnerable to infections (primarily due to compromised immune function),[4, 5] infection and inflammation can also affect iron homeostasis[6] and the risk of iron deficiency.[7] Iron status can usually be improved through food fortification, or supplementation; however, evidence from a large randomized trial

conducted in a malaria endemic area (Pemba, Zanzibar) indicated that supplementing young children with iron may increase their risk of malaria and infection-related morbidity and mortality, particularly if they are iron replete.[8] Since iron supplements or fortificants would likely be withheld from children with malaria or other infections, assessing the risk of infection is an important component of developing safe and effective means of administering iron to children in LMICs.

Measuring biomarkers of infection status (such as C-reactive protein) among children in a low-resource context may have limited feasibility, due to the requirement of blood samples, and the potential for inflammation prevalence to vary over relatively short periods of time (e.g. within seasons). As such, there is a need to identify indicators or risk factors associated with infection in LMICs that are more feasible to measure, and thus provide an efficient means of identifying high-risk populations. This need could be addressed with geographical factors (or "geo-indicators"), such as the environmental or spatial characteristics of a village or region.[9] Geo-indicators could provide additional insight into the dynamics and distribution of infection among children that informs treatment needs and the prophylactic use of iron supplementation or fortification.[10-13] Collecting geo-spatial data is not invasive and comparatively less costly than biological measures, as geographical datasets are often publicly available on the internet. This also improves the access to and comparability of population-level statistics within and across national borders.

We previously conducted a secondary spatial analysis to determine the geo-spatial factors associated with infection status among iron deficient and sufficient children in rural Ghana (Aimone, Brown, Zlotkin, Cole, and Owusu-Agyei 2016). The results of these analyses suggested that the risk of infection may be related to elevation, and distance to the nearest health facility. The objective of the current analysis was to determine the geo-spatial factors associated with malaria and non-malaria infection status among Ghanaian children at the end of a 5-month randomized iron intervention trial.

Methods

Study population

The data used in these analyses were generated from a study population of young children (6-35 months of age) who participated in a community-based cluster-randomized trial conducted in the Brong-Ahafo Region of Ghana in 2010.[14] At the time of the trial, the estimated incidence of malaria in Ghana was 7.2 million cases per year, and the prevalence of anaemia among preschool aged children was 76.1% (95% CI 73.9-78.2%).[15, 16] Details of the clinical trial, [14] and geographical layout of the study area have been described elsewhere (Aimone, Brown, Zlotkin, Cole, Owusu-Agyei 2016).

Measures from trial data

Biological samples collected at baseline and endline were analysed for plasma ferritin (Spectro Ferritin S-22, Ramco Laboratories Inc., Stafford, USA), C-reactive protein (QuickRead CRP, Orion Diagnostica, Espoo, Finland) , and malaria parasite density (microscopy).[14]. Malaria screening was also performed on a weekly basis throughout the intervention period. If a child had a history of fever (within 48 hours) or an axillary temperature >37.5^{0}C, a blood sample was drawn and analysed in the field via antigen test (Paracheck Pf®), and in the lab using microscopy (thin and thick smears). Parasite density was combined with fever information to calculate clinical malaria incidence (episode counts). Demographic and nutrition-related information was also collected at baseline at and included household assets, maternal education, and child body weight and length. Weight-for-length and length-for-age z-scores were calculated using the WHO Child Growth Standards.[17]

Independent variable preparation

Geographical coordinates for the compounds of 1943 trial participants (representing 1539 clusters), surrounding health facilities and major road networks were collected using handheld global positioning system (GPS) units (WGS 1984 coordinate system, universal transverse Mercator zone 30N projection, EPSG code: 32630). Satellite-derived data were downloaded as global datasets[18-20] and cropped according to the geographical boundaries of the trial area. Elevation had a range of 116-530 meters, and values were centred by subtracting 250. Land cover type (LC) consisted of 3 categorical values, representing woody savannah (LC=8, n=21/1943 observations), urban and built up land (LC=13, n=243/1943 observations), and cropland/natural vegetation mosaic (LC=14, n=1679/1943 observations). NDVI, included as a proxy for soil moisture,[21] was averaged over the year that the trial was conducted (2010), and ranged in value from 0.22 to 0.62. Two NDVI-LC interaction terms were created (NDVI*LC8 and NDVI*LC13) by overlaying the final NDVI and LC rasters, and masking the LC cells except where they had a value of 8 (or 13). These unmasked cells were given a value of zero.

Baseline age, in months, was calculated using the reported date of birth and trial enrolment date with a change point at 24 months. Household asset score was generated using a principal component analysis of 6 economic indicators (farm ownership, size and type of crops grown, type of toilet facility, and house ownership). Maternal education was included as a binary variable representing “no” (0) versus “any” (1) level of education. Baseline iron status was defined as serum ferritin concentration corrected for inflammation (baseline CRP) using a regression-based method (Namaste, Rohner, Suchdev, Kupka, Mei, Bhushan, Williams, Rowat, Raiten, Flores-Ayala, Clewes, 2016), and re-scaled by multiplying the corrected values by the inter-quartile range. Straight-line (Euclidean) distance to the nearest health facility was measured using the Near Table tool in ArcMap (ArcGIS 10.2, Environmental Systems Resource Institute, Redlands, California).

Spatial modelling

The data were analysed using generalized linear geostatistical models (GLGM) [22, 23] with Bayesian inference via an Integrated Nested Laplace Approximation (INLA) algorithm.[24] Weak or uninformative priors were used for all model parameters with the exception of the Matern shape parameter (fixed at 2). Four different endline infection outcomes were modelled separately: 1) inflammation (CRP >5 mg/L) with/without malaria parasitaemia; 2) inflammation (CRP >5 mg/L) without parasitaemia; 3) parasitaemia with measured concurrent fever (axillary temperature $>37.5^0$C) or reported history of fever within 48 hours (i.e. clinical malaria)); and 4) parasitaemia with or without concurrent fever or history of fever. All variables were binary-valued (coded as '1' for positive infection status at endline) and analysed using logistic regression, with the exception of the third definition (parasitaemia with fever), which was a count variable (number of new clinical malaria episodes during the intervention period) analysed using Poisson regression. Median infection probabilities (and 95% credible intervals) were modelled as the sum of the contributions of the independent variables, residual spatial variation, and a compound-level random effect term. The *glgm* function from the "geostatsp" package in R was used for all spatial modelling.[25, 26]

The spatial analyses were conducted in three modelling steps: 1) No-iron group only; 2) Iron group only; 3) both intervention groups combined. The interventions groups were analysed separately in order to differentiate the effects of time and the iron treatment. Infection probabilities from the combined-group models were plotted on a base map of the trial area, with study compounds, and major road networks. The maps depicted a spatial risk surface of predicted infection probabilities, which were computed as the posterior means of the odds or risk of infection. The posterior means were estimated assuming baseline values for individual-level covariates and location-specific values for spatial covariates. The posterior mean of the spatial random effect was also plotted, representing the residual spatial variation that corresponded to the difference between the predicted and expected odds or risk of infection at each location.

Ethics

The original clinical trial was approved by the Kintampo Health Research Centre (KHRC) Institutional Ethics Committee, the Ghana Health Service (GHS) Ethical Review Committee, the Hospital for Sick Children Research Ethics Board, and the Food and Drugs Authority of Ghana. Approval for conducting the secondary analyses was obtained from the Hospital for Sick Children and University of Toronto Health Sciences Research Ethics Boards.

Results

Baseline and endline characteristics of the study sample are presented in **Table 1**. A total of 1780 trial participants were included in the endline analyses, representing those with geocoded residences (compounds), who provided blood samples at endline, and had both baseline and endline CRP and parasitaemia values (see online supplementary file Figure S1). For the infection outcome defined as parasitaemia with fever, there were 1939 observations included in the Poisson regression analysis, which corresponded to the number of children with geocoded compounds who had at least one recorded follow-up visit during the intervention period, and thus contributed data to the malaria count outcome.

The proportion of children with evidence of infection (elevated CRP and/or parasitaemia) increased from baseline to endline (37.0% at baseline versus 41.6% at endline). This increase may have been driven primarily by a greater prevalence of parasitaemia at endline (27.1% versus 23.0% at baseline), though the difference between intervention groups was small (26.3% versus 28.0% in the No-iron and Iron groups, respectively). The prevalence of non-malaria inflammation (CRP>5 mg/L without parasitaemia) remained relatively stable over the course of the trial; however, the proportion in the Iron group was slightly higher at endline (7.64% in the Iron group versus 6.58% in the No-iron group). After correcting ferritin concentration for inflammation (CRP) using the regression method, the prevalence of iron deficiency (ferritin <12 µg/L) was 25.5% (496/1943) at baseline and 12.7% (226/1781) at endline (95/886 in the Iron group, 131/895 in the No-iron group).

Table 1: Baseline and endline characteristics of the Ghana trial participants

	Baseline	Endline
Trial participants with a geocoded residence (n)	1943	1780
Males (%)	992 (51.1)	900 (50.5)
Age at enrolment (months), mean (SD)[a]	19.2 (8.5)	19.3 (8.5)
Serum ferritin (µg/L), geometric mean (SD)	35.1 (3.65)	73.9 (3.58)
Parasite density (count/µL), geometric mean (SD)	3003.0 (5.35)	4160.4 (6.30)
C-reactive protein (mg/L), mean (SD)	3.34 (4.96)	3.86 (5.10)
Infection status		
Inflammation without parasitaemia[b], n (%)	272 (14.0)	258 (14.5)
Inflammation and/or parasitaemia[c], n (%)	719 (37.0)	741 (41.6)
Parasitaemia with fever[dǂ], n (%)	150 (7.72)	555 (28.6)
All parasitaemia[e], n (%)	447 (23.0)	483 (27.1)
Anthropometric status[f]		
Weight-for-length z-score, mean (SD)	-0.63 (0.97)	-0.62 (0.97)
Length-for-age z-score, mean (SD)	-0.81 (1.21)	-0.80 (1.29)
Maternal Education, n (%)[g]		
None	586 (33.5)	543 (33.0)
Any	1166 (66.5)	1105 (67.0)
Household Asset Score, n (%)[h]		
Low	900 (49.0)	817 (47.7)
High	938 (51.0)	897 (52.3)

[ǂ]Endline value represents the period prevalence for 1939 participants
[a]Measured at baseline only
[b]Inflammation without parasitaemia (n=1780) = CRP > 5 mg/L without malaria parasitaemia;
[c]Inflammation and/or parasitaemia (n=1780) = CRP > 5 mg/L and/or any malaria parasitaemia;
[d]Parasitaemia with fever (n=1939) = any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
[e]All parasitaemia (n=1780) = any malaria parasitaemia (with/without fever)
[f]Measured at baseline; z-scores estimated using the WHO Child Growth Standards [23]
[g] Measured at baseline only; total n=1752 (74 respondents were not mothers, 117 missing due to incomplete surveys)
[h] Measured at baseline only; Low= below median, High = above median; reduced sample size (approximately 1825) due to incomplete surveys and "unknown" responses

Results from the by-group analyses indicated that the risk of elevated CRP (without parasitaemia) at endline among children in the No-iron group was positively associated with baseline weight-for-length z-score (OR 1.30, 95% credible interval (CrI) 1.06, 1.60), and negatively associated with age between 24 and 36 months (OR 0.92, 95% CrI 0.84, 1.006). When infection was defined as all parasitaemia, baseline infection status became a significant factor (OR for definition 4: 2.86, 95% CrI 1.97, 4.17), indicating that children in the No-iron

group with any parasitaemia (with or without CRP or fever) at baseline were up to 186% more likely to have parasitaemia at endline (see online supplementary file Table S1). In the Iron group, when infection was defined as inflammation and/or parasitaemia, baseline infection status was the only factor associated with infection risk at endline (OR 2.27, 95% CrI 1.66, 3.11). Similar results were found when infection was defined as all parasitaemia (OR 2.54, 95% CrI 1.75, 3.68) (see online supplementary file Table S2).

In the combined-group analyses, the risk of elevated CRP and/or parasitaemia at endline was negatively associated with household asset score, and positively associated with baseline infection status (OR for definition 1: 0.88, 95% CrI 0.78, 0.98) (**Table 2**). Similar to the Iron group analyses, baseline infection status was significantly and positively associated with the corresponding endline outcome when defined as inflammation and/or parasitaemia (OR 1.84, 95% CrI 1.36, 2.50) or all parasitaemia (2.75, 95% CrI 1.91, 3.95).

Table 2: Geo-spatial and non-spatial risk factors of endline infection status[1] among Ghanaian children in the *Iron* and *No-iron* groups (Brong-Ahafo Region, Sept-Nov 2010)

Covariates	Estimate (95% CrI) on the exponential scale[2]		Range parameter in km (95% CrI)	Standard deviations of random effects (95% CrI)	
				Spatial	Compound
Outcome 1: Inflammation and/or parasitaemia (n=1780)					
Intercept	0.589	(0.337, 1.072)	7.341	0.525	0.008
Age per month			(2.928,	(0.306,	(0.004,
6-23 months	1.004	(0.984, 1.026)	15.84)	0.944)	0.029)
24-35 months	1.000	(0.962, 1.038)			
Sex (male reference)	1.087	(0.886, 1.333)			
Length-for-age z-score	0.982	(0.896, 1.075)			
Weight-for-length z-score	1.088	(0.978, 1.211)			
Asset score	0.875	(0.779, 0.980)*			
Distance to health facility (km)	1.034	(0.933, 1.136)			
Elevation (m)	0.996	(0.991, 1.001)			
Baseline infection status	1.843	(1.359, 2.500)*			
Group	1.094	(0.826, 1.448)			
Baseline iron status	1.068	(0.956, 1.194)			
Baseline infection status*Group	1.192	(0.775, 1.833)			
Baseline iron status*Group	0.983	(0.847, 1.144)			
Outcome 2: Inflammation without parasitaemia (n=1780)					
Intercept	0.183	(0.106, 0.309)*	10.77	0.407	0.007
Age per month			(8.767,	(0.208,	(0.004,
6-23 months	1.009	(0.982, 1.038)	25.54)	0.830)	0.028)
24-35 months	0.956	(0.904, 1.009)			
Sex (male reference)	1.139	(0.865, 1.502)			
Length-for-age z-score	1.092	(0.968, 1.230)			
Weight-for-length z-score	1.054	(0.912, 1.217)			
Asset score	0.871	(0.750, 1.010)			
Distance to health facility (km)	1.012	(0.924, 1.093)			
Elevation (m)	0.999	(0.994, 1.003)			
Baseline infection status	1.055	(0.574, 1.826)			
Group	1.009	(0.713, 1.427)			
Baseline iron status	1.001	(0.844, 1.156)			
Baseline infection status*Group	1.523	(0.718, 3.300)			
Baseline iron status*Group	1.033	(0.856, 1.261)			
Outcome 3: Parasitaemia with fever (n=1939)					
Intercept	0.003	(0.002, 0.004)*	7.365	0.464	0.007
Age per month			(3.225,	(0.273,	(0.004,
6-23 months	1.008	(0.991, 1.025)	15.18)	0.825)	0.028)
24-35 months	0.978	(0.948, 1.008)			

Sex (male reference)	0.939	(0.799, 1.104)			
Length-for-age z-score	0.992	(0.923, 1.064)			
Weight-for-length z-score	0.966	(0.886, 1.053)			
Asset score	1.029	(0.937, 1.130)			
Distance to health facility (km)	1.057	(0.972, 1.149)			
Elevation (m)	0.997	(0.993, 1.002)			
Group	0.891	(0.729, 1.087)			
Baseline infection status	0.730	(0.452, 1.114)			
Baseline iron status	1.028	(0.942, 1.112)			
Baseline infection status*Group	1.209	(0.639, 2.282)			
Baseline iron status*Group	0.954	(0.849, 1.069)			
Outcome 4: All parasitaemia (n=1780)					
Intercept	0.271	(0.135, 0.571)*	7.846	0.712	0.008
Age per month			(3.384,	(0.406,	(0.004,
6-23 months	0.993	(0.966, 1.021)	16.06)	1.291)	0.029)
24-35 months	1.022	(0.994, 1.050)			
Sex (male reference)	1.024	(0.814, 1.287)			
Length-for-age z-score	0.926	(0.835, 1.026)			
Weight-for-length z-score	1.068	(0.947, 1.204)			
Asset score	0.916	(0.802, 1.044)			
Distance to health facility (km)	1.022	(0.902, 1.164)			
Elevation (m)	0.996	(0.990, 1.003)			
Baseline infection status	2.746	(1.910, 3.951)*			
Group	1.169	(0.862, 1.587)			
Baseline iron status	1.061	(0.940, 1.193)			
Baseline infection status*Group	0.915	(0.555, 1.508)			
Baseline iron status*Group	0.980	(0.839, 1.145)			

[1]Infection status definitions:
Outcome 1: Inflammation and/or parasitaemia (binary): 1 = CRP > 5 mg/L and/or any malaria parasitaemia, 0 = CRP ≤ 5 mg/L and absence of parasitaemia;
Outcome 2: Inflammation without parasitaemia (binary): 1 = CRP > 5 mg/L without malaria parasitaemia, 0 = CRP ≤ 5 mg/L without parasitaemia;
Outcome 3: Parasitaemia with fever (count): any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
Outcome 4: All parasitaemia (binary): 1 = any malaria parasitaemia (with/without fever), 0 = absence of parasitaemia
Model prior shape = 1.117, model prior rate = 0.157
[2]Exponentials of posterior medians and 2.5% and 97.5% posterior quantiles of model parameters, with a value of 1.05 indicating that a variable increases the risk of infection by 5% (odds ratio from logistic model for all binary outcomes; relative risk from Poisson model for count outcome)
CrI = credible interval
Baseline infection status = baseline infection status (yes/no) according to corresponding definition
Baseline iron status = baseline serum ferritin concentration (µg/dL) corrected for CRP using the regression method (Namaste et al.) and re-scaled by multiplying by the inverse of the inter-quartile range
*Statistical significance at the 0.05 level

Plots of the predicted risk and residual spatial variation from all combined-group models are illustrated in **Figure 1**. The maps show a defined low-risk area around the District centre, regardless of how infection was defined. Conversely, the location of high-risk areas seemed to vary across models and, in some cases, roughly approximated elevation (**Figure 2**). The maps depicting residual spatial variation also show defined high- and low-risk areas, indicating that a large amount of spatial variation was not explained by the variables included in the analyses and further supported the significant spatial random effects observed across all models.

Discussion

The analyses presented herein explored the geo-spatial variation and associated factors of infection, defined using both inflammatory and parasitic biomarkers, among children in a malaria endemic area (rural Ghana) after a randomized iron intervention trial. Although none of the spatial variables included in the by-group or combined-group models demonstrated significant associations with endline infection status (regardless of how infection was defined), the plots of predicted infection probabilities and spatial random effects showed defined high- and low-risk areas across the study region, particularly around the District centre. In terms of individual-level effects, both the by-group and combined-group analyses showed that baseline infection status was a consistent predictor of endline infection risk, particularly when infection was defined using parasitaemia (with or without inflammation).

The consistency of the relationship with baseline infection status across intervention groups is particularly relevant, as current evidence might lead one to expect that providing iron to children with malaria parasitaemia would increase their risk of subsequent parasitic infections to a greater extent than those who do not receive iron.[8] Another factor to consider is that all children in both intervention groups were monitored and treated for identified clinical malaria episodes (parasitaemia plus fever) throughout the trial. As shown in Table 1, most parasitaemia at baseline was asymptomatic (without fever), which may have reflected chronic infection or treatment

failure of a previous infection. In either case, these asymptomatic parasitic infections would not have been treated as clinical malaria episodes, and thus may have persisted throughout the intervention period of the trial, regardless of whether iron was received.

After stratifying the analyses by group, we found that children in the No-iron group with a higher weight-for-length z-score (a measure of nutritional status) at baseline were 30% more likely to have high CRP (>5 mg/L) at endline (OR 1.30, 95% CrI 1.06, 1.60) (Table S1). This positive association was not expected, given that well-nourished children (those with higher weight-length z-scores) are expected have a lower risk of infection.[4] Since CRP becomes elevated during the first 48 hours of the inflammatory response,[27] it is unlikely that anthropometric measures at baseline influenced inflammatory status 5 months later at endline. Rather, it is more likely that the association was influenced through another unmeasured factor, such as immune function. High CRP was also inversely associated with age in the No-iron group for children between 24 and 36 months (OR 0.92, 95% CrI (0.84, 1.00)), indicating an 8% lower odds of elevated CRP at endline for every month of age older. Assuming that elevated CRP without parasitaemia represented acute non-malaria infection, it is plausible that the inverse relationship reflected the development of the immune system with age, thus resulting in older children being more resilient to infection exposure.

In the combined-group models (**Table 2**), higher asset score was associated with a 12% decreased odds of endline infection, defined as CRP >5 mg/L and/or parasitaemia (OR 0.88, 95% CrI 0.78, 0.98), suggesting that children from wealthier households had a lower risk of malaria or non-malaria infection at endline (regardless of whether they received MNPs with or without iron). This finding was not surprising given the well-documented association between household wealth and child health in low- and middle-income settings;[28] however, the inconsistency of this relationship across infection definitions may have been a reflection of other socioeconomic or behavioural factors that were not included in the analyses.[29] Despite any differences in (or lack of) variable associations across infection outcomes, the residual spatial variation in infection risk remained significant in all combined-group models. The importance of

accounting for residual spatial variation was further illustrated when the spatial random effects were plotted. For example, when infection was defined using CRP only, the model output plots looked similar (**Figure 1B**); suggesting that the spatial random effect encompassed a large amount of the variation in predicted risk, while the variables included in the model did not.

For the infection outcomes that included parasitaemia, we observed similarities between the geographical variation in predicted mean risk and elevation (**Figure 2**). This finding is consistent with other studies conducted in malaria endemic areas showing a higher prevalence of malaria among populations living at lower elevations and vice versa.[30-33] The association between malaria and elevation is related to temperature, as the early stages of parasite development are inhibited in colder environments, which are found at higher altitudes.[34] The model output plots also demonstrated larger low-risk areas around villages, and particularly around the District centre. Other studies have also shown that living in a capital or municipal centre is associated with a reduced risk of adverse infection- or nutrition-related health outcomes,[35, 36] likely due to increased access to health care services.

A potential limitation of the present analyses was the use of straight-line (Euclidean) distance for estimating proximity to a health facility. While distance by road may have been a more appropriate indicator of travel impedance or access, incomplete or missing vector information (e.g. miss-aligned junctions, missing or disconnected road segments) made it difficult to generate accurate measures. Nesbitt and colleagues (2014) compared different measures of travel impedance to estimate access to delivery care in the Brong-Ahafo region of Ghana[37] and concluded that straight-line distance was as informative as distance by road for determining spatial access in rural Ghana. Therefore, we felt it was justified to use Euclidean distance in the present analyses.

Non-malaria infections were identified using CRP, an acute phase protein that rises in accordance with the early phase of the inflammatory response (approximately 48 hours). Other

acute phase proteins (such as alpha-1-acid glycoprotein) reach their peak concentration during the late phase (approximately 2-8 days), which more closely approximates the change in ferritin concentration in response to inflammation.[27] Since CRP was the only inflammatory biomarker available for the current analysis, and the original trial protocol was not designed to include diagnoses of other infection types, it is possible that the prevalence of non-malaria infection among participants was underestimated.

Conclusions

The analyses presented herein explored the spatial dynamics of infection risk in the context of an iron intervention trial in a malaria endemic area among children with varying levels of iron status, where all children received bed nets and access to malaria treatment. Geographical variation in the risk of malaria and non-malaria infections was observed with distinct high- and low-risk areas, particularly around the District centre. Future research should include biomarkers of non-malaria infection that represent both the early and late stages of the inflammatory response. Overall, our findings emphasize the importance of considering geographical distribution when assessing infection risk and planning intervention trials in LMIC paediatric populations.

Acknowledgments

The authors would like to acknowledge Seeba Amenga-Etego and the members of the GIS team at the Kintampo Health Research Centre in (Kintampo, Ghana) who collected the GPS data that were used in the secondary analysis.

Author contribution

AA, SHZ and SOA conducted the original trial in Ghana. AA and SOA coordinated the acquisition of geographical data. AA conceived and conducted the secondary analysis with substantial contribution from PEB. DCC and SHZ were also involved in the conception and design of the secondary analysis, and interpretation of data. AA drafted the manuscript. All authors read and approved the final manuscript.

Funding

Funding for this manuscript was provided by AA's Doctoral Research Award from the Canadian Institutes of Health Research (CIHR).

Competing interests

None declared

Data sharing

Any unpublished data are available upon request by emailing the corresponding author.

References

1 WHO. Children: reducing mortality. *Fact sheet No 178*. World Health Organization 2014.

2 WHO. Micronutrient Deficiencies: Iron deficiency anaemia. World Health Organization 2015.

3 Stoltzfus RJ. Iron deficiency: global prevalence and consequences. *Food Nutr Bull* 2003;**24**:S99-103.

4 Katona P, Katona-Apte J. The interaction between nutrition and infection. *Clin Infect Dis* 2008;**46**:1582-8.

5 Raiten DJ, Sakr Ashour FA, Ross AC*, et al.* Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE). *J Nutr* 2015;**145**:1039S-108S.

6 Spottiswoode N, Duffy PE, Drakesmith H. Iron, anemia and hepcidin in malaria. *Front Pharmacol* 2014;**5**:125.

7 Nemeth E, Tuttle MS, Powelson J*, et al.* Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004;**306**:2090-3.

8 Sazawal Z, Black, RE., Ramsan, M., Chwaya, HM., Stoltzfus, RJ., Dutta, A ., et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet* 2006;**367**:133-43.

9 Rezaeian M, Dunn G, St Leger S*, et al.* Geographical epidemiology, spatial analysis and geographical information systems: a multidisciplinary glossary. *J Epidemiol Community Health* 2007;**61**:98-102.

10 Schur N, Vounatsou P, Utzinger J. Determining treatment needs at different spatial scales using geostatistical model-based risk estimates of schistosomiasis. *PLoS Negl Trop Dis* 2012;**6**:e1773.

11 Pullan RL, Gething PW, Smith JL*, et al.* Spatial modelling of soil-transmitted helminth infections in Kenya: a disease control planning tool. *PLoS Negl Trop Dis* 2011;**5**:e958.

12 Soares Magalhães RJ, Langa A, Pedro JM*, et al.* Role of malnutrition and parasite infections in the spatial variation in children's anaemia risk in northern Angola. *Geospat Health* 2013;**7**:341-54.

13 Sasaki S, Igarashi K, Fujino Y, *et al.* The impact of community-based outreach immunisation services on immunisation coverage with GIS network accessibility analysis in peri-urban areas, Zambia. *J Epidemiol Community Health* 2011;**65**:1171-8.

14 Zlotkin S, Newton S, Aimone AM, *et al.* Effect of iron fortification on malaria incidence in infants and young children in Ghana: a randomized trial. *JAMA* 2013;**310**:938-47.

15 WHO. World Malaria Report 2009. Geneva, Switzerland: World Health Organization 2009.

16 WHO. Worldwide prevalence of anaemia 1993-2005. *WHO Global Database on Anaemia*. Geneva, Switzerland: World Health Organization 2008.

17 WHO/UNICEF. WHO child growth standards and the identification of severe acute malnutrition in infants and children: A Joint Statement by the World Health Organization and the United Nations Children's Fund. Geneva, Switzerland: World Health Organization 2009.

18 USGS. United States Geological Survey. 2012.

19 USGS. Land Processes Distributed Active Archive Center. 2014.

20 Hengl T. Worldgrids — a public repository and a WPS for global environmental layers. ISRIC - World Soil Information 2014.

21 Samadoulougou S, Maheu-Giroux M, Kirakoya-Samadoulougou F, *et al.* Multilevel and geo-statistical modeling of malaria risk in children of Burkina Faso. *Parasit Vectors* 2014;**7**:350.

22 Diggle PJ, Moyeed RA, Tawn JA. Model-based geostatistics. *Applied Statistics* 1998;**47**:299-350.

23 Diggle PJ, Ribeiro PJ. *Model-based geostatistics*. New York: Springer-Verlag 2006.

24 Rue H, Martino S. Approximate Bayesian inference for latent Gaussian models by using integrated nested Laplace approximations. *Journal of the Royal Statistical Society: Series B* 2009;**71**:319-92.

25 Brown PE. Model-based geostatistics the easy way. *Journal of Statistical Software* 2015;**63**:1-24.

26 R. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing 2015.

27 Thurnham DI, McCabe GP. Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron. In: Organization WH, ed. *Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15-17 September 2010*. Geneva: World Health Organization 2012:63-80.

28 Black RE, Allen LH, Bhutta ZA*, et al.* Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008;**371**:243-60.

29 Ezzati M, Utzinger J, Cairncross S*, et al.* Environmental risks in the developing world: exposure indicators for evaluating interventions, programmes, and policies. *J Epidemiol Community Health* 2005;**59**:15-22.

30 Cohen JM, Ernst KC, Lindblade KA*, et al.* Topography-derived wetness indices are associated with household-level malaria risk in two communities in the western Kenyan highlands. *Malar J* 2008;**7**:40.

31 Drakeley CJ, Carneiro I, Reyburn H*, et al.* Altitude-dependent and -independent variations in Plasmodium falciparum prevalence in northeastern Tanzania. *J Infect Dis* 2005;**191**:1589-98.

32 Brooker S, Clarke S, Njagi JK*, et al.* Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. *Trop Med Int Health* 2004;**9**:757-66.

33 Reid H, Vallely A, Taleo G*, et al.* Baseline spatial distribution of malaria prior to an elimination programme in Vanuatu. *Malar J* 2010;**9**:150.

34 Hay SI, Omumbo JA, Craig MH*, et al.* Earth observation, geographic information systems and Plasmodium falciparum malaria in sub-Saharan Africa. *Adv Parasitol* 2000;**47**:173-215.

35 Shirayama Y, Phompida S, Shibuya K. Geographic information system (GIS) maps and malaria control monitoring: intervention coverage and health outcome in distal villages of Khammouane province, Laos. *Malar J* 2009;**8**:217.

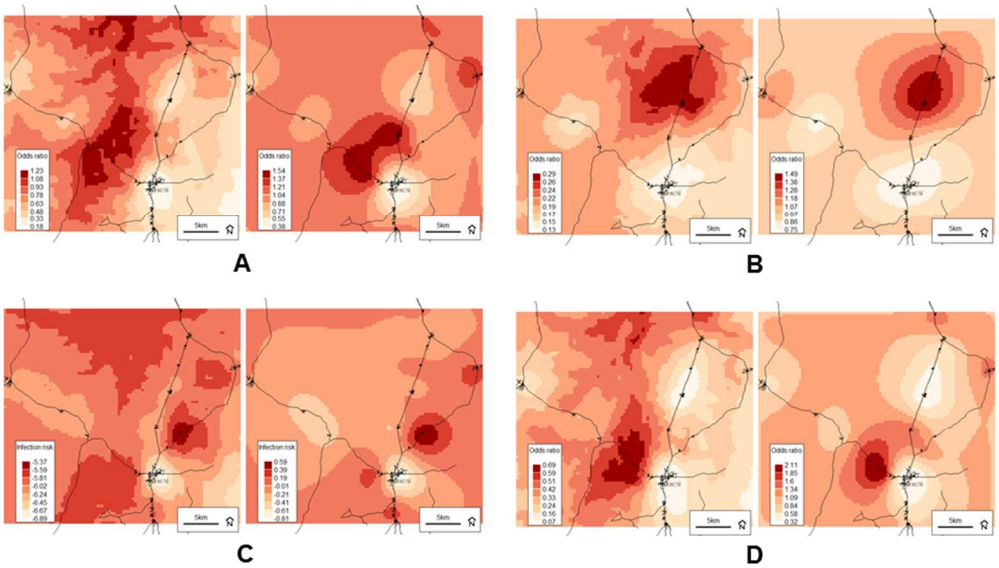
36 Kandala NB, Madungu TP, Emina JB*, et al.* Malnutrition among children under the age of five in the Democratic Republic of Congo (DRC): does geographic location matter? *BMC Public Health* 2011;**11**:261.

37 Nesbitt RC, Gabrysch S, Laub A, *et al.* Methods to measure potential spatial access to delivery care in low- and middle-income countries: a case study in rural Ghana. *Int J Health Geogr* 2014;**13**:25.

Figure legends

Figure 1: Predicted probabilities (left), and residual spatial variation (right) from the final combined-group models for the odds of inflammation (CRP>5 mg/L) *and/or any* malaria parasitaemia (A); the odds of inflammation (CRP > 5 mg/L) *without* malaria parasitaemia (B); the risk of malaria parasitaemia *with* concurrent fever (axillary temperature >37.5^{0}C - or history of reported fever within 48 hours) (C); and the odds of malaria parasitaemia *with or without* fever (D). Darker colour indicates higher risk at endline. Background © Stamen Design.

Figure 2: Elevation (meters) across the study area. Green colour indicates higher elevation.



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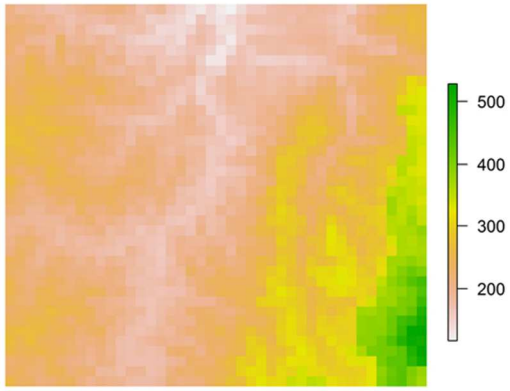

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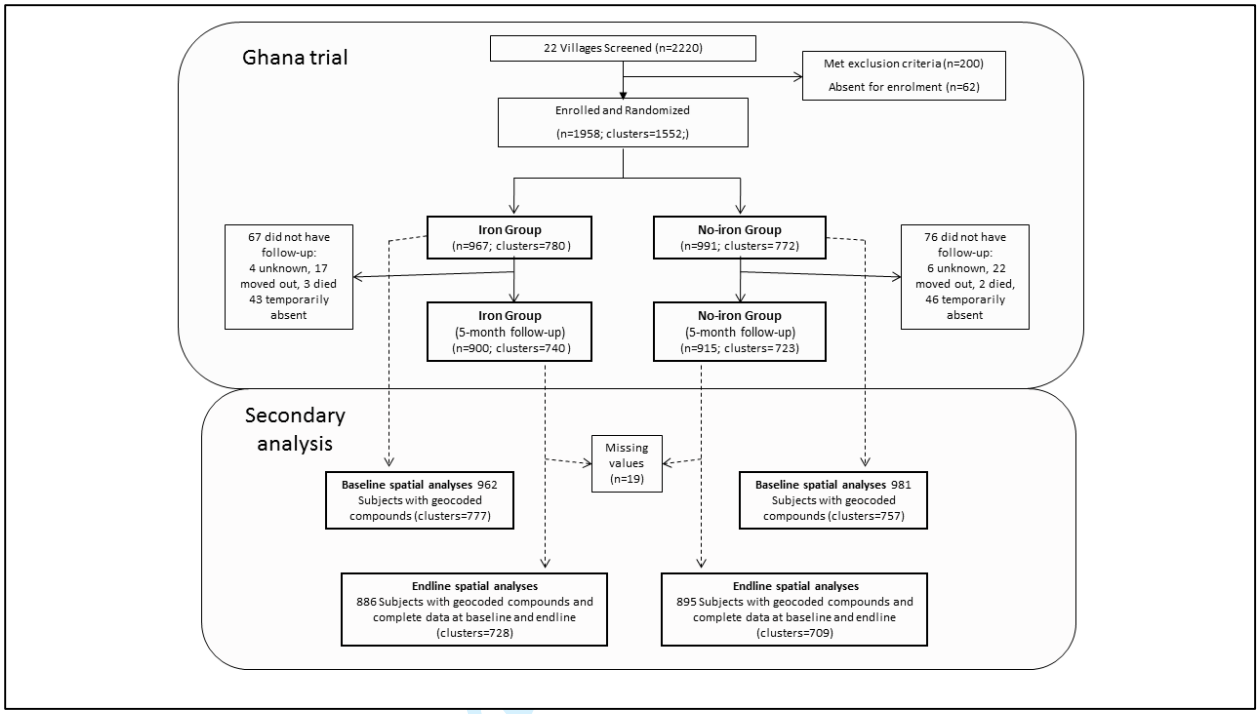

Figure S1: Study flow for the Ghana trial (top section) and secondary analyses (bottom section). Out of the 1958 participants from the Ghana trial, a total of 1943 with geocoded compounds were included in the baseline secondary spatial analyses (13 compounds were untraceable, corresponding to 15 participants not included in the secondary analyses). The endline spatial analyses included a total of 1781 observation, representing trial participants with geocoded compounds who provided blood samples at the end of the intervention period (5-month follow-up), and after removing 19 observations due to missing baseline or endline ferritin values.

Table S1: Geo-spatial and non-spatial risk factors of endline infection status[1] among Ghanaian children in the *No-iron* group (Brong-Ahafo Region, Sept-Nov 2010)

Covariates	Estimate (95% CrI) on the exponential scale[2]		Range parameter in km (95% CrI)	Standard deviations of random effects (95% CrI)	
				Spatial	Compound
Outcome 1: Inflammation and/or parasitaemia (n=894)					
Intercept	0.550	(0.298, 1.034)	7.050	0.506	0.008
Age per month			(2.016,	(0.271,	(0.004,
6-23 months	1.004	(0.975, 1.035)	18.26)	0.979)	0.030)
24-35 months	1.018	(0.964, 1.074)			
Sex (male reference)	1.016	(0.760, 1.357)			
Length-for-age z-score	0.984	(0.869, 1.113)			
Weight-for-length z-score	1.229	(1.056, 1.431)*			
Asset score	0.893	(0.760, 1.047)			
Distance to health facility (km)	1.072	(0.963, 1.188)			
Elevation (m)	0.995	(0.990, 1.001)			
Baseline infection status	1.887	(1.384, 2.576)*			
Baseline iron status	1.051	(0.938, 1.179)			
Outcome 2: Inflammation without parasitaemia (n=894)					
Intercept	0.219	(0.109, 0.426)*	9.458	0.442	0.007
Age per month			(2.389,	(0.206,	(0.004,
6-23 months	1.011	(0.971, 1.052)	23.57)	0.965)	0.029)
24-35 months	0.920	(0.843, 0.996)*			
Sex (male reference)	1.001	(0.671, 1.493)			
Length-for-age z-score	1.153	(0.977, 1.360)			
Weight-for-length z-score	1.302	(1.058, 1.603)*			
Asset score	0.872	(0.705, 1.075)			
Distance to health facility (km)	1.065	(0.953, 1.176)			
Elevation (m)	0.997	(0.991, 1.003)			
Baseline infection status	1.034	(0.556, 1.813)			
Baseline iron status	1.003	(0.841, 1.166)			
Outcome 3: Parasitaemia with fever (n=979)					
Intercept	0.003	(0.002, 0.004)*	7.690	0.448	0.007
Age per month			(2.821,	(0.243,	(0.004,
6-23 months	1.001	(0.979, 1.023)	18.01)	0.858)	0.028)
24-35 months	0.970	(0.927, 1.013)			
Sex (male reference)	0.896	(0.718, 1.116)			
Length-for-age z-score	1.009	(0.919, 1.107)			
Weight-for-length z-score	0.896	(0.797, 1.007)			
Asset score	1.075	(0.947, 1.220)			
Distance to health facility (km)	1.047	(0.959, 1.142)			

Elevation (m)	0.998	(0.993, 1.003)			
Baseline infection status	0.757	(0.467, 1.161)			
Baseline iron status	1.040	(0.953, 1.125)			
Outcome 4: All parasitaemia (n=894)					
Intercept	0.186	(0.089, 0.400)*	6.535	0.652	0.008
Age per month			(2.037,	(0.344,	(0.004,
6-23 months	0.974	(0.936, 1.014)	15.08)	1.255)	0.029)
24-35 months	1.067	(1.026, 1.110)*			
Sex (male reference)	1.050	(0.756, 1.459)			
Length-for-age z-score	0.901	(0.780, 1.037)			
Weight-for-length z-score	1.095	(0.923, 1.298)			
Asset score	0.941	(0.781, 1.130)			
Distance to health facility (km)	1.023	(0.896, 1.165)			
Elevation (m)	0.996	(0.989, 1.004)			
Baseline infection status	2.864	(1.968, 4.172)*			
Baseline iron status	1.036	(0.914, 1.169)			

[1]Infection status definitions:
Outcome 1: Inflammation and/or parasitaemia (binary): 1 = CRP > 5 mg/L and/or any malaria parasitaemia, 0 = CRP ≤ 5 mg/L and absence of parasitaemia;
Outcome 2: Inflammation without parasitaemia (binary): 1 = CRP > 5 mg/L without malaria parasitaemia, 0 = CRP ≤ 5 mg/L without parasitaemia;
Outcome 3: Parasitaemia with fever (count): any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
Outcome 4: All parasitaemia (binary): 1 = any malaria parasitaemia (with/without fever), 0 = absence of parasitaemia with/without fever
Model prior shape = 1.117, model prior rate = 0.157
[2]Exponentials of posterior medians and 2.5% and 97.5% posterior quantiles of model parameters, with a value of 1.05 indicating that a variable increases the risk of infection by 5% (odds ratio from logistic model for all binary outcomes; relative risk from Poisson model for count outcome)
CrI = credible interval
Baseline infection status = baseline infection status (yes/no) according to corresponding definition
Baseline iron status = baseline serum ferritin concentration (µg/dL) corrected for CRP using the regression method (Namaste et al.) and re-scaled by multiplying by the inverse of the inter-quartile range
*Statistical significance at the 0.05 level

Table S2: Geo-spatial and non-spatial risk factors of endline infection status[1] among Ghanaian children in the *Iron* group (Brong-Ahafo Region, Sept-Nov 2010)

Covariates	Estimate (95% CrI) on the exponential scale[2]		Range parameter in km (95% CrI)	Standard deviations of random effects (95% CrI)	
				Spatial	Compound
Outcome 1: Inflammation and/or parasitaemia (n=886)					
Intercept	0.647	(0.354, 1.263)	7.761	0.487	0.007
Age per month			(2.682,	(0.263,	(0.004,
6-23 months	1.004	(0.975, 1.035)	17.96)	0.931)	0.028)
24-35 months	0.977	(0.925, 1.031)			
Sex (male reference)	1.162	(0.871, 1.551)			
Length-for-age z-score	0.980	(0.857, 1.119)			
Weight-for-length z-score	0.971	(0.833, 1.130)			
Asset score	0.906	(0.769, 1.064)			
Distance to health facility (km)	1.003	(0.904, 1.106)			
Elevation (m)	0.995	(0.989, 1.001)			
Baseline infection status	2.272	(1.664, 3.108)*			
Baseline iron status	1.053	(0.953, 1.172)			
Outcome 2: Inflammation without parasitaemia (n=886)					
Intercept	0.143	(0.073, 0.274)*	12.56	0.428	0.007
Age per month			(4.318,	(0.203,	(0.004,
6-23 months	1.007	(0.969, 1.048)	27.27)	0.923)	0.028)
24-35 months	0.983	(0.910, 1.056)			
Sex (male reference)	1.330	(0.905, 1.962)			
Length-for-age z-score	1.054	(0.884, 1.255)			
Weight-for-length z-score	0.890	(0.726, 1.090)			
Asset score	0.888	(0.725, 1.087)			
Distance to health facility (km)	0.984	(0.883, 1.080)			
Elevation (m)	1.001	(0.995, 1.006)			
Baseline infection status	1.508	(0.897, 2.459)			
Baseline iron status	1.044	(0.918, 1.166)			
Outcome 3: Parasitaemia with fever (n=960)					
Intercept	0.002	(0.001, 0.004)*	8.352	0.466	0.007
Age per month			(3.203,	(0.244,	(0.004,
6-23 months	1.017	(0.992, 1.043)	17.99)	0.896)	0.028)
24-35 months	0.987	(0.945, 1.029)			
Sex (male reference)	0.991	(0.780, 1.257)			
Length-for-age z-score	0.969	(0.867, 1.082)			
Weight-for-length z-score	1.052	(0.925, 1.197)			
Asset score	1.002	(0.872, 1.152)			
Distance to health facility (km)	1.064	(0.970, 1.163)			
Elevation (m)	0.998	(0.993, 1.003)			

Baseline infection status	0.849	(0.516, 1.321)			
Baseline iron status	0.981	(0.895, 1.060)			
Outcome 4: All parasitaemia (n=886)					
Intercept	0.412	(0.199, 0.938)*	7.293	0.666	0.008
Age per month			(2.486,	(0.376,	(0.004,
6-23 months	1.015	(0.975, 1.056)	17.67)	1.230)	0.030)
24-35 months	0.977	(0.938, 1.016)			
Sex (male reference)	0.977	(0.707, 1.347)			
Length-for-age z-score	0.946	(0.812, 1.098)			
Weight-for-length z-score	1.034	(0.872, 1.225)			
Asset score	0.946	(0.785, 1.137)			
Distance to health facility (km)	1.009	(0.889, 1.149)			
Elevation (m)	0.994	(0.987, 1.001)			
Baseline infection status	2.597	(1.791, 3.768)*			
Baseline iron status	1.042	(0.939, 1.153)			

[1]Infection status definitions:
Outcome 1: Inflammation and/or parasitaemia (binary): 1 = CRP > 5 mg/L and/or any malaria parasitaemia, 0 = CRP ≤ 5 mg/L and absence of parasitaemia;
Outcome2: Inflammation without parasitaemia (binary): 1 = CRP > 5 mg/L without malaria parasitaemia, 0 = CRP ≤ 5 mg/L without parasitaemia;
Outcome3: Parasitaemia with fever (count): any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
Outcome4: All parasitaemia (binary): 1 = any malaria parasitaemia (with/without fever), 0 = absence of parasitaemia with/without fever
Model prior shape = 1.117, model prior rate = 0.157
[2]Exponentials of posterior medians and 2.5% and 97.5% posterior quantiles of model parameters, with a value of 1.05 indicating that a variable increases the risk of infection by 5% (odds ratio from logistic model for all binary outcomes; relative risk from Poisson model for count outcome)
CrI = credible interval
Baseline infection status = baseline infection status (yes/no) according to corresponding definition
Baseline iron status = baseline ferritin concentration (µg/dL) corrected for CRP using the regression method (Namaste et al.) and re-scaled by multiplying by the inverse of the inter-quartile range
*Statistical significance at the 0.05 level

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	NA
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5-7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	5
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	5

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	5
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6-7
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	6-7
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure S1
	13b	For each group, losses and exclusions after randomisation, together with reasons	8
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	9
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	9-13
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	9-13
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	9-10
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13-16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13-16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13-16
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	17

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

RESEARCH PROPOSAL

Project Title: Seasonal Impact of Iron Fortification on Malaria Incidence in Ghanaian Children

Principal Investigator: Dr. Stanley Zlotkin (The Hospital for Sick Children)
Co-Investigator: Dr. Seth Owusu-Agyei (Kintampo Health Research Centre)

1. SPECIFIC AIMS

This RFA is intended to address "those factors affecting the safe and effective use of iron interventions for the prevention and treatment of iron deficiency and anemia in women of reproductive age (including adolescent girls), infants, and children, particularly in areas of endemic malaria". One of the three core priority areas described in the RFA is the category of "interventions". The RFA asks the question, "What are the safest and most effective interventions to prevent and treat iron deficiency in women, infants and children in areas of endemic malaria?" The current proposal specifically addresses the terms of the RFA by proposing the use of microencapsulated ferrous fumarate, supplied in a powder form with other essential micronutrients ("Sprinkles"), as an effective and potentially safer alternative to syrups or drops for providing iron to infants and young children living in regions where the burden of malaria is high.

In 2006, The World Health Organization (WHO) and United Nations Children's Fund (UNICEF) released a joint statement recommending that in malaria endemic areas, iron supplementation be targeted only to those children who are anemic and at risk of iron deficiency. As well, these children should receive concurrent protection from malaria and other infectious diseases through prevention and effective case management. The statement went on to state that "these conclusions should not be extrapolated to fortification or food-based approaches for delivering iron, where the patterns of iron absorption and metabolism may be substantially different. The Joint Statement did not provide recommendations regarding 'point-of-use' fortificants (i.e. single-dose powdered mineral and vitamin supplements to be sprinkled on to home-made foods at the table).

In reality, it is neither practical nor feasible to meet the population-wide screening requirements as outlined in the WHO/UNICEF Joint Statement (above), especially in under-developed countries. There simply are no screening tools for anemia and iron deficiency that can be practically used and, indeed, the cost of screening for hemoglobin alone is often higher than the cost of providing iron supplements. As a result, for the past 2-3 years, iron deficiency anemia has remained largely an unresolved nutritional problem in areas of endemic malaria. It is estimated, for example, that the prevalence of iron-deficiency anemia is as high as 60-65% in children under age 24 months in West Africa. In our past research, we have demonstrated in Ghana that the use of powdered minerals and vitamins, including microencapsulated iron will reduce anemia rates by up to 60%. However, because of the uncertainty around the use of iron in high malaria burden areas, the implementation (scaling-up) of these results is virtually at a stand-still.

Given that we have already demonstrated the efficacy of encapsulated iron (as ferrous fumarate) in lowering the burden of anemia, the primary objective of this research proposal is to determine the impact of providing Sprinkles (including microencapsulated iron as a powder added to complementary foods) on the susceptibility to clinical malaria among anemic and non-anemic infants and young children (6-35 months of age) living in a high malaria burden area. Due to the well documented difference in mosquito bite-rates between the wet and dry seasons, we will conduct the study during the rainy season only (when bite rates are usually higher). Certain secondary variables, such as iron and anemia status and breastfeeding rates may influence the primary outcome, thus we will concurrently collect data on these variables throughout the intervention.

Our secondary objectives are to determine the impact of this iron intervention on the *severity* of clinical malaria by documenting parasite counts and hospital admission rates, as well as differences in secondary complications of malaria infections, such as death, cerebral malaria, pneumonia, dehydration, and diarrhea. Although the current study is not powered to show causation between the intervention and the secondary outcomes, it is anticipated that the proposed research will help address the confusion that has been generated regarding the safe and appropriate use of iron supplements in high malaria transmission areas. If it is demonstrated that the provision of iron as a powder added to food does not have an adverse effect on malaria incidence and has a positive impact on anemia rates, then this type of iron delivery system can possibly be recommended for scale-up in Ghana and in other countries with a similar malaria burden. Conversely, if we demonstrate adverse effects, especially in iron replete children, then further research is needed to identify inexpensive and non-invasive methods to screen children for anemia before iron is provided. **The long term goal of the proposed research is to develop new evidence to inform global policy and ultimately guide the implementation of programs to prevent and treat iron deficiency disorders in malaria endemic regions.** These goals coincide directly with the stated objectives of this RFA.

2. BACKGROUND AND SIGNIFICANCE

Ghana as the Proposed Study Site

Ghana, with an estimated population of 23 million, is located in Western Africa, bordering the Gulf of Guinea, between Cote d'Ivoire and Togo. Well endowed with natural resources, Ghana has roughly twice the per capita output of the poorest countries in West Africa. Even so, Ghana remains heavily dependent on international financial and technical assistance. Gold and cocoa production and individual remittances are major sources of foreign exchange. The domestic economy continues to revolve around agriculture, which accounts for about 35% of the gross domestic product and employs about 55% of the work force, which is mainly made up of small landholders.

Approximately 37.8% of the population is between 0-14 years of age (male 4,470,382/female 4,360,359). The birth rate is 29.22 births/1,000 population, while the death rate is 9.39 deaths/1,000 population (2008 est.). The total infant mortality rate is 52.31 deaths/1,000 live births (male: 56.64 deaths/1,000 live births; female: 47.85 deaths/1,000 live births [2008 est.]). Ghana is considered to be one of the most progressive countries in sub-Saharan Africa.

The value of performing this research in Ghana is three fold:

(i) There has been a well-established and successful research collaboration between the Government of Ghana (Kintampo Health Research Centre) and the Hospital for Sick Children, University of Toronto since 1998;

(ii) Malaria and anemia remain the most important causes of death and morbidity in Ghana; and

(iii) The vital capacity-building potential of joint projects, such as the one described in this application, goes well beyond the life of this project.

2.1 Background and Gaps

Global prevalence and impact of iron deficiency anemia

Iron deficiency and iron deficiency anemia (IDA) are the most prevalent micronutrient deficiencies on a worldwide basis, especially in developing countries. The impact of severe IDA can have mortal consequences, since without adequate hemoglobin, the brain and body become deprived of oxygen and, if allowed to continue, death may ensue. While the impact of mild and moderate IDA on child development and immune function remain areas of fertile research, there are currently no firm conclusions on either short- or long-term effects (1). There is agreement, however, that the prevention of iron deficiency and iron deficiency anemia (from mild to severe) are public health priorities, based on their potential negative impact on the health of children. There are experimental animal models that have examined potential mechanisms for the role of iron in brain development, and human cohort studies which have documented short- and long-term effects of mild-moderate IDA on impaired cognitive and motor development. Although the human cohort studies all suffer from an unavoidable design bias, since one cannot purposely assign (randomize) children to become anemic, those that have been conducted in different countries under different conditions have generally shown similar adverse outcomes on school achievement and measures of cognition and learning. It has also been suggested that in terms of the impact of iron on the developing brain, the first two years of life are a 'window of opportunity', and once the window is closed the impact may not be reversible.

Despite the uncertainties around the impact of IDA on the health of children, governments and United Nations agencies continue to place high priority on the prevention and treatment of IDA. Even private 'think-tanks' like the Copenhagen Consensus have recognized the importance of controlling IDA. For example, in their most recent session, they ranked the control of micronutrient deficiencies as the number one global challenge, and placed iron fortification as the third most important solution due to their extremely high ratio of benefits to costs (2). And finally, the fourth Millennium Development Goal of "reducing the under-five child mortality to one third by 2015" is at least indirectly related to the control of IDA (3).

Global prevalence and impact of malaria

Nutritional intervention programs have generally demonstrated that the provision of iron supplements can enhance child development (4, 5) and reduce the prevalence of severe anemia; however, there is some evidence to suggest that iron supplementation (in the form of syrups, drops or pills often provided in a post-prandial state) results in high levels of malaria parasitemia (6, 7), increased rates of malaria, as well as pneumonia and diarrhea (7-10). In contrast, the most recent systematic review (2002) does not generally support an increased risk of malaria attack rate or severity associated with iron supplementation (pooled OR significant for malaria + smear = 1.43 [1.08-1.91], but not significant when adjusted for baseline malaria smear = 1.24 [0.98-1.57]) (10). Clearly multiple factors contribute to the complex etiology of anemia in high malaria burden areas, but iron status and malaria infection are the strongest predictors of hemoglobin concentration (11). Recent research has suggested that haptoglobin might also influence hemoglobin levels in an environment of malaria-induced hemolytic stress. Research by Atkinson and colleagues suggest that the $Hp^{2/2}$ genotype is a risk factor for childhood anemia in malaria-endemic countries. The authors found that average hemoglobin levels fell over the malaria season, and children who had the $Hp^{2/2}$ genotype had the greatest drop compared to other children (11). These findings suggest that a child's haptoglobin type may be an important influence on whether that child gets anemia in areas where malaria is very common

The relationship between iron and malaria has important implications because malaria is a tropical parasitic disease that contributes significantly to morbidity and mortality rates in many parts of the world. Recent estimates from the 2005 World Malaria Report were around 350-500 million clinical disease episodes per year (12). A large proportion of the global malaria burden is concentrated in Africa, where approximately 60% of

clinical cases and over 80% of malaria-related deaths occur. Further, most of the Africans who die from malaria each year are children under five years of age (13). Studies conducted in the north-western African country of Ghana have revealed that malaria can account for more than 44% of reported outpatient visits (12), and that the majority of these cases tend to occur during the wet season (June-October) (14, 15).

Impact of iron supplementation on malaria incidence and/or severity in Pemba, Zanzibar

A recent community-based randomized controlled trial, by Sazawal et al, demonstrated increased morbidity and mortality in infants provided with an iron and folic acid supplement in a highly malaria endemic region in Pemba, Zanzibar (16). In this large study, infants were provided with a multivitamin-mineral tablet with and without iron, that was dissolvable in either water or breast milk. While iron supplementation was effective for the reduction of iron deficiency and anemia in iron deficient children, it was associated with increased rates of hospitalization (primarily due to malaria and infectious disease), and mortality when given to individuals who were iron replete (with or without anemia). On advice from the Data Safety Monitoring Board, the iron-supplemented arms of the trial were discontinued after approximately 20 months. A subsequent critical review of the data from this trial led to the release of a joint statement by the WHO and UNICEF with the following recommendations:

> "Caution should be exercised in settings where the prevalence of malaria and other infectious diseases is high. Until the WHO recommendations are revised it is advised that iron and folic acid supplementation to be targeted to those who are anemic and at risk of iron deficiency. They should receive concurrent protection from malaria and other infectious diseases through prevention and effective case management. The conclusions drawn from the Zanzibar trial should not be extrapolated to fortification or food-based approaches for delivering iron, where the patterns of iron absorption and metabolism may be substantially different (3)."

The UN agencies also recommended that additional research was urgently needed to develop the most effective strategies for controlling iron deficiency and anemia in regions where malaria transmission is high.

Iron Homeostasis and infectious disease risk

The body's ability to maintain a safe equilibrium of iron is crucial and complex. Iron homeostasis is regulated at the level of absorption, unlike other minerals that are regulated through excretory mechanisms. The human gastrointestinal tract is very sensitive to iron stores and oxygen carrying capacity and has the molecular and biochemical capacity to increase (up-regulate) absorption when iron stores are becoming depleted (or hemoglobin concentration is low), and to down-regulate absorption when iron stores are replete. In an iron deficient individual, iron absorption may be as high as 40-50%, while in an iron-replete individual, iron absorption is between 5-10%. One can imagine that humans developed this sensitive and sophisticated regulatory capacity since too little iron can lead to inadequate oxygenation of vital tissues (and ultimately death), while too much iron can overwhelm the capacity of the body to safely bind the iron to protein, leaving potentially toxic free (non protein-bound) iron. In the case of malaria, it is thought that the parasite can proliferate when there is a labile pool of non protein-bound iron available. On the other hand, it has been postulated that iron deficiency might protect the host organism through increased zinc protoporphyrin in red blood cells, which inhibits parasite haemozoin formation, similar to the mechanism of certain anti-malarial drugs (17).

Potential Adverse Impact of 'Excess' Iron

Iron excess has been associated with oxidative damage in animal models, through the impact of free iron on lipid membrane peroxidation as well as DNA damage. There is a suggestion of lipid peroxidative damage from excess iron in humans, but the studies are limited in number and design. A study by Dewey et al described a degree of growth impairment in iron replete infants provided with a liquid iron supplement (18). In this study, iron supplementation was investigated in two cohorts of infants, one in Sweden and the other in Honduras. It was observed that iron supplementation (with iron syrup) of iron replete Swedish infants between the ages of 4 – 9 months demonstrated significantly decreased length and head circumference growth compared to those receiving no iron supplement. In the Honduran infants, decreased length was observed in iron replete infants between the ages of 4-6 months provided with a similar iron supplement, compared to those receiving a placebo. The results of this study were confirmed in a similar study in India, also using liquid iron supplements (19).

Overall, the findings from the above studies, as well as the one conducted in Pemba, have generated much discussion on the possible deleterious impact of iron supplementation in otherwise healthy iron-replete infants and young children. A plausible biological explanation for these observations is related to the absorption kinetics of iron provided as a supplement. With supplementation, a concentrated form of iron is provided, often in a post-prandial state, with resulting high peak serum iron concentrations (C-max) and a shorter 'time to maximum serum concentration' (T-max). It has been suggested that, under these conditions, the rate of iron absorption may be greater than the child's capacity to bind the absorbed iron (with transferrin), resulting in increased 'free iron' if only for a short period of time. As mentioned above, 'free iron' may benefit any eukaryotic pathogens concomitantly present (like malaria parasites, Yersinia, etc), with resulting proliferation of the pathogens and an adverse clinical outcome. We believe that there may be varying outcomes associated with the form of iron and mode of delivery, leading us to ask whether the form and mode of iron delivery have an impact on the incidence of malaria among children at risk.

In the current grant, we are proposing to use a form of iron and a delivery system that differ from those used in the Pemba study in four major ways: Firstly, we plan to use a powdered mineral 'fortificant' as the iron source. This source differs from a typical iron supplement in a number of ways which may protect the recipient against the suggested generation of free iron. The iron source is microencapsulated ferrous fumarate. As will be discussed (below), microencapsulation protects the iron from the food matrix (thus preventing oxidation of the iron) and likely results in a lower C-max and longer T-max. Secondly, we will provide the powdered iron source for 5 months in the wet season, while the Pemba study provided the supplement for a full year. Although the length of supplementation did not have an impact on the results in Pemba, results from previous studies completed by our research group have demonstrated that the use of powdered iron as a fortificant for relatively short periods of time (as short as two months) resulted in a significant increase in hemoglobin and a significant decrease in rates of anemia that lasted for as long as six months after the end of the intervention period (20). Thus we believe that a period shorter than 1 year will be sufficient to have a significant impact on anemia rates. Thirdly, although the dose of iron in the current study (12.5 mg/day) is similar to that provided to the older infants and young children in the Pemba study, as previously discussed, it will be provided in a food matrix, rather than as a supplement. We believe that this dose will be adequate to have an impact on anemia rates in children with iron deficiency, but not excessive for children whose iron stores are replete. Finally, the minerals and vitamins (including iron as microencapsulated ferrous fumarate, zinc, vitamins A, and C) will be provided in single-dose sachets (like small packets of sugar), which are easily sprinkled once daily onto any semi-solid or 'soft' foods. Although folic acid was included in the multimicronutrient supplement in the Pemba study, it is possible that folic acid may inhibit the action of certain anti-malarial drugs, thus it will **not** be included in the sachet. Because the powdered formulation is

taken with food and due to the microencapsulation of the iron, it is likely that the absorption characteristics will be different from that of non-microencapsulated iron given in a post-prandial state – potentially reducing the amount of available free iron. **By reducing the peak labile pool of free iron, it is possible that the safety of using powdered fortificants in areas with a high incidence of infection may be increased.**

Microencapsulation

Microencapsulation is a process by which tiny parcels of a gas, liquid, or solids are packaged within a second material for the purpose of shielding the active ingredient from the surrounding environment. There can be numerous reasons for microencapsulation. These include isolation of the contents from the environment (e.g.. preventing iron from oxidizing with food), improving handling properties (e.g. preventing dangerous pesticides from coming in contact with hands) and/or controlling the release of the contents (e.g. reducing the rate of release of drugs in the gastrointestinal tract).

Effect of micoencapsulation on pharmacokinetic properties

Microencapsulation has has been shown to have an effect on the absorption characteristics of a drug by significantly reducing and delaying peak plasma concentrations (Cmax and Tmax) (21-23). Further, studies examining the effect of giving a microencapsulated drug with or without food have shown that, post-prandially, the maximum plasma concentration tends to be lower as compared to the fasted state (21, 24). Although not tested directly, the findings from these pharmacokinetic studies suggest that the use of microencapsulated iron as proposed in the current study (i.e. mixed with food) may result in a reduced Cmax (peak plasma level) and longer Tmax with a potentially reduced peak labile pool of free iron and thus a higher safety profile in a malaria endemic region.

2.2 Importance and relevance of research

Notwithstanding the debate around the adverse impact of iron deficiency anemia on malaria and possibly growth (in iron replete children), all countries, as well as the UN agencies, UNICEF and the WHO, recognize iron as an essential nutrient and recommend a daily intake of iron between 5 -10 mg/day depending on the age of the child. It is also recommended that, whenever possible, iron should come from the diet alone; however, when dietary sources are inadequate, either supplementation or fortification is recommended. As such, the international nutrition community has been exploring ways to treat and prevent IDA through food diversification, supplementation and fortification. Unfortunately for children under age 2 years in developing countries, neither diversification nor supplementation has proven to be an effective means of coping with the problem. Although food fortification of commodities like wheat flour are used to prevent IDA in adult populations, they have not been successful for young children since the level of fortification is aimed at the adult male, and the total amount of the fortified food that is eaten is too low in the young infant or small child to meet their iron intake requirements. We and others have demonstrated over the past 10 year that 'point of use fortification' with powdered minerals and vitamins is both efficacious and effective (25-34), and this intervention is beginning to be scaled up in a number of countries where malaria is not present. To our knowledge, the safety and efficacy of providing children with iron, in the form of a powdered 'point of use' fortificant, in areas with a high prevalence of malaria has not been investigated. **We are confident that the outcome of the current proposal, combined with the results from the Pemba study, should enable more effective decision making and policy formulation regarding the use of powdered minerals and vitamins in malaria endemic regions globally**.

2.3 Significance

Overall, this investigation will provide important information on how health-related policies and programs can be improved to ensure that infants and young children in high malaria burden countries are safely protected from micronutrient malnutrition, and thus able to better achieve their growth and development potential. Specifically, the protocol will contribute the following to the literature on this topic:

1. We will determine whether the form of the iron used in the present study, and the delivery mode (powdered iron sprinkled on to food), results in an increase or decrease in malaria incidence. The Pemba study reported increased morbidity and mortality with a dissolvable iron tablet supplement which may have been rapidly absorbed from the proximal gastrointestinal tract. The form of iron to be used in the proposed study is microencapsulated ferrous fumarate, mixed in a food matrix. It is anticipated that the digestion and absorption of this form and delivery of iron is slower (lower Cmax [peak serum concentration] and higher Tmax [longer time to peak absorption]) and thus safer than a supplement.

2. As was the case in the Pemba study, we will determine whether iron replete children are more likely to have an adverse outcome, compared to iron deficient children. In the proposed protocol, we plan to enroll all otherwise healthy children without severe anemia. Based on our previous studies in Ghana, we expect that approximately 40 – 50% of children will be iron replete. Our sample size is large enough to allow for statistical evaluation of the primary outcome based on the iron status of the subjects at baseline.

It is anticipated that the proposed research will help address the confusion that has been generated regarding the safe and appropriate use of iron supplements in high malaria transmission areas. If it is demonstrated that the provision of iron, as a powder added to food, does not have an adverse effect on malaria incidence and a positive impact on anemia rates, then this type of iron delivery system can possibly be recommended for scale-up in Ghana, and in other countries with a similar malaria burden. If we demonstrate adverse effects, especially in iron replete children, then further research is needed to identify inexpensive and non-invasive methods to screen children for anemia before iron is provided. With either outcome, this investigation will provide important information on how health-related policies and programs can be improved to ensure that infants and young children in underdeveloped countries are protected from infectious diseases and micronutrient malnutrition, and thus able to better achieve their growth and development potential.

2.4 Potential effect of these studies on the concepts, methods, treatments, services or preventative interventions that drive this field

These studies will have a major impact on interventions to treat and prevent iron deficiency anemia in malaria endemic areas. The use of powdered mineral and vitamin fortificants is being scaled-up in a number of zero or low malaria burden developing countries as a means to prevent micronutrient deficiencies, including iron deficiency. Drs. Zlotkin and Owusu-Agyei organized a one day symposium with the Ministry of Health of Ghana in 2006, including key United Nations agencies and NGOs. The government of Ghana has an Anemia Subcommittee dedicated to finding interventions to prevent anemia in Ghanaian children. The progress of the Subcommittee, however, has been markedly impaired by the results of the Pemba study and the subsequent UNICEF/WHO guidance on the use of iron in malaria endemic areas. The results of the proposed research study will provide the government of Ghana (as well as governments in other high malaria burden countries) with new and significant information for planning safe and effective anemia prevention programs.

3. PRELIMINARY STUDIES

The Research Institute, Hospital for Sick Children in Toronto and the Kintampo Health Research Centre in Ghana have a long standing and collaborative working relationship. This provides the proposed project with a well-established and unique team of expertise in global health generally, and specifically in the area of iron deficiency anemia, malaria control and research (clinical trials) methodology.

Both Drs. Owusu-Agyei at the Kintampo Health Research Centre (KHRC) and Zlotkin (Research Institute, Hospital for Sick Children and University of Toronto) have extensive experience as researchers in the fields of malaria and in infant and young child nutrition. Zlotkin, a professor of Pediatrics, Nutritional Sciences and Public Health Sciences at the University of Toronto has worked collaboratively with the Kintampo Health Research Centre since the late 1990s, originally with Dr. Paul Arthur, Director at the time (now passed away) and for the past six years with Dr. Owusu-Agyei who has been the Director of KHRC since 2002. Zlotkin's original efficacy trials on 'Sprinkles' (a powdered mineral and vitamin fortificant) were completed at KHRC. The first collaborative manuscript was published in 2002 (29) the most recent publications from Ghana were published with Dr. Owusu-Agyei in 2006 (30, 35).

Sprinkles are sachets (like small packets of sugar) containing a blend of vitamins and minerals in powder form, which are easily sprinkled onto different foods. The single-serving sachets enable families without access to commercially fortified foods to add essential vitamins and minerals directly to traditional foods prepared in the home. They are inexpensive to produce, have no special storage requirements and are simple to use, even by those who cannot read. Because the iron is microencapsulated, there is no staining of a young child's teeth as may be the case with iron syrup or drops. A major advantage of the 'sprinkles' concept is that local foods can continue to be used and, thus, there is no need to teach caregivers how to prepare new and often expensive store-bought foods. Sprinkles were invented by the PI, Dr. Stanley Zlotkin, who, over the past ten years, has led a collaborative research team in multiple countries globally. Together these collaborative teams have demonstrated the absorption, efficacy, and effectiveness of microencapsulated iron, as well as the acceptability of the sprinkles concept (powdered minerals and vitamins) to treat and prevent iron and other micronutrient deficiencies (28, 29, 31, 35, 36).

As previously mentioned, the first randomized controlled trials were conducted in Ghana at KHRC. A total of 5 studies were completed at KHRC and published in peer-reviewed journals, including the American Journal of Clinical Nutrition and the Journal of Nutrition. More recently Dr. Zlotkin has collaborated with a research team from the University of California at Davis (led by Dr. Kay Dewey), and partners at the University of Ghana at Legon, to examine the relative merits of a powdered mineral and vitamin fortificant versus a fortified spread for the treatment and prevention of micronutrient deficiencies. Two recent publications describe that work (37, 38).

As well as having worked in Ghana with KHRC, Dr. Zlotkin has experience with a number of other countries and research organizations. He has a long-standing research collaboration with the Research and Evaluation Division of BRAC in Bangladesh. He and his collaborators at BRAC have evaluated the effectiveness and acceptability of powdered mineral and vitamin preparations when provided on a weekly basis (versus daily) and with a flexible regimen (20, 39). Dr. Zlotkin has also had successful research collaborations with the Chinese Centre for Disease Control (40), the Swiss Red Cross in Kyrgyzstan, World Vision International in Mongolia, CARE International in Benin (41), Agha Khan University in Pakistan (32, 42) and the King Edward Medical Hospital in India (25, 33, 43). In addition, he has collaborated with health economists to assess the cost effectiveness of 'point of use' powdered mineral and vitamin products (44). In northern Canada, powdered minerals and vitamins were examined in 'First Nations' populations (45-47). Powdered minerals and vitamins

have also been used, in collaboration with the World Food Program, World health Organization and Helen Keller International in relief situations (48).

Dr. Stanley Zlotkin has beneficial interests in certain intellectual property rights to his invention known as "Sprinkles". These interests include (i) patent rights for the United States and Canada only, which are held by Ped-Med Limited, a Canadian corporation, of which Dr. Zlotkin is the sole shareholder; and (ii) trade-marks rights in various jurisdictions to the name "Sprinkles" which are held either by Ped-Med Limited, or by the Sprinkles Global Health Initiative Inc. a Canadian not-for-profit corporation of which Dr. Zlotkin is a member.

Global recognition of a 'powdered mineral and vitamin fortificant' as an important, new tool for improving the health of children

The concept of using powdered mineral and vitamins packaged in a single-serving package has been recognized by international agencies such as UNICEF, the World Health Organization (WHO) and the World Food Program (WFP). Its success has been widely reported in both academic journals and the popular press. A number of government ministries of health have enacted legislation to include home fortification with powdered mineral and vitamin fortificants in recently updated national nutrition policies (Mongolia, Bolivia, Bangladesh, etc)

About the Kintampo Health Research Centre

Kintampo Health Research Centre (KHRC) is a well-established, African-based, research centre. The mission of KHRC is to conduct public health research and develop health research capacity which will contribute to a significant reduction in ill-health and the achievement of the Millennium Development Goals for Africa's most disadvantaged communities. The African identity of KHRC is important as it emphasizes African solutions to African health challenges. KHRC is one of three field research centers of the Health Research Unit of Ghana Health Service established in 1994. KHRC is situated in the middle belt of Ghana in the Brong Ahafo Region with a mandate to serve health policy and practice throughout Ghana and Africa.

There are over 500 employees at KHRC. KHRC has over 12 years of extensive experience in health research in Ghana. Among many areas of activity in which it has demonstrated skill, knowledge and core competency it has developed one of the largest and most reliable district surveillance systems (DSS) and study populations in Africa. KHRC has an established reputation for quality research and personnel. KHRC is highly regarded and is the preferred partner of governments, organizations and donors in health research initiatives in particular large-scale health research trials. KHRC funding collaborators include government, bilateral and multilateral institutions, private corporations, private charities and international organizations from Africa, Europe, Canada and the USA

KHRC Priority Research Areas

- Communicable diseases (CDs), particularly Malaria, TB and HIV/AIDS
- Sexual and Reproductive Health
- Maternal, Neonatal and Child Health
- Mental Health
- Non-communicable diseases (NCDs) such as hypertension and cancer
- Health Systems

- Using the DSS to track progress towards the Millennium Development Goals (MDGs) using indicators such as mortality levels, patterns and trends

Since 2003, Dr Owusu-Agyei and his research teams at KHRC have characterized the epidemiology of malaria in the Kintampo area in the middle belt of Ghana (49-51). He has documented the incidence of malaria in children less than 5 years in this area as 7 attacks per child per year (Owusu-Agyei et al., in press). The transmission levels have been high throughout the year with an average inoculation rate of 269 infective bites per person per year (Owusu-Agyei et al., in press). Dr Owusu-Agyei and his team of researchers are documenting the manifestations (both clinical and laboratory indicators) of severe and complicated malaria. Several antimalarials have been tested and evaluated in this area (52) and currently the most advanced malaria vaccine is being tested in Kintampo as one of several African countries. For more details on the KHRC laboratory and clinical facilities, please see pages 22-23 of this application (Resources Format Page).

The Kintampo Health Research Centre (KHRC) was awarded the 2008 Prince of Asturias Award for International Cooperation in recognition of its contribution to the fight against malaria in sub-Saharan Africa. The Prince of Asturias Foundation was formed by His Royal Highness, the Prince of Asturias, heir to the throne of Spain. It has conferred its awards since 1981. The awards are intended to acknowledge scientific, technical, cultural, social and humanitarian work carried out internationally by individuals, groups or in the categories of communication and humanities, social sciences, arts, letters, scientific and technical research, international cooperation, concord and sports.

The collaboration between the Research Institute at the Hospital for Sick Children in Toronto and KHRC in Ghana provides this project with a well-established and highly experienced team of experts in iron deficiency anemia, malaria control and Sprinkles-based research methodology. Given the past working relationship between the two partners, the protocol can be put into action without the need for preliminary relationship and trust-building activities between the two organizations.

Global Health at the University of Toronto, the Hospital for Sick Children and its Research Institute
One of the strategic directions of the Hospital for Sick Children in Toronto is "to lead nationally and internationally". Operationally this strategic goal is being met through programs within the Hospital, its Research Institute and its affiliation with the University of Toronto. For example, the Program for Global Pediatric Research at the Hospital was formed to address the disparity between the scientific research resources available in high-income countries and the quantity of scientific research focused on the health of children in mid- and low-income countries. This Program works at the centre of a global network to inform, educate, and facilitate international research cooperation and collaboration, as well as advocate for research to improve the health of all children. Zlotkin is a member of that program.

The Research Institute at The Hospital for Sick Children undertakes child-centered research across the life continuum from fetal origins to adult outcomes, including fundamental discovery, applied research, and outcomes and impact. Support for clinical trials at the Research Institute is through the Clinical Research Support Unit (CRSU). This Unit is a consultation service operated by the Child Health Evaluative Sciences Research Program of the Research Institute. The mandate of the CRSU is to improve the quality of clinical research at the Hospital by providing consultation in the areas of study design and methodology, statistical analysis, and data management.

Dr. Zlotkin is Head of the Division of Gastroenterology, Hepatology and Nutrition at the Hospital for Sick Children, a Senior Scientist in the Research Institute in the Child Health and Evaluative Sciences Research Program, and a Professor (of Pediatrics, Nutritional Sciences and Public health Sciences) at the University of Toronto. As a Professor, he has full access to the research facilities at the University. The University of Toronto is the largest university in Canada with more than 50,000 undergraduate students and 10,000 graduate students. It has more than 18,000 staff and faculty in 520 graduate programs and 42 professional programs. Its library system has over 18 million holdings, one of the top five research libraries in North America and its research grant and contract support is over $800 million.

The combined infrastructure of the Hospital, the Research Institute and the University of Toronto provide more than ample infrastructure support for the collaborative project described in this application.

4. RESEARCH DESIGN AND METHODS

4.1 Design Conceptual or Clinical Framework

Study Design

As stated previously, the efficacy of sprinkles in reducing iron deficiency anemia among infants and young children has been well documented; however the safety of this product in malaria endemic areas has not been investigated at the community level. The proposed study is a community-based blinded randomized controlled trial with the primary objective of determining the impact of providing Sprinkles (including microencapsulated iron as a powder added to complementary foods) on the susceptibility to clinical malaria among anemic and non-anemic infants and young children (6-35 months of age) living in a high malaria burden area. In order to achieve this objective, it is necessary to include a group that receives iron and a group that does not. Therefore, the trial has 2 study arms:

- Iron group: Eligible subjects will be randomized by compound to receive a daily dose of a powdered vitamin/mineral fortificant (Sprinkles) containing 12.5 mg of iron (plus ascorbic acid, vitamin A and zinc), added to complementary foods, for a period of 5 months during the wet season (between March and November). At the end of the 5-month intervention period, subject will discontinue Sprinkles use and be followed up for one additional month.

- Placebo group: Eligible subjects will be randomized by compound to receive a daily dose of a powdered vitamin/mineral fortificant (Sprinkles) containing ascorbic acid, vitamin A and zinc only (no iron), added to complementary foods, for a period of 5 months during the wet season (between March and November). At the end of the 5-month intervention period, subject will discontinue Sprinkles use and be followed up for one additional month.

Primary and Secondary Outcomes

The primary outcome will be ***clinical malaria***, defined (according to WHO criteria) as parasitemia (malaria parasites detected on a blood smear) of any density plus history of fever (within 48 hours) or axillary temperature >37.5^{0}C. Criteria for clinical malaria will be taken from the UNICEF Case Management Series, entitled, "Promoting Rational Use of Drugs and Correct Case Management in Basic Health Services – Malaria Prevention and Treatment" published by UNICEF's Programme Division in cooperation with the World Health Organization in 2000. To distinguish unique malaria episodes, treatment will be supervised and follow-up blood smears will be collected from the child as described below in Section 5.2(c).

Secondary outcomes will include: (i) changes in anemia status; (ii) the severity of the clinical malaria (based on level of parasitemia); (iii) cerebral malaria; (iv) hospitalization (from any cause) (v) death; (vi) pneumonia; (vii) diarrhea and dehydration.

Subjects/study population

Infants and young children (6-35 months of age), living in the Brong Ahafo Region of northern Ghana, will be included in the study if, at baseline, they are ingesting weaning foods in addition to breastmilk; free from major illness; afebrile; living in the study area for the duration of the intervention and follow-up period; and if parental consent is obtained. Inclusion of a child into the study will proceed after the possible risks and benefits are discussed with the parents (in the appropriate local language), and a signed consent is obtained. The exclusion criteria are as follows: severe anemia (hemoglobin <70g/L), weight-for-height <-3 z-score (severe wasting), kwashiorkor (defined as evidence of edema), congenital abnormality, treatment with iron supplements in the past 6 months, or any chronic illness. Children that are severely anemic (Hb<70 g/L) and/or severely malnourished will be excluded from entry to the study and referred to the local health provider for treatment according to Ghana Ministry of Health guidelines.

Sample Size

We hypothesize that the incidence of malaria will be significantly higher in children receiving iron versus a placebo of micronutrients without iron. It was decided not to use the change in anemia status as a primary outcome since, in all previous studies with powdered mineral and vitamin fortificants, anemia rates declined compared to placebo controls, or were no different from the improvement in anemia rates observed with iron drops.

Based on the 2006 estimate of "cases and deaths from fevers suspected of being malaria" for children under 5 years of age in all of Ghana (53), the baseline rate of 3.44 episodes/child/year was assumed. We estimated that a total of 351 person-years would be required to detect a 15% increase in malaria incidence rates with 80% power and at a 5% type I error. Based on the experience and expertise of the PI and co-investigator, a difference of 15% in malaria incidence was considered to be clinically significant. Knowing that all children enrolled in the trial will begin the 5-month intervention period at a similar risk level, and accounting for 15% loss to follow-up, as well as the need to test the hypothesis twice (due to the interim analysis by the Data Safety and Monitoring committee), the sample size has been calculated as 1940 children (970 per group) (see Table 1 below). All calculations were reviewed by 2 statisticians who will be involved in subsequent analyses.

Table 1: Sample size calculation adjusted for testing the hypothesis twice

Least Meaningful Difference	**2006 rates**	**Adjustment for use of bed-net**	**Rate unexposed**	**Rate exposed**	**Person-years**	**Follow-up years**	**n**	**Loss to follow-up**	**Sample size per group***
0.05	3.44	0.75	2.58	2.71	3017	0.42	7240	0.15	8326
0.1	3.44	0.75	2.58	2.84	772	0.42	1854	0.15	2132
0.15	**3.44**	**0.75**	**2.58**	**2.97**	**351**	**0.42**	**843**	**0.15**	**970**
0.2	3.44	0.75	2.58	3.10	202	0.42	485	0.15	558

*Adjusted for estimated bed net use and loss to follow-up

4.2 Procedures

Recruitment, Randomization Process and Blinding

Subjects will be recruited from villages in the Wenchi District once permission has been obtained from the village assemblies and elders, as well as the parent or guardian of the eligible child. Field researchers, employed by the KHRC, will visit individual compounds and interview parents or caregivers regarding the inclusion criteria. Once consent has been obtained and documented via signature or thumbprint, eligible children will be enrolled and randomized (at the compound level), using a computer-generated model, to either the iron (Fe) or placebo (P) group.

Sachets containing the powdered minerals with and without iron will look and taste identical with the exception that each package will be marked with an 'A' or 'B' denoting packages with or without iron. The study team and caretakers of children will be blinded to the 'A' or 'B' designation. Only the manufacturer of the powdered fortificant (in Ghana) and a research pharmacist each in Toronto and Kintampo will hold the key to the randomization and 'sachet' code lists. The key will be revealed to the 'Data and Safety Monitoring Committee (DSMC)' if a significant difference in outcomes is observed, and to the researchers after the database is closed and statistical analyses completed.

Supply and content of the powdered mineral and vitamin fortificant.

A powdered mineral and vitamin fortificant product, "Sprinkles", will be used in the proposed study. The Sprinkles formulation contains a specific combination of minerals and vitamins, including iron in the form of microencapsulated ferrous fumarate. The applicant (SZ) has extensive experience with the Sprinkles product both in research protocols and in working with governments, UN agencies and NGOs for scaling up the intervention. The product will be procured from a production facility in Canada, India or Bangladesh. Each of these facilities has supplied a reliable high quality product to the applicant for past research projects. The production facilities in India and Bangladesh are UNICEF approved facilities. The dose of micronutrients including iron is shown in Table 2 below. The dose of individual nutrients is based on WHO or IOM-DRI guidelines, or estimates based on these guidelines.

Table 2: Justification of a powdered mineral and vitamin supplement dose for infants and young children

	6-11 mo		12-24 mo		Powdered fortificant
	WHO[1]	IOM DRI[2]	WHO	IOM DRI	
Vitamin A, µg RE	400	500*	400	300	400
Vitamin C, mg	30	50*	30	15	30
Folic Acid, µg	80	80*	160	150	OMIT
Iron, mg[3]	9.3	11	5.8	7	12.5
Zinc mg[4]	4.1	3	1.1	3	5

[1] Recommended Nutrient Intakes. Source: Joint FAO/WHO Expert Consultation. (2002) Vitamin and mineral requirements in human nutrition. World Health Organization, Geneva, Switzerland.

[2] Recommended Dietary Allowances (RDA). Sources: Institute of Medicine, Dietary Reference Intakes. National Academy Press, Washington D.C.

[3] The dose of 12.5 mg is the current INACG/WHO/UNICEF recommendation for children 6-35 months of age for large scale distribution. It assumes a medium bioavailability (5-10%).

[4] Assuming moderate bioavailability (30%)
* Based on Adequate Intake (AI) estimates

Clinical protocol

a) Baseline: After consent is provided, subjects will be screened for anemia (based on hemoglobin) and parasitemia. Enough blood will be taken to determine C-reactive protein (CRP), serum ferritin (SF), zinc protoporphyrin (ZnPP) and serum transferrin receptor (TfR). Those who are severely anemic (Hb <70 g/L), or have clinical signs of severe malnourishment will be referred to the study clinician who will offer the child the standard care, as defined by the Ghana Ministry of Health. All other children will be enrolled and randomized to either the iron (Fe) or placebo (P) group. The child's weight and length measurements will be recorded, as well as any relevant demographic (age, gender) and historical health information (birth weight and previous hospitalizations). Caregivers will also be asked to provide information regarding their usual feeding practices and health-seeking behaviours. All households will be provided with a treated bed net and a supply of powdered fortificant with oral instructions on how to use each.

b) Monitoring visits: Field researchers will visit each subject at their home every week for the duration of the intervention and follow-up periods. At each visit, the field researcher will conduct a health assessment (including axillary temperature) and collect information on supplement compliance, bed net use and morbidity. At the beginning of the intervention period, 7 new sachets will be provided at each weekly visit. In the following weeks, the number of sachets supplied at one visit will be increased in a stepwise manner until a monthly distribution schedule (30 new sachets) is obtained. Clear instructions for storage and administration of sachets will be provided to families. Sprinkles adherence will be assessed by counting and recording all used and unused sachets at each home visit. It has been our experience in other similar studies in Ghana and elsewhere that by providing pre-emptive information on the impact of the fortificant to parents (like telling parents that stools will darken from the iron, and that children will become more active and have enhanced appetites), the adherence to the use of the sachets is generally in the range of 70 – 98%. We will provide this pre-emptive information in the current study.

c) Tracking the subjects: All subjects who become ill and need to visit a health facility will be identified and tracked through their study identity cards. The use of such cards is standard practice at the Kintampo Health Research Centre (KHRC). If a fever has been reported or recorded, a blood sample will be taken to determine parasite species and count. If the child is admitted to the hospital or health centre, blood parasite level will similarly be determined, and further tests will be conducted (as needed) to rule out cerebral malaria, pneumonia, diarrhea, and/or dehydration. All treatments will follow the treatment guidelines in Ghana (Ghana Ministry of Health). For those assessed to have malaria, the first-line antimalarial (Artesunate-Amodiaquine) will be prescribed and provided. To determine if the treatment has been successful, and from the perspective of the study, to distinguish unique malaria episodes, treatment will be supervised and follow-up blood smears will be collected from the child on the 7th, and 14th day following treatment (this process has been successfully used in past trials including "A multi-centre, randomized, double-blind, double dummy study comparing the efficacy and safety of chlorproguanil-dapsone-artesunate versus artemether-lumefantrine in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in children and adolescents in Africa Protocol #: SB-714703/005. Collaborations among KHRC/LSHTM/GSK/MMV/WHO"). All slides (thick and thin films) will be stained with Giemsa following fixing of the thin film and read twice by independent microscopists blinded to each other's reading. A third microscopist will be asked to read the slide if there is disagreement between the first two. The readings will be used to determine if the treatment has been successful or not.

d) Endline: At the end of 5 months, subjects will again be screened for anemia, CRP, ferritin, TfR, zinc protoporphyrin and a blood smear for their malaria status. The subjects will be weighed and measured, and caregivers will again be asked about supplement use, and bed net use. Any remaining sachets will be collected and counted. For those children who are anemic, based on their end-line hemoglobin assessment, an additional 60 sachets of iron-containing powdered fortificant will be provided at the end of the post-intervention follow-up period. Providing iron to anemic children is not a safety issue in this case because they will be eligible for treatment according to the WHO statement on the use of iron in malaria endemic areas (3).

e) Post-intervention Follow up: After the 5-month intervention period, subjects will discontinue Sprinkles use and be followed up on a weekly basis for one additional month. At these visits, the field researcher will continue to conduct health assessments (including axillary temperature) and collect information on bed net use and morbidity. If a subject becomes ill and/or needs to visit a health facility, the same protocol as described above will be used for tracking and monitoring these cases. All families that have completed the intervention and follow up periods will be provided with a study closure package (including a bar of soap) as has been the standard goodwill practice of KHRC to participants from the communities.

f) *Discontinuation of supplementation*. Supplementation will be stopped if any of the following is reported: admission to the hospital ward for >5 days; evidence of chronic diarrhea (defined as loose stools lasting >4 weeks); prolonged fever of unknown origin; emergence of any exclusion criteria after randomization. Prompt, daily disclosure by field workers of any concerns will be encouraged. If necessary, children will be referred to local physicians for treatment according to local standards. Reasons for stopping supplementation voluntarily will be recorded.

4.3 Analyses

Laboratory and Clinical Analyses

All laboratory analyses will be conducted at the Kintampo Health Research Centre by qualified laboratory technicians.

Screening for malaria will be performed in the field using rapid diagnostic tests (RDTs) to help decide on treatment and blood smears for microscopy to provide counts. All clinical signs and symptoms will be interpreted using standardized 'case definitions' as described in previous studies (16, 54). For example, cerebral malaria (defined by a parasite count >5000/μL blood and a concurrent score of $\leq$2 on the Blantyre coma scale, with or without convulsions), pneumonia (defined by the presence of a cough or breathing difficulties, tachypnea, lower chest wall indrawing, and the appearance of consolidation or pleural effusion on a chest X-ray), diarrhea (defined by $\geq$3 loose or watery stools in the previous 24 hours), and dehydration (defined by lethargy, sunken eyes, and decreased skin turgor [>2 seconds for skin to return following a skin pinch]) will be assessed in the hospital or health centre using standard procedures/methods. Severe malaria disease will be diagnosed based on symptoms and signs occurring at presentation or developing during admission according to generally accepted case definitions as have been used in all previous studies in Ghana. These 'case definitions' are available on request.

For identifying and analyzing cause-specific deaths or admissions to hospital, we will use exclusive categories to ensure that independent events are not classified more than once. As such, we will first allocate malaria related causes, then pneumonia and other infection related causes, and finally diarrhea and others.

Blood samples will be analyzed for full blood counts (CBC) using a hematology auto-analyzer (Horiba ABX Micros 60-OT). This machine is a recent addition to Kintampo Health Research Centre laboratory, and needs only drop of blood (a few micro-litres) to perform analyses for Hemoglobin, hematocrit, and various red blood cell (RBC) indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW). Red blood cell indices are the reference standard for identifying anemic subjects, and can help to differentiate between types of anemias. Therefore, measuring the RBC indices permit us to narrow down the possible causes of an anemia. For example, the MCV is an index of the size of the red blood cells. When the MCV is below normal, the RBCs will be smaller than normal (microcytic), and when the MCV is elevated, the RBCs will be larger than normal (macrocytic). Red blood cells of normal size are termed normocytic. Failure to produce hemoglobin results in smaller than normal cells (microcytosis). This occurs in many diseases, including iron deficiency anemia and thalassemia. Macrocytic cells occur when division of RBC precursor cells in the bone marrow is impaired. The most common causes of macrocytic anemia are vitamin B_{12} deficiency, folate deficiency, and liver disease. Normocytic anemia may be caused by decreased production of RBCs (e.g., malignancy and other causes of bone marrow failure), increased destruction (hemolytic anemia), or blood loss. For example, with malaria there is red blood cell hemolysis, therefore the RBC count is low, but the size and amount of hemoglobin in the cells tends to be normal.

A low MCH indicates that cells have too little hemoglobin. This is caused by deficient hemoglobin production. Such cells will be pale when examined under the microscope and are termed hypochromic. Iron deficiency is the most common cause of a hypochromic anemia. The MCHC is the ratio of hemoglobin mass in the RBC to cell volume. Cells with too little hemoglobin are lighter in color and have a low MCHC. The MCHC is low in microcytic, hypochromic anemias such as iron deficiency, but is usually normal in macrocytic anemias.

Finally, the red cell distribution width (RDW) is a measure of the variance in red blood cell size. It is calculated by dividing the standard deviation (a measure of variation) of RBC volume by the MCV and multiplying by 100. A large RDW indicates abnormal variation in cell size, termed anisocytosis. The RDW aids in differentiating anemias that have similar indices. For example, thalassemia minor and iron deficiency anemia are both microcytic and hypochromic anemias, and overlap in MCV and MCH. However, iron deficiency anemia has an abnormally high RDW, but thalassemia minor does not. Therefore, by using a combination of the RBC indices, it will be possible to differentiate between iron deficiency anemia and thalassemia, and between iron deficiency and the anemia of malaria.

Plasma CRP, ferritin, transferrin receptor, and red blood cell zinc protoporphyrin will also be assayed using standard methods, including internal and external reference standards. Since CRP is a measure of inflammation, blood samples from subjects with elevated CRP values (>8 mg/L) will be excluded from further analysis (55). It should be noted however, that even in a malaria endemic area, albeit in school children (in Zanzibar), iron status assessment using these indicators may not be seriously influenced by malarial infection (4). Zinc protoporphyrin is a metabolic intermediate of the hemoglobin synthetic pathway which accumulates in red blood cells when iron supply is limited. It can be easily measured fluorometrically and is expressed as a ratio to heme (ZPP/H). In adults, ZPP/H correlates inversely with plasma ferritin across a wide range of ferritin concentrations (56), and is inversely related to the amount of stainable iron in the marrow (57). In adults (56, 58, 59) and children (60) ZPP/H has been shown to be more sensitive than the packed cell volume or hemoglobin concentration in detecting iron deficiency. It is particularly suited as a screening test because it is cheap, convenient (61) and can be carried out on a single drop of blood (62). ZPP/H ratios are expressed as μg/g hemoglobin (Hb) and plasma

ferritin as μg/L. ZPP/H and plasma ferritin will be log transformed (to the base 10) before analysis to normalize the distribution, as both are usually positively skewed.

TfR and ZnPP are sensitive measures of iron-deficient erythropoiesis and have been used to define iron status in children in developing countries (63-67). TfR may have an advantage over SF because it is unaffected by the acute phase response (63, 68, 69). However, the specificity of TfR may be low because it can be increased by malaria (70), megaloblastic anemia due to vitamin deficiencies (71), and hemoglobinopathies such as sickle cell disease (72), hemoglobin H disease, and the thalassemias (73, 74). ZnPP has advantages of low cost and simplicity, but its specificity may be low as it also can be increased by malaria and other infections, chronic inflammation, and hemoglobinopathies (60, 63, 64, 75-77). To improve specificity, SF is often combined with TfR, ZnPP, or both (78). In the current protocol, we will include all three measurements in our assessment of iron status.

Zimmerman et al recently studied the use of serum transferrin receptor and zinc protoporphyrin as indicators of iron status in African children (Côte d'Ivoire). He determined the most sensitive and specific markers for iron status and their 'optimal' diagnostic cutoffs (TfR >9.4 mg/L or ZnPP > 52 μmol/mol heme) after correcting for inflammation (79). We plan to use these cutoffs to define iron status as iron replete and anemic, iron deficient and anemic, of iron replete and not anemic. Zimmerman defined anemia as a hemoglobin concentration below the WHO cutoff values (110 g/L) minus 10 g/L, and normal ferritin as > 30 μg/L (79). Several investigators have argued that a SF concentration >30 μg/L should be used to define adequate iron stores in developing countries with a high prevalence of infection (63, 80). Therefore, we have chosen a SF cutoff of >30 μg/L as being indicative of iron sufficiency.

To summarize: anemia will be defined as Hb <100 g/L; IDA will be defined as Hb <100 g/L; plus two of low MCV (<80 μm^3), MCH (<26.5 pg), MCHC (<31.5 g/dL), Hct (<35 %) or high RDW (>10 %); plus one of low ferritin (<30 μg/L), high TfR (>9.4 mg/L) or high ZnPP (>52 μmol/mol heme); and iron deficiency will be defined as one of low ferritin (<30 μg/L) or high ZnPP (>52 μmol/mol heme). We recognize that infection and inflammation will confound the interpretation of ZnPP; thus we will exclude and/or adjust this indicator for those children who have signs of infection or inflammation, as defined by an elevated CRP concentration (>8 mg/L) (55, 74).

4.4 Data Collection and Management

Baseline demographic, anthropometric, and health history data, as well as information pertaining to feeding practices and health-seeking behaviours, will be collected by field researchers employed by the KHRC. All blood samples will be collected by field researchers or other qualified individuals, trained in pediatric phlebotomy techniques.

Visual Basic will be used to manage data. All data obtained in the field will be entered by the end of the next day. The systems used will have extensive range and checking facilities. Possible errors will be verified with field or hospital staff on a daily basis. Data collection and supplement allocation will be rigorously controlled with the help of computer monitoring. For all outcomes, a double-data entry will be used to detect errors. Flow of information, distribution of supplements, and collection of samples between households and villages in Wenchi and the central office at the Kintampo Health Research Centre will be ensured by supervisors and the study coordinator (by motorbike or car) on the same day.

4.5 Data Analysis

All variables will be first explored and summarized using descriptive statistics such as number of events in person-time, incidence rates, means and standard deviations, medians and ranges, counts and proportions, and various graphs, as appropriate.

Primary analysis

The primary outcome, incidence of malaria, will be compared between the two groups using Poisson regression. A 95% confidence interval for the ratio between the incidence in the iron group and the incidence in the placebo group will be calculated. The upper boundary of this confidence interval will be compared to the 10% tolerance limit. If it is lower, then non-inferiority can be concluded.

Secondary analysis

Univariate Poisson regression with the same outcome will be used to evaluate associations with other variables: iron status at baseline, breast-feeding, age, gender and use of bed-net. These will then be introduced and re-evaluated in a multiple Poisson regression model, which will allow for adjusted group comparisons. Most importantly, by including the baseline iron status variable and the interaction with the main group variable, we can focus on the iron sufficient children that are receiving iron supplements and compare them against the others. The Poisson models will be validated by checking the model fit and for over-dispersion. Adjustments or transformations will be used if necessary.

The number of hospital admissions will be analyzed in a similar fashion, using Poisson regression. The parasite count outcome will be compared between the two groups, using a t-test. Univariate associations with other factors will be verified here as well, using t-tests, correlation coefficients and univariate regression. Ultimately a multiple regression model will be developed, again for the purpose of adjusted comparisons between groups.

The normality of the data will be verified and, in the case of any departures, a log-transformation will be used and the data reanalyzed. If the log-transformation fails to achieve normality, a nonparametric alternative will be used instead. All statistical analyses will be carried out using SAS 9.1. The Analysis will be carried out on an intention-to-treat basis including all randomized children.

Upon the completion of the first intervention phase, an interim analysis will be performed by a Data and Safety Monitoring Committee (DSMC) as described above (primary analysis). The study will be stopped if the incidence of malaria is higher in one group.

4.6 Data Interpretation

Findings from the proposed research will provide evidence regarding the safety and efficacy of providing daily iron supplementation (12.5 mg/day), in the form of a micronutrient powder that is added to complementary foods, during the wet season in a malaria endemic area.

4.7 Data Sharing Plan

Findings from the proposed research will be shared according to the applicable NIH policy for foreign institutions.

4.8 Potential Difficulties and Limitations

a) Blinding: Although research staff will be blinded to the intervention, the stools of children receiving the iron containing fortificant will likely be darker (blacker) than those receiving the placebo. The applicants are unable to control for this likelihood.

b) It will not be possible to compare the results of the proposed current study to many of the results from the Pemba study. In Pemba, the primary outcome was mortality, which necessitated a sample size of more than 30,000 subjects. Also, in the current protocol, folic acid will not be included in the powdered mineral and vitamin supplement. Based on the estimated dietary folic acid intake of older infants and children in West Africa, folic acid deficiency should not be a problem (fruit is plentiful and inexpensive). Lastly, in the current protocol all children will receive and be encouraged to use bed-nets. This was not the case in the Pemba study.

c) Although studies in adults have suggested that elevations in ZnPP (68) can be definitive indicators of iron deficiency, there is large overlap in the distribution of this indicator in children with iron deficiency anemia as well as those with normal iron status. This overlap may be explained by a greater variability in the erythroid mass in children than in adults (75) together with the many variables affecting children in developing countries that influence ZnPP independent of iron status. Because of this overlap, the sensitivity and specificity of ZnPP in identifying iron deficiency and IDA may not be as high as we would prefer regardless of the diagnostic cutoff chosen. Despite this limitation. ZnPP will be statistically evaluated individually and in conjunction with CBC values since this measure is very important in the context of the current study.

4.9 Tentative Project Timetable

TASKS	Nov 2009	Dec 2009	Jan 2010	Feb 2010	Mar 2010	Apr 2010	May 2010	Jun 2010	Jul 2010	Aug 2010	Sep 2010	Oct 2010	Nov 2010	Dec 2010	Jan 2011	Feb 2011	Mar 2011
Ethics and FDB approval																	
Sprinkles procurment																	
Community Meetings																	
DSMC preliminary meeting																	
Training of all field staff																	
Subject screening & recruitment																	
Intervention Period																	
Post-intervention follow-up																	
Sprinkles (+Fe) distribution to subjects with anemia at endline																	
DSMC assessment																	
Biochemical analyses																	
Data entry and cleaning																	
Data analysis																	
Manuscript writing and submission																	

5. BIBLIOGRAPHY AND REFERENCES

1. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr 2007;85(4):931-45.
2. Bhagwati J, Bourguignon F, Kydland F, Mundell R, North D, Schelling T, et al. Copenhagen Consensus 2008 Frederiksberg, Denmark: Copenhagen Concensus Centre; 2008.
3. WHO/UNICEF. Joint statement: Iron supplementation of young children in regions where malaria transmission is intense and infectious disease highly prevalent; 2006.
4. Stoltzfus RJ, Kvalsvig JD, Chwaya HM, Montresor A, Albonico M, Tielsch JM, et al. Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: double blind, placebo controlled study. Bmj 2001;323(7326):1389-93.

5. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr 2001;131(2S-2):649S-666S; discussion 666S-668S.
6. Bates CJ, Powers HJ, Lamb WH, Gelman W, Webb E. Effect of supplementary vitamins and iron on malaria indices in rural Gambian children. Trans R Soc Trop Med Hyg 1987;81(2):286-91.
7. Smith AW, Hendrickse RG, Harrison C, Hayes RJ, Greenwood BM. The effects on malaria of treatment of iron-deficiency anaemia with oral iron in Gambian children. Ann Trop Paediatr 1989;9(1):17-23.
8. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. J Nutr 2001;131(2S-2):616S-633S; discussion 633S-635S.
9. van den Hombergh J, Dalderop E, Smit Y. Does iron therapy benefit children with severe malaria-associated anaemia? A clinical trial with 12 weeks supplementation of oral iron in young children from the Turiani Division, Tanzania. J Trop Pediatr 1996;42(4):220-7.
10. Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. Bmj 2002;325(7373):1142.
11. Atkinson SH, Rockett K, Sirugo G, Bejon PA, Fulford A, O'Connell MA, et al. Seasonal childhood anaemia in West Africa is associated with the haptoglobin 2-2 genotype. PLoS Med 2006;3(5):e172.
12. WHO/UNICEF. World Malaria Report 2005. In. Geneva: http://www.rbm.who.int/wmr2005/
13. WHO. World Health Report 2003: Shaping the future; 2003.
14. Appawu M, Owusu-Agyei S, Dadzie S, Asoala V, Anto F, Koram K, et al. Malaria transmission dynamics at a site in northern Ghana proposed for testing malaria vaccines. Trop Med Int Health 2004;9(1):164-70.
15. Koram KA, Owusu-Agyei S, Fryauff DJ, Anto F, Atuguba F, Hodgson A, et al. Seasonal profiles of malaria infection, anaemia, and bednet use among age groups and communities in northern Ghana. Trop Med Int Health 2003;8(9):793-802.
16. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. Lancet 2006;367(9505):133-43.
17. Iyer JK, Shi L, Shankar AH, Sullivan DJ, Jr. Zinc protoporphyrin IX binds heme crystals to inhibit the process of crystallization in Plasmodium falciparum. Mol Med 2003;9(5-8):175-82.
18. Dewey KG, Domellof M, Cohen RJ, Landa Rivera L, Hernell O, Lonnerdal B. Iron supplementation affects growth and morbidity of breast-fed infants: results of a randomized trial in Sweden and Honduras. J Nutr 2002;132(11):3249-55.
19. Majumdar I, Paul P, Talib VH, Ranga S. The effect of iron therapy on the growth of iron-replete and iron-deplete children. J Trop Pediatr 2003;49(2):84-8.
20. Ip H, Hyder SM, Haseen F, Rahman M, Zlotkin SH. Improved adherence and anaemia cure rates with flexible administration of micronutrient Sprinkles: a new public health approach to anaemia control. Eur J Clin Nutr 2007.
21. Bergdahl B, Bogentoft C, Jonsson UE, Magnusson JO. Fasting and postprandial absorption of digoxin from a microencapsulated formulation. Eur J Clin Pharmacol 1983;25(2):207-10.
22. Olver JS, Burrows GD, Norman TR. The treatment of depression with different formulations of venlafaxine: a comparative analysis. Hum Psychopharmacol 2004;19(1):9-16.
23. Baldi A, Bontempo V, Cheli F, Carli S, Sgoifo Rossi C, Dell'Orto V. Relative bioavailability of vitamin E in dairy cows following intraruminal administration of three different preparations of DL-alpha-tocopheryl acetate. Vet Res 1997;28(6):517-24.

24. Lakritz J, Wilson WD, Marsh AE, Mihalyi JE. Effects of prior feeding on pharmacokinetics and estimated bioavailability after oral administration of a single dose of microencapsulated erythromycin base in healthy foals. Am J Vet Res 2000;61(9):1011-5.
25. Zlotkin SH, Christofides AL, Hyder SM, Schauer CS, Tondeur MC, Sharieff W. Controlling iron deficiency anemia through the use of home-fortified complementary foods. Indian J Pediatr 2004;71(11):1015-9.
26. Zlotkin SH, Schauer C, Christofides A, Sharieff W, Tondeur MC, Hyder SM. Micronutrient sprinkles to control childhood anaemia. PLoS Med 2005;2(1):e1.
27. Schauer C, Zlotkin SH. Home fortification with micronutrient sprinkles - A new approach for the prevention and treatment of nutritional anemias. Paediatric Child Health 2003;8(2):87-90.
28. Zlotkin S, Antwi KY, Schauer C, Yeung G. Use of microencapsulated iron(II) fumarate sprinkles to prevent recurrence of anaemia in infants and young children at high risk. Bull World Health Organ 2003;81(2):108-15.
29. Zlotkin S, Arthur P, Antwi KY, Yeung G. Treatment of anemia with microencapsulated ferrous fumarate plus ascorbic acid supplied as sprinkles to complementary (weaning) foods. Am J Clin Nutr 2001;74(6):791-5.
30. Christofides A, Asante KP, Schauer C, Sharieff W, Owusu-Agyei S, Zlotkin S. Multi-micronutrient Sprinkles including a low dose of iron provided as microencapsulated ferrous fumarate improves haematologic indices in anaemic children: a randomized clinical trial. Matern Child Nutr 2006;2(3):169-80.
31. Zlotkin S, Arthur P, Schauer C, Antwi KY, Yeung G, Piekarz A. Home-fortification with iron and zinc sprinkles or iron sprinkles alone successfully treats anemia in infants and young children. J Nutr 2003;133(4):1075-80.
32. Khan E, Hyder SMZ, Tondeur MC, Raza S, Khan NA, Zlotkin S. Home fortification with Sprinkles to reduce childhood anemia: Lessons learned in North West Frontier Province, Pakistan. Pakistan J of Med Res 2006;45(2):35-40.
33. Hirve S, Bhave S, Bavdekar A, Naik S, Pandit A, Schauer C, et al. Low dose 'Sprinkles'-- an innovative approach to treat iron deficiency anemia in infants and young children. Indian Pediatr 2007;44(2):91-100.
34. Menon P, Ruel MT, Loechl CU, Arimond M, Habicht JP, Pelto G, et al. Micronutrient Sprinkles reduce anemia among 9- to 24-mo-old children when delivered through an integrated health and nutrition program in rural Haiti. J Nutr 2007;137(4):1023-30.
35. Zlotkin SH, Schauer C, Owusu Agyei S, Wolfson J, Tondeur MC, Asante KP, et al. Demonstrating zinc and iron bioavailability from intrinsically labeled microencapsulated ferrous fumarate and zinc gluconate Sprinkles in young children. J Nutr 2006;136(4):920-5.
36. Tondeur MC, Schauer CS, Christofides AL, Asante KP, Newton S, Serfass RE, et al. Determination of iron absorption from intrinsically labeled microencapsulated ferrous fumarate (sprinkles) in infants with different iron and hematologic status by using a dual-stable-isotope method. Am J Clin Nutr 2004;80(5):1436-44.
37. Adu-Afarwuah S, Lartey A, Brown KH, Zlotkin S, Briend A, Dewey KG. Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: effects on growth and motor development. Am J Clin Nutr 2007;86(2):412-20.
38. Adu-Afarwuah S, Lartey A, Brown KH, Zlotkin S, Briend A, Dewey KG. Home fortification of complementary foods with micronutrient supplements is well accepted and has positive effects on infant iron status in Ghana. Am J Clin Nutr 2008;87(4):929-38.
39. Hyder Z, Zlotkin S. Effect of daily versus once weekly home fortification with sprinkles on haematological and iron status among young children in rural Bangladesh. Food and Nutrition Bulletin 2007;28(2):156-164.
40. Sharieff W, Yin SA, Wu M, Yang Q, Schauer C, Tomlinson G, et al. Short-term daily or weekly administration of micronutrient Sprinkles has high compliance and does not cause iron overload in Chinese schoolchildren: a cluster-randomised trial. Public Health Nutr 2006;9(3):336-44.

41. Sharieff W, Dofonsou J, Zlotkin S. Is cooking food in iron pots an appropriate solution for the control of anaemia in developing countries? A randomised clinical trial in Benin. Public Health Nutr 2008;11(9):971-7.
42. Sharieff W, Bhutta Z, Schauer C, Tomlinson G, Zlotkin S. Micronutrients (including zinc) reduce diarrhoea in children: the Pakistan Sprinkles Diarrhoea Study. Arch Dis Child 2006;91(7):573-9.
43. Zlotkin S. Control of anemia: the time to act is now. Indian Pediatr 2007;44(2):84-6.
44. Sharieff W, Horton SE, Zlotkin S. Economic gains of a home fortification program: evaluation of "Sprinkles" from the provider's perspective. Can J Public Health 2006;97(1):20-3.
45. Christofides A, Schauer C, Zlotkin S. Iron deficiency anemia among children: Addressing a global public health problem within a Canadian context. Paediatric Child Health 2005;10(10):597-601.
46. Christofides A, Schauer C, Zlotkin SH. Iron deficiency and anemia prevalence and associated etiologic risk factors in First Nations and Inuit communities in Northern Ontario and Nunavut. Can J Public Health 2005;96(4):304-7.
47. Christofides A, Schauer C, Sharieff W, Zlotkin SH. Acceptability of micronutrient sprinkles: a new food-based approach for delivering iron to First Nations and Inuit children in Northern Canada. Chronic Dis Can 2005;26(4):114-20.
48. De Pee S, Moench-Pfanner R, Martini E, Zlotkin S, Darton-Hill I, Bloem MW. Home-fortification in emergency response and transition programming: Experiences in Aceh and Nias, Indonesia. Food and Nutrition Bulletin 2007;28(2):189-197.
49. Chandramohan D, Hodgson A, Coleman P, Baiden R, Asante K, Awine E, et al. An evaluation of the immunogenicity and safety of a new trivalent meningococcal polysaccharide vaccine. Vaccine 2007;25 Suppl 1:A83-91.
50. Edmond KM, Kirkwood BR, Amenga-Etego S, Owusu-Agyei S, Hurt LS. Effect of early infant feeding practices on infection-specific neonatal mortality: an investigation of the causal links with observational data from rural Ghana. Am J Clin Nutr 2007;86(4):1126-31.
51. Owusu-Agyei S, Awini E, Anto F, Mensah-Afful T, Adjuik M, Hodgson A, et al. Assessing malaria control in the Kassena-Nankana district of northern Ghana through repeated surveys using the RBM tools. Malar J 2007;6:103.
52. Owusu-Agyei S, Asante KP, Owusu R, Adjuik M, Amenga-Etego S, Dosoo DK, et al. An open label, randomised trial of artesunate+amodiaquine, artesunate+chlorproguanil-dapsone and artemether-lumefantrine for the treatment of uncomplicated malaria. PLoS ONE 2008;3(6):e2530.
53. WHO. World Malaria Report 2008. Geneva, Switzerland; 2008.
54. Chandramohan D, Owusu-Agyei S, Carneiro I, Awine T, Amponsa-Achiano K, Mensah N, et al. Cluster randomised trial of intermittent preventive treatment for malaria in infants in area of high, seasonal transmission in Ghana. Bmj 2005;331(7519):727-33.
55. Verhoef H, West CE, Ndeto P, Burema J, Beguin Y, Kok FJ. Serum transferrin receptor concentration indicates increased erythropoiesis in Kenyan children with asymptomatic malaria. Am J Clin Nutr 2001;74(6):767-75.
56. Labbe RF, Finch CA, Smith NJ, Doan RN, Sood SK, Madan N. Erythrocyte protoporphyrin/heme ratio in the assessment of iron status. Clin Chem 1979;25(1):87-92.
57. McLaren GD, Carpenter JT, Jr., nino HV. Erythrocyte protoporphyrin in the detection of iron deficiency. Clin Chem 1975;21(8):1121-7.
58. Jensen BM, Sando SH, Grandjean P, Wiggers P, Dalhoj J. Screening with zinc protoporphyrin for iron deficiency in non-anemic female blood donors. Clin Chem 1990;36(6):846-8.
59. Labbe RF, Rettmer RL, Shah AG, Turnlund JR. Zinc protoporphyrin. Past, present, and future. Ann N Y Acad Sci 1987;514:7-14.

60. Siegel RM, LaGrone DH. The use of zinc protoporphyrin in screening young children for iron deficiency. Clin Pediatr (Phila) 1994;33(8):473-9.
61. Schifman RB. Red cell protoporphyrin: an alternatie to serum ferritin for assessing iron depletion [Abstract]. Clin Chem 1982;28:1628.
62. Cavill I, Jacobs A, Worwood M. Diagnostic methods for iron status. Ann Clin Biochem 1986;23 (Pt 2):168-71.
63. Asobayire FS, Adou P, Davidsson L, Cook JD, Hurrell RF. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Cote d'Ivoire. Am J Clin Nutr 2001;74(6):776-82.
64. Stoltzfus RJ, Chwaya HM, Montresor A, Albonico M, Savioli L, Tielsch JM. Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0- to 5-y old Zanzibari children and these relationships change with age. J Nutr 2000;130(7):1724-33.
65. Stoltzfus RJ, Chwaya HM, Albonico M, Schulze KJ, Savioli L, Tielsch JM. Serum ferritin, erythrocyte protoporphyrin and hemoglobin are valid indicators of iron status of school children in a malaria-holoendemic population. J Nutr 1997;127(2):293-8.
66. Rettmer RL, Carlson TH, Origenes ML, Jack RM, Labb RF. Zinc protoporphyrin/heme ratio for diagnosis of preanemic iron deficiency. Pediatrics 1999;104(3):e37.
67. Dimitriou H, Stiakaki E, Markaki EA, Bolonaki I, Giannakopoulou C, Kalmanti M. Soluble transferrin receptor levels and soluble transferrin receptor/log ferritin index in the evaluation of erythropoietic status in childhood infections and malignancy. Acta Paediatr 2000;89(10):1169-73.
68. Hastka J, Lasserre JJ, Schwarzbeck A, Strauch M, Hehlmann R. Washing erythrocytes to remove interferents in measurements of zinc protoporphyrin by front-face hematofluorometry. Clin Chem 1992;38(11):2184-9.
69. Junca J, Oriol A. Serum transferrin receptor. Am J Clin Pathol 2001;116(3):446; author reply 447-9.
70. Ritchie B, McNeil Y, Brewster DR. Soluble transferrin receptor in Aboriginal children with a high prevalence of iron deficiency and infection. Trop Med Int Health 2004;9(1):96-105.
71. Cook JD, Baynes RD, Skikne BS. Iron deficiency and the measurement of iron status. Nutr Res Rev 1992;5:189-202.
72. Zimmermann MB, Molinari L, Staubli-Asobayire F, Hess SY, Chaouki N, Adou P, et al. Serum transferrin receptor and zinc protoporphyrin as indicators of iron status in African children. Am J Clin Nutr 2005;81(3):615-23.
73. van den Broek N. Anaemia in pregnancy in developing countries. Br J Obstet Gynaecol 1998;105(4):385-90.
74. Pepys MB. C-reactive protein fifty years on. Lancet 1981;1(8221):653-7.
75. Olivares M, Walter T, Cook JD, Hertrampf E, Pizarro F. Usefulness of serum transferrin receptor and serum ferritin in diagnosis of iron deficiency in infancy. Am J Clin Nutr 2000;72(5):1191-5.

6. PROTECTION OF HUMAN SUBJECTS

6.1 Risks to Human Subjects

6.1a Human Subjects Involvement and Characteristics

- Proposed involvement of human subjects
    i. Studies using animals are not appropriate to answer the research questions posed in this proposal, namely to determine the impact of the provision of iron on the susceptibility to clinical malaria among infants and young children (6-35 months of age) living in a high

malaria burden area, using a powdered vitamin and mineral 'sprinkle' added to complementary foods.

- Characteristics of the subject population – number, age range, health status
  i. Number: Approximately 970 in the intervention group and 970 in the control group
  ii. Age: 6 – 35 months
  iii. Health Status – generally healthy
- Inclusion/exclusion criteria for any subpopulation
  i. Children to be included must meet the criteria described above. They must be free of major illnesses at the time of enrolment (malaria infections will be treated before enrollment). They must have plans to remain in the study area for the duration of the intervention and follow-up periods. Informed consent must be freely given by a parent of the children enrolled for the study. Both boys and girls will be recruited without bias. Girl children will specifically be encouraged to participate, as appropriate.
  ii. Children will be excluded if they fail to meet the inclusion criteria shown above.
- Rationale for involvement of special classes of subjects (e.g. children)
  i. The highest malaria burden is in children in the first 3-4 years of life and the highest iron deficiency anemia burden is in the age range 6 – 24 months of age. Thus, we choose to include children who are at highest risk of anemia and malaria.
- List any collaborating sites where human subjects research will be performed, describe role of those sites and collaborating investigators in performing proposed research
  i. The research field work will be conducted in a northern district of Ghana (Wenchi). Wenchi district is within the catchment area of the Kintampo Health Research Centre (KHRC), one of three Ministry of Health Research Centres in Ghana.
  ii. Research Ethics Board approval will be from two sources: the University of Toronto, the home institution of the PI and the Ghana Ministry of Health.
  iii. The University of Toronto, Hospital for Sick Children PI will provide oversight of the project, while the co-investigator at KHRC will supervise all aspects of the field research.

6.1b Sources of Materials

- Describe research material obtained from individuals in form of specimens, records, or data
  i. Capillary blood samples will be collected from study subjects. Individuals collecting the samples will be trained in pediatric phlebotomy techniques. Results of analysis of blood samples will be collated and recorded to be used for statistical analysis.
- Describe any data to be collected from human subjects
  i. Individual subject records will be collected. This information includes general demographic data (age and gender) and information specifically related to diet, health and use of health care facilities.
- Indicate who will have access to individually identifiable private information
  i. In Ghana, field workers, field supervisors and the study co-investigator will have access to individually identifiable private information.
  ii. Statisticians, the research coordinator and the PI in Canada will not have access to individually identifiable private information.
- Provide information about how specimens, records, or data are collected and whether will be collected specifically for proposed project
  i. Only data to be included in the statistical analysis to meet primary and secondary objectives will be collected.
  ii. Blood samples are collected as described above.

iii. Individual data will be collected from the parents or guardians of the study subjects.
iv. No previous records on study subjects will be collected with the exception of birth weight and previous hospitalizations.

6.1c Potential Risks

- Describe potential risks to subjects (physical, psychological, financial, legal, or other), and assess likelihood and seriousness to subjects
  - *i.* Subjects in the intervention group receiving the powdered mineral and vitamin 'sprinkle' *with iron* may be at risk of higher rates of clinical malaria or more severe attacks of malaria or other infections *if they are not anemic* at baseline.
  - *ii.* Subjects in the control group receiving the powdered mineral and vitamin 'sprinkle' *without iron* may be at risk of higher rates of clinical malaria or more severe attacks of malaria or other infections *if they are anemic at baseline.*
  - iii. There are no other psychological, financial, legal or other risks.
- Where appropriate, describe alternative treatments and procedures (including risks and benefits of each)
  - i. All subjects (in both groups) will be provided with treated bed-nets and instructions for their appropriate use. If used, they may decrease the rates of mosquito bites and thus malaria.

6.2 Adequacy of Protection Against Risks

6.2a Recruitment and Informed Consent

- Describe plans for recruitment and process for obtaining informed consent (parental permission and child assent)
  - i. Children between the ages of 6 – 35 months are too young to provide assent.
  - ii. Parents or guardians will be informed of the objectives of the study and the protocol in a setting and language appropriate to rural Ghana. Members of the field research team have worked in the study communities (or neighboring communities) for many years, thus have experience in conducting research in this population.
  - iii. The parent or guardian of the recruited child will be given to opportunity to sign the consent form, or not, in a totally non-coercive manner. Non-participation will have no effect on the provision of care to the child.
  - iv. Permission to recruit in villages in the KHRC catchment area will be obtained from the village assemblies and village elders prior to the start of the recruitment phase.
- Describe circumstances under which consent will be sought and obtained, who will seek it, nature of info provided to subjects, method of documenting consent (justification for waiver if used)
  - i. After permission is granted to recruit from a village (see previous paragraph), individual households will be visited and parents interviewed with regard to inclusion criteria. The study will take place in a very rural environment where telephone and other communication tools are not common. Thus recruitment will be face-to-face.
  - ii. Recruitment will be done by field researchers employed by the Kintampo Health Research Centre. All are trained in health research techniques and many already have experience in recruitment for research projects involving children. Field workers will be supervised by 'field supervisors' who have experience in field research including proper methods of recruitment.
  - iii. Parents of subjects will be provided with oral as well as written material explaining the nature of trial, including procedures, risks and benefits.

iv. Consent will be documented through the signing of the name of a parent, or if a signature is not possible, a thumb print will be used.

6.2b Protections Against Risk

- Describe planned procedures for protecting against or minimizing potential risks (e.g. to privacy or confidentiality of data) and assess likely effectiveness
  i. Data will be kept confidential by ensuring that named documents are locked in cabinets, or if electronically stored, it will be password protected.
  ii. All data will be identified with a numeric-alphabetic code, linked to a master list. It is this master list that will be stored as described above.
  iii. These procedures have been successfully used by the research team in past collaborate research projects without breaches of confidentiality or privacy (to the best of our knowledge).
- Additional protections for children (OHRP subpart D Guidance)
  i. Additional protections for children will be implemented in compliance with NIH policy and as outlined in the applicable sections of 45 CFR Part 46 Subpart D, as well as that deemed necessary by the applicant's Institutional Review Board.
- Plans for ensuring necessary medical or professional intervention in event of adverse effect to subjects
  i. Kintampo Health Research Centre (KHRC) has a well-established clinical facility and referral system to manage all cases (solicited and unsolicited) of adverse reactions. The Wenchi District Hospital has all of the emergency/resuscitation equipment and items to support clinical trials such as those that KHRC has been embarking on since 2002. For any complicated cases of malaria or anemia beyond the capacity of the Wenchi District Hospital to handle, an ambulance service is available to facilitate referral to the Komfo-Anokye Teaching Hospital, the second largest Teaching Hospital in Ghana (two hours away). This referral system has been successfully used over the years and will be available for all children participating in this trial.

6.3 Potential Benefits of the Proposed Research to Human Subjects and Others

- Discuss potential benefits of research to participants and others
  i. Subjects in the intervention group may benefit from the intervention by having fewer bouts of anemia or less severe attacks of malaria or other infections *if they are anemic* at baseline.
  ii. Subject in the control group may benefit from the intervention by having fewer bouts of anemia or less severe attacks of malaria or other infections *if they are non-anemic* at baseline.
  iii. The results of this research may ultimately benefit the children of Ghana (and other West African countries) by informing policy on the appropriate use of iron in children in high malaria burden areas at risk of iron deficiency anemia.
- Discuss why risks to subjects reasonable in relation to anticipated benefits
  i. There is relative equipoise for subjects in the control and intervention groups for many reasons. Subjects with iron deficiency anemia may benefit from the powdered iron supplement and subjects without iron deficiency may benefit from being in the control group. Risks are similarly equal among the two groups.
  ii. All subjects will receive treated bed nets which will mitigate the risk of contracting malaria.

6.4 Importance of the Knowledge to be Gained

- Discuss importance of knowledge to be gained
    i. Currently there is great confusion regarding the safety of providing iron to children at high risk of iron deficiency anemia in a high burden malaria area. If a child with severe iron deficiency anemia remains untreated, death may ensue. With moderate or even mild iron deficiency anemia, there is documentation of adverse developmental consequences including delayed motor and cognitive development that may not be reversible.
    ii. The decision to withhold iron supplements (UNICEF/WHO guidance) was based primarily on a single study from Zanzibar in a highly malaria endemic region, where bed nets were neither provided nor widely used. In that study it was the children without anemia who fared the worst.
    iii. The knowledge to be gained from this study may help inform public policy on this important issue.
- Discuss why risks to subjects are reasonable in relation to importance of knowledge that may be expected to result
    i. In most developing countries it is not possible to screen children for iron deficiency anemia. The currently available tools for screening are insensitive and often more costly than the intervention. Thus, it is recommended that when the prevalence of anemia is greater than 40%, blanket fortification or supplementation is warranted.
    ii. As previously noted, it is the children who do NOT have iron deficiency anemia that seem to be at higher risk of adverse outcomes if they are given iron and concomitantly have malaria. But this observation is primarily from a single study.
    iii. In the current study, because of the design, there is a risk of giving iron or not giving iron depending on the individual circumstances of the child. However, the form of iron (microencapsulated iron) and the delivery system (as a powder and added to food), may protect all children from adverse effects of iron, even if they have malaria.
    iv. The information that hopefully will be obtained from the results of this study has the potential to be very important for forming public policy regarding the use of iron in a high malaria burden area.

6.5 Data and Safety Monitoring Plan

- General description of a monitoring plan that will establish as the overall framework for data and safety monitoring. Describe entity that will be responsible for monitoring and the process by which Adverse Events will be reported to the Institutional Review Board, the funding I/C, the NIH Office of Biotechnology Activities (OBA), and the Food and Drug Administration (FDA) in accordance with Investigational New Drug (IND) or Investigational Device Exemption (IDE) regulations.
    i. Field workers will describe any possible adverse events to the field supervisors. At weekly meetings with the study site coordinator, potential adverse events will be reviewed. A written summary of this weekly meeting will be shared with the co-investigator who will decide if the information should be shared with the PI. Any adverse events reported to the PI will be reported to the IRBs in Ghana and Canada.
- Options for monitoring trials include, but not limited to: PD/PI (required); Institutional Review Board (required); Independent individual/safety officer; Designated medical monitor; Internal Committee or Board with explicit guidelines; Data and Safety Monitoring Board (required by NIH

for multi-site clinical trials involving interventions that entail potential risk to participants, and generally for Phase III clinical trials)

i. A detailed Data and Safety Monitoring Plan will be submitted to IRBs in both Toronto and Ghana and (subsequently) the funding IC for approval prior to the accrual of human subjects.

6.6 ClinicalTrials.gov Requirements

- NIH encourages registration of ALL trials in ClinicalTrials.gov whether required under the law or not.
  - i This trial will be registered at ClinicalTrials.gov

7. INCLUSION OF WOMEN AND MINORITIES

This project is specifically directed to children in the age range 6 – 35 months, thus, women will not be recruited to be included as subjects. In most cases, however, the mothers of the children will contacted as the surrogate to provide consent for their children to be included in the study. All ethnic, racial and minority groups will be included in the recruitment and the study.

8. TARGETED/PLANNED ENROLMENT TABLE

Not applicable

9. INCLUSION OF CHILDREN

This project is specifically directed to children in the age range 6 – 35 months. This age range was chosen because it is considered to be both the most vulnerable period of life for the infant and young child, and also a 'window of opportunity'. It is vulnerable because growth is faster during this period than at any other time in the life of the child, thus the need for nutrients is higher during this time. If individual nutrients are missing from the child's diet during this time period, the consequences can be long-lasting. For example, there is documentation that young children diagnosed with iron deficiency anemia during the first year of life were at risk of retarded development in certain key areas, including some emotional development and educational attainment. This age range is also specifically pertinent to the questions posed in the RFA, since it refers to the recent study conducted in Pemba, which included only infants and young children.

Both investigators (Owusu and Zlotkin) have experience in performing research in infants and young children. Zlotkin specifically is a pediatrician. His clinical and research focus is on children. His laboratory is at the Hospital for Sick Children in Toronto, and the IRB is located at the Hospital for Sick Children. The Hospital for Sick Children is the largest children's hospital in Canada, and possibly in North America, and has one of the largest Research Institutes associated with it. The IRB at the Hospital and Research Institute is thus extremely well-suited to evaluate the ethical aspects of research involving children. Field researchers at the Kintampo Health Research Centre (KHRC) have experience working with children and their parents, and phlebotomists at KHRC are experienced in taking blood samples from children. Further, there are sufficient numbers of children in the catchment area of KHRC to meet the sampling needs of the study. The fertility rate in Ghana is 3.78 children born/women (2008 estimate) (https://www.cia.gov/library/publications/the-world-factbook/geos/gh.html).

10. VERTEBRATE ANIMALS

Not applicable

11. SELECT AGENT RESEARCH

Not applicable

12. MULTIPLE PD/PI LEADERSHIP PLAN

This project will be a joint effort between the University of Toronto, the Research Institute at Hospital for Sick Children, and the Ghanaian Ministry of Health, through the Kintampo Health Research Centre (KHRC). The PI from the University of Toronto, Department of Paediatrics, Nutritional Sciences and Public Health Sciences has extensive experience in research involving children, specifically research on iron and anemia in developing countries, including Ghana. The co-investigator is the Director of the KHRC. He has extensive research experience dealing with malaria, including iron and malaria, in Ghana. Drs. Zlotkin and Owusu have worked successfully together in the past, thus one of the advantages of this collaboration is the trust between the two institutions and individuals running the study.

As has been the case with past studies involving the two institutions, there will be a clear *a priori* communications plan. For the weeks preceding the start of the study, there will be an on-site presence of the PI and/or his delegate. There will be weekly conference calls for the first 4-6 weeks of the study, and bi-weekly calls thereafter. There will be routine e-mail communication between the study co-coordinator in Toronto and her equivalent in Kintampo. All important decisions about the protocol and procedures will have been made before the protocol is submitted to the NIH. Ongoing major decisions will be made jointly by the PI and co-investigator, as has been the case in past studies. Any potential differences in opinion will be worked out through discussion and compromise. Publications will be jointly authored; with Dr. Owusu as the primary author on those publications pertaining to malaria, and Dr. Zlotkin as the primary author on those pertaining to anemia.

IRB reviews will be completed in both Ghana and Canada. In Canada, Dr. Zlotkin will be responsible for shepherding the review, while Dr. Owusu will be responsible in Ghana. The budget allocation (Ghana vs. Canada) is clearly shown in the budget component of this application.

13. CONSORTIUM/CONTRACTUAL ARRANGEMENTS

An agreement including programmatic, fiscal and administrative arrangements will be developed and signed by both organizations (the Kintampo Health Research Centre and the Research Institute at the Hospital for Sick Children). Similar agreements have been used in past collaborative projects between the two organizations.

14. LETTERS OF SUPPORT

See the attached letter of support from the consortium.

15. RESOURCE/DATA SHARING PLAN

Findings from the proposed research will be shared according to the applicable NIH policy for foreign institutions.

BMJ Open

Impact of iron fortification on the geo-spatial patterns of malaria and non-malaria infection risk among young children in rural Ghana

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-013192.R1
Article Type:	Research
Date Submitted by the Author:	01-Dec-2016
Complete List of Authors:	Aimone, Ashley; University of Toronto, Dalla Lana School of Public Health Brown, Patrick; University of Toronto, Dalla Lana School of Public Health; Cancer Care Ontario, Analytics and Informatics Owusu-Agyei, Seth; Kintampo Health Research Centre Zlotkin, Stanley H.; Hospital for Sick Children, Centre for Global Child Health; University of Toronto, Dalla Lana School of Public Health Cole, Donald; University of Toronto, Dalla Lana School of Public Health
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Global health, Paediatrics
Keywords:	Nutrition < TROPICAL MEDICINE, Community child health < PAEDIATRICS, Geographical mapping < TROPICAL MEDICINE

Impact of iron fortification on the geo-spatial patterns of malaria and non-malaria infection risk among young children in rural Ghana

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Keywords
Spatial analysis, infection, child health, GIS, nutrition

Word count: 5,714 (including tables and references)

Abstract

Objectives: Patterns of infection among children with varying levels of iron status in a malaria endemic area may vary spatially in ways requiring integrated infection and iron deficiency control programs. The objective of this secondary analysis was to determine the geo-spatial factors associated with malaria and non-malaria infection status among young Ghanaian children at the end of a 5-month iron intervention trial.

Design: Cluster-randomised controlled trial

Setting: Rural Ghana

Participants: 1943 children (6-35 months of age) with geocoded compounds.

Interventions: Point-of-use fortification with micronutrient powders containing vitamins and minerals with or without iron.

Primary and secondary outcome measures: Generalized linear geostatistical models with a Matern spatial correlation function were used to analyse four infection response variables, defined using different combinations of inflammation (C-reactive protein, CRP >5 mg/L) and malaria parasitaemia. Analyses were also stratified by treatment group to assess the independent effects of the iron intervention.

Results: The by-group and combined-group analyses both showed that baseline infection status was the most consistent predictor of endline infection risk, particularly when infection was defined using parasitaemia. In the No-iron group, age above 24 months and weight-for-length z-score at baseline were associated with high CRP at endline. Higher asset score was associated with a 12% decreased odds of endline infection, defined as CRP >5 mg/L and/or parasitaemia (OR 0.88, 95% CrI 0.78, 0.98), regardless of group. Maps of the predicted risk and spatial random effects showed a defined low-risk area around the District centre, regardless of how infection was defined.

Conclusions: In a clinical trial setting of iron fortification, where all children receive treated bed nets and access to malaria treatment, there may be geographical variation in the risk of infection with distinct high- and low-risk areas, particularly around municipal centres.

Trial registration: clinicaltrials.gov, NCT01001871.

Strengths and limitations of this study

- The geostatistical analyses conducted in this study are the first of their kind to use model-based geostatistics with Bayesian inference and integrated nested Laplace approximations (INLA) to explore the spatial variation and associated risk factors of malaria and non-malaria infection risk among children in rural Ghana after a 5-month randomized iron intervention trial.
- These analyses also provide input into the potential utility of geographical indicators, particularly for assessing infection risk potential, which could help guide the implementation of iron interventions in areas where infectious diseases are prevalent.
- Since satellite-derived spatial data often vary at a higher level than the individual (e.g. village or region), the use of these data in statistical models with individual-level outcomes may increase the risk of a change of support or ecological inference problem.
- The use of C-reactive protein (CRP) as an indicator of non-malaria infection may have led to the underestimation of this outcome, since CRP is an acute phase protein that only rises in accordance with the first 48 hours of the inflammatory response.
- Straight-line distance, rather than distance by road, was used to estimate proximity to a health facility or the district centre, and thus may not have fully accounted for travel impedance.

Background

The leading causes of death in children less than 5 years of age are infection-related, and child mortality rates are highest in low- and middle-income countries (LMICs).[1] Malnutrition is also a large contributor to mortality (45% of all deaths),[1] including micronutrient deficiencies. The most common nutritional disorder worldwide is iron deficiency,[2] which is estimated to account for 2.2 million disability-adjusted life years lost per year among children less than five years of age.[3] While iron deficient children are more vulnerable to infections (primarily due to compromised immune function),[4, 5] infection and inflammation can also affect iron homeostasis[6] and the risk of iron deficiency.[7] Iron status can usually be improved through food fortification, or supplementation; however, evidence from a large randomized trial

conducted in a malaria endemic area (Pemba, Zanzibar) indicated that supplementing young children with iron may increase their risk of malaria and infection-related morbidity and mortality, particularly if they are iron replete.[8] Since iron supplements or fortificants would likely be withheld from children with malaria or other infections, assessing the risk of infection is an important component of developing safe and effective means of administering iron to children in LMICs.

Measuring biomarkers of infection status (such as C-reactive protein) among children in a low-resource context may have limited feasibility, due to the requirement of blood samples, and the potential for inflammation prevalence to vary over relatively short periods of time (e.g. within seasons). As such, there is a need to identify indicators or risk factors associated with infection in LMICs that are more feasible to measure, and thus provide an efficient means of identifying high-risk populations. This need could be addressed with geographical factors (or "geo-indicators"), such as the environmental or spatial characteristics of a village or region.[9] Geo-indicators could provide additional insight into the dynamics and distribution of infection among children that informs treatment needs and the prophylactic use of iron supplementation or fortification.[10-13] Collecting geo-spatial data is not invasive and comparatively less costly than biological measures, as geographical datasets are often publicly available on the internet. This also improves the access to and comparability of population-level statistics within and across national borders.

We previously conducted a secondary spatial analysis to determine the geo-spatial factors associated with infection status among iron deficient and sufficient children in rural Ghana.[14] The results of these analyses suggested that the risk of infection may be related to elevation, and distance to the nearest health facility. The objective of the current analysis was to determine the geo-spatial factors associated with malaria and non-malaria infection status among Ghanaian children at the end of a 5-month randomized iron intervention trial.

Methods

Study population

The data used in these analyses were generated from a study population of young children (6-35 months of age) who participated in a 6-month community-based cluster-randomized trial conducted in the Brong-Ahafo Region of Ghana in 2010.[15] At the time of the trial, the estimated incidence of malaria in Ghana was 7.2 million cases per year, and the prevalence of anaemia among preschool aged children was 76.1% (95% CI 73.9-78.2%).[16, 17] Details of the clinical trial, [15] and geographical layout of the study area have been described elsewhere (Aimone, Brown, Zlotkin, Cole, Owusu-Agyei 2016).

Measures from trial data

Biological samples collected at baseline and endline were analysed for plasma ferritin (Spectro Ferritin S-22, Ramco Laboratories Inc., Stafford, USA), C-reactive protein (QuickRead CRP, Orion Diagnostica, Espoo, Finland), and malaria parasite density (microscopy).[15] Malaria screening was also performed on a weekly basis throughout the intervention period. If a child had a history of fever (within 48 hours) or an axillary temperature >37.5^0C, a blood sample was drawn and analysed in the field via antigen test (Paracheck Pf®), and in the lab using microscopy (thin and thick smears). Parasite density was combined with fever information to calculate clinical malaria incidence (episode counts). Demographic and nutrition-related information was also collected at baseline and included household assets, maternal education, and child body weight and length. Weight-for-length and length-for-age z-scores were calculated using the WHO Child Growth Standards.[18]

Variable preparation

Geographical coordinates for the compounds of 1943 trial participants (representing 1539 clusters), surrounding health facilities and major road networks were collected using handheld global positioning system (GPS) units (WGS 1984 coordinate system, universal transverse Mercator zone 30N projection, EPSG code: 32630). Satellite-derived data were downloaded as global datasets[19-21] and cropped according to the geographical boundaries of the trial area. Elevation had a range of 116-530 meters, and values were centred by subtracting 250. Land cover type (LC) consisted of 3 categorical values, representing woody savannah (LC=8, n=21/1943 observations), urban and built up land (LC=13, n=243/1943 observations), and cropland/natural vegetation mosaic (LC=14, n=1679/1943 observations). NDVI, a vegetation index included as a proxy for moisture,[22] was averaged over the year that the trial was conducted (2010), and ranged in value from 0.22 to 0.62. NDVI has also been used to create malaria risk distribution maps through the characterization of vector habitat potential (e.g. closed forest versus open forest).[23] Two NDVI-LC interaction terms were created (NDVI*LC8 and NDVI*LC13) by overlaying the final NDVI and LC rasters, and masking the LC cells except where they had a value of 8 (or 13). These unmasked cells were given a value of zero.

Baseline age, in months, was calculated using the reported date of birth and trial enrolment date with a change point at 24 months. Household asset score was generated using a principal component analysis of 6 economic indicators (farm ownership, size and type of crops grown, type of toilet facility, and house ownership). Maternal education was included as a binary variable representing “no” (0) versus “any” (1) level of education. Baseline iron status was defined as serum ferritin concentration corrected for inflammation (baseline CRP) using a regression-based method (Namaste, Rohner, Suchdev, Kupka, Mei, Bhushan, Williams, Rowat, Raiten, Flores-Ayala, Clewes, 2016), and re-scaled by multiplying the corrected values by the inter-quartile range. Straight-line (Euclidean) distance to the nearest health facility was measured using the Near Table tool in ArcMap (ArcGIS 10.2, Environmental Systems Resource Institute, Redlands, California).

Spatial modelling

The data were analysed using generalized linear geostatistical models (GLGM) [24, 25] with Bayesian inference via an Integrated Nested Laplace Approximation (INLA) algorithm.[26] Weak or uninformative priors were used for all model parameters with the exception of the Matern shape parameter (fixed at 2). Four different endline infection outcomes were modelled separately: 1) inflammation (CRP >5 mg/L) with/without malaria parasitaemia; 2) inflammation (CRP >5 mg/L) without parasitaemia; 3) parasitaemia with measured concurrent fever (axillary temperature >37.5^{0}C) or reported history of fever within 48 hours (i.e. clinical malaria)); and 4) parasitaemia with or without concurrent fever or history of fever. All variables were binary-valued (coded as '1' for positive infection status at endline) and analysed using logistic regression, with the exception of the third definition (parasitaemia with fever), which was a count variable (number of new clinical malaria episodes during the intervention period) analysed using Poisson regression. Median infection probabilities (and 95% credible intervals) were modelled as the sum of the contributions of the independent variables, residual spatial variation, and a compound-level random effect term. The *glgm* function from the "geostatsp" package in R was used for all spatial modelling.[27, 28]

The spatial analyses were conducted in three modelling steps: 1) No-iron group only; 2) Iron group only; 3) both intervention groups combined. The interventions groups were analysed separately in order to differentiate the effects of time and the iron treatment. Infection probabilities from the combined-group models were plotted on a base map of the trial area, with study compounds, and major road networks. The maps depicted a spatial risk surface of predicted infection probabilities, which were computed as the posterior means of the odds or risk of infection. The posterior means were estimated assuming baseline values for individual-level covariates and location-specific values for spatial covariates. The posterior mean of the spatial random effect was also plotted, representing the residual spatial variation that corresponded to the difference between the predicted and expected odds or risk of infection at each location.

Ethics

The original clinical trial was approved by the Kintampo Health Research Centre (KHRC) Institutional Ethics Committee, the Ghana Health Service (GHS) Ethical Review Committee, the Hospital for Sick Children Research Ethics Board, and the Food and Drugs Authority of Ghana. Approval for conducting the secondary analyses was obtained from the Hospital for Sick Children and University of Toronto Health Sciences Research Ethics Boards.

Results

Baseline and endline characteristics of the study sample are presented in **Table 1**. A total of 1780 trial participants were included in the endline analyses, representing those with geocoded residences (compounds), who provided blood samples at endline, and had both baseline and endline CRP and parasitaemia values (see online supplementary file Figure S1). For the infection outcome defined as parasitaemia with fever, there were 1939 observations included in the Poisson regression analysis, which corresponded to the number of children with geocoded compounds who had at least one recorded follow-up visit during the intervention period, and thus contributed data to the malaria count outcome.

The proportion of children with evidence of infection (elevated CRP and/or parasitaemia) increased from baseline to endline (37.0% at baseline versus 41.6% at endline). This increase may have been driven primarily by a greater prevalence of parasitaemia at endline (27.1% versus 23.0% at baseline), though the difference between intervention groups was small (26.3% versus 28.0% in the No-iron and Iron groups, respectively). The prevalence of non-malaria inflammation (CRP>5 mg/L without parasitaemia) remained relatively stable over the course of the trial; however, the proportion in the Iron group was slightly higher at endline (7.64% in the Iron group versus 6.58% in the No-iron group). After correcting ferritin concentration for inflammation (CRP) using the regression method, the prevalence of iron deficiency (ferritin <12 µg/L) was 25.5% (496/1943) at baseline and 12.7% (226/1781) at endline (95/886 in the Iron group, 131/895 in the No-iron group).

Table 1: Baseline and endline characteristics of the Ghana trial participants

	Baseline	Endline
Trial participants with a geocoded residence (n)	1943	1780
Males (%)	992 (51.1)	900 (50.5)
Age at enrolment (months), mean (SD)[a]	19.2 (8.5)	19.3 (8.5)
Serum ferritin (µg/L), geometric mean (SD)	35.1 (3.65)	73.9 (3.58)
Parasite density (count/µL), geometric mean (SD)	3003.0 (5.35)	4160.4 (6.30)
C-reactive protein (mg/L), mean (SD)	3.34 (4.96)	3.86 (5.10)
Infection status		
Inflammation without parasitaemia[b], n (%)	272 (14.0)	258 (14.5)
Inflammation and/or parasitaemia[c], n (%)	719 (37.0)	741 (41.6)
Parasitaemia with fever[d‡], n (%)	150 (7.72)	555 (28.6)
All parasitaemia[e], n (%)	447 (23.0)	483 (27.1)
Anthropometric status[f]		
Weight-for-length z-score, mean (SD)	-0.63 (0.97)	-0.62 (0.97)
Length-for-age z-score, mean (SD)	-0.81 (1.21)	-0.80 (1.29)
Maternal Education, n (%)[g]		
None	586 (33.5)	543 (33.0)
Any	1166 (66.5)	1105 (67.0)
Household Asset Score, n (%)[h]		
Low	900 (49.0)	817 (47.7)
High	938 (51.0)	897 (52.3)

[‡]Endline value represents the period prevalence for 1939 participants
[a]Measured at baseline only
[b]Inflammation without parasitaemia (n=1780) = CRP > 5 mg/L without malaria parasitaemia;
[c]Inflammation and/or parasitaemia (n=1780) = CRP > 5 mg/L and/or any malaria parasitaemia;
[d]Parasitaemia with fever (n=1939) = any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
[e]All parasitaemia (n=1780) = any malaria parasitaemia (with/without fever)
[f]Measured at baseline; z-scores estimated using the WHO Child Growth Standards [23]
[g] Measured at baseline only; total n=1752 (74 respondents were not mothers, 117 missing due to incomplete surveys)
[h] Measured at baseline only; Low= below median, High = above median; reduced sample size (approximately 1825) due to incomplete surveys and "unknown" responses

In the combined-group analyses, the risk of elevated CRP and/or parasitaemia at endline was negatively associated with household asset score, and positively associated with baseline infection status (OR for definition 1: 0.88, 95% CrI 0.78, 0.98) (**Table 2**). Baseline infection status was also significantly and positively associated with the corresponding endline outcome when defined as inflammation and/or parasitaemia (OR 1.84, 95% CrI 1.36, 2.50) or all parasitaemia (2.75, 95% CrI 1.91, 3.95). Although not statistically significant, the effect of the

"Group" variable seemed to suggest that the iron intervention was positively associated with endline infection risk, except in the case of outcome definition 3 (parasitaemia with fever) where the iron treatment may have had a protective effect.

Results from the by-group analyses indicated that the risk of elevated CRP (without parasitaemia) at endline among children in the No-iron group was positively associated with baseline weight-for-length z-score (OR 1.30, 95% credible interval (CrI) 1.06, 1.60), and negatively associated with age between 24 and 36 months (OR 0.92, 95% CrI 0.84, 1.006). When infection was defined as all parasitaemia, baseline infection status became a significant factor (OR for definition 4: 2.86, 95% CrI 1.97, 4.17), indicating that children in the No-iron group with any parasitaemia (with or without CRP or fever) at baseline were up to 186% more likely to have parasitaemia at endline (see online supplementary file Table S1). In the Iron group, when infection was defined as inflammation and/or parasitaemia, baseline infection status was the only factor associated with infection risk at endline (OR 2.27, 95% CrI 1.66, 3.11). Similar results were found when infection was defined as all parasitaemia (OR 2.54, 95% CrI 1.75, 3.68) (see online supplementary file Table S2).

Table 2: Geo-spatial and non-spatial risk factors of endline infection status[1] among Ghanaian children in the combined *Iron* and *No-iron* groups (Brong-Ahafo Region 2010)

Covariates	Estimate (95% CrI) on the exponential scale[2]		Range parameter in km (95% CrI)	Standard deviations of random effects (95% CrI)	
				Spatial	Compound
Outcome 1: Inflammation and/or parasitaemia (n=1780)					
Intercept	0.589	(0.337, 1.072)	7.341	0.525	0.008
Age per month			(2.928,	(0.306,	(0.004,
6-23 months	1.004	(0.984, 1.026)	15.84)	0.944)	0.029)
24-35 months	1.000	(0.962, 1.038)			
Sex (male reference)	1.087	(0.886, 1.333)			
Length-for-age z-score	0.982	(0.896, 1.075)			
Weight-for-length z-score	1.088	(0.978, 1.211)			
Asset score	0.875	(0.779, 0.980)*			
Distance to health facility (km)	1.034	(0.933, 1.136)			
Elevation (m)	0.996	(0.991, 1.001)			
Baseline infection status	1.843	(1.359, 2.500)*			
Group	1.094	(0.826, 1.448)			
Baseline iron status	1.068	(0.956, 1.194)			
Baseline infection status*Group	1.192	(0.775, 1.833)			
Baseline iron status*Group	0.983	(0.847, 1.144)			
Outcome 2: Inflammation without parasitaemia (n=1780)					
Intercept	0.183	(0.106, 0.309)*	10.77	0.407	0.007
Age per month			(8.767,	(0.208,	(0.004,
6-23 months	1.009	(0.982, 1.038)	25.54)	0.830)	0.028)
24-35 months	0.956	(0.904, 1.009)			
Sex (male reference)	1.139	(0.865, 1.502)			
Length-for-age z-score	1.092	(0.968, 1.230)			
Weight-for-length z-score	1.054	(0.912, 1.217)			
Asset score	0.871	(0.750, 1.010)			
Distance to health facility (km)	1.012	(0.924, 1.093)			
Elevation (m)	0.999	(0.994, 1.003)			
Baseline infection status	1.055	(0.574, 1.826)			
Group	1.009	(0.713, 1.427)			
Baseline iron status	1.001	(0.844, 1.156)			
Baseline infection status*Group	1.523	(0.718, 3.300)			
Baseline iron status*Group	1.033	(0.856, 1.261)			
Outcome 3: Parasitaemia with fever (n=1939)					
Intercept	0.003	(0.002, 0.004)*	7.365	0.464	0.007
Age per month			(3.225,	(0.273,	(0.004,
6-23 months	1.008	(0.991, 1.025)	15.18)	0.825)	0.028)
24-35 months	0.978	(0.948, 1.008)			

Sex (male reference)	0.939	(0.799, 1.104)			
Length-for-age z-score	0.992	(0.923, 1.064)			
Weight-for-length z-score	0.966	(0.886, 1.053)			
Asset score	1.029	(0.937, 1.130)			
Distance to health facility (km)	1.057	(0.972, 1.149)			
Elevation (m)	0.997	(0.993, 1.002)			
Group	0.891	(0.729, 1.087)			
Baseline infection status	0.730	(0.452, 1.114)			
Baseline iron status	1.028	(0.942, 1.112)			
Baseline infection status*Group	1.209	(0.639, 2.282)			
Baseline iron status*Group	0.954	(0.849, 1.069)			
Outcome 4: All parasitaemia (n=1780)					
Intercept	0.271	(0.135, 0.571)*	7.846	0.712	0.008
Age per month			(3.384,	(0.406,	(0.004,
6-23 months	0.993	(0.966, 1.021)	16.06)	1.291)	0.029)
24-35 months	1.022	(0.994, 1.050)			
Sex (male reference)	1.024	(0.814, 1.287)			
Length-for-age z-score	0.926	(0.835, 1.026)			
Weight-for-length z-score	1.068	(0.947, 1.204)			
Asset score	0.916	(0.802, 1.044)			
Distance to health facility (km)	1.022	(0.902, 1.164)			
Elevation (m)	0.996	(0.990, 1.003)			
Baseline infection status	2.746	(1.910, 3.951)*			
Group	1.169	(0.862, 1.587)			
Baseline iron status	1.061	(0.940, 1.193)			
Baseline infection status*Group	0.915	(0.555, 1.508)			
Baseline iron status*Group	0.980	(0.839, 1.145)			

[1]Infection status definitions:
Outcome 1: Inflammation and/or parasitaemia (binary): 1 = CRP > 5 mg/L and/or any malaria parasitaemia, 0 = CRP ≤ 5 mg/L and absence of parasitaemia;
Outcome 2: Inflammation without parasitaemia (binary): 1 = CRP > 5 mg/L without malaria parasitaemia, 0 = CRP ≤ 5 mg/L without parasitaemia;
Outcome 3: Parasitaemia with fever (count): any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
Outcome 4: All parasitaemia (binary): 1 = any malaria parasitaemia (with/without fever), 0 = absence of parasitaemia
Model prior shape = 1.117, model prior rate = 0.157
[2]Exponentials of posterior medians and 2.5% and 97.5% posterior quantiles of model parameters, with a value of 1.05 indicating that a variable increases the risk of infection by 5% (odds ratio from logistic model for all binary outcomes; relative risk from Poisson model for count outcome)
CrI = credible interval
Baseline infection status = baseline infection status (yes/no) according to corresponding definition
Baseline iron status = baseline serum ferritin concentration (µg/dL) corrected for CRP using the regression method (Namaste et al.) and re-scaled by multiplying by the inverse of the inter-quartile range
*Statistical significance at the 0.05 level

Plots of the predicted risk and residual spatial variation from all combined-group models are illustrated in **Figure 1**. The maps show a defined low-risk area around the District centre, regardless of how infection was defined. Conversely, the location of high-risk areas seemed to vary across models and, in some cases, roughly approximated elevation (**Figure 2**). The maps depicting residual spatial variation also show defined high- and low-risk areas, indicating that a large amount of spatial variation was not explained by the variables included in the analyses and further supported the significant spatial random effects observed across all models.

Discussion

The analyses presented herein explored the geo-spatial variation and associated factors of infection, defined using both inflammatory and parasitic biomarkers, among children in a malaria endemic area (rural Ghana) after a randomized iron intervention trial. Although none of the spatial variables included in the by-group or combined-group models demonstrated significant associations with endline infection status (regardless of how infection was defined), the plots of predicted infection probabilities and spatial random effects showed defined high- and low-risk areas across the study region, particularly around the District centre. In terms of individual-level effects, baseline infection status was a consistent predictor of endline infection risk, particularly in the Iron group and when infection was defined using parasitaemia (with or without inflammation).

The consistency of the relationship with baseline parasitaemia in both intervention groups is somewhat surprising, as current evidence might lead one to expect that providing iron to children with malaria would increase their risk of subsequent parasitic infections to a greater extent than those who do not receive iron.[8] Interestingly, the Iron group analyses also revealed a significant effect of baseline infection status when acute non-malaria inflammation (high CRP without parasitaemia) was included in the definition. This observation is supported by recent research in mice demonstrating that iron supplementation may exacerbate non-malaria bacterial

infections, and that co-infection with *Plasmodium yoelli* and *Salmonella typhimurium* can produced overwhelming *Salmonella* sepsis [29]. Findings from the current analyses that also suggested a potential adverse effect of the iron intervention were the positive group effects across all infection definitions with the exception of clinical malaria (parasitaemia with fever). It should be noted however, that these were non-significant associations only. This discrepancy is likely related to the fact that all children in both groups were monitored and treated for identified clinical malaria episodes throughout the intervention period. Therefore, any cases of asymptomatic malaria (parasitaemia without fever) may have persisted over the course of the trial, potentially interacting with the iron intervention, as well as other opportunistic non-malaria infections.

After stratifying the analyses by group, we found that children in the No-iron group with a higher weight-for-length z-score (a measure of nutritional status) at baseline were 30% more likely to have high CRP (>5 mg/L) at endline (OR 1.30, 95% CrI 1.06, 1.60) (Table S1). This positive association was not expected, given that well-nourished children (those with higher weight-length z-scores) are expected have a lower risk of infection.[4] Since CRP becomes elevated during the first 48 hours of the inflammatory response,[30] it is unlikely that anthropometric measures at baseline influenced inflammatory status 5 months later at endline. Rather, it is more likely that the association was influenced through another unmeasured factor, such as immune function. High CRP was also inversely associated with age in the No-iron group for children between 24 and 36 months (OR 0.92, 95% CrI (0.84, 1.00)), indicating an 8% lower odds of elevated CRP at endline for every month of age older. Assuming that elevated CRP without parasitaemia represented acute non-malaria infection, it is plausible that the inverse relationship reflected the development of the immune system with age, thus resulting in older children being more resilient to infection exposure.

In the combined-group models (**Table 2**), higher asset score was associated with a 12% decreased odds of endline infection, defined as CRP >5 mg/L and/or parasitaemia (OR 0.88, 95% CrI 0.78, 0.98), suggesting that children from wealthier households had a lower risk of

malaria or non-malaria infection at endline (regardless of whether they received MNPs with or without iron). This finding was not surprising given the well-documented association between household wealth and child health in low- and middle-income settings;[31] however, the inconsistency of this relationship across infection definitions may have been a reflection of other socioeconomic or behavioural factors that were not included in the analyses.[32] Despite any differences in (or lack of) variable associations across infection outcomes, the residual spatial variation in infection risk remained significant in all combined-group models. The importance of accounting for residual spatial variation was further illustrated when the spatial random effects were plotted. For example, when infection was defined using CRP only, the model output plots looked similar (**Figure 1B**); suggesting that the spatial random effect encompassed a large amount of the variation in predicted risk, while the variables included in the model did not.

For the infection outcomes that included parasitaemia, we observed similarities between the geographical variation in predicted mean risk and elevation (**Figure 2**). This finding is consistent with our baseline analyses,[14] as well as other studies conducted in malaria endemic areas, where a higher prevalence of malaria is observed among populations living at lower elevations and vice versa.[33-36] The association between malaria and elevation is related to temperature, as the early stages of parasite development are inhibited in colder environments, which are found at higher altitudes.[37] Our baseline analyses also demonstrated a significant effect of distance to the nearest health facility, indicating that those children living farther from a health facility had an increased risk of malaria parasitaemia at baseline. The current endline analyses also suggested a potential risk-enhancing effect of distance to a health facility across all infection definitions, although the effect was not significant. This trend was further supported by the model output plots, which demonstrated larger low-risk areas around villages, and particularly around the District centre. Other studies have shown that living in a capital or municipal centre is associated with a reduced risk of adverse infection- or nutrition-related health outcomes,[38, 39] likely due to increased access to health care services and social amenities.

Overall, it appears that the effect of the intervention may have diminished the spatial associations observed at baseline; however, with the current data, it is difficult to differentiate between the effects of the iron intervention itself and the trial-related malaria mitigation activities (e.g. universal provision of treated bed nets, and malaria treatment when indicated). Further research involving additional secondary analyses with expanded spatial datasets[40] could shed more light on these relationships, as well as their implications on the safety of iron interventions in malaria endemic areas. Until that time, the potential utility of geo-spatial analysis lies primarily in the assessment or planning stages of micronutrient programs, in terms of where malaria and/or non-malaria infection risk is likely a concern; and thus where infection control efforts should be focused if iron fortification or supplementation is to be implemented.

A potential limitation of the present analyses was the use of straight-line (Euclidean) distance for estimating proximity to a health facility. While distance by road may have been a more appropriate indicator of travel impedance or access, incomplete or missing vector information (e.g. miss-aligned junctions, missing or disconnected road segments) made it difficult to generate accurate measures. Nesbitt and colleagues (2014) compared different measures of travel impedance to estimate access to delivery care in the Brong-Ahafo region of Ghana[41] and concluded that straight-line distance was as informative as distance by road for determining spatial access in rural Ghana. Therefore, we felt it was justified to use Euclidean distance in the present analyses.

Non-malaria infections were identified using CRP, an acute phase protein that rises in accordance with the early phase of the inflammatory response (approximately 48 hours). Other acute phase proteins (such as alpha-1-acid glycoprotein) reach their peak concentration during

the late phase (approximately 2-8 days), which more closely approximates the change in ferritin concentration in response to inflammation.[30] Since CRP was the only inflammatory biomarker available for the current analysis, and the original trial protocol was not designed to include diagnoses of other infection types, it is possible that the prevalence of non-malaria infection among participants was underestimated.

Conclusions

The analyses presented herein explored the spatial dynamics of infection risk in the context of an iron intervention trial in a malaria endemic area among children with varying levels of iron status, where all children received bed nets and access to malaria treatment. Geographical variation in the risk of malaria and non-malaria infections was observed with distinct high- and low-risk areas, particularly around the District centre. Overall, our findings emphasize the importance of considering geographical distribution when assessing infection risk and planning intervention trials in LMIC paediatric populations.

Acknowledgments

The authors would like to acknowledge Seeba Amenga-Etego and the members of the GIS team at the Kintampo Health Research Centre in (Kintampo, Ghana) who collected the GPS data that were used in the secondary analysis.

Author contribution

AA, SHZ and SOA conducted the original trial in Ghana. AA and SOA coordinated the acquisition of geographical data. AA conceived and conducted the secondary analysis with substantial contribution from PEB. DCC and SHZ were also involved in the conception and design of the secondary analysis, and interpretation of data. AA drafted the manuscript. All authors read and approved the final manuscript.

Funding

Funding for this manuscript was provided by AA's Doctoral Research Award from the Canadian Institutes of Health Research (CIHR).

Competing interests

None declared

Data sharing

Any unpublished data are available upon request by emailing the corresponding author.

References

1 WHO. Children: reducing mortality. *Fact sheet No 178*. World Health Organization 2016.
2 WHO. Micronutrient Deficiencies: Iron deficiency anaemia. World Health Organization 2015.
3 Stoltzfus RJ. Iron deficiency: global prevalence and consequences. *Food Nutr Bull* 2003;**24**:S99-103.
4 Katona P, Katona-Apte J. The interaction between nutrition and infection. *Clin Infect Dis* 2008;**46**:1582-8.
5 Raiten DJ, Sakr Ashour FA, Ross AC*, et al.* Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE). *J Nutr* 2015;**145**:1039S-108S.
6 Spottiswoode N, Duffy PE, Drakesmith H. Iron, anemia and hepcidin in malaria. *Front Pharmacol* 2014;**5**:125.
7 Nemeth E, Tuttle MS, Powelson J*, et al.* Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004;**306**:2090-3.
8 Sazawal Z, Black, RE., Ramsan, M., Chwaya, HM., Stoltzfus, RJ., Dutta, A ., et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet* 2006;**367**:133-43.
9 Rezaeian M, Dunn G, St Leger S*, et al.* Geographical epidemiology, spatial analysis and geographical information systems: a multidisciplinary glossary. *J Epidemiol Community Health* 2007;**61**:98-102.
10 Schur N, Vounatsou P, Utzinger J. Determining treatment needs at different spatial scales using geostatistical model-based risk estimates of schistosomiasis. *PLoS Negl Trop Dis* 2012;**6**:e1773.
11 Pullan RL, Gething PW, Smith JL*, et al.* Spatial modelling of soil-transmitted helminth infections in Kenya: a disease control planning tool. *PLoS Negl Trop Dis* 2011;**5**:e958.
12 Soares Magalhães RJ, Langa A, Pedro JM*, et al.* Role of malnutrition and parasite infections in the spatial variation in children's anaemia risk in northern Angola. *Geospat Health* 2013;**7**:341-54.
13 Sasaki S, Igarashi K, Fujino Y*, et al.* The impact of community-based outreach immunisation services on immunisation coverage with GIS network accessibility analysis in peri-urban areas, Zambia. *J Epidemiol Community Health* 2011;**65**:1171-8.
14 Aimone AM, Patrick BE, Zlotkin SH*, et al.* Geo-spatial factors associated with infection risk among young children in rural Ghana: a secondary spatial analysis. *Malaria Journal* 2016;**15**.
15 Zlotkin S, Newton S, Aimone AM*, et al.* Effect of iron fortification on malaria incidence in infants and young children in Ghana: a randomized trial. *JAMA* 2013;**310**:938-47.
16 WHO. World Malaria Report 2009. Geneva, Switzerland: World Health Organization 2009.
17 WHO. Worldwide prevalence of anaemia 1993-2005. *WHO Global Database on Anaemia*. Geneva, Switzerland: World Health Organization 2008.
18 WHO/UNICEF. WHO child growth standards and the identification of severe acute malnutrition in infants and children: A Joint Statement by the World Health Organization and the United Nations Children's Fund. Geneva, Switzerland: World Health Organization 2009.
19 USGS. United States Geological Survey. 2012.

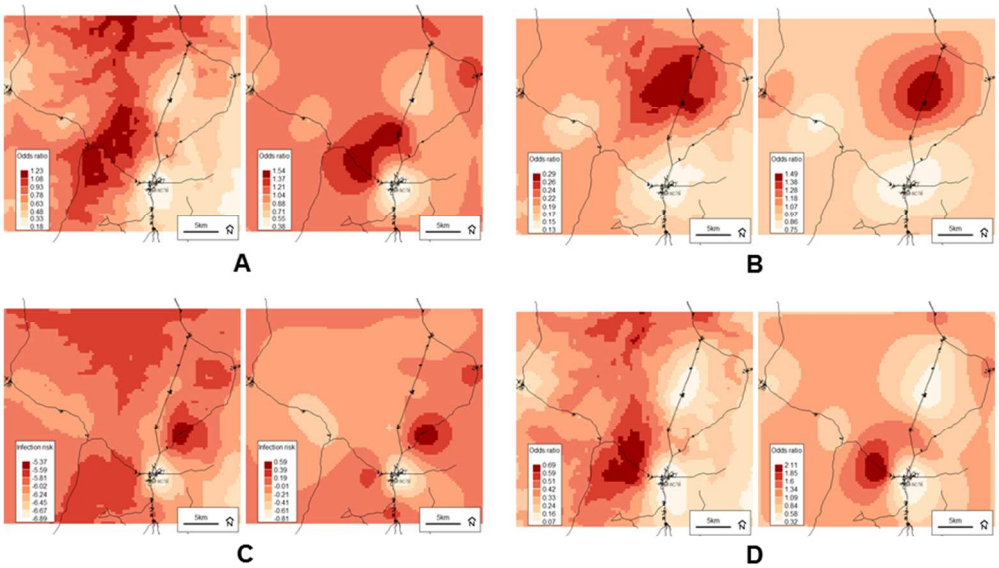
20 USGS. Land Processes Distributed Active Archive Center. 2014.
21 Hengl T. Land cover types for 2010 based on the MODIS IGBP classes. In: Center LPDAA, ed: Worldgrids (ISRIC - World Soil Information) 2013.
22 Samadoulougou S, Maheu-Giroux M, Kirakoya-Samadoulougou F, *et al.* Multilevel and geo-statistical modeling of malaria risk in children of Burkina Faso. *Parasit Vectors* 2014;**7**:350.
23 Guerra CA, Snow RW, Hay SI. A global assessment of closed forests, deforestation and malaria risk. *Ann Trop Med Parasitol* 2006;**100**:189-204.
24 Diggle PJ, Moyeed RA, Tawn JA. Model-based geostatistics. *Applied Statistics* 1998;**47**:299-350.
25 Diggle PJ, Ribeiro PJ. *Model-based geostatistics*. New York: Springer-Verlag 2006.
26 Rue H, Martino S. Approximate Bayesian inference for latent Gaussian models by using integrated nested Laplace approximations. *Journal of the Royal Statistical Society: Series B* 2009;**71**:319-92.
27 Brown PE. Model-based geostatistics the easy way. *Journal of Statistical Software* 2015;**63**:1-24.
28 R. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing 2015.
29 Prestia K, Bandyopadhyay S, Slate A, *et al.* Transfusion of stored blood impairs host defenses against Gram-negative pathogens in mice. *Transfusion* 2014;**54**:2842-51.
30 Thurnham DI, McCabe GP. Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron. In: Organization WH, ed. *Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15-17 September 2010*. Geneva: World Health Organization 2012:63-80.
31 Black RE, Allen LH, Bhutta ZA, *et al.* Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008;**371**:243-60.
32 Ezzati M, Utzinger J, Cairncross S, *et al.* Environmental risks in the developing world: exposure indicators for evaluating interventions, programmes, and policies. *J Epidemiol Community Health* 2005;**59**:15-22.
33 Cohen JM, Ernst KC, Lindblade KA, *et al.* Topography-derived wetness indices are associated with household-level malaria risk in two communities in the western Kenyan highlands. *Malar J* 2008;**7**:40.
34 Drakeley CJ, Carneiro I, Reyburn H, *et al.* Altitude-dependent and -independent variations in Plasmodium falciparum prevalence in northeastern Tanzania. *J Infect Dis* 2005;**191**:1589-98.
35 Brooker S, Clarke S, Njagi JK, *et al.* Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. *Trop Med Int Health* 2004;**9**:757-66.
36 Reid H, Vallely A, Taleo G, *et al.* Baseline spatial distribution of malaria prior to an elimination programme in Vanuatu. *Malar J* 2010;**9**:150.
37 Hay SI, Omumbo JA, Craig MH, *et al.* Earth observation, geographic information systems and Plasmodium falciparum malaria in sub-Saharan Africa. *Adv Parasitol* 2000;**47**:173-215.
38 Shirayama Y, Phompida S, Shibuya K. Geographic information system (GIS) maps and malaria control monitoring: intervention coverage and health outcome in distal villages of Khammouane province, Laos. *Malar J* 2009;**8**:217.

39 Kandala NB, Madungu TP, Emina JB*, et al.* Malnutrition among children under the age of five in the Democratic Republic of Congo (DRC): does geographic location matter? *BMC Public Health* 2011;**11**:261.
40 Weiss DJ, Mappin B, Dalrymple U*, et al.* Re-examining environmental correlates of Plasmodium falciparum malaria endemicity: a data-intensive variable selection approach. *Malar J* 2015;**14**:68.
41 Nesbitt RC, Gabrysch S, Laub A*, et al.* Methods to measure potential spatial access to delivery care in low- and middle-income countries: a case study in rural Ghana. *Int J Health Geogr* 2014;**13**:25.

Figure legends

Figure 1: Predicted probabilities (left), and residual spatial variation (right) from the final combined-group models for the odds of inflammation (CRP>5 mg/L) *and/or any* malaria parasitaemia (A); the odds of inflammation (CRP > 5 mg/L) *without* malaria parasitaemia (B); the risk of malaria parasitaemia *with* concurrent fever (axillary temperature >37.5^{0}C - or history of reported fever within 48 hours) (C); and the odds of malaria parasitaemia *with or without* fever (D). Darker colour indicates higher risk at endline. Background © Stamen Design.

Figure 2: Elevation (meters) across the study area. Green colour indicates higher elevation.



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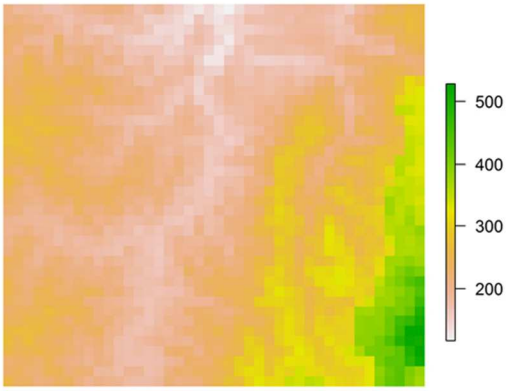

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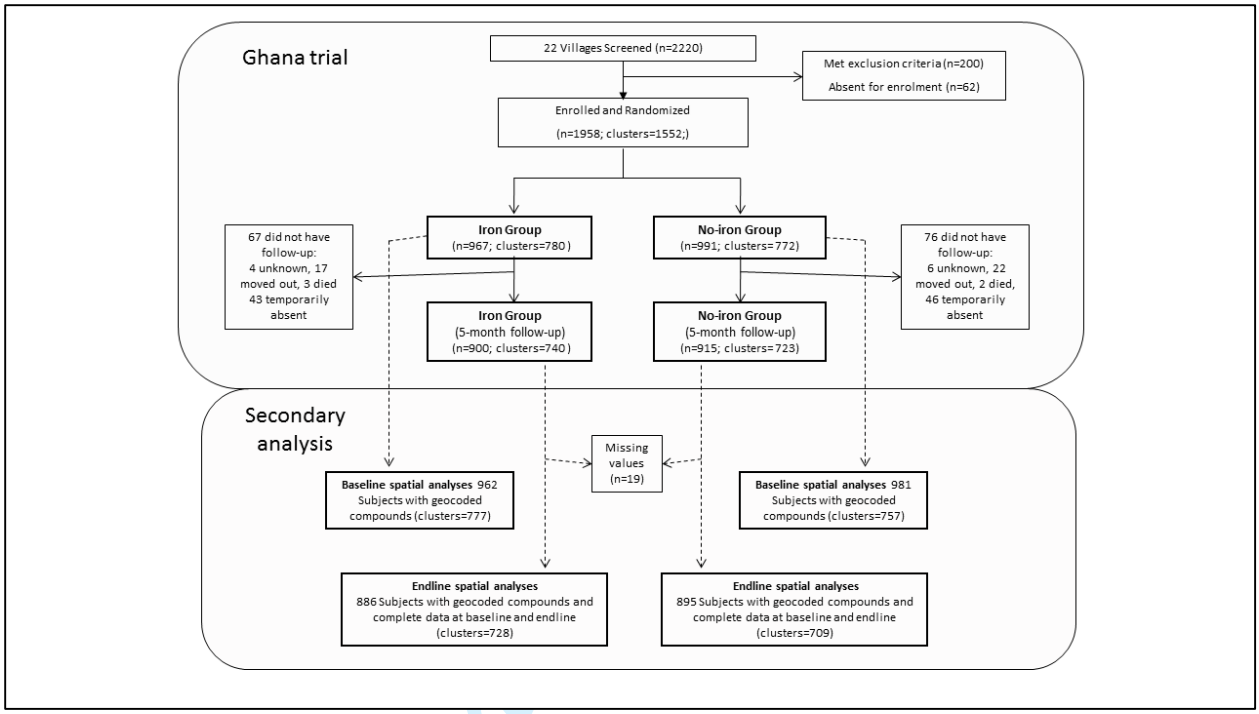

Figure S1: Study flow for the Ghana trial (top section) and secondary analyses (bottom section). Out of the 1958 participants from the Ghana trial, a total of 1943 with geocoded compounds were included in the baseline secondary spatial analyses (13 compounds were untraceable, corresponding to 15 participants not included in the secondary analyses). The endline spatial analyses included a total of 1781 observation, representing trial participants with geocoded compounds who provided blood samples at the end of the intervention period (5-month follow-up), and after removing 19 observations due to missing baseline or endline ferritin values.

Table S1: Geo-spatial and non-spatial risk factors of endline infection status[1] among Ghanaian children in the *No-iron* group (Brong-Ahafo Region, Sept-Nov 2010)

Covariates	Estimate (95% CrI) on the exponential scale[2]		Range parameter in km (95% CrI)	Standard deviations of random effects (95% CrI)	
				Spatial	Compound
Outcome 1: Inflammation and/or parasitaemia (n=894)					
Intercept	0.550	(0.298, 1.034)	7.050	0.506	0.008
Age per month			(2.016,	(0.271,	(0.004,
6-23 months	1.004	(0.975, 1.035)	18.26)	0.979)	0.030)
24-35 months	1.018	(0.964, 1.074)			
Sex (male reference)	1.016	(0.760, 1.357)			
Length-for-age z-score	0.984	(0.869, 1.113)			
Weight-for-length z-score	1.229	(1.056, 1.431)*			
Asset score	0.893	(0.760, 1.047)			
Distance to health facility (km)	1.072	(0.963, 1.188)			
Elevation (m)	0.995	(0.990, 1.001)			
Baseline infection status	1.887	(1.384, 2.576)*			
Baseline iron status	1.051	(0.938, 1.179)			
Outcome 2: Inflammation without parasitaemia (n=894)					
Intercept	0.219	(0.109, 0.426)*	9.458	0.442	0.007
Age per month			(2.389,	(0.206,	(0.004,
6-23 months	1.011	(0.971, 1.052)	23.57)	0.965)	0.029)
24-35 months	0.920	(0.843, 0.996)*			
Sex (male reference)	1.001	(0.671, 1.493)			
Length-for-age z-score	1.153	(0.977, 1.360)			
Weight-for-length z-score	1.302	(1.058, 1.603)*			
Asset score	0.872	(0.705, 1.075)			
Distance to health facility (km)	1.065	(0.953, 1.176)			
Elevation (m)	0.997	(0.991, 1.003)			
Baseline infection status	1.034	(0.556, 1.813)			
Baseline iron status	1.003	(0.841, 1.166)			
Outcome 3: Parasitaemia with fever (n=979)					
Intercept	0.003	(0.002, 0.004)*	7.690	0.448	0.007
Age per month			(2.821,	(0.243,	(0.004,
6-23 months	1.001	(0.979, 1.023)	18.01)	0.858)	0.028)
24-35 months	0.970	(0.927, 1.013)			
Sex (male reference)	0.896	(0.718, 1.116)			
Length-for-age z-score	1.009	(0.919, 1.107)			
Weight-for-length z-score	0.896	(0.797, 1.007)			
Asset score	1.075	(0.947, 1.220)			
Distance to health facility (km)	1.047	(0.959, 1.142)			

Elevation (m)	0.998	(0.993, 1.003)			
Baseline infection status	0.757	(0.467, 1.161)			
Baseline iron status	1.040	(0.953, 1.125)			
Outcome 4: All parasitaemia (n=894)					
Intercept	0.186	(0.089, 0.400)*	6.535	0.652	0.008
Age per month			(2.037,	(0.344,	(0.004,
6-23 months	0.974	(0.936, 1.014)	15.08)	1.255)	0.029)
24-35 months	1.067	(1.026, 1.110)*			
Sex (male reference)	1.050	(0.756, 1.459)			
Length-for-age z-score	0.901	(0.780, 1.037)			
Weight-for-length z-score	1.095	(0.923, 1.298)			
Asset score	0.941	(0.781, 1.130)			
Distance to health facility (km)	1.023	(0.896, 1.165)			
Elevation (m)	0.996	(0.989, 1.004)			
Baseline infection status	2.864	(1.968, 4.172)*			
Baseline iron status	1.036	(0.914, 1.169)			

[1]Infection status definitions:
Outcome 1: Inflammation and/or parasitaemia (binary): 1 = CRP > 5 mg/L and/or any malaria parasitaemia, 0 = CRP ≤ 5 mg/L and absence of parasitaemia;
Outcome 2: Inflammation without parasitaemia (binary): 1 = CRP > 5 mg/L without malaria parasitaemia, 0 = CRP ≤ 5 mg/L without parasitaemia;
Outcome 3: Parasitaemia with fever (count): any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
Outcome 4: All parasitaemia (binary): 1 = any malaria parasitaemia (with/without fever), 0 = absence of parasitaemia with/without fever
Model prior shape = 1.117, model prior rate = 0.157
[2]Exponentials of posterior medians and 2.5% and 97.5% posterior quantiles of model parameters, with a value of 1.05 indicating that a variable increases the risk of infection by 5% (odds ratio from logistic model for all binary outcomes; relative risk from Poisson model for count outcome)
CrI = credible interval
Baseline infection status = baseline infection status (yes/no) according to corresponding definition
Baseline iron status = baseline serum ferritin concentration (µg/dL) corrected for CRP using the regression method (Namaste et al.) and re-scaled by multiplying by the inverse of the inter-quartile range
*Statistical significance at the 0.05 level

Table S2: Geo-spatial and non-spatial risk factors of endline infection status[1] among Ghanaian children in the *Iron* group (Brong-Ahafo Region, Sept-Nov 2010)

Covariates	Estimate (95% CrI) on the exponential scale[2]		Range parameter in km (95% CrI)	Standard deviations of random effects (95% CrI)	
				Spatial	Compound
Outcome 1: Inflammation and/or parasitaemia (n=886)					
Intercept	0.647	(0.354, 1.263)	7.761	0.487	0.007
Age per month			(2.682,	(0.263,	(0.004,
6-23 months	1.004	(0.975, 1.035)	17.96)	0.931)	0.028)
24-35 months	0.977	(0.925, 1.031)			
Sex (male reference)	1.162	(0.871, 1.551)			
Length-for-age z-score	0.980	(0.857, 1.119)			
Weight-for-length z-score	0.971	(0.833, 1.130)			
Asset score	0.906	(0.769, 1.064)			
Distance to health facility (km)	1.003	(0.904, 1.106)			
Elevation (m)	0.995	(0.989, 1.001)			
Baseline infection status	2.272	(1.664, 3.108)*			
Baseline iron status	1.053	(0.953, 1.172)			
Outcome 2: Inflammation without parasitaemia (n=886)					
Intercept	0.143	(0.073, 0.274)*	12.56	0.428	0.007
Age per month			(4.318,	(0.203,	(0.004,
6-23 months	1.007	(0.969, 1.048)	27.27)	0.923)	0.028)
24-35 months	0.983	(0.910, 1.056)			
Sex (male reference)	1.330	(0.905, 1.962)			
Length-for-age z-score	1.054	(0.884, 1.255)			
Weight-for-length z-score	0.890	(0.726, 1.090)			
Asset score	0.888	(0.725, 1.087)			
Distance to health facility (km)	0.984	(0.883, 1.080)			
Elevation (m)	1.001	(0.995, 1.006)			
Baseline infection status	1.508	(0.897, 2.459)			
Baseline iron status	1.044	(0.918, 1.166)			
Outcome 3: Parasitaemia with fever (n=960)					
Intercept	0.002	(0.001, 0.004)*	8.352	0.466	0.007
Age per month			(3.203,	(0.244,	(0.004,
6-23 months	1.017	(0.992, 1.043)	17.99)	0.896)	0.028)
24-35 months	0.987	(0.945, 1.029)			
Sex (male reference)	0.991	(0.780, 1.257)			
Length-for-age z-score	0.969	(0.867, 1.082)			
Weight-for-length z-score	1.052	(0.925, 1.197)			
Asset score	1.002	(0.872, 1.152)			
Distance to health facility (km)	1.064	(0.970, 1.163)			
Elevation (m)	0.998	(0.993, 1.003)			

Baseline infection status	0.849	(0.516, 1.321)			
Baseline iron status	0.981	(0.895, 1.060)			
Outcome 4: All parasitaemia (n=886)					
Intercept	0.412	(0.199, 0.938)*	7.293	0.666	0.008
Age per month			(2.486,	(0.376,	(0.004,
6-23 months	1.015	(0.975, 1.056)	17.67)	1.230)	0.030)
24-35 months	0.977	(0.938, 1.016)			
Sex (male reference)	0.977	(0.707, 1.347)			
Length-for-age z-score	0.946	(0.812, 1.098)			
Weight-for-length z-score	1.034	(0.872, 1.225)			
Asset score	0.946	(0.785, 1.137)			
Distance to health facility (km)	1.009	(0.889, 1.149)			
Elevation (m)	0.994	(0.987, 1.001)			
Baseline infection status	2.597	(1.791, 3.768)*			
Baseline iron status	1.042	(0.939, 1.153)			

[1]Infection status definitions:
Outcome 1: Inflammation and/or parasitaemia (binary): 1 = CRP > 5 mg/L and/or any malaria parasitaemia, 0 = CRP ≤ 5 mg/L and absence of parasitaemia;
Outcome2: Inflammation without parasitaemia (binary): 1 = CRP > 5 mg/L without malaria parasitaemia, 0 = CRP ≤ 5 mg/L without parasitaemia;
Outcome3: Parasitaemia with fever (count): any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
Outcome4: All parasitaemia (binary): 1 = any malaria parasitaemia (with/without fever), 0 = absence of parasitaemia with/without fever
Model prior shape = 1.117, model prior rate = 0.157
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CrI = credible interval
Baseline infection status = baseline infection status (yes/no) according to corresponding definition
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*Statistical significance at the 0.05 level

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	NA
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5-7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	5
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	5

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	5
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6-7
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	6-7
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure S1
	13b	For each group, losses and exclusions after randomisation, together with reasons	8
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	9
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	9-13
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	9-13
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	9-10
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13-16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13-16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13-16
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	17

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

Impact of iron fortification on the geo-spatial patterns of malaria and non-malaria infection risk among young children: a secondary spatial analysis of clinical trial data from Ghana

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-013192.R2
Article Type:	Research
Date Submitted by the Author:	24-Jan-2017
Complete List of Authors:	Aimone, Ashley; University of Toronto, Dalla Lana School of Public Health Brown, Patrick; University of Toronto, Dalla Lana School of Public Health; Cancer Care Ontario, Analytics and Informatics Owusu-Agyei, Seth; Kintampo Health Research Centre Zlotkin, Stanley H.; Hospital for Sick Children, Centre for Global Child Health; University of Toronto, Dalla Lana School of Public Health Cole, Donald; University of Toronto, Dalla Lana School of Public Health
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Global health, Paediatrics
Keywords:	Nutrition < TROPICAL MEDICINE, Community child health < PAEDIATRICS, Geographical mapping < TROPICAL MEDICINE

Impact of iron fortification on the geo-spatial patterns of malaria and non-malaria infection risk among young children: a secondary spatial analysis of clinical trial data from Ghana

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Keywords
Spatial analysis, infection, child health, GIS, nutrition

Word count: 5,714 (including tables and references)

Abstract

Objectives: Patterns of infection among children with varying levels of iron status in a malaria endemic area may vary spatially in ways requiring integrated infection and iron deficiency control programs. The objective of this secondary analysis was to determine the geo-spatial factors associated with malaria and non-malaria infection status among young Ghanaian children at the end of a 5-month iron intervention trial.

Design: Cluster-randomised controlled trial

Setting: Rural Ghana

Participants: 1943 children (6-35 months of age) with geocoded compounds.

Interventions: Point-of-use fortification with micronutrient powders containing vitamins and minerals with or without iron.

Primary and secondary outcome measures: Generalized linear geostatistical models with a Matern spatial correlation function were used to analyse four infection response variables, defined using different combinations of inflammation (C-reactive protein, CRP >5 mg/L) and malaria parasitaemia. Analyses were also stratified by treatment group to assess the independent effects of the iron intervention.

Results: The by-group and combined-group analyses both showed that baseline infection status was the most consistent predictor of endline infection risk, particularly when infection was defined using parasitaemia. In the No-iron group, age above 24 months and weight-for-length z-score at baseline were associated with high CRP at endline. Higher asset score was associated with a 12% decreased odds of endline infection, defined as CRP >5 mg/L and/or parasitaemia (OR 0.88, 95% CrI 0.78, 0.98), regardless of group. Maps of the predicted risk and spatial random effects showed a defined low-risk area around the District centre, regardless of how infection was defined.

Conclusions: In a clinical trial setting of iron fortification, where all children receive treated bed nets and access to malaria treatment, there may be geographical variation in the risk of infection with distinct high- and low-risk areas, particularly around municipal centres.

Trial registration: clinicaltrials.gov, NCT01001871.

Strengths and limitations of this study

- The geostatistical analyses conducted in this study are the first of their kind to use model-based geostatistics with Bayesian inference and integrated nested Laplace approximations (INLA) to explore the spatial variation and associated risk factors of malaria and non-malaria infection risk among children in rural Ghana after a 5-month randomized iron intervention trial.
- These analyses also provide input into the potential utility of geographical indicators, particularly for assessing infection risk potential, which could help guide the implementation of iron interventions in areas where infectious diseases are prevalent.
- Since satellite-derived spatial data often vary at a higher level than the individual (e.g. village or region), the use of these data in statistical models with individual-level outcomes may increase the risk of a change of support or ecological inference problem.
- The use of C-reactive protein (CRP) as an indicator of non-malaria infection may have led to the underestimation of this outcome, since CRP is an acute phase protein that only rises in accordance with the first 48 hours of the inflammatory response.
- Straight-line distance, rather than distance by road, was used to estimate proximity to a health facility or the district centre, and thus may not have fully accounted for travel impedance.

Background

The leading causes of death in children less than 5 years of age are infection-related, and child mortality rates are highest in low- and middle-income countries (LMICs).[1] Malnutrition is also a large contributor to mortality (45% of all deaths),[1] including micronutrient deficiencies. The most common nutritional disorder worldwide is iron deficiency,[2] which is estimated to account for 2.2 million disability-adjusted life years lost per year among children less than five years of age.[3] While iron deficient children are more vulnerable to infections (primarily due to compromised immune function),[4, 5] infection and inflammation can also affect iron homeostasis[6] and the risk of iron deficiency.[7] Iron status can usually be improved through food fortification, or supplementation; however, evidence from a large randomized trial

conducted in a malaria endemic area (Pemba, Zanzibar) indicated that supplementing young children with iron may increase their risk of malaria and infection-related morbidity and mortality, particularly if they are iron replete.[8] Since iron supplements or fortificants would likely be withheld from children with malaria or other infections, assessing the risk of infection is an important component of developing safe and effective means of administering iron to children in LMICs.

Measuring biomarkers of infection status (such as C-reactive protein) among children in a low-resource context may have limited feasibility, due to the requirement of blood samples, and the potential for inflammation prevalence to vary over relatively short periods of time (e.g. within seasons). As such, there is a need to identify indicators or risk factors associated with infection in LMICs that are more feasible to measure, and thus provide an efficient means of identifying high-risk populations. This need could be addressed with geographical factors (or "geo-indicators"), such as the environmental or spatial characteristics of a village or region.[9] Geo-indicators could provide additional insight into the dynamics and distribution of infection among children that informs treatment needs and the prophylactic use of iron supplementation or fortification.[10-13] Collecting geo-spatial data is not invasive and comparatively less costly than biological measures, as geographical datasets are often publicly available on the internet. This also improves the access to and comparability of population-level statistics within and across national borders.

We previously conducted a secondary spatial analysis to determine the geo-spatial factors associated with infection status among iron deficient and sufficient children in rural Ghana.[14] The results of these analyses suggested that the risk of infection may be related to elevation, and distance to the nearest health facility. The objective of the current analysis was to determine the geo-spatial factors associated with malaria and non-malaria infection status among Ghanaian children at the end of a 5-month randomized iron intervention trial.

Methods

Study population

The data used in these analyses were generated from a study population of young children (6-35 months of age) who participated in a community-based cluster-randomized trial conducted in the Brong-Ahafo Region of Ghana in 2010.[15] The trial consisted of a 5-month intervention period, where participants received micronutrient powders with or without iron, followed by a 1-month post-intervention follow-up period (6 months in total). At the time of the trial, the estimated prevalence of malaria in Ghana was 7.2 million cases per year, and the prevalence of anaemia among preschool aged children was 76.1% (95% CI 73.9-78.2%).[16, 17] Details of the clinical trial, [15] and geographical layout of the study area have been described elsewhere (Aimone, Brown, Zlotkin, Cole, Owusu-Agyei 2016).

Measures from trial data

Biological samples collected at the beginning and end of the 5-month intervention period were analysed for plasma ferritin (Spectro Ferritin S-22, Ramco Laboratories Inc., Stafford, USA), C-reactive protein (QuickRead CRP, Orion Diagnostica, Espoo, Finland), and malaria parasite density (microscopy).[15] Malaria screening was also performed on a weekly basis throughout the intervention period. If a child had a history of fever (within 48 hours) or an axillary temperature >37.5^{0}C, a blood sample was drawn and analysed in the field via antigen test (Paracheck Pf®), and in the lab using microscopy (thin and thick smears). Parasite density was combined with fever information to calculate clinical malaria incidence (episode counts). Demographic and nutrition-related information was also collected at baseline and included household assets, maternal education, and child body weight and length. Weight-for-length and length-for-age z-scores were calculated using the WHO Child Growth Standards.[18]

Variable preparation

Geographical coordinates for the compounds of 1943 trial participants (representing 1539 clusters), surrounding health facilities and major road networks were collected using handheld global positioning system (GPS) units (WGS 1984 coordinate system, universal transverse Mercator zone 30N projection, EPSG code: 32630). Satellite-derived data were downloaded as global datasets[19-21] and cropped according to the geographical boundaries of the trial area. Elevation had a range of 116-530 meters, and values were centred by subtracting 250. Land cover type (LC) consisted of 3 categorical values, representing woody savannah (LC=8, n=21/1943 observations), urban and built up land (LC=13, n=243/1943 observations), and cropland/natural vegetation mosaic (LC=14, n=1679/1943 observations). NDVI, a vegetation index included as a proxy for moisture,[22] was averaged over the year that the trial was conducted (2010), and ranged in value from 0.22 to 0.62. NDVI has also been used to create malaria risk distribution maps through the characterization of vector habitat potential (e.g. closed forest versus open forest).[23] Two NDVI-LC interaction terms were created (NDVI*LC8 and NDVI*LC13) by overlaying the final NDVI and LC rasters, and masking the LC cells except where they had a value of 8 (or 13). These unmasked cells were given a value of zero.

Baseline age, in months, was calculated using the reported date of birth and trial enrolment date with a change point at 24 months. Household asset score was generated using a principal component analysis of 6 economic indicators (farm ownership, size and type of crops grown, type of toilet facility, and house ownership). Maternal education was included as a binary variable representing “no” (0) versus “any” (1) level of education. Baseline iron status was defined as serum ferritin concentration corrected for inflammation (baseline CRP) using a regression-based method (Namaste, Rohner, Suchdev, Kupka, Mei, Bhushan, Williams, Rowat, Raiten, Flores-Ayala, Clewes, 2016), and re-scaled by multiplying the corrected values by the inter-quartile range. Straight-line (Euclidean) distance to the nearest health facility was measured using the Near Table tool in ArcMap (ArcGIS 10.2, Environmental Systems Resource Institute, Redlands, California).

Spatial modelling

The endline data were analysed using generalized linear geostatistical models (GLGM) [24, 25] with Bayesian inference via an Integrated Nested Laplace Approximation (INLA) algorithm.[26] Weak or uninformative priors were used for all model parameters with the exception of the Matern shape parameter (fixed at 2). Four different endline infection outcomes were modelled separately: 1) inflammation (CRP >5 mg/L) with/without malaria parasitaemia; 2) inflammation (CRP >5 mg/L) without parasitaemia; 3) parasitaemia with measured concurrent fever (axillary temperature >37.5^{0}C) or reported history of fever within 48 hours (i.e. clinical malaria)); and 4) parasitaemia with or without concurrent fever or history of fever. All variables were binary-valued (coded as '1' for positive infection status at endline) and analysed using logistic regression, with the exception of the third definition (parasitaemia with fever), which was a count variable (number of new clinical malaria episodes during the intervention period) analysed using Poisson regression. Median infection probabilities (and 95% credible intervals) were modelled as the sum of the contributions of the independent variables, residual spatial variation, and a compound-level random effect term. The *glgm* function from the "geostatsp" package in R was used for all spatial modelling.[27, 28]

The spatial analyses were conducted in three modelling steps: 1) No-iron group only; 2) Iron group only; 3) both intervention groups combined. The interventions groups were analysed separately in order to differentiate the effects of time and the iron treatment. Infection probabilities from the combined-group models were plotted on a base map of the trial area, with study compounds, and major road networks. The maps depicted a spatial risk surface of predicted infection probabilities, which were computed as the posterior means of the odds or risk of infection. The posterior means were estimated assuming baseline values for individual-level covariates and location-specific values for spatial covariates. The posterior mean of the spatial random effect was also plotted, representing the residual spatial variation that corresponded to the difference between the predicted and expected odds or risk of infection at each location.

Ethics

The original clinical trial was approved by the Kintampo Health Research Centre (KHRC) Institutional Ethics Committee, the Ghana Health Service (GHS) Ethical Review Committee, the Hospital for Sick Children Research Ethics Board, and the Food and Drugs Authority of Ghana. Approval for conducting the secondary analyses was obtained from the Hospital for Sick Children and University of Toronto Health Sciences Research Ethics Boards.

Results

Baseline and endline characteristics of the study sample are presented in **Table 1**. A total of 1780 trial participants were included in the endline analyses, representing those with geocoded residences (compounds), who provided blood samples at endline, and had both baseline and endline CRP and parasitaemia values (see online supplementary file Figure S1). For the infection outcome defined as parasitaemia with fever, there were 1939 observations included in the Poisson regression analysis, which corresponded to the number of children with geocoded compounds who had at least one recorded follow-up visit during the intervention period, and thus contributed data to the malaria count outcome.

The proportion of children with evidence of infection (elevated CRP and/or parasitaemia) increased from baseline to endline (37.0% at baseline versus 41.6% at endline). This increase may have been driven primarily by a greater prevalence of parasitaemia at endline (27.1% versus 23.0% at baseline), though the difference between intervention groups was small (26.3% versus 28.0% in the No-iron and Iron groups, respectively). The prevalence of non-malaria inflammation (CRP>5 mg/L without parasitaemia) remained relatively stable over the course of the trial; however, the proportion in the Iron group was slightly higher at endline (7.64% in the Iron group versus 6.58% in the No-iron group). After correcting ferritin concentration for inflammation (CRP) using the regression method, the prevalence of iron deficiency (ferritin <12 µg/L) was 25.5% (496/1943) at baseline and 12.7% (226/1781) at endline (95/886 in the Iron group, 131/895 in the No-iron group).

Table 1: Baseline and endline characteristics of the Ghana trial participants

	Baseline	Endline
Trial participants with a geocoded residence (n)	1943	1780
Males (%)	992 (51.1)	900 (50.5)
Age at enrolment (months), mean (SD)[a]	19.2 (8.5)	19.3 (8.5)
Serum ferritin (µg/L), geometric mean (SD)	35.1 (3.65)	73.9 (3.58)
Parasite density (count/µL), geometric mean (SD)	3003.0 (5.35)	4160.4 (6.30)
C-reactive protein (mg/L), mean (SD)	3.34 (4.96)	3.86 (5.10)
Infection status		
Inflammation without parasitaemia[b], n (%)	272 (14.0)	258 (14.5)
Inflammation and/or parasitaemia[c], n (%)	719 (37.0)	741 (41.6)
Parasitaemia with fever[d‡], n (%)	150 (7.72)	555 (28.6)
All parasitaemia[e], n (%)	447 (23.0)	483 (27.1)
Anthropometric status[f]		
Weight-for-length z-score, mean (SD)	-0.63 (0.97)	-0.62 (0.97)
Length-for-age z-score, mean (SD)	-0.81 (1.21)	-0.80 (1.29)
Maternal Education, n (%)[g]		
None	586 (33.5)	543 (33.0)
Any	1166 (66.5)	1105 (67.0)
Household Asset Score, n (%)[h]		
Low	900 (49.0)	817 (47.7)
High	938 (51.0)	897 (52.3)

[‡]Endline value represents the period prevalence for 1939 participants
[a]Measured at baseline only
[b]Inflammation without parasitaemia (n=1780) = CRP > 5 mg/L without malaria parasitaemia;
[c]Inflammation and/or parasitaemia (n=1780) = CRP > 5 mg/L and/or any malaria parasitaemia;
[d]Parasitaemia with fever (n=1939) = any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
[e]All parasitaemia (n=1780) = any malaria parasitaemia (with/without fever)
[f]Measured at baseline; z-scores estimated using the WHO Child Growth Standards [23]
[g] Measured at baseline only; total n=1752 (74 respondents were not mothers, 117 missing due to incomplete surveys)
[h] Measured at baseline only; Low= below median, High = above median; reduced sample size (approximately 1825) due to incomplete surveys and "unknown" responses

In the combined-group analyses, the risk of elevated CRP and/or parasitaemia at endline was negatively associated with household asset score, and positively associated with baseline infection status (OR for definition 1: 0.88, 95% CrI 0.78, 0.98) (**Table 2**). Baseline infection status was also significantly and positively associated with the corresponding endline outcome when defined as inflammation and/or parasitaemia (OR 1.84, 95% CrI 1.36, 2.50) or all parasitaemia (2.75, 95% CrI 1.91, 3.95). Although not statistically significant, the effect of the

"Group" variable seemed to suggest that the iron intervention was positively associated with endline infection risk, except in the case of outcome definition 3 (parasitaemia with fever) where the iron treatment may have had a protective effect.

Results from the by-group analyses indicated that the risk of elevated CRP (without parasitaemia) at endline among children in the No-iron group was positively associated with baseline weight-for-length z-score (OR 1.30, 95% credible interval (CrI) 1.06, 1.60), and negatively associated with age between 24 and 36 months (OR 0.92, 95% CrI 0.84, 1.006). When infection was defined as all parasitaemia, baseline infection status became a significant factor (OR for definition 4: 2.86, 95% CrI 1.97, 4.17), indicating that children in the No-iron group with any parasitaemia (with or without CRP or fever) at baseline were up to 186% more likely to have parasitaemia at endline (see online supplementary file Table S1). In the Iron group, when infection was defined as inflammation and/or parasitaemia, baseline infection status was the only factor associated with infection risk at endline (OR 2.27, 95% CrI 1.66, 3.11). Similar results were found when infection was defined as all parasitaemia (OR 2.54, 95% CrI 1.75, 3.68) (see online supplementary file Table S2).

Table 2: Geo-spatial and non-spatial risk factors of endline infection status[1] among Ghanaian children in the combined *Iron* and *No-iron* groups (Brong-Ahafo Region 2010)

Covariates	Estimate (95% CrI) on the exponential scale[2]		Range parameter in km (95% CrI)	Standard deviations of random effects (95% CrI)	
				Spatial	Compound
Outcome 1: Inflammation and/or parasitaemia (n=1780)					
Intercept	0.589	(0.337, 1.072)	7.341	0.525	0.008
Age per month			(2.928,	(0.306,	(0.004,
6-23 months	1.004	(0.984, 1.026)	15.84)	0.944)	0.029)
24-35 months	1.000	(0.962, 1.038)			
Sex (male reference)	1.087	(0.886, 1.333)			
Length-for-age z-score	0.982	(0.896, 1.075)			
Weight-for-length z-score	1.088	(0.978, 1.211)			
Asset score	0.875	(0.779, 0.980)*			
Distance to health facility (km)	1.034	(0.933, 1.136)			
Elevation (m)	0.996	(0.991, 1.001)			
Baseline infection status	1.843	(1.359, 2.500)*			
Group	1.094	(0.826, 1.448)			
Baseline iron status	1.068	(0.956, 1.194)			
Baseline infection status*Group	1.192	(0.775, 1.833)			
Baseline iron status*Group	0.983	(0.847, 1.144)			
Outcome 2: Inflammation without parasitaemia (n=1780)					
Intercept	0.183	(0.106, 0.309)*	10.77	0.407	0.007
Age per month			(8.767,	(0.208,	(0.004,
6-23 months	1.009	(0.982, 1.038)	25.54)	0.830)	0.028)
24-35 months	0.956	(0.904, 1.009)			
Sex (male reference)	1.139	(0.865, 1.502)			
Length-for-age z-score	1.092	(0.968, 1.230)			
Weight-for-length z-score	1.054	(0.912, 1.217)			
Asset score	0.871	(0.750, 1.010)			
Distance to health facility (km)	1.012	(0.924, 1.093)			
Elevation (m)	0.999	(0.994, 1.003)			
Baseline infection status	1.055	(0.574, 1.826)			
Group	1.009	(0.713, 1.427)			
Baseline iron status	1.001	(0.844, 1.156)			
Baseline infection status*Group	1.523	(0.718, 3.300)			
Baseline iron status*Group	1.033	(0.856, 1.261)			
Outcome 3: Parasitaemia with fever (n=1939)					
Intercept	0.003	(0.002, 0.004)*	7.365	0.464	0.007
Age per month			(3.225,	(0.273,	(0.004,
6-23 months	1.008	(0.991, 1.025)	15.18)	0.825)	0.028)
24-35 months	0.978	(0.948, 1.008)			

Sex (male reference)	0.939	(0.799, 1.104)			
Length-for-age z-score	0.992	(0.923, 1.064)			
Weight-for-length z-score	0.966	(0.886, 1.053)			
Asset score	1.029	(0.937, 1.130)			
Distance to health facility (km)	1.057	(0.972, 1.149)			
Elevation (m)	0.997	(0.993, 1.002)			
Group	0.891	(0.729, 1.087)			
Baseline infection status	0.730	(0.452, 1.114)			
Baseline iron status	1.028	(0.942, 1.112)			
Baseline infection status*Group	1.209	(0.639, 2.282)			
Baseline iron status*Group	0.954	(0.849, 1.069)			
Outcome 4: All parasitaemia (n=1780)					
Intercept	0.271	(0.135, 0.571)*	7.846	0.712	0.008
Age per month			(3.384,	(0.406,	(0.004,
6-23 months	0.993	(0.966, 1.021)	16.06)	1.291)	0.029)
24-35 months	1.022	(0.994, 1.050)			
Sex (male reference)	1.024	(0.814, 1.287)			
Length-for-age z-score	0.926	(0.835, 1.026)			
Weight-for-length z-score	1.068	(0.947, 1.204)			
Asset score	0.916	(0.802, 1.044)			
Distance to health facility (km)	1.022	(0.902, 1.164)			
Elevation (m)	0.996	(0.990, 1.003)			
Baseline infection status	2.746	(1.910, 3.951)*			
Group	1.169	(0.862, 1.587)			
Baseline iron status	1.061	(0.940, 1.193)			
Baseline infection status*Group	0.915	(0.555, 1.508)			
Baseline iron status*Group	0.980	(0.839, 1.145)			

[1]Infection status definitions:
Outcome 1: Inflammation and/or parasitaemia (binary): 1 = CRP > 5 mg/L and/or any malaria parasitaemia, 0 = CRP ≤ 5 mg/L and absence of parasitaemia;
Outcome 2: Inflammation without parasitaemia (binary): 1 = CRP > 5 mg/L without malaria parasitaemia, 0 = CRP ≤ 5 mg/L without parasitaemia;
Outcome 3: Parasitaemia with fever (count): any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
Outcome 4: All parasitaemia (binary): 1 = any malaria parasitaemia (with/without fever), 0 = absence of parasitaemia
Model prior shape = 1.117, model prior rate = 0.157
[2]Exponentials of posterior medians and 2.5% and 97.5% posterior quantiles of model parameters, with a value of 1.05 indicating that a variable increases the risk of infection by 5% (odds ratio from logistic model for all binary outcomes; relative risk from Poisson model for count outcome)
CrI = credible interval
Baseline infection status = baseline infection status (yes/no) according to corresponding definition
Baseline iron status = baseline serum ferritin concentration (µg/dL) corrected for CRP using the regression method (Namaste et al.) and re-scaled by multiplying by the inverse of the inter-quartile range
*Statistical significance at the 0.05 level

Plots of the predicted risk and residual spatial variation from all combined-group models are illustrated in **Figure 1**. The maps show a defined low-risk area around the District centre, regardless of how infection was defined. Conversely, the location of high-risk areas seemed to vary across models and, in some cases, roughly approximated elevation (**Figure 2**). The maps depicting residual spatial variation also show defined high- and low-risk areas, indicating that a large amount of spatial variation was not explained by the variables included in the analyses and further supported the significant spatial random effects observed across all models.

Discussion

The analyses presented herein explored the geo-spatial variation and associated factors of infection, defined using both inflammatory and parasitic biomarkers, among children in a malaria endemic area (rural Ghana) after a randomized iron intervention trial. Although none of the spatial variables included in the by-group or combined-group models demonstrated significant associations with endline infection status (regardless of how infection was defined), the plots of predicted infection probabilities and spatial random effects showed defined high- and low-risk areas across the study region, particularly around the District centre. In terms of individual-level effects, baseline infection status was a consistent predictor of endline infection risk, particularly in the Iron group and when infection was defined using parasitaemia (with or without inflammation).

The consistency of the relationship with baseline parasitaemia in both intervention groups is somewhat surprising, as current evidence might lead one to expect that providing iron to children with malaria would increase their risk of subsequent parasitic infections to a greater extent than those who do not receive iron.[8] Interestingly, the Iron group analyses also revealed a significant effect of baseline infection status when acute non-malaria inflammation (high CRP without parasitaemia) was included in the definition. This observation is supported by recent research in mice demonstrating that iron supplementation may exacerbate non-malaria bacterial

infections, and that co-infection with *Plasmodium yoelli* and *Salmonella typhimurium* can produced overwhelming *Salmonella* sepsis [29]. Findings from the current analyses that also suggested a potential adverse effect of the iron intervention were the positive group effects across all infection definitions with the exception of clinical malaria (parasitaemia with fever). It should be noted however, that these were non-significant associations only. This discrepancy is likely related to the fact that all children in both groups were monitored and treated for identified clinical malaria episodes throughout the intervention period. Therefore, any cases of asymptomatic malaria (parasitaemia without fever) may have persisted over the course of the trial, potentially interacting with the iron intervention, as well as other opportunistic non-malaria infections.

After stratifying the analyses by group, we found that children in the No-iron group with a higher weight-for-length z-score (a measure of nutritional status) at baseline were 30% more likely to have high CRP (>5 mg/L) at endline (OR 1.30, 95% CrI 1.06, 1.60) (Table S1). This positive association was not expected, given that well-nourished children (those with higher weight-length z-scores) are expected have a lower risk of infection.[4] Since CRP becomes elevated during the first 48 hours of the inflammatory response,[30] it is unlikely that anthropometric measures at baseline influenced inflammatory status 5 months later at endline. Rather, it is more likely that the association was influenced through another unmeasured factor, such as immune function. High CRP was also inversely associated with age in the No-iron group for children between 24 and 36 months (OR 0.92, 95% CrI (0.84, 1.00)), indicating an 8% lower odds of elevated CRP at endline for every month of age older. Assuming that elevated CRP without parasitaemia represented acute non-malaria infection, it is plausible that the inverse relationship reflected the development of the immune system with age, thus resulting in older children being more resilient to infection exposure.

In the combined-group models (**Table 2**), higher asset score was associated with a 12% decreased odds of endline infection, defined as CRP >5 mg/L and/or parasitaemia (OR 0.88, 95% CrI 0.78, 0.98), suggesting that children from wealthier households had a lower risk of

malaria or non-malaria infection at endline (regardless of whether they received MNPs with or without iron). This finding was not surprising given the well-documented association between household wealth and child health in low- and middle-income settings;[31] however, the inconsistency of this relationship across infection definitions may have been a reflection of other socioeconomic or behavioural factors that were not included in the analyses.[32] Despite any differences in (or lack of) variable associations across infection outcomes, the residual spatial variation in infection risk remained significant in all combined-group models. The importance of accounting for residual spatial variation was further illustrated when the spatial random effects were plotted. For example, when infection was defined using CRP only, the model output plots looked similar (**Figure 1B**); suggesting that the spatial random effect encompassed a large amount of the variation in predicted risk, while the variables included in the model did not.

For the infection outcomes that included parasitaemia, we observed similarities between the geographical variation in predicted mean risk and elevation (**Figure 2**). This finding is consistent with our baseline analyses,[14] as well as other studies conducted in malaria endemic areas, where a higher prevalence of malaria is observed among populations living at lower elevations and vice versa.[33-36] The association between malaria and elevation is related to temperature, as the early stages of parasite development are inhibited in colder environments, which are found at higher altitudes.[37] Our baseline analyses also demonstrated a significant effect of distance to the nearest health facility, indicating that those children living farther from a health facility had an increased risk of malaria parasitaemia at baseline. The current endline analyses also suggested a potential risk-enhancing effect of distance to a health facility across all infection definitions, although the effect was not significant. This trend was further supported by the model output plots, which demonstrated larger low-risk areas around villages, and particularly around the District centre. Other studies have shown that living in a capital or municipal centre is associated with a reduced risk of adverse infection- or nutrition-related health outcomes,[38, 39] likely due to increased access to health care services and social amenities.

Overall, it appears that the effect of the intervention may have diminished the spatial associations observed at baseline; however, with the current data, it is difficult to differentiate between the effects of the iron intervention itself and the trial-related malaria mitigation activities (e.g. universal provision of treated bed nets, and malaria treatment when indicated). Further research involving additional secondary analyses with expanded spatial datasets[40] could shed more light on these relationships, as well as their implications on the safety of iron interventions in malaria endemic areas. Until that time, the potential utility of geo-spatial analysis lies primarily in the assessment or planning stages of micronutrient programs, in terms of where malaria and/or non-malaria infection risk is likely a concern; and thus where infection control efforts should be focused if iron fortification or supplementation is to be implemented.

A potential limitation of the present analyses was the use of straight-line (Euclidean) distance for estimating proximity to a health facility. While distance by road may have been a more appropriate indicator of travel impedance or access, incomplete or missing vector information (e.g. miss-aligned junctions, missing or disconnected road segments) made it difficult to generate accurate measures. Nesbitt and colleagues (2014) compared different measures of travel impedance to estimate access to delivery care in the Brong-Ahafo region of Ghana[41] and concluded that straight-line distance was as informative as distance by road for determining spatial access in rural Ghana. Therefore, we felt it was justified to use Euclidean distance in the present analyses.

Non-malaria infections were identified using CRP, an acute phase protein that rises in accordance with the early phase of the inflammatory response (approximately 48 hours). Other acute phase proteins (such as alpha-1-acid glycoprotein) reach their peak concentration during

the late phase (approximately 2-8 days), which more closely approximates the change in ferritin concentration in response to inflammation.[30] Since CRP was the only inflammatory biomarker available for the current analysis, and the original trial protocol was not designed to include diagnoses of other infection types, it is possible that the prevalence of non-malaria infection among participants was underestimated.

Conclusions

The analyses presented herein explored the spatial dynamics of infection risk in the context of an iron intervention trial in a malaria endemic area among children with varying levels of iron status, where all children received bed nets and access to malaria treatment. Geographical variation in the risk of malaria and non-malaria infections was observed with distinct high- and low-risk areas, particularly around the District centre. Overall, our findings emphasize the importance of considering geographical distribution when assessing infection risk and planning intervention trials in LMIC paediatric populations.

Acknowledgments

The authors would like to acknowledge Seeba Amenga-Etego and the members of the GIS team at the Kintampo Health Research Centre in (Kintampo, Ghana) who collected the GPS data that were used in the secondary analysis.

Author contribution

AA, SHZ and SOA conducted the original trial in Ghana. AA and SOA coordinated the acquisition of geographical data. AA conceived and conducted the secondary analysis with substantial contribution from PEB. DCC and SHZ were also involved in the conception and design of the secondary analysis, and interpretation of data. AA drafted the manuscript. All authors read and approved the final manuscript.

Funding

Funding for this manuscript was provided by AA's Doctoral Research Award from the Canadian Institutes of Health Research (CIHR).

Competing interests

None declared

Data sharing

Any unpublished data are available upon request by emailing the corresponding author.

References

1 WHO. Children: reducing mortality. *Fact sheet No 178*. World Health Organization 2016.
2 WHO. Micronutrient Deficiencies: Iron deficiency anaemia. World Health Organization 2015.
3 Stoltzfus RJ. Iron deficiency: global prevalence and consequences. *Food Nutr Bull* 2003;**24**:S99-103.
4 Katona P, Katona-Apte J. The interaction between nutrition and infection. *Clin Infect Dis* 2008;**46**:1582-8.
5 Raiten DJ, Sakr Ashour FA, Ross AC*, et al.* Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE). *J Nutr* 2015;**145**:1039S-108S.
6 Spottiswoode N, Duffy PE, Drakesmith H. Iron, anemia and hepcidin in malaria. *Front Pharmacol* 2014;**5**:125.
7 Nemeth E, Tuttle MS, Powelson J*, et al.* Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004;**306**:2090-3.
8 Sazawal Z, Black, RE., Ramsan, M., Chwaya, HM., Stoltzfus, RJ., Dutta, A ., et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet* 2006;**367**:133-43.
9 Rezaeian M, Dunn G, St Leger S*, et al.* Geographical epidemiology, spatial analysis and geographical information systems: a multidisciplinary glossary. *J Epidemiol Community Health* 2007;**61**:98-102.
10 Schur N, Vounatsou P, Utzinger J. Determining treatment needs at different spatial scales using geostatistical model-based risk estimates of schistosomiasis. *PLoS Negl Trop Dis* 2012;**6**:e1773.
11 Pullan RL, Gething PW, Smith JL*, et al.* Spatial modelling of soil-transmitted helminth infections in Kenya: a disease control planning tool. *PLoS Negl Trop Dis* 2011;**5**:e958.
12 Soares Magalhães RJ, Langa A, Pedro JM*, et al.* Role of malnutrition and parasite infections in the spatial variation in children's anaemia risk in northern Angola. *Geospat Health* 2013;**7**:341-54.
13 Sasaki S, Igarashi K, Fujino Y*, et al.* The impact of community-based outreach immunisation services on immunisation coverage with GIS network accessibility analysis in peri-urban areas, Zambia. *J Epidemiol Community Health* 2011;**65**:1171-8.
14 Aimone AM, Patrick BE, Zlotkin SH*, et al.* Geo-spatial factors associated with infection risk among young children in rural Ghana: a secondary spatial analysis. *Malaria Journal* 2016;**15**.
15 Zlotkin S, Newton S, Aimone AM*, et al.* Effect of iron fortification on malaria incidence in infants and young children in Ghana: a randomized trial. *JAMA* 2013;**310**:938-47.
16 WHO. World Malaria Report 2009. Geneva, Switzerland: World Health Organization 2009.
17 WHO. Worldwide prevalence of anaemia 1993-2005. *WHO Global Database on Anaemia*. Geneva, Switzerland: World Health Organization 2008.
18 WHO/UNICEF. WHO child growth standards and the identification of severe acute malnutrition in infants and children: A Joint Statement by the World Health Organization and the United Nations Children's Fund. Geneva, Switzerland: World Health Organization 2009.
19 USGS. United States Geological Survey. 2012.

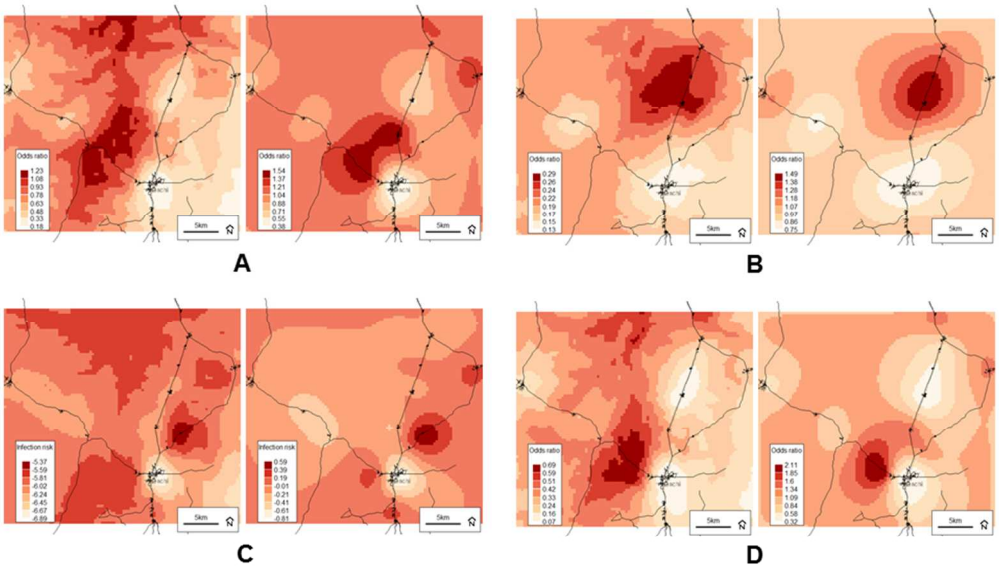
20 USGS. Land Processes Distributed Active Archive Center. 2014.
21 Hengl T. Land cover types for 2010 based on the MODIS IGBP classes. In: Center LPDAA, ed: Worldgrids (ISRIC - World Soil Information) 2013.
22 Samadoulougou S, Maheu-Giroux M, Kirakoya-Samadoulougou F, *et al.* Multilevel and geo-statistical modeling of malaria risk in children of Burkina Faso. *Parasit Vectors* 2014;**7**:350.
23 Guerra CA, Snow RW, Hay SI. A global assessment of closed forests, deforestation and malaria risk. *Ann Trop Med Parasitol* 2006;**100**:189-204.
24 Diggle PJ, Moyeed RA, Tawn JA. Model-based geostatistics. *Applied Statistics* 1998;**47**:299-350.
25 Diggle PJ, Ribeiro PJ. *Model-based geostatistics*. New York: Springer-Verlag 2006.
26 Rue H, Martino S. Approximate Bayesian inference for latent Gaussian models by using integrated nested Laplace approximations. *Journal of the Royal Statistical Society: Series B* 2009;**71**:319-92.
27 Brown PE. Model-based geostatistics the easy way. *Journal of Statistical Software* 2015;**63**:1-24.
28 R. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing 2015.
29 Prestia K, Bandyopadhyay S, Slate A, *et al.* Transfusion of stored blood impairs host defenses against Gram-negative pathogens in mice. *Transfusion* 2014;**54**:2842-51.
30 Thurnham DI, McCabe GP. Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron. In: Organization WH, ed. *Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15-17 September 2010*. Geneva: World Health Organization 2012:63-80.
31 Black RE, Allen LH, Bhutta ZA, *et al.* Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008;**371**:243-60.
32 Ezzati M, Utzinger J, Cairncross S, *et al.* Environmental risks in the developing world: exposure indicators for evaluating interventions, programmes, and policies. *J Epidemiol Community Health* 2005;**59**:15-22.
33 Cohen JM, Ernst KC, Lindblade KA, *et al.* Topography-derived wetness indices are associated with household-level malaria risk in two communities in the western Kenyan highlands. *Malar J* 2008;**7**:40.
34 Drakeley CJ, Carneiro I, Reyburn H, *et al.* Altitude-dependent and -independent variations in Plasmodium falciparum prevalence in northeastern Tanzania. *J Infect Dis* 2005;**191**:1589-98.
35 Brooker S, Clarke S, Njagi JK, *et al.* Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. *Trop Med Int Health* 2004;**9**:757-66.
36 Reid H, Vallely A, Taleo G, *et al.* Baseline spatial distribution of malaria prior to an elimination programme in Vanuatu. *Malar J* 2010;**9**:150.
37 Hay SI, Omumbo JA, Craig MH, *et al.* Earth observation, geographic information systems and Plasmodium falciparum malaria in sub-Saharan Africa. *Adv Parasitol* 2000;**47**:173-215.
38 Shirayama Y, Phompida S, Shibuya K. Geographic information system (GIS) maps and malaria control monitoring: intervention coverage and health outcome in distal villages of Khammouane province, Laos. *Malar J* 2009;**8**:217.

39 Kandala NB, Madungu TP, Emina JB*, et al.* Malnutrition among children under the age of five in the Democratic Republic of Congo (DRC): does geographic location matter? *BMC Public Health* 2011;**11**:261.
40 Weiss DJ, Mappin B, Dalrymple U*, et al.* Re-examining environmental correlates of Plasmodium falciparum malaria endemicity: a data-intensive variable selection approach. *Malar J* 2015;**14**:68.
41 Nesbitt RC, Gabrysch S, Laub A*, et al.* Methods to measure potential spatial access to delivery care in low- and middle-income countries: a case study in rural Ghana. *Int J Health Geogr* 2014;**13**:25.

Figure legends

Figure 1: Predicted probabilities (left), and residual spatial variation (right) from the final combined-group models for the odds of inflammation (CRP>5 mg/L) *and/or any* malaria parasitaemia (A); the odds of inflammation (CRP > 5 mg/L) *without* malaria parasitaemia (B); the risk of malaria parasitaemia *with* concurrent fever (axillary temperature >37.5^{0}C - or history of reported fever within 48 hours) (C); and the odds of malaria parasitaemia *with or without* fever (D). Darker colour indicates higher risk at endline. Background © Stamen Design.

Figure 2: Elevation (meters) across the study area. Green colour indicates higher elevation.



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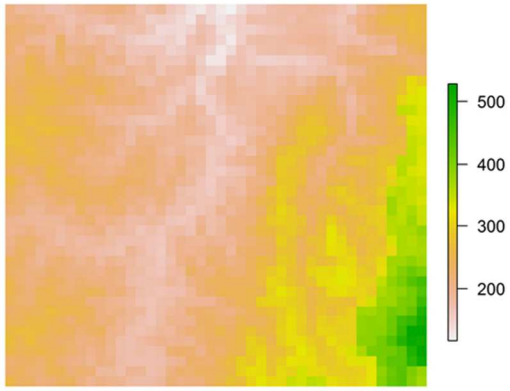

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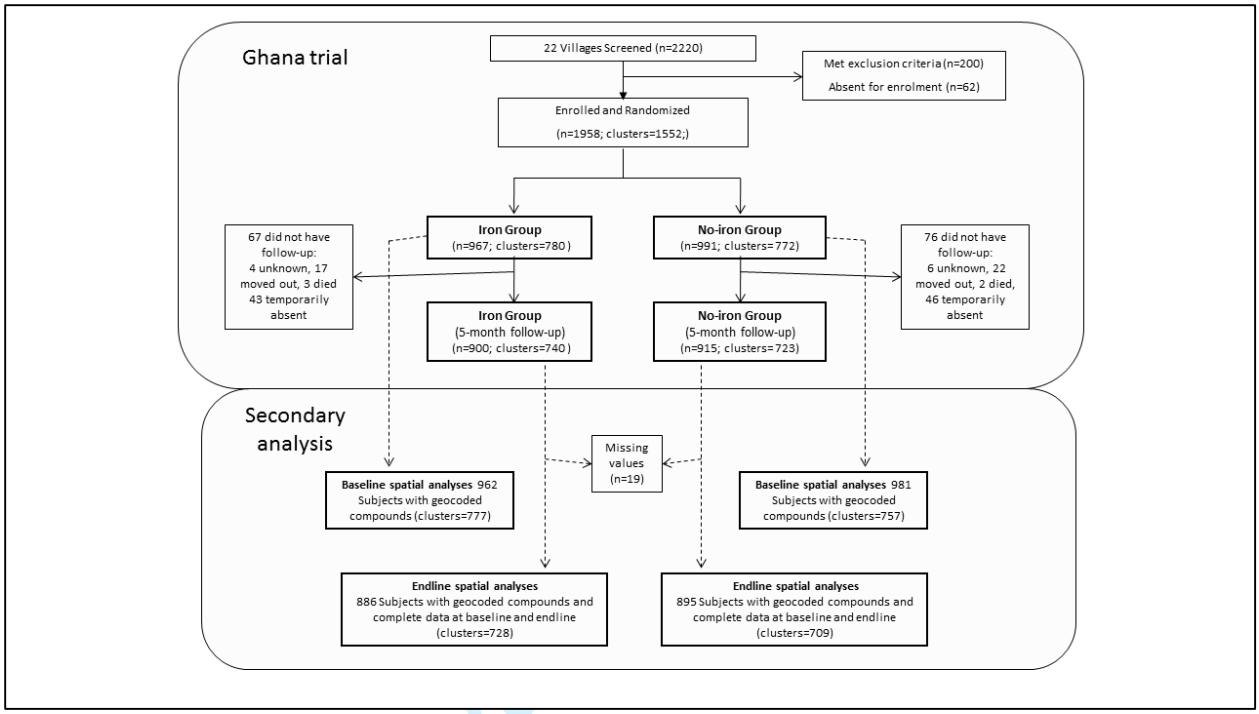

Figure S1: Study flow for the Ghana trial (top section) and secondary analyses (bottom section). Out of the 1958 participants from the Ghana trial, a total of 1943 with geocoded compounds were included in the baseline secondary spatial analyses (13 compounds were untraceable, corresponding to 15 participants not included in the secondary analyses). The endline spatial analyses included a total of 1781 observation, representing trial participants with geocoded compounds who provided blood samples at the end of the intervention period (5-month follow-up), and after removing 19 observations due to missing baseline or endline ferritin values.

Table S1: Geo-spatial and non-spatial risk factors of endline infection status[1] among Ghanaian children in the *No-iron* group (Brong-Ahafo Region, Sept-Nov 2010)

Covariates	Estimate (95% CrI) on the exponential scale[2]		Range parameter in km (95% CrI)	Standard deviations of random effects (95% CrI)	
				Spatial	Compound
Outcome 1: Inflammation and/or parasitaemia (n=894)					
Intercept	0.550	(0.298, 1.034)	7.050	0.506	0.008
Age per month			(2.016,	(0.271,	(0.004,
6-23 months	1.004	(0.975, 1.035)	18.26)	0.979)	0.030)
24-35 months	1.018	(0.964, 1.074)			
Sex (male reference)	1.016	(0.760, 1.357)			
Length-for-age z-score	0.984	(0.869, 1.113)			
Weight-for-length z-score	1.229	(1.056, 1.431)*			
Asset score	0.893	(0.760, 1.047)			
Distance to health facility (km)	1.072	(0.963, 1.188)			
Elevation (m)	0.995	(0.990, 1.001)			
Baseline infection status	1.887	(1.384, 2.576)*			
Baseline iron status	1.051	(0.938, 1.179)			
Outcome 2: Inflammation without parasitaemia (n=894)					
Intercept	0.219	(0.109, 0.426)*	9.458	0.442	0.007
Age per month			(2.389,	(0.206,	(0.004,
6-23 months	1.011	(0.971, 1.052)	23.57)	0.965)	0.029)
24-35 months	0.920	(0.843, 0.996)*			
Sex (male reference)	1.001	(0.671, 1.493)			
Length-for-age z-score	1.153	(0.977, 1.360)			
Weight-for-length z-score	1.302	(1.058, 1.603)*			
Asset score	0.872	(0.705, 1.075)			
Distance to health facility (km)	1.065	(0.953, 1.176)			
Elevation (m)	0.997	(0.991, 1.003)			
Baseline infection status	1.034	(0.556, 1.813)			
Baseline iron status	1.003	(0.841, 1.166)			
Outcome 3: Parasitaemia with fever (n=979)					
Intercept	0.003	(0.002, 0.004)*	7.690	0.448	0.007
Age per month			(2.821,	(0.243,	(0.004,
6-23 months	1.001	(0.979, 1.023)	18.01)	0.858)	0.028)
24-35 months	0.970	(0.927, 1.013)			
Sex (male reference)	0.896	(0.718, 1.116)			
Length-for-age z-score	1.009	(0.919, 1.107)			
Weight-for-length z-score	0.896	(0.797, 1.007)			
Asset score	1.075	(0.947, 1.220)			
Distance to health facility (km)	1.047	(0.959, 1.142)			

Elevation (m)	0.998	(0.993, 1.003)			
Baseline infection status	0.757	(0.467, 1.161)			
Baseline iron status	1.040	(0.953, 1.125)			
Outcome 4: All parasitaemia (n=894)					
Intercept	0.186	(0.089, 0.400)*	6.535	0.652	0.008
Age per month			(2.037,	(0.344,	(0.004,
6-23 months	0.974	(0.936, 1.014)	15.08)	1.255)	0.029)
24-35 months	1.067	(1.026, 1.110)*			
Sex (male reference)	1.050	(0.756, 1.459)			
Length-for-age z-score	0.901	(0.780, 1.037)			
Weight-for-length z-score	1.095	(0.923, 1.298)			
Asset score	0.941	(0.781, 1.130)			
Distance to health facility (km)	1.023	(0.896, 1.165)			
Elevation (m)	0.996	(0.989, 1.004)			
Baseline infection status	2.864	(1.968, 4.172)*			
Baseline iron status	1.036	(0.914, 1.169)			

[1]Infection status definitions:
Outcome 1: Inflammation and/or parasitaemia (binary): 1 = CRP > 5 mg/L and/or any malaria parasitaemia, 0 = CRP ≤ 5 mg/L and absence of parasitaemia;
Outcome 2: Inflammation without parasitaemia (binary): 1 = CRP > 5 mg/L without malaria parasitaemia, 0 = CRP ≤ 5 mg/L without parasitaemia;
Outcome 3: Parasitaemia with fever (count): any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
Outcome 4: All parasitaemia (binary): 1 = any malaria parasitaemia (with/without fever), 0 = absence of parasitaemia with/without fever
Model prior shape = 1.117, model prior rate = 0.157
[2]Exponentials of posterior medians and 2.5% and 97.5% posterior quantiles of model parameters, with a value of 1.05 indicating that a variable increases the risk of infection by 5% (odds ratio from logistic model for all binary outcomes; relative risk from Poisson model for count outcome)
CrI = credible interval
Baseline infection status = baseline infection status (yes/no) according to corresponding definition
Baseline iron status = baseline serum ferritin concentration (µg/dL) corrected for CRP using the regression method (Namaste et al.) and re-scaled by multiplying by the inverse of the inter-quartile range
*Statistical significance at the 0.05 level

Table S2: Geo-spatial and non-spatial risk factors of endline infection status[1] among Ghanaian children in the *Iron* group (Brong-Ahafo Region, Sept-Nov 2010)

Covariates	Estimate (95% CrI) on the exponential scale[2]		Range parameter in km (95% CrI)	Standard deviations of random effects (95% CrI)	
				Spatial	Compound
Outcome 1: Inflammation and/or parasitaemia (n=886)					
Intercept	0.647	(0.354, 1.263)	7.761	0.487	0.007
Age per month			(2.682,	(0.263,	(0.004,
6-23 months	1.004	(0.975, 1.035)	17.96)	0.931)	0.028)
24-35 months	0.977	(0.925, 1.031)			
Sex (male reference)	1.162	(0.871, 1.551)			
Length-for-age z-score	0.980	(0.857, 1.119)			
Weight-for-length z-score	0.971	(0.833, 1.130)			
Asset score	0.906	(0.769, 1.064)			
Distance to health facility (km)	1.003	(0.904, 1.106)			
Elevation (m)	0.995	(0.989, 1.001)			
Baseline infection status	2.272	(1.664, 3.108)*			
Baseline iron status	1.053	(0.953, 1.172)			
Outcome 2: Inflammation without parasitaemia (n=886)					
Intercept	0.143	(0.073, 0.274)*	12.56	0.428	0.007
Age per month			(4.318,	(0.203,	(0.004,
6-23 months	1.007	(0.969, 1.048)	27.27)	0.923)	0.028)
24-35 months	0.983	(0.910, 1.056)			
Sex (male reference)	1.330	(0.905, 1.962)			
Length-for-age z-score	1.054	(0.884, 1.255)			
Weight-for-length z-score	0.890	(0.726, 1.090)			
Asset score	0.888	(0.725, 1.087)			
Distance to health facility (km)	0.984	(0.883, 1.080)			
Elevation (m)	1.001	(0.995, 1.006)			
Baseline infection status	1.508	(0.897, 2.459)			
Baseline iron status	1.044	(0.918, 1.166)			
Outcome 3: Parasitaemia with fever (n=960)					
Intercept	0.002	(0.001, 0.004)*	8.352	0.466	0.007
Age per month			(3.203,	(0.244,	(0.004,
6-23 months	1.017	(0.992, 1.043)	17.99)	0.896)	0.028)
24-35 months	0.987	(0.945, 1.029)			
Sex (male reference)	0.991	(0.780, 1.257)			
Length-for-age z-score	0.969	(0.867, 1.082)			
Weight-for-length z-score	1.052	(0.925, 1.197)			
Asset score	1.002	(0.872, 1.152)			
Distance to health facility (km)	1.064	(0.970, 1.163)			
Elevation (m)	0.998	(0.993, 1.003)			

Baseline infection status	0.849	(0.516, 1.321)			
Baseline iron status	0.981	(0.895, 1.060)			
Outcome 4: All parasitaemia (n=886)					
Intercept	0.412	(0.199, 0.938)*	7.293	0.666	0.008
Age per month			(2.486,	(0.376,	(0.004,
6-23 months	1.015	(0.975, 1.056)	17.67)	1.230)	0.030)
24-35 months	0.977	(0.938, 1.016)			
Sex (male reference)	0.977	(0.707, 1.347)			
Length-for-age z-score	0.946	(0.812, 1.098)			
Weight-for-length z-score	1.034	(0.872, 1.225)			
Asset score	0.946	(0.785, 1.137)			
Distance to health facility (km)	1.009	(0.889, 1.149)			
Elevation (m)	0.994	(0.987, 1.001)			
Baseline infection status	2.597	(1.791, 3.768)*			
Baseline iron status	1.042	(0.939, 1.153)			

[1]Infection status definitions:
Outcome 1: Inflammation and/or parasitaemia (binary): 1 = CRP > 5 mg/L and/or any malaria parasitaemia, 0 = CRP ≤ 5 mg/L and absence of parasitaemia;
Outcome2: Inflammation without parasitaemia (binary): 1 = CRP > 5 mg/L without malaria parasitaemia, 0 = CRP ≤ 5 mg/L without parasitaemia;
Outcome3: Parasitaemia with fever (count): any malaria parasitaemia with concurrent fever (axillary temperature >37.5^{0}C) or history of reported fever (within 48 hours);
Outcome4: All parasitaemia (binary): 1 = any malaria parasitaemia (with/without fever), 0 = absence of parasitaemia with/without fever
Model prior shape = 1.117, model prior rate = 0.157
[2]Exponentials of posterior medians and 2.5% and 97.5% posterior quantiles of model parameters, with a value of 1.05 indicating that a variable increases the risk of infection by 5% (odds ratio from logistic model for all binary outcomes; relative risk from Poisson model for count outcome)
CrI = credible interval
Baseline infection status = baseline infection status (yes/no) according to corresponding definition
Baseline iron status = baseline ferritin concentration (µg/dL) corrected for CRP using the regression method (Namaste et al.) and re-scaled by multiplying by the inverse of the inter-quartile range
*Statistical significance at the 0.05 level

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	NA
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5-7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	5
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	5

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	5
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6-7
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	6-7
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure S1
	13b	For each group, losses and exclusions after randomisation, together with reasons	8
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	9
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	9-13
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	9-13
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	9-10
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13-16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13-16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13-16
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	17

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

RESEARCH PROPOSAL

Project Title: Seasonal Impact of Iron Fortification on Malaria Incidence in Ghanaian Children

Principal Investigator: Dr. Stanley Zlotkin (The Hospital for Sick Children)
Co-Investigator: Dr. Seth Owusu-Agyei (Kintampo Health Research Centre)

1. SPECIFIC AIMS

This RFA is intended to address "those factors affecting the safe and effective use of iron interventions for the prevention and treatment of iron deficiency and anemia in women of reproductive age (including adolescent girls), infants, and children, particularly in areas of endemic malaria". One of the three core priority areas described in the RFA is the category of "interventions". The RFA asks the question, "What are the safest and most effective interventions to prevent and treat iron deficiency in women, infants and children in areas of endemic malaria?" The current proposal specifically addresses the terms of the RFA by proposing the use of microencapsulated ferrous fumarate, supplied in a powder form with other essential micronutrients ("Sprinkles"), as an effective and potentially safer alternative to syrups or drops for providing iron to infants and young children living in regions where the burden of malaria is high.

In 2006, The World Health Organization (WHO) and United Nations Children's Fund (UNICEF) released a joint statement recommending that in malaria endemic areas, iron supplementation be targeted only to those children who are anemic and at risk of iron deficiency. As well, these children should receive concurrent protection from malaria and other infectious diseases through prevention and effective case management. The statement went on to state that "these conclusions should not be extrapolated to fortification or food-based approaches for delivering iron, where the patterns of iron absorption and metabolism may be substantially different. The Joint Statement did not provide recommendations regarding 'point-of-use' fortificants (i.e. single-dose powdered mineral and vitamin supplements to be sprinkled on to home-made foods at the table).

In reality, it is neither practical nor feasible to meet the population-wide screening requirements as outlined in the WHO/UNICEF Joint Statement (above), especially in under-developed countries. There simply are no screening tools for anemia and iron deficiency that can be practically used and, indeed, the cost of screening for hemoglobin alone is often higher than the cost of providing iron supplements. As a result, for the past 2-3 years, iron deficiency anemia has remained largely an unresolved nutritional problem in areas of endemic malaria. It is estimated, for example, that the prevalence of iron-deficiency anemia is as high as 60-65% in children under age 24 months in West Africa. In our past research, we have demonstrated in Ghana that the use of powdered minerals and vitamins, including microencapsulated iron will reduce anemia rates by up to 60%. However, because of the uncertainty around the use of iron in high malaria burden areas, the implementation (scaling-up) of these results is virtually at a stand-still.

Given that we have already demonstrated the efficacy of encapsulated iron (as ferrous fumarate) in lowering the burden of anemia, the primary objective of this research proposal is to determine the impact of providing Sprinkles (including microencapsulated iron as a powder added to complementary foods) on the susceptibility to clinical malaria among anemic and non-anemic infants and young children (6-35 months of age) living in a high malaria burden area. Due to the well documented difference in mosquito bite-rates between the wet and dry seasons, we will conduct the study during the rainy season only (when bite rates are usually higher). Certain secondary variables, such as iron and anemia status and breastfeeding rates may influence the primary outcome, thus we will concurrently collect data on these variables throughout the intervention.

Our secondary objectives are to determine the impact of this iron intervention on the *severity* of clinical malaria by documenting parasite counts and hospital admission rates, as well as differences in secondary complications of malaria infections, such as death, cerebral malaria, pneumonia, dehydration, and diarrhea. Although the current study is not powered to show causation between the intervention and the secondary outcomes, it is anticipated that the proposed research will help address the confusion that has been generated regarding the safe and appropriate use of iron supplements in high malaria transmission areas. If it is demonstrated that the provision of iron as a powder added to food does not have an adverse effect on malaria incidence and has a positive impact on anemia rates, then this type of iron delivery system can possibly be recommended for scale-up in Ghana and in other countries with a similar malaria burden. Conversely, if we demonstrate adverse effects, especially in iron replete children, then further research is needed to identify inexpensive and non-invasive methods to screen children for anemia before iron is provided. **The long term goal of the proposed research is to develop new evidence to inform global policy and ultimately guide the implementation of programs to prevent and treat iron deficiency disorders in malaria endemic regions.** These goals coincide directly with the stated objectives of this RFA.

2. BACKGROUND AND SIGNIFICANCE

Ghana as the Proposed Study Site

Ghana, with an estimated population of 23 million, is located in Western Africa, bordering the Gulf of Guinea, between Cote d'Ivoire and Togo. Well endowed with natural resources, Ghana has roughly twice the per capita output of the poorest countries in West Africa. Even so, Ghana remains heavily dependent on international financial and technical assistance. Gold and cocoa production and individual remittances are major sources of foreign exchange. The domestic economy continues to revolve around agriculture, which accounts for about 35% of the gross domestic product and employs about 55% of the work force, which is mainly made up of small landholders.

Approximately 37.8% of the population is between 0-14 years of age (male 4,470,382/female 4,360,359). The birth rate is 29.22 births/1,000 population, while the death rate is 9.39 deaths/1,000 population (2008 est.). The total infant mortality rate is 52.31 deaths/1,000 live births (male: 56.64 deaths/1,000 live births; female: 47.85 deaths/1,000 live births [2008 est.]). Ghana is considered to be one of the most progressive countries in sub-Saharan Africa.

The value of performing this research in Ghana is three fold:

(i) There has been a well-established and successful research collaboration between the Government of Ghana (Kintampo Health Research Centre) and the Hospital for Sick Children, University of Toronto since 1998;

(ii) Malaria and anemia remain the most important causes of death and morbidity in Ghana; and

(iii) The vital capacity-building potential of joint projects, such as the one described in this application, goes well beyond the life of this project.

2.1 Background and Gaps

Global prevalence and impact of iron deficiency anemia

Iron deficiency and iron deficiency anemia (IDA) are the most prevalent micronutrient deficiencies on a worldwide basis, especially in developing countries. The impact of severe IDA can have mortal consequences, since without adequate hemoglobin, the brain and body become deprived of oxygen and, if allowed to continue, death may ensue. While the impact of mild and moderate IDA on child development and immune function remain areas of fertile research, there are currently no firm conclusions on either short- or long-term effects (1). There is agreement, however, that the prevention of iron deficiency and iron deficiency anemia (from mild to severe) are public health priorities, based on their potential negative impact on the health of children. There are experimental animal models that have examined potential mechanisms for the role of iron in brain development, and human cohort studies which have documented short- and long-term effects of mild-moderate IDA on impaired cognitive and motor development. Although the human cohort studies all suffer from an unavoidable design bias, since one cannot purposely assign (randomize) children to become anemic, those that have been conducted in different countries under different conditions have generally shown similar adverse outcomes on school achievement and measures of cognition and learning. It has also been suggested that in terms of the impact of iron on the developing brain, the first two years of life are a 'window of opportunity', and once the window is closed the impact may not be reversible.

Despite the uncertainties around the impact of IDA on the health of children, governments and United Nations agencies continue to place high priority on the prevention and treatment of IDA. Even private 'think-tanks' like the Copenhagen Consensus have recognized the importance of controlling IDA. For example, in their most recent session, they ranked the control of micronutrient deficiencies as the number one global challenge, and placed iron fortification as the third most important solution due to their extremely high ratio of benefits to costs (2). And finally, the fourth Millennium Development Goal of "reducing the under-five child mortality to one third by 2015" is at least indirectly related to the control of IDA (3).

Global prevalence and impact of malaria

Nutritional intervention programs have generally demonstrated that the provision of iron supplements can enhance child development (4, 5) and reduce the prevalence of severe anemia; however, there is some evidence to suggest that iron supplementation (in the form of syrups, drops or pills often provided in a post-prandial state) results in high levels of malaria parasitemia (6, 7), increased rates of malaria, as well as pneumonia and diarrhea (7-10). In contrast, the most recent systematic review (2002) does not generally support an increased risk of malaria attack rate or severity associated with iron supplementation (pooled OR significant for malaria + smear = 1.43 [1.08-1.91], but not significant when adjusted for baseline malaria smear = 1.24 [0.98-1.57]) (10). Clearly multiple factors contribute to the complex etiology of anemia in high malaria burden areas, but iron status and malaria infection are the strongest predictors of hemoglobin concentration (11). Recent research has suggested that haptoglobin might also influence hemoglobin levels in an environment of malaria-induced hemolytic stress. Research by Atkinson and colleagues suggest that the $Hp^{2/2}$ genotype is a risk factor for childhood anemia in malaria-endemic countries. The authors found that average hemoglobin levels fell over the malaria season, and children who had the $Hp^{2/2}$ genotype had the greatest drop compared to other children (11). These findings suggest that a child's haptoglobin type may be an important influence on whether that child gets anemia in areas where malaria is very common

The relationship between iron and malaria has important implications because malaria is a tropical parasitic disease that contributes significantly to morbidity and mortality rates in many parts of the world. Recent estimates from the 2005 World Malaria Report were around 350-500 million clinical disease episodes per year (12). A large proportion of the global malaria burden is concentrated in Africa, where approximately 60% of

clinical cases and over 80% of malaria-related deaths occur. Further, most of the Africans who die from malaria each year are children under five years of age (13). Studies conducted in the north-western African country of Ghana have revealed that malaria can account for more than 44% of reported outpatient visits (12), and that the majority of these cases tend to occur during the wet season (June-October) (14, 15).

Impact of iron supplementation on malaria incidence and/or severity in Pemba, Zanzibar

A recent community-based randomized controlled trial, by Sazawal et al, demonstrated increased morbidity and mortality in infants provided with an iron and folic acid supplement in a highly malaria endemic region in Pemba, Zanzibar (16). In this large study, infants were provided with a multivitamin-mineral tablet with and without iron, that was dissolvable in either water or breast milk. While iron supplementation was effective for the reduction of iron deficiency and anemia in iron deficient children, it was associated with increased rates of hospitalization (primarily due to malaria and infectious disease), and mortality when given to individuals who were iron replete (with or without anemia). On advice from the Data Safety Monitoring Board, the iron-supplemented arms of the trial were discontinued after approximately 20 months. A subsequent critical review of the data from this trial led to the release of a joint statement by the WHO and UNICEF with the following recommendations:

> "Caution should be exercised in settings where the prevalence of malaria and other infectious diseases is high. Until the WHO recommendations are revised it is advised that iron and folic acid supplementation to be targeted to those who are anemic and at risk of iron deficiency. They should receive concurrent protection from malaria and other infectious diseases through prevention and effective case management. The conclusions drawn from the Zanzibar trial should not be extrapolated to fortification or food-based approaches for delivering iron, where the patterns of iron absorption and metabolism may be substantially different (3)."

The UN agencies also recommended that additional research was urgently needed to develop the most effective strategies for controlling iron deficiency and anemia in regions where malaria transmission is high.

Iron Homeostasis and infectious disease risk

The body's ability to maintain a safe equilibrium of iron is crucial and complex. Iron homeostasis is regulated at the level of absorption, unlike other minerals that are regulated through excretory mechanisms. The human gastrointestinal tract is very sensitive to iron stores and oxygen carrying capacity and has the molecular and biochemical capacity to increase (up-regulate) absorption when iron stores are becoming depleted (or hemoglobin concentration is low), and to down-regulate absorption when iron stores are replete. In an iron deficient individual, iron absorption may be as high as 40-50%, while in an iron-replete individual, iron absorption is between 5-10%. One can imagine that humans developed this sensitive and sophisticated regulatory capacity since too little iron can lead to inadequate oxygenation of vital tissues (and ultimately death), while too much iron can overwhelm the capacity of the body to safely bind the iron to protein, leaving potentially toxic free (non protein-bound) iron. In the case of malaria, it is thought that the parasite can proliferate when there is a labile pool of non protein-bound iron available. On the other hand, it has been postulated that iron deficiency might protect the host organism through increased zinc protoporphyrin in red blood cells, which inhibits parasite haemozoin formation, similar to the mechanism of certain anti-malarial drugs (17).

Potential Adverse Impact of 'Excess' Iron

Iron excess has been associated with oxidative damage in animal models, through the impact of free iron on lipid membrane peroxidation as well as DNA damage. There is a suggestion of lipid peroxidative damage from excess iron in humans, but the studies are limited in number and design. A study by Dewey et al described a degree of growth impairment in iron replete infants provided with a liquid iron supplement (18). In this study, iron supplementation was investigated in two cohorts of infants, one in Sweden and the other in Honduras. It was observed that iron supplementation (with iron syrup) of iron replete Swedish infants between the ages of 4 – 9 months demonstrated significantly decreased length and head circumference growth compared to those receiving no iron supplement. In the Honduran infants, decreased length was observed in iron replete infants between the ages of 4-6 months provided with a similar iron supplement, compared to those receiving a placebo. The results of this study were confirmed in a similar study in India, also using liquid iron supplements (19).

Overall, the findings from the above studies, as well as the one conducted in Pemba, have generated much discussion on the possible deleterious impact of iron supplementation in otherwise healthy iron-replete infants and young children. A plausible biological explanation for these observations is related to the absorption kinetics of iron provided as a supplement. With supplementation, a concentrated form of iron is provided, often in a post-prandial state, with resulting high peak serum iron concentrations (C-max) and a shorter 'time to maximum serum concentration' (T-max). It has been suggested that, under these conditions, the rate of iron absorption may be greater than the child's capacity to bind the absorbed iron (with transferrin), resulting in increased 'free iron' if only for a short period of time. As mentioned above, 'free iron' may benefit any eukaryotic pathogens concomitantly present (like malaria parasites, Yersinia, etc), with resulting proliferation of the pathogens and an adverse clinical outcome. We believe that there may be varying outcomes associated with the form of iron and mode of delivery, leading us to ask whether the form and mode of iron delivery have an impact on the incidence of malaria among children at risk.

In the current grant, we are proposing to use a form of iron and a delivery system that differ from those used in the Pemba study in four major ways: Firstly, we plan to use a powdered mineral 'fortificant' as the iron source. This source differs from a typical iron supplement in a number of ways which may protect the recipient against the suggested generation of free iron. The iron source is microencapsulated ferrous fumarate. As will be discussed (below), microencapsulation protects the iron from the food matrix (thus preventing oxidation of the iron) and likely results in a lower C-max and longer T-max. Secondly, we will provide the powdered iron source for 5 months in the wet season, while the Pemba study provided the supplement for a full year. Although the length of supplementation did not have an impact on the results in Pemba, results from previous studies completed by our research group have demonstrated that the use of powdered iron as a fortificant for relatively short periods of time (as short as two months) resulted in a significant increase in hemoglobin and a significant decrease in rates of anemia that lasted for as long as six months after the end of the intervention period (20). Thus we believe that a period shorter than 1 year will be sufficient to have a significant impact on anemia rates. Thirdly, although the dose of iron in the current study (12.5 mg/day) is similar to that provided to the older infants and young children in the Pemba study, as previously discussed, it will be provided in a food matrix, rather than as a supplement. We believe that this dose will be adequate to have an impact on anemia rates in children with iron deficiency, but not excessive for children whose iron stores are replete. Finally, the minerals and vitamins (including iron as microencapsulated ferrous fumarate, zinc, vitamins A, and C) will be provided in single-dose sachets (like small packets of sugar), which are easily sprinkled once daily onto any semi-solid or 'soft' foods. Although folic acid was included in the multimicronutrient supplement in the Pemba study, it is possible that folic acid may inhibit the action of certain anti-malarial drugs, thus it will **not** be included in the sachet. Because the powdered formulation is

taken with food and due to the microencapsulation of the iron, it is likely that the absorption characteristics will be different from that of non-microencapsulated iron given in a post-prandial state – potentially reducing the amount of available free iron. **By reducing the peak labile pool of free iron, it is possible that the safety of using powdered fortificants in areas with a high incidence of infection may be increased.**

Microencapsulation

Microencapsulation is a process by which tiny parcels of a gas, liquid, or solids are packaged within a second material for the purpose of shielding the active ingredient from the surrounding environment. There can be numerous reasons for microencapsulation. These include isolation of the contents from the environment (e.g.. preventing iron from oxidizing with food), improving handling properties (e.g. preventing dangerous pesticides from coming in contact with hands) and/or controlling the release of the contents (e.g. reducing the rate of release of drugs in the gastrointestinal tract).

Effect of micoencapsulation on pharmacokinetic properties

Microencapsulation has has been shown to have an effect on the absorption characteristics of a drug by significantly reducing and delaying peak plasma concentrations (Cmax and Tmax) (21-23). Further, studies examining the effect of giving a microencapsulated drug with or without food have shown that, post-prandially, the maximum plasma concentration tends to be lower as compared to the fasted state (21, 24). Although not tested directly, the findings from these pharmacokinetic studies suggest that the use of microencapsulated iron as proposed in the current study (i.e. mixed with food) may result in a reduced Cmax (peak plasma level) and longer Tmax with a potentially reduced peak labile pool of free iron and thus a higher safety profile in a malaria endemic region.

2.2 Importance and relevance of research

Notwithstanding the debate around the adverse impact of iron deficiency anemia on malaria and possibly growth (in iron replete children), all countries, as well as the UN agencies, UNICEF and the WHO, recognize iron as an essential nutrient and recommend a daily intake of iron between 5 -10 mg/day depending on the age of the child. It is also recommended that, whenever possible, iron should come from the diet alone; however, when dietary sources are inadequate, either supplementation or fortification is recommended. As such, the international nutrition community has been exploring ways to treat and prevent IDA through food diversification, supplementation and fortification. Unfortunately for children under age 2 years in developing countries, neither diversification nor supplementation has proven to be an effective means of coping with the problem. Although food fortification of commodities like wheat flour are used to prevent IDA in adult populations, they have not been successful for young children since the level of fortification is aimed at the adult male, and the total amount of the fortified food that is eaten is too low in the young infant or small child to meet their iron intake requirements. We and others have demonstrated over the past 10 year that 'point of use fortification' with powdered minerals and vitamins is both efficacious and effective (25-34), and this intervention is beginning to be scaled up in a number of countries where malaria is not present. To our knowledge, the safety and efficacy of providing children with iron, in the form of a powdered 'point of use' fortificant, in areas with a high prevalence of malaria has not been investigated. **We are confident that the outcome of the current proposal, combined with the results from the Pemba study, should enable more effective decision making and policy formulation regarding the use of powdered minerals and vitamins in malaria endemic regions globally**.

2.3 Significance

Overall, this investigation will provide important information on how health-related policies and programs can be improved to ensure that infants and young children in high malaria burden countries are safely protected from micronutrient malnutrition, and thus able to better achieve their growth and development potential. Specifically, the protocol will contribute the following to the literature on this topic:

1. We will determine whether the form of the iron used in the present study, and the delivery mode (powdered iron sprinkled on to food), results in an increase or decrease in malaria incidence. The Pemba study reported increased morbidity and mortality with a dissolvable iron tablet supplement which may have been rapidly absorbed from the proximal gastrointestinal tract. The form of iron to be used in the proposed study is microencapsulated ferrous fumarate, mixed in a food matrix. It is anticipated that the digestion and absorption of this form and delivery of iron is slower (lower Cmax [peak serum concentration] and higher Tmax [longer time to peak absorption]) and thus safer than a supplement.

2. As was the case in the Pemba study, we will determine whether iron replete children are more likely to have an adverse outcome, compared to iron deficient children. In the proposed protocol, we plan to enroll all otherwise healthy children without severe anemia. Based on our previous studies in Ghana, we expect that approximately 40 – 50% of children will be iron replete. Our sample size is large enough to allow for statistical evaluation of the primary outcome based on the iron status of the subjects at baseline.

It is anticipated that the proposed research will help address the confusion that has been generated regarding the safe and appropriate use of iron supplements in high malaria transmission areas. If it is demonstrated that the provision of iron, as a powder added to food, does not have an adverse effect on malaria incidence and a positive impact on anemia rates, then this type of iron delivery system can possibly be recommended for scale-up in Ghana, and in other countries with a similar malaria burden. If we demonstrate adverse effects, especially in iron replete children, then further research is needed to identify inexpensive and non-invasive methods to screen children for anemia before iron is provided. With either outcome, this investigation will provide important information on how health-related policies and programs can be improved to ensure that infants and young children in underdeveloped countries are protected from infectious diseases and micronutrient malnutrition, and thus able to better achieve their growth and development potential.

2.4 Potential effect of these studies on the concepts, methods, treatments, services or preventative interventions that drive this field

These studies will have a major impact on interventions to treat and prevent iron deficiency anemia in malaria endemic areas. The use of powdered mineral and vitamin fortificants is being scaled-up in a number of zero or low malaria burden developing countries as a means to prevent micronutrient deficiencies, including iron deficiency. Drs. Zlotkin and Owusu-Agyei organized a one day symposium with the Ministry of Health of Ghana in 2006, including key United Nations agencies and NGOs. The government of Ghana has an Anemia Subcommittee dedicated to finding interventions to prevent anemia in Ghanaian children. The progress of the Subcommittee, however, has been markedly impaired by the results of the Pemba study and the subsequent UNICEF/WHO guidance on the use of iron in malaria endemic areas. The results of the proposed research study will provide the government of Ghana (as well as governments in other high malaria burden countries) with new and significant information for planning safe and effective anemia prevention programs.

3. PRELIMINARY STUDIES

The Research Institute, Hospital for Sick Children in Toronto and the Kintampo Health Research Centre in Ghana have a long standing and collaborative working relationship. This provides the proposed project with a well-established and unique team of expertise in global health generally, and specifically in the area of iron deficiency anemia, malaria control and research (clinical trials) methodology.

Both Drs. Owusu-Agyei at the Kintampo Health Research Centre (KHRC) and Zlotkin (Research Institute, Hospital for Sick Children and University of Toronto) have extensive experience as researchers in the fields of malaria and in infant and young child nutrition. Zlotkin, a professor of Pediatrics, Nutritional Sciences and Public Health Sciences at the University of Toronto has worked collaboratively with the Kintampo Health Research Centre since the late 1990s, originally with Dr. Paul Arthur, Director at the time (now passed away) and for the past six years with Dr. Owusu-Agyei who has been the Director of KHRC since 2002. Zlotkin's original efficacy trials on 'Sprinkles' (a powdered mineral and vitamin fortificant) were completed at KHRC. The first collaborative manuscript was published in 2002 (29) the most recent publications from Ghana were published with Dr. Owusu-Agyei in 2006 (30, 35).

Sprinkles are sachets (like small packets of sugar) containing a blend of vitamins and minerals in powder form, which are easily sprinkled onto different foods. The single-serving sachets enable families without access to commercially fortified foods to add essential vitamins and minerals directly to traditional foods prepared in the home. They are inexpensive to produce, have no special storage requirements and are simple to use, even by those who cannot read. Because the iron is microencapsulated, there is no staining of a young child's teeth as may be the case with iron syrup or drops. A major advantage of the 'sprinkles' concept is that local foods can continue to be used and, thus, there is no need to teach caregivers how to prepare new and often expensive store-bought foods. Sprinkles were invented by the PI, Dr. Stanley Zlotkin, who, over the past ten years, has led a collaborative research team in multiple countries globally. Together these collaborative teams have demonstrated the absorption, efficacy, and effectiveness of microencapsulated iron, as well as the acceptability of the sprinkles concept (powdered minerals and vitamins) to treat and prevent iron and other micronutrient deficiencies (28, 29, 31, 35, 36).

As previously mentioned, the first randomized controlled trials were conducted in Ghana at KHRC. A total of 5 studies were completed at KHRC and published in peer-reviewed journals, including the American Journal of Clinical Nutrition and the Journal of Nutrition. More recently Dr. Zlotkin has collaborated with a research team from the University of California at Davis (led by Dr. Kay Dewey), and partners at the University of Ghana at Legon, to examine the relative merits of a powdered mineral and vitamin fortificant versus a fortified spread for the treatment and prevention of micronutrient deficiencies. Two recent publications describe that work (37, 38).

As well as having worked in Ghana with KHRC, Dr. Zlotkin has experience with a number of other countries and research organizations. He has a long-standing research collaboration with the Research and Evaluation Division of BRAC in Bangladesh. He and his collaborators at BRAC have evaluated the effectiveness and acceptability of powdered mineral and vitamin preparations when provided on a weekly basis (versus daily) and with a flexible regimen (20, 39). Dr. Zlotkin has also had successful research collaborations with the Chinese Centre for Disease Control (40), the Swiss Red Cross in Kyrgyzstan, World Vision International in Mongolia, CARE International in Benin (41), Agha Khan University in Pakistan (32, 42) and the King Edward Medical Hospital in India (25, 33, 43). In addition, he has collaborated with health economists to assess the cost effectiveness of 'point of use' powdered mineral and vitamin products (44). In northern Canada, powdered minerals and vitamins were examined in 'First Nations' populations (45-47). Powdered minerals and vitamins

have also been used, in collaboration with the World Food Program, World health Organization and Helen Keller International in relief situations (48).

Dr. Stanley Zlotkin has beneficial interests in certain intellectual property rights to his invention known as "Sprinkles". These interests include (i) patent rights for the United States and Canada only, which are held by Ped-Med Limited, a Canadian corporation, of which Dr. Zlotkin is the sole shareholder; and (ii) trade-marks rights in various jurisdictions to the name "Sprinkles" which are held either by Ped-Med Limited, or by the Sprinkles Global Health Initiative Inc. a Canadian not-for-profit corporation of which Dr. Zlotkin is a member.

Global recognition of a 'powdered mineral and vitamin fortificant' as an important, new tool for improving the health of children

The concept of using powdered mineral and vitamins packaged in a single-serving package has been recognized by international agencies such as UNICEF, the World Health Organization (WHO) and the World Food Program (WFP). Its success has been widely reported in both academic journals and the popular press. A number of government ministries of health have enacted legislation to include home fortification with powdered mineral and vitamin fortificants in recently updated national nutrition policies (Mongolia, Bolivia, Bangladesh, etc)

About the Kintampo Health Research Centre

Kintampo Health Research Centre (KHRC) is a well-established, African-based, research centre. The mission of KHRC is to conduct public health research and develop health research capacity which will contribute to a significant reduction in ill-health and the achievement of the Millennium Development Goals for Africa's most disadvantaged communities. The African identity of KHRC is important as it emphasizes African solutions to African health challenges. KHRC is one of three field research centers of the Health Research Unit of Ghana Health Service established in 1994. KHRC is situated in the middle belt of Ghana in the Brong Ahafo Region with a mandate to serve health policy and practice throughout Ghana and Africa.

There are over 500 employees at KHRC. KHRC has over 12 years of extensive experience in health research in Ghana. Among many areas of activity in which it has demonstrated skill, knowledge and core competency it has developed one of the largest and most reliable district surveillance systems (DSS) and study populations in Africa. KHRC has an established reputation for quality research and personnel. KHRC is highly regarded and is the preferred partner of governments, organizations and donors in health research initiatives in particular large-scale health research trials. KHRC funding collaborators include government, bilateral and multilateral institutions, private corporations, private charities and international organizations from Africa, Europe, Canada and the USA

KHRC Priority Research Areas

- Communicable diseases (CDs), particularly Malaria, TB and HIV/AIDS
- Sexual and Reproductive Health
- Maternal, Neonatal and Child Health
- Mental Health
- Non-communicable diseases (NCDs) such as hypertension and cancer
- Health Systems

- Using the DSS to track progress towards the Millennium Development Goals (MDGs) using indicators such as mortality levels, patterns and trends

Since 2003, Dr Owusu-Agyei and his research teams at KHRC have characterized the epidemiology of malaria in the Kintampo area in the middle belt of Ghana (49-51). He has documented the incidence of malaria in children less than 5 years in this area as 7 attacks per child per year (Owusu-Agyei et al., in press). The transmission levels have been high throughout the year with an average inoculation rate of 269 infective bites per person per year (Owusu-Agyei et al., in press). Dr Owusu-Agyei and his team of researchers are documenting the manifestations (both clinical and laboratory indicators) of severe and complicated malaria. Several antimalarials have been tested and evaluated in this area (52) and currently the most advanced malaria vaccine is being tested in Kintampo as one of several African countries. For more details on the KHRC laboratory and clinical facilities, please see pages 22-23 of this application (Resources Format Page).

The Kintampo Health Research Centre (KHRC) was awarded the 2008 Prince of Asturias Award for International Cooperation in recognition of its contribution to the fight against malaria in sub-Saharan Africa. The Prince of Asturias Foundation was formed by His Royal Highness, the Prince of Asturias, heir to the throne of Spain. It has conferred its awards since 1981. The awards are intended to acknowledge scientific, technical, cultural, social and humanitarian work carried out internationally by individuals, groups or in the categories of communication and humanities, social sciences, arts, letters, scientific and technical research, international cooperation, concord and sports.

The collaboration between the Research Institute at the Hospital for Sick Children in Toronto and KHRC in Ghana provides this project with a well-established and highly experienced team of experts in iron deficiency anemia, malaria control and Sprinkles-based research methodology. Given the past working relationship between the two partners, the protocol can be put into action without the need for preliminary relationship and trust-building activities between the two organizations.

Global Health at the University of Toronto, the Hospital for Sick Children and its Research Institute
One of the strategic directions of the Hospital for Sick Children in Toronto is "to lead nationally and internationally". Operationally this strategic goal is being met through programs within the Hospital, its Research Institute and its affiliation with the University of Toronto. For example, the Program for Global Pediatric Research at the Hospital was formed to address the disparity between the scientific research resources available in high-income countries and the quantity of scientific research focused on the health of children in mid- and low-income countries. This Program works at the centre of a global network to inform, educate, and facilitate international research cooperation and collaboration, as well as advocate for research to improve the health of all children. Zlotkin is a member of that program.

The Research Institute at The Hospital for Sick Children undertakes child-centered research across the life continuum from fetal origins to adult outcomes, including fundamental discovery, applied research, and outcomes and impact. Support for clinical trials at the Research Institute is through the Clinical Research Support Unit (CRSU). This Unit is a consultation service operated by the Child Health Evaluative Sciences Research Program of the Research Institute. The mandate of the CRSU is to improve the quality of clinical research at the Hospital by providing consultation in the areas of study design and methodology, statistical analysis, and data management.

Dr. Zlotkin is Head of the Division of Gastroenterology, Hepatology and Nutrition at the Hospital for Sick Children, a Senior Scientist in the Research Institute in the Child Health and Evaluative Sciences Research Program, and a Professor (of Pediatrics, Nutritional Sciences and Public health Sciences) at the University of Toronto. As a Professor, he has full access to the research facilities at the University. The University of Toronto is the largest university in Canada with more than 50,000 undergraduate students and 10,000 graduate students. It has more than 18,000 staff and faculty in 520 graduate programs and 42 professional programs. Its library system has over 18 million holdings, one of the top five research libraries in North America and its research grant and contract support is over $800 million.

The combined infrastructure of the Hospital, the Research Institute and the University of Toronto provide more than ample infrastructure support for the collaborative project described in this application.

4. RESEARCH DESIGN AND METHODS

4.1 Design Conceptual or Clinical Framework

Study Design

As stated previously, the efficacy of sprinkles in reducing iron deficiency anemia among infants and young children has been well documented; however the safety of this product in malaria endemic areas has not been investigated at the community level. The proposed study is a community-based blinded randomized controlled trial with the primary objective of determining the impact of providing Sprinkles (including microencapsulated iron as a powder added to complementary foods) on the susceptibility to clinical malaria among anemic and non-anemic infants and young children (6-35 months of age) living in a high malaria burden area. In order to achieve this objective, it is necessary to include a group that receives iron and a group that does not. Therefore, the trial has 2 study arms:

- Iron group: Eligible subjects will be randomized by compound to receive a daily dose of a powdered vitamin/mineral fortificant (Sprinkles) containing 12.5 mg of iron (plus ascorbic acid, vitamin A and zinc), added to complementary foods, for a period of 5 months during the wet season (between March and November). At the end of the 5-month intervention period, subject will discontinue Sprinkles use and be followed up for one additional month.

- Placebo group: Eligible subjects will be randomized by compound to receive a daily dose of a powdered vitamin/mineral fortificant (Sprinkles) containing ascorbic acid, vitamin A and zinc only (no iron), added to complementary foods, for a period of 5 months during the wet season (between March and November). At the end of the 5-month intervention period, subject will discontinue Sprinkles use and be followed up for one additional month.

Primary and Secondary Outcomes

The primary outcome will be ***clinical malaria***, defined (according to WHO criteria) as parasitemia (malaria parasites detected on a blood smear) of any density plus history of fever (within 48 hours) or axillary temperature >37.5^{0}C. Criteria for clinical malaria will be taken from the UNICEF Case Management Series, entitled, "Promoting Rational Use of Drugs and Correct Case Management in Basic Health Services – Malaria Prevention and Treatment" published by UNICEF's Programme Division in cooperation with the World Health Organization in 2000. To distinguish unique malaria episodes, treatment will be supervised and follow-up blood smears will be collected from the child as described below in Section 5.2(c).

Secondary outcomes will include: (i) changes in anemia status; (ii) the severity of the clinical malaria (based on level of parasitemia); (iii) cerebral malaria; (iv) hospitalization (from any cause) (v) death; (vi) pneumonia; (vii) diarrhea and dehydration.

Subjects/study population

Infants and young children (6-35 months of age), living in the Brong Ahafo Region of northern Ghana, will be included in the study if, at baseline, they are ingesting weaning foods in addition to breastmilk; free from major illness; afebrile; living in the study area for the duration of the intervention and follow-up period; and if parental consent is obtained. Inclusion of a child into the study will proceed after the possible risks and benefits are discussed with the parents (in the appropriate local language), and a signed consent is obtained. The exclusion criteria are as follows: severe anemia (hemoglobin <70g/L), weight-for-height <-3 z-score (severe wasting), kwashiorkor (defined as evidence of edema), congenital abnormality, treatment with iron supplements in the past 6 months, or any chronic illness. Children that are severely anemic (Hb<70 g/L) and/or severely malnourished will be excluded from entry to the study and referred to the local health provider for treatment according to Ghana Ministry of Health guidelines.

Sample Size

We hypothesize that the incidence of malaria will be significantly higher in children receiving iron versus a placebo of micronutrients without iron. It was decided not to use the change in anemia status as a primary outcome since, in all previous studies with powdered mineral and vitamin fortificants, anemia rates declined compared to placebo controls, or were no different from the improvement in anemia rates observed with iron drops.

Based on the 2006 estimate of "cases and deaths from fevers suspected of being malaria" for children under 5 years of age in all of Ghana (53), the baseline rate of 3.44 episodes/child/year was assumed. We estimated that a total of 351 person-years would be required to detect a 15% increase in malaria incidence rates with 80% power and at a 5% type I error. Based on the experience and expertise of the PI and co-investigator, a difference of 15% in malaria incidence was considered to be clinically significant. Knowing that all children enrolled in the trial will begin the 5-month intervention period at a similar risk level, and accounting for 15% loss to follow-up, as well as the need to test the hypothesis twice (due to the interim analysis by the Data Safety and Monitoring committee), the sample size has been calculated as 1940 children (970 per group) (see Table 1 below). All calculations were reviewed by 2 statisticians who will be involved in subsequent analyses.

Table 1: Sample size calculation adjusted for testing the hypothesis twice

Least Meaningful Difference	**2006 rates**	**Adjustment for use of bed-net**	**Rate unexposed**	**Rate exposed**	**Person-years**	**Follow-up years**	**n**	**Loss to follow-up**	**Sample size per group***
0.05	3.44	0.75	2.58	2.71	3017	0.42	7240	0.15	8326
0.1	3.44	0.75	2.58	2.84	772	0.42	1854	0.15	2132
0.15	**3.44**	**0.75**	**2.58**	**2.97**	**351**	**0.42**	**843**	**0.15**	**970**
0.2	3.44	0.75	2.58	3.10	202	0.42	485	0.15	558

*Adjusted for estimated bed net use and loss to follow-up

4.2 Procedures

Recruitment, Randomization Process and Blinding

Subjects will be recruited from villages in the Wenchi District once permission has been obtained from the village assemblies and elders, as well as the parent or guardian of the eligible child. Field researchers, employed by the KHRC, will visit individual compounds and interview parents or caregivers regarding the inclusion criteria. Once consent has been obtained and documented via signature or thumbprint, eligible children will be enrolled and randomized (at the compound level), using a computer-generated model, to either the iron (Fe) or placebo (P) group.

Sachets containing the powdered minerals with and without iron will look and taste identical with the exception that each package will be marked with an 'A' or 'B' denoting packages with or without iron. The study team and caretakers of children will be blinded to the 'A' or 'B' designation. Only the manufacturer of the powdered fortificant (in Ghana) and a research pharmacist each in Toronto and Kintampo will hold the key to the randomization and 'sachet' code lists. The key will be revealed to the 'Data and Safety Monitoring Committee (DSMC)' if a significant difference in outcomes is observed, and to the researchers after the database is closed and statistical analyses completed.

Supply and content of the powdered mineral and vitamin fortificant.

A powdered mineral and vitamin fortificant product, "Sprinkles", will be used in the proposed study. The Sprinkles formulation contains a specific combination of minerals and vitamins, including iron in the form of microencapsulated ferrous fumarate. The applicant (SZ) has extensive experience with the Sprinkles product both in research protocols and in working with governments, UN agencies and NGOs for scaling up the intervention. The product will be procured from a production facility in Canada, India or Bangladesh. Each of these facilities has supplied a reliable high quality product to the applicant for past research projects. The production facilities in India and Bangladesh are UNICEF approved facilities. The dose of micronutrients including iron is shown in Table 2 below. The dose of individual nutrients is based on WHO or IOM-DRI guidelines, or estimates based on these guidelines.

Table 2: Justification of a powdered mineral and vitamin supplement dose for infants and young children

	6-11 mo		12-24 mo		Powdered fortificant
	WHO[1]	IOM DRI[2]	WHO	IOM DRI	
Vitamin A, µg RE	400	500*	400	300	400
Vitamin C, mg	30	50*	30	15	30
Folic Acid, µg	80	80*	160	150	OMIT
Iron, mg[3]	9.3	11	5.8	7	12.5
Zinc mg[4]	4.1	3	1.1	3	5

[1] Recommended Nutrient Intakes. Source: Joint FAO/WHO Expert Consultation. (2002) Vitamin and mineral requirements in human nutrition. World Health Organization, Geneva, Switzerland.

[2] Recommended Dietary Allowances (RDA). Sources: Institute of Medicine, Dietary Reference Intakes. National Academy Press, Washington D.C.

[3] The dose of 12.5 mg is the current INACG/WHO/UNICEF recommendation for children 6-35 months of age for large scale distribution. It assumes a medium bioavailability (5-10%).

[4] Assuming moderate bioavailability (30%)
* Based on Adequate Intake (AI) estimates

Clinical protocol

a) Baseline: After consent is provided, subjects will be screened for anemia (based on hemoglobin) and parasitemia. Enough blood will be taken to determine C-reactive protein (CRP), serum ferritin (SF), zinc protoporphyrin (ZnPP) and serum transferrin receptor (TfR). Those who are severely anemic (Hb <70 g/L), or have clinical signs of severe malnourishment will be referred to the study clinician who will offer the child the standard care, as defined by the Ghana Ministry of Health. All other children will be enrolled and randomized to either the iron (Fe) or placebo (P) group. The child's weight and length measurements will be recorded, as well as any relevant demographic (age, gender) and historical health information (birth weight and previous hospitalizations). Caregivers will also be asked to provide information regarding their usual feeding practices and health-seeking behaviours. All households will be provided with a treated bed net and a supply of powdered fortificant with oral instructions on how to use each.

b) Monitoring visits: Field researchers will visit each subject at their home every week for the duration of the intervention and follow-up periods. At each visit, the field researcher will conduct a health assessment (including axillary temperature) and collect information on supplement compliance, bed net use and morbidity. At the beginning of the intervention period, 7 new sachets will be provided at each weekly visit. In the following weeks, the number of sachets supplied at one visit will be increased in a stepwise manner until a monthly distribution schedule (30 new sachets) is obtained. Clear instructions for storage and administration of sachets will be provided to families. Sprinkles adherence will be assessed by counting and recording all used and unused sachets at each home visit. It has been our experience in other similar studies in Ghana and elsewhere that by providing pre-emptive information on the impact of the fortificant to parents (like telling parents that stools will darken from the iron, and that children will become more active and have enhanced appetites), the adherence to the use of the sachets is generally in the range of 70 – 98%. We will provide this pre-emptive information in the current study.

c) Tracking the subjects: All subjects who become ill and need to visit a health facility will be identified and tracked through their study identity cards. The use of such cards is standard practice at the Kintampo Health Research Centre (KHRC). If a fever has been reported or recorded, a blood sample will be taken to determine parasite species and count. If the child is admitted to the hospital or health centre, blood parasite level will similarly be determined, and further tests will be conducted (as needed) to rule out cerebral malaria, pneumonia, diarrhea, and/or dehydration. All treatments will follow the treatment guidelines in Ghana (Ghana Ministry of Health). For those assessed to have malaria, the first-line antimalarial (Artesunate-Amodiaquine) will be prescribed and provided. To determine if the treatment has been successful, and from the perspective of the study, to distinguish unique malaria episodes, treatment will be supervised and follow-up blood smears will be collected from the child on the 7th, and 14th day following treatment (this process has been successfully used in past trials including "A multi-centre, randomized, double-blind, double dummy study comparing the efficacy and safety of chlorproguanil-dapsone-artesunate versus artemether-lumefantrine in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in children and adolescents in Africa Protocol #: SB-714703/005. Collaborations among KHRC/LSHTM/GSK/MMV/WHO"). All slides (thick and thin films) will be stained with Giemsa following fixing of the thin film and read twice by independent microscopists blinded to each other's reading. A third microscopist will be asked to read the slide if there is disagreement between the first two. The readings will be used to determine if the treatment has been successful or not.

d) Endline: At the end of 5 months, subjects will again be screened for anemia, CRP, ferritin, TfR, zinc protoporphyrin and a blood smear for their malaria status. The subjects will be weighed and measured, and caregivers will again be asked about supplement use, and bed net use. Any remaining sachets will be collected and counted. For those children who are anemic, based on their end-line hemoglobin assessment, an additional 60 sachets of iron-containing powdered fortificant will be provided at the end of the post-intervention follow-up period. Providing iron to anemic children is not a safety issue in this case because they will be eligible for treatment according to the WHO statement on the use of iron in malaria endemic areas (3).

e) Post-intervention Follow up: After the 5-month intervention period, subjects will discontinue Sprinkles use and be followed up on a weekly basis for one additional month. At these visits, the field researcher will continue to conduct health assessments (including axillary temperature) and collect information on bed net use and morbidity. If a subject becomes ill and/or needs to visit a health facility, the same protocol as described above will be used for tracking and monitoring these cases. All families that have completed the intervention and follow up periods will be provided with a study closure package (including a bar of soap) as has been the standard goodwill practice of KHRC to participants from the communities.

f) *Discontinuation of supplementation*. Supplementation will be stopped if any of the following is reported: admission to the hospital ward for >5 days; evidence of chronic diarrhea (defined as loose stools lasting >4 weeks); prolonged fever of unknown origin; emergence of any exclusion criteria after randomization. Prompt, daily disclosure by field workers of any concerns will be encouraged. If necessary, children will be referred to local physicians for treatment according to local standards. Reasons for stopping supplementation voluntarily will be recorded.

4.3 Analyses

Laboratory and Clinical Analyses

All laboratory analyses will be conducted at the Kintampo Health Research Centre by qualified laboratory technicians.

Screening for malaria will be performed in the field using rapid diagnostic tests (RDTs) to help decide on treatment and blood smears for microscopy to provide counts. All clinical signs and symptoms will be interpreted using standardized 'case definitions' as described in previous studies (16, 54). For example, cerebral malaria (defined by a parasite count >5000/μL blood and a concurrent score of $\leq$2 on the Blantyre coma scale, with or without convulsions), pneumonia (defined by the presence of a cough or breathing difficulties, tachypnea, lower chest wall indrawing, and the appearance of consolidation or pleural effusion on a chest X-ray), diarrhea (defined by $\geq$3 loose or watery stools in the previous 24 hours), and dehydration (defined by lethargy, sunken eyes, and decreased skin turgor [>2 seconds for skin to return following a skin pinch]) will be assessed in the hospital or health centre using standard procedures/methods. Severe malaria disease will be diagnosed based on symptoms and signs occurring at presentation or developing during admission according to generally accepted case definitions as have been used in all previous studies in Ghana. These 'case definitions' are available on request.

For identifying and analyzing cause-specific deaths or admissions to hospital, we will use exclusive categories to ensure that independent events are not classified more than once. As such, we will first allocate malaria related causes, then pneumonia and other infection related causes, and finally diarrhea and others.

Blood samples will be analyzed for full blood counts (CBC) using a hematology auto-analyzer (Horiba ABX Micros 60-OT). This machine is a recent addition to Kintampo Health Research Centre laboratory, and needs only drop of blood (a few micro-litres) to perform analyses for Hemoglobin, hematocrit, and various red blood cell (RBC) indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW). Red blood cell indices are the reference standard for identifying anemic subjects, and can help to differentiate between types of anemias. Therefore, measuring the RBC indices permit us to narrow down the possible causes of an anemia. For example, the MCV is an index of the size of the red blood cells. When the MCV is below normal, the RBCs will be smaller than normal (microcytic), and when the MCV is elevated, the RBCs will be larger than normal (macrocytic). Red blood cells of normal size are termed normocytic. Failure to produce hemoglobin results in smaller than normal cells (microcytosis). This occurs in many diseases, including iron deficiency anemia and thalassemia. Macrocytic cells occur when division of RBC precursor cells in the bone marrow is impaired. The most common causes of macrocytic anemia are vitamin B_{12} deficiency, folate deficiency, and liver disease. Normocytic anemia may be caused by decreased production of RBCs (e.g., malignancy and other causes of bone marrow failure), increased destruction (hemolytic anemia), or blood loss. For example, with malaria there is red blood cell hemolysis, therefore the RBC count is low, but the size and amount of hemoglobin in the cells tends to be normal.

A low MCH indicates that cells have too little hemoglobin. This is caused by deficient hemoglobin production. Such cells will be pale when examined under the microscope and are termed hypochromic. Iron deficiency is the most common cause of a hypochromic anemia. The MCHC is the ratio of hemoglobin mass in the RBC to cell volume. Cells with too little hemoglobin are lighter in color and have a low MCHC. The MCHC is low in microcytic, hypochromic anemias such as iron deficiency, but is usually normal in macrocytic anemias.

Finally, the red cell distribution width (RDW) is a measure of the variance in red blood cell size. It is calculated by dividing the standard deviation (a measure of variation) of RBC volume by the MCV and multiplying by 100. A large RDW indicates abnormal variation in cell size, termed anisocytosis. The RDW aids in differentiating anemias that have similar indices. For example, thalassemia minor and iron deficiency anemia are both microcytic and hypochromic anemias, and overlap in MCV and MCH. However, iron deficiency anemia has an abnormally high RDW, but thalassemia minor does not. Therefore, by using a combination of the RBC indices, it will be possible to differentiate between iron deficiency anemia and thalassemia, and between iron deficiency and the anemia of malaria.

Plasma CRP, ferritin, transferrin receptor, and red blood cell zinc protoporphyrin will also be assayed using standard methods, including internal and external reference standards. Since CRP is a measure of inflammation, blood samples from subjects with elevated CRP values (>8 mg/L) will be excluded from further analysis (55). It should be noted however, that even in a malaria endemic area, albeit in school children (in Zanzibar), iron status assessment using these indicators may not be seriously influenced by malarial infection (4). Zinc protoporphyrin is a metabolic intermediate of the hemoglobin synthetic pathway which accumulates in red blood cells when iron supply is limited. It can be easily measured fluorometrically and is expressed as a ratio to heme (ZPP/H). In adults, ZPP/H correlates inversely with plasma ferritin across a wide range of ferritin concentrations (56), and is inversely related to the amount of stainable iron in the marrow (57). In adults (56, 58, 59) and children (60) ZPP/H has been shown to be more sensitive than the packed cell volume or hemoglobin concentration in detecting iron deficiency. It is particularly suited as a screening test because it is cheap, convenient (61) and can be carried out on a single drop of blood (62). ZPP/H ratios are expressed as μg/g hemoglobin (Hb) and plasma

ferritin as μg/L. ZPP/H and plasma ferritin will be log transformed (to the base 10) before analysis to normalize the distribution, as both are usually positively skewed.

TfR and ZnPP are sensitive measures of iron-deficient erythropoiesis and have been used to define iron status in children in developing countries (63-67). TfR may have an advantage over SF because it is unaffected by the acute phase response (63, 68, 69). However, the specificity of TfR may be low because it can be increased by malaria (70), megaloblastic anemia due to vitamin deficiencies (71), and hemoglobinopathies such as sickle cell disease (72), hemoglobin H disease, and the thalassemias (73, 74). ZnPP has advantages of low cost and simplicity, but its specificity may be low as it also can be increased by malaria and other infections, chronic inflammation, and hemoglobinopathies (60, 63, 64, 75-77). To improve specificity, SF is often combined with TfR, ZnPP, or both (78). In the current protocol, we will include all three measurements in our assessment of iron status.

Zimmerman et al recently studied the use of serum transferrin receptor and zinc protoporphyrin as indicators of iron status in African children (Côte d'Ivoire). He determined the most sensitive and specific markers for iron status and their 'optimal' diagnostic cutoffs (TfR >9.4 mg/L or ZnPP > 52 μmol/mol heme) after correcting for inflammation (79). We plan to use these cutoffs to define iron status as iron replete and anemic, iron deficient and anemic, of iron replete and not anemic. Zimmerman defined anemia as a hemoglobin concentration below the WHO cutoff values (110 g/L) minus 10 g/L, and normal ferritin as > 30 μg/L (79). Several investigators have argued that a SF concentration >30 μg/L should be used to define adequate iron stores in developing countries with a high prevalence of infection (63, 80). Therefore, we have chosen a SF cutoff of >30 μg/L as being indicative of iron sufficiency.

To summarize: anemia will be defined as Hb <100 g/L; IDA will be defined as Hb <100 g/L; plus two of low MCV (<80 μm^3), MCH (<26.5 pg), MCHC (<31.5 g/dL), Hct (<35 %) or high RDW (>10 %); plus one of low ferritin (<30 μg/L), high TfR (>9.4 mg/L) or high ZnPP (>52 μmol/mol heme); and iron deficiency will be defined as one of low ferritin (<30 μg/L) or high ZnPP (>52 μmol/mol heme). We recognize that infection and inflammation will confound the interpretation of ZnPP; thus we will exclude and/or adjust this indicator for those children who have signs of infection or inflammation, as defined by an elevated CRP concentration (>8 mg/L) (55, 74).

4.4 Data Collection and Management

Baseline demographic, anthropometric, and health history data, as well as information pertaining to feeding practices and health-seeking behaviours, will be collected by field researchers employed by the KHRC. All blood samples will be collected by field researchers or other qualified individuals, trained in pediatric phlebotomy techniques.

Visual Basic will be used to manage data. All data obtained in the field will be entered by the end of the next day. The systems used will have extensive range and checking facilities. Possible errors will be verified with field or hospital staff on a daily basis. Data collection and supplement allocation will be rigorously controlled with the help of computer monitoring. For all outcomes, a double-data entry will be used to detect errors. Flow of information, distribution of supplements, and collection of samples between households and villages in Wenchi and the central office at the Kintampo Health Research Centre will be ensured by supervisors and the study coordinator (by motorbike or car) on the same day.

4.5 Data Analysis

All variables will be first explored and summarized using descriptive statistics such as number of events in person-time, incidence rates, means and standard deviations, medians and ranges, counts and proportions, and various graphs, as appropriate.

Primary analysis

The primary outcome, incidence of malaria, will be compared between the two groups using Poisson regression. A 95% confidence interval for the ratio between the incidence in the iron group and the incidence in the placebo group will be calculated. The upper boundary of this confidence interval will be compared to the 10% tolerance limit. If it is lower, then non-inferiority can be concluded.

Secondary analysis

Univariate Poisson regression with the same outcome will be used to evaluate associations with other variables: iron status at baseline, breast-feeding, age, gender and use of bed-net. These will then be introduced and re-evaluated in a multiple Poisson regression model, which will allow for adjusted group comparisons. Most importantly, by including the baseline iron status variable and the interaction with the main group variable, we can focus on the iron sufficient children that are receiving iron supplements and compare them against the others. The Poisson models will be validated by checking the model fit and for over-dispersion. Adjustments or transformations will be used if necessary.

The number of hospital admissions will be analyzed in a similar fashion, using Poisson regression. The parasite count outcome will be compared between the two groups, using a t-test. Univariate associations with other factors will be verified here as well, using t-tests, correlation coefficients and univariate regression. Ultimately a multiple regression model will be developed, again for the purpose of adjusted comparisons between groups.

The normality of the data will be verified and, in the case of any departures, a log-transformation will be used and the data reanalyzed. If the log-transformation fails to achieve normality, a nonparametric alternative will be used instead. All statistical analyses will be carried out using SAS 9.1. The Analysis will be carried out on an intention-to-treat basis including all randomized children.

Upon the completion of the first intervention phase, an interim analysis will be performed by a Data and Safety Monitoring Committee (DSMC) as described above (primary analysis). The study will be stopped if the incidence of malaria is higher in one group.

4.6 Data Interpretation

Findings from the proposed research will provide evidence regarding the safety and efficacy of providing daily iron supplementation (12.5 mg/day), in the form of a micronutrient powder that is added to complementary foods, during the wet season in a malaria endemic area.

4.7 Data Sharing Plan

Findings from the proposed research will be shared according to the applicable NIH policy for foreign institutions.

4.8 Potential Difficulties and Limitations

a) Blinding: Although research staff will be blinded to the intervention, the stools of children receiving the iron containing fortificant will likely be darker (blacker) than those receiving the placebo. The applicants are unable to control for this likelihood.

b) It will not be possible to compare the results of the proposed current study to many of the results from the Pemba study. In Pemba, the primary outcome was mortality, which necessitated a sample size of more than 30,000 subjects. Also, in the current protocol, folic acid will not be included in the powdered mineral and vitamin supplement. Based on the estimated dietary folic acid intake of older infants and children in West Africa, folic acid deficiency should not be a problem (fruit is plentiful and inexpensive). Lastly, in the current protocol all children will receive and be encouraged to use bed-nets. This was not the case in the Pemba study.

c) Although studies in adults have suggested that elevations in ZnPP (68) can be definitive indicators of iron deficiency, there is large overlap in the distribution of this indicator in children with iron deficiency anemia as well as those with normal iron status. This overlap may be explained by a greater variability in the erythroid mass in children than in adults (75) together with the many variables affecting children in developing countries that influence ZnPP independent of iron status. Because of this overlap, the sensitivity and specificity of ZnPP in identifying iron deficiency and IDA may not be as high as we would prefer regardless of the diagnostic cutoff chosen. Despite this limitation. ZnPP will be statistically evaluated individually and in conjunction with CBC values since this measure is very important in the context of the current study.

4.9 Tentative Project Timetable

TASKS	Nov 2009	Dec 2009	Jan 2010	Feb 2010	Mar 2010	Apr 2010	May 2010	Jun 2010	Jul 2010	Aug 2010	Sep 2010	Oct 2010	Nov 2010	Dec 2010	Jan 2011	Feb 2011	Mar 2011
Ethics and FDB approval																	
Sprinkles procurment																	
Community Meetings																	
DSMC preliminary meeting																	
Training of all field staff																	
Subject screening & recruitment																	
Intervention Period																	
Post-intervention follow-up																	
Sprinkles (+Fe) distribution to subjects with anemia at endline																	
DSMC assessment																	
Biochemical analyses																	
Data entry and cleaning																	
Data analysis																	
Manuscript writing and submission																	

5. BIBLIOGRAPHY AND REFERENCES

1. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr 2007;85(4):931-45.
2. Bhagwati J, Bourguignon F, Kydland F, Mundell R, North D, Schelling T, et al. Copenhagen Consensus 2008 Frederiksberg, Denmark: Copenhagen Concensus Centre; 2008.
3. WHO/UNICEF. Joint statement: Iron supplementation of young children in regions where malaria transmission is intense and infectious disease highly prevalent; 2006.
4. Stoltzfus RJ, Kvalsvig JD, Chwaya HM, Montresor A, Albonico M, Tielsch JM, et al. Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: double blind, placebo controlled study. Bmj 2001;323(7326):1389-93.

5. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr 2001;131(2S-2):649S-666S; discussion 666S-668S.
6. Bates CJ, Powers HJ, Lamb WH, Gelman W, Webb E. Effect of supplementary vitamins and iron on malaria indices in rural Gambian children. Trans R Soc Trop Med Hyg 1987;81(2):286-91.
7. Smith AW, Hendrickse RG, Harrison C, Hayes RJ, Greenwood BM. The effects on malaria of treatment of iron-deficiency anaemia with oral iron in Gambian children. Ann Trop Paediatr 1989;9(1):17-23.
8. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. J Nutr 2001;131(2S-2):616S-633S; discussion 633S-635S.
9. van den Hombergh J, Dalderop E, Smit Y. Does iron therapy benefit children with severe malaria-associated anaemia? A clinical trial with 12 weeks supplementation of oral iron in young children from the Turiani Division, Tanzania. J Trop Pediatr 1996;42(4):220-7.
10. Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. Bmj 2002;325(7373):1142.
11. Atkinson SH, Rockett K, Sirugo G, Bejon PA, Fulford A, O'Connell MA, et al. Seasonal childhood anaemia in West Africa is associated with the haptoglobin 2-2 genotype. PLoS Med 2006;3(5):e172.
12. WHO/UNICEF. World Malaria Report 2005. In. Geneva: http://www.rbm.who.int/wmr2005/
13. WHO. World Health Report 2003: Shaping the future; 2003.
14. Appawu M, Owusu-Agyei S, Dadzie S, Asoala V, Anto F, Koram K, et al. Malaria transmission dynamics at a site in northern Ghana proposed for testing malaria vaccines. Trop Med Int Health 2004;9(1):164-70.
15. Koram KA, Owusu-Agyei S, Fryauff DJ, Anto F, Atuguba F, Hodgson A, et al. Seasonal profiles of malaria infection, anaemia, and bednet use among age groups and communities in northern Ghana. Trop Med Int Health 2003;8(9):793-802.
16. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. Lancet 2006;367(9505):133-43.
17. Iyer JK, Shi L, Shankar AH, Sullivan DJ, Jr. Zinc protoporphyrin IX binds heme crystals to inhibit the process of crystallization in Plasmodium falciparum. Mol Med 2003;9(5-8):175-82.
18. Dewey KG, Domellof M, Cohen RJ, Landa Rivera L, Hernell O, Lonnerdal B. Iron supplementation affects growth and morbidity of breast-fed infants: results of a randomized trial in Sweden and Honduras. J Nutr 2002;132(11):3249-55.
19. Majumdar I, Paul P, Talib VH, Ranga S. The effect of iron therapy on the growth of iron-replete and iron-deplete children. J Trop Pediatr 2003;49(2):84-8.
20. Ip H, Hyder SM, Haseen F, Rahman M, Zlotkin SH. Improved adherence and anaemia cure rates with flexible administration of micronutrient Sprinkles: a new public health approach to anaemia control. Eur J Clin Nutr 2007.
21. Bergdahl B, Bogentoft C, Jonsson UE, Magnusson JO. Fasting and postprandial absorption of digoxin from a microencapsulated formulation. Eur J Clin Pharmacol 1983;25(2):207-10.
22. Olver JS, Burrows GD, Norman TR. The treatment of depression with different formulations of venlafaxine: a comparative analysis. Hum Psychopharmacol 2004;19(1):9-16.
23. Baldi A, Bontempo V, Cheli F, Carli S, Sgoifo Rossi C, Dell'Orto V. Relative bioavailability of vitamin E in dairy cows following intraruminal administration of three different preparations of DL-alpha-tocopheryl acetate. Vet Res 1997;28(6):517-24.

24. Lakritz J, Wilson WD, Marsh AE, Mihalyi JE. Effects of prior feeding on pharmacokinetics and estimated bioavailability after oral administration of a single dose of microencapsulated erythromycin base in healthy foals. Am J Vet Res 2000;61(9):1011-5.
25. Zlotkin SH, Christofides AL, Hyder SM, Schauer CS, Tondeur MC, Sharieff W. Controlling iron deficiency anemia through the use of home-fortified complementary foods. Indian J Pediatr 2004;71(11):1015-9.
26. Zlotkin SH, Schauer C, Christofides A, Sharieff W, Tondeur MC, Hyder SM. Micronutrient sprinkles to control childhood anaemia. PLoS Med 2005;2(1):e1.
27. Schauer C, Zlotkin SH. Home fortification with micronutrient sprinkles - A new approach for the prevention and treatment of nutritional anemias. Paediatric Child Health 2003;8(2):87-90.
28. Zlotkin S, Antwi KY, Schauer C, Yeung G. Use of microencapsulated iron(II) fumarate sprinkles to prevent recurrence of anaemia in infants and young children at high risk. Bull World Health Organ 2003;81(2):108-15.
29. Zlotkin S, Arthur P, Antwi KY, Yeung G. Treatment of anemia with microencapsulated ferrous fumarate plus ascorbic acid supplied as sprinkles to complementary (weaning) foods. Am J Clin Nutr 2001;74(6):791-5.
30. Christofides A, Asante KP, Schauer C, Sharieff W, Owusu-Agyei S, Zlotkin S. Multi-micronutrient Sprinkles including a low dose of iron provided as microencapsulated ferrous fumarate improves haematologic indices in anaemic children: a randomized clinical trial. Matern Child Nutr 2006;2(3):169-80.
31. Zlotkin S, Arthur P, Schauer C, Antwi KY, Yeung G, Piekarz A. Home-fortification with iron and zinc sprinkles or iron sprinkles alone successfully treats anemia in infants and young children. J Nutr 2003;133(4):1075-80.
32. Khan E, Hyder SMZ, Tondeur MC, Raza S, Khan NA, Zlotkin S. Home fortification with Sprinkles to reduce childhood anemia: Lessons learned in North West Frontier Province, Pakistan. Pakistan J of Med Res 2006;45(2):35-40.
33. Hirve S, Bhave S, Bavdekar A, Naik S, Pandit A, Schauer C, et al. Low dose 'Sprinkles'-- an innovative approach to treat iron deficiency anemia in infants and young children. Indian Pediatr 2007;44(2):91-100.
34. Menon P, Ruel MT, Loechl CU, Arimond M, Habicht JP, Pelto G, et al. Micronutrient Sprinkles reduce anemia among 9- to 24-mo-old children when delivered through an integrated health and nutrition program in rural Haiti. J Nutr 2007;137(4):1023-30.
35. Zlotkin SH, Schauer C, Owusu Agyei S, Wolfson J, Tondeur MC, Asante KP, et al. Demonstrating zinc and iron bioavailability from intrinsically labeled microencapsulated ferrous fumarate and zinc gluconate Sprinkles in young children. J Nutr 2006;136(4):920-5.
36. Tondeur MC, Schauer CS, Christofides AL, Asante KP, Newton S, Serfass RE, et al. Determination of iron absorption from intrinsically labeled microencapsulated ferrous fumarate (sprinkles) in infants with different iron and hematologic status by using a dual-stable-isotope method. Am J Clin Nutr 2004;80(5):1436-44.
37. Adu-Afarwuah S, Lartey A, Brown KH, Zlotkin S, Briend A, Dewey KG. Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: effects on growth and motor development. Am J Clin Nutr 2007;86(2):412-20.
38. Adu-Afarwuah S, Lartey A, Brown KH, Zlotkin S, Briend A, Dewey KG. Home fortification of complementary foods with micronutrient supplements is well accepted and has positive effects on infant iron status in Ghana. Am J Clin Nutr 2008;87(4):929-38.
39. Hyder Z, Zlotkin S. Effect of daily versus once weekly home fortification with sprinkles on haematological and iron status among young children in rural Bangladesh. Food and Nutrition Bulletin 2007;28(2):156-164.
40. Sharieff W, Yin SA, Wu M, Yang Q, Schauer C, Tomlinson G, et al. Short-term daily or weekly administration of micronutrient Sprinkles has high compliance and does not cause iron overload in Chinese schoolchildren: a cluster-randomised trial. Public Health Nutr 2006;9(3):336-44.

41. Sharieff W, Dofonsou J, Zlotkin S. Is cooking food in iron pots an appropriate solution for the control of anaemia in developing countries? A randomised clinical trial in Benin. Public Health Nutr 2008;11(9):971-7.
42. Sharieff W, Bhutta Z, Schauer C, Tomlinson G, Zlotkin S. Micronutrients (including zinc) reduce diarrhoea in children: the Pakistan Sprinkles Diarrhoea Study. Arch Dis Child 2006;91(7):573-9.
43. Zlotkin S. Control of anemia: the time to act is now. Indian Pediatr 2007;44(2):84-6.
44. Sharieff W, Horton SE, Zlotkin S. Economic gains of a home fortification program: evaluation of "Sprinkles" from the provider's perspective. Can J Public Health 2006;97(1):20-3.
45. Christofides A, Schauer C, Zlotkin S. Iron deficiency anemia among children: Addressing a global public health problem within a Canadian context. Paediatric Child Health 2005;10(10):597-601.
46. Christofides A, Schauer C, Zlotkin SH. Iron deficiency and anemia prevalence and associated etiologic risk factors in First Nations and Inuit communities in Northern Ontario and Nunavut. Can J Public Health 2005;96(4):304-7.
47. Christofides A, Schauer C, Sharieff W, Zlotkin SH. Acceptability of micronutrient sprinkles: a new food-based approach for delivering iron to First Nations and Inuit children in Northern Canada. Chronic Dis Can 2005;26(4):114-20.
48. De Pee S, Moench-Pfanner R, Martini E, Zlotkin S, Darton-Hill I, Bloem MW. Home-fortification in emergency response and transition programming: Experiences in Aceh and Nias, Indonesia. Food and Nutrition Bulletin 2007;28(2):189-197.
49. Chandramohan D, Hodgson A, Coleman P, Baiden R, Asante K, Awine E, et al. An evaluation of the immunogenicity and safety of a new trivalent meningococcal polysaccharide vaccine. Vaccine 2007;25 Suppl 1:A83-91.
50. Edmond KM, Kirkwood BR, Amenga-Etego S, Owusu-Agyei S, Hurt LS. Effect of early infant feeding practices on infection-specific neonatal mortality: an investigation of the causal links with observational data from rural Ghana. Am J Clin Nutr 2007;86(4):1126-31.
51. Owusu-Agyei S, Awini E, Anto F, Mensah-Afful T, Adjuik M, Hodgson A, et al. Assessing malaria control in the Kassena-Nankana district of northern Ghana through repeated surveys using the RBM tools. Malar J 2007;6:103.
52. Owusu-Agyei S, Asante KP, Owusu R, Adjuik M, Amenga-Etego S, Dosoo DK, et al. An open label, randomised trial of artesunate+amodiaquine, artesunate+chlorproguanil-dapsone and artemether-lumefantrine for the treatment of uncomplicated malaria. PLoS ONE 2008;3(6):e2530.
53. WHO. World Malaria Report 2008. Geneva, Switzerland; 2008.
54. Chandramohan D, Owusu-Agyei S, Carneiro I, Awine T, Amponsa-Achiano K, Mensah N, et al. Cluster randomised trial of intermittent preventive treatment for malaria in infants in area of high, seasonal transmission in Ghana. Bmj 2005;331(7519):727-33.
55. Verhoef H, West CE, Ndeto P, Burema J, Beguin Y, Kok FJ. Serum transferrin receptor concentration indicates increased erythropoiesis in Kenyan children with asymptomatic malaria. Am J Clin Nutr 2001;74(6):767-75.
56. Labbe RF, Finch CA, Smith NJ, Doan RN, Sood SK, Madan N. Erythrocyte protoporphyrin/heme ratio in the assessment of iron status. Clin Chem 1979;25(1):87-92.
57. McLaren GD, Carpenter JT, Jr., nino HV. Erythrocyte protoporphyrin in the detection of iron deficiency. Clin Chem 1975;21(8):1121-7.
58. Jensen BM, Sando SH, Grandjean P, Wiggers P, Dalhoj J. Screening with zinc protoporphyrin for iron deficiency in non-anemic female blood donors. Clin Chem 1990;36(6):846-8.
59. Labbe RF, Rettmer RL, Shah AG, Turnlund JR. Zinc protoporphyrin. Past, present, and future. Ann N Y Acad Sci 1987;514:7-14.

60. Siegel RM, LaGrone DH. The use of zinc protoporphyrin in screening young children for iron deficiency. Clin Pediatr (Phila) 1994;33(8):473-9.
61. Schifman RB. Red cell protoporphyrin: an alternatie to serum ferritin for assessing iron depletion [Abstract]. Clin Chem 1982;28:1628.
62. Cavill I, Jacobs A, Worwood M. Diagnostic methods for iron status. Ann Clin Biochem 1986;23 (Pt 2):168-71.
63. Asobayire FS, Adou P, Davidsson L, Cook JD, Hurrell RF. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Cote d'Ivoire. Am J Clin Nutr 2001;74(6):776-82.
64. Stoltzfus RJ, Chwaya HM, Montresor A, Albonico M, Savioli L, Tielsch JM. Malaria, hookworms and recent fever are related to anemia and iron status indicators in 0- to 5-y old Zanzibari children and these relationships change with age. J Nutr 2000;130(7):1724-33.
65. Stoltzfus RJ, Chwaya HM, Albonico M, Schulze KJ, Savioli L, Tielsch JM. Serum ferritin, erythrocyte protoporphyrin and hemoglobin are valid indicators of iron status of school children in a malaria-holoendemic population. J Nutr 1997;127(2):293-8.
66. Rettmer RL, Carlson TH, Origenes ML, Jack RM, Labb RF. Zinc protoporphyrin/heme ratio for diagnosis of preanemic iron deficiency. Pediatrics 1999;104(3):e37.
67. Dimitriou H, Stiakaki E, Markaki EA, Bolonaki I, Giannakopoulou C, Kalmanti M. Soluble transferrin receptor levels and soluble transferrin receptor/log ferritin index in the evaluation of erythropoietic status in childhood infections and malignancy. Acta Paediatr 2000;89(10):1169-73.
68. Hastka J, Lasserre JJ, Schwarzbeck A, Strauch M, Hehlmann R. Washing erythrocytes to remove interferents in measurements of zinc protoporphyrin by front-face hematofluorometry. Clin Chem 1992;38(11):2184-9.
69. Junca J, Oriol A. Serum transferrin receptor. Am J Clin Pathol 2001;116(3):446; author reply 447-9.
70. Ritchie B, McNeil Y, Brewster DR. Soluble transferrin receptor in Aboriginal children with a high prevalence of iron deficiency and infection. Trop Med Int Health 2004;9(1):96-105.
71. Cook JD, Baynes RD, Skikne BS. Iron deficiency and the measurement of iron status. Nutr Res Rev 1992;5:189-202.
72. Zimmermann MB, Molinari L, Staubli-Asobayire F, Hess SY, Chaouki N, Adou P, et al. Serum transferrin receptor and zinc protoporphyrin as indicators of iron status in African children. Am J Clin Nutr 2005;81(3):615-23.
73. van den Broek N. Anaemia in pregnancy in developing countries. Br J Obstet Gynaecol 1998;105(4):385-90.
74. Pepys MB. C-reactive protein fifty years on. Lancet 1981;1(8221):653-7.
75. Olivares M, Walter T, Cook JD, Hertrampf E, Pizarro F. Usefulness of serum transferrin receptor and serum ferritin in diagnosis of iron deficiency in infancy. Am J Clin Nutr 2000;72(5):1191-5.

6. PROTECTION OF HUMAN SUBJECTS

6.1 Risks to Human Subjects

6.1a Human Subjects Involvement and Characteristics

- Proposed involvement of human subjects
    i. Studies using animals are not appropriate to answer the research questions posed in this proposal, namely to determine the impact of the provision of iron on the susceptibility to clinical malaria among infants and young children (6-35 months of age) living in a high

malaria burden area, using a powdered vitamin and mineral 'sprinkle' added to complementary foods.

- Characteristics of the subject population – number, age range, health status
  i. Number: Approximately 970 in the intervention group and 970 in the control group
  ii. Age: 6 – 35 months
  iii. Health Status – generally healthy
- Inclusion/exclusion criteria for any subpopulation
  i. Children to be included must meet the criteria described above. They must be free of major illnesses at the time of enrolment (malaria infections will be treated before enrollment). They must have plans to remain in the study area for the duration of the intervention and follow-up periods. Informed consent must be freely given by a parent of the children enrolled for the study. Both boys and girls will be recruited without bias. Girl children will specifically be encouraged to participate, as appropriate.
  ii. Children will be excluded if they fail to meet the inclusion criteria shown above.
- Rationale for involvement of special classes of subjects (e.g. children)
  i. The highest malaria burden is in children in the first 3-4 years of life and the highest iron deficiency anemia burden is in the age range 6 – 24 months of age. Thus, we choose to include children who are at highest risk of anemia and malaria.
- List any collaborating sites where human subjects research will be performed, describe role of those sites and collaborating investigators in performing proposed research
  i. The research field work will be conducted in a northern district of Ghana (Wenchi). Wenchi district is within the catchment area of the Kintampo Health Research Centre (KHRC), one of three Ministry of Health Research Centres in Ghana.
  ii. Research Ethics Board approval will be from two sources: the University of Toronto, the home institution of the PI and the Ghana Ministry of Health.
  iii. The University of Toronto, Hospital for Sick Children PI will provide oversight of the project, while the co-investigator at KHRC will supervise all aspects of the field research.

6.1b Sources of Materials

- Describe research material obtained from individuals in form of specimens, records, or data
  i. Capillary blood samples will be collected from study subjects. Individuals collecting the samples will be trained in pediatric phlebotomy techniques. Results of analysis of blood samples will be collated and recorded to be used for statistical analysis.
- Describe any data to be collected from human subjects
  i. Individual subject records will be collected. This information includes general demographic data (age and gender) and information specifically related to diet, health and use of health care facilities.
- Indicate who will have access to individually identifiable private information
  i. In Ghana, field workers, field supervisors and the study co-investigator will have access to individually identifiable private information.
  ii. Statisticians, the research coordinator and the PI in Canada will not have access to individually identifiable private information.
- Provide information about how specimens, records, or data are collected and whether will be collected specifically for proposed project
  i. Only data to be included in the statistical analysis to meet primary and secondary objectives will be collected.
  ii. Blood samples are collected as described above.

iii. Individual data will be collected from the parents or guardians of the study subjects.
iv. No previous records on study subjects will be collected with the exception of birth weight and previous hospitalizations.

6.1c Potential Risks

- Describe potential risks to subjects (physical, psychological, financial, legal, or other), and assess likelihood and seriousness to subjects
  - *i.* Subjects in the intervention group receiving the powdered mineral and vitamin 'sprinkle' *with iron* may be at risk of higher rates of clinical malaria or more severe attacks of malaria or other infections *if they are not anemic* at baseline.
  - *ii.* Subjects in the control group receiving the powdered mineral and vitamin 'sprinkle' *without iron* may be at risk of higher rates of clinical malaria or more severe attacks of malaria or other infections *if they are anemic at baseline.*
  - iii. There are no other psychological, financial, legal or other risks.
- Where appropriate, describe alternative treatments and procedures (including risks and benefits of each)
  - i. All subjects (in both groups) will be provided with treated bed-nets and instructions for their appropriate use. If used, they may decrease the rates of mosquito bites and thus malaria.

6.2 Adequacy of Protection Against Risks

6.2a Recruitment and Informed Consent

- Describe plans for recruitment and process for obtaining informed consent (parental permission and child assent)
  - i. Children between the ages of 6 – 35 months are too young to provide assent.
  - ii. Parents or guardians will be informed of the objectives of the study and the protocol in a setting and language appropriate to rural Ghana. Members of the field research team have worked in the study communities (or neighboring communities) for many years, thus have experience in conducting research in this population.
  - iii. The parent or guardian of the recruited child will be given to opportunity to sign the consent form, or not, in a totally non-coercive manner. Non-participation will have no effect on the provision of care to the child.
  - iv. Permission to recruit in villages in the KHRC catchment area will be obtained from the village assemblies and village elders prior to the start of the recruitment phase.
- Describe circumstances under which consent will be sought and obtained, who will seek it, nature of info provided to subjects, method of documenting consent (justification for waiver if used)
  - i. After permission is granted to recruit from a village (see previous paragraph), individual households will be visited and parents interviewed with regard to inclusion criteria. The study will take place in a very rural environment where telephone and other communication tools are not common. Thus recruitment will be face-to-face.
  - ii. Recruitment will be done by field researchers employed by the Kintampo Health Research Centre. All are trained in health research techniques and many already have experience in recruitment for research projects involving children. Field workers will be supervised by 'field supervisors' who have experience in field research including proper methods of recruitment.
  - iii. Parents of subjects will be provided with oral as well as written material explaining the nature of trial, including procedures, risks and benefits.

iv. Consent will be documented through the signing of the name of a parent, or if a signature is not possible, a thumb print will be used.

6.2b Protections Against Risk

- Describe planned procedures for protecting against or minimizing potential risks (e.g. to privacy or confidentiality of data) and assess likely effectiveness
  i. Data will be kept confidential by ensuring that named documents are locked in cabinets, or if electronically stored, it will be password protected.
  ii. All data will be identified with a numeric-alphabetic code, linked to a master list. It is this master list that will be stored as described above.
  iii. These procedures have been successfully used by the research team in past collaborate research projects without breaches of confidentiality or privacy (to the best of our knowledge).
- Additional protections for children (OHRP subpart D Guidance)
  i. Additional protections for children will be implemented in compliance with NIH policy and as outlined in the applicable sections of 45 CFR Part 46 Subpart D, as well as that deemed necessary by the applicant's Institutional Review Board.
- Plans for ensuring necessary medical or professional intervention in event of adverse effect to subjects
  i. Kintampo Health Research Centre (KHRC) has a well-established clinical facility and referral system to manage all cases (solicited and unsolicited) of adverse reactions. The Wenchi District Hospital has all of the emergency/resuscitation equipment and items to support clinical trials such as those that KHRC has been embarking on since 2002. For any complicated cases of malaria or anemia beyond the capacity of the Wenchi District Hospital to handle, an ambulance service is available to facilitate referral to the Komfo-Anokye Teaching Hospital, the second largest Teaching Hospital in Ghana (two hours away). This referral system has been successfully used over the years and will be available for all children participating in this trial.

6.3 Potential Benefits of the Proposed Research to Human Subjects and Others

- Discuss potential benefits of research to participants and others
  i. Subjects in the intervention group may benefit from the intervention by having fewer bouts of anemia or less severe attacks of malaria or other infections *if they are anemic* at baseline.
  ii. Subject in the control group may benefit from the intervention by having fewer bouts of anemia or less severe attacks of malaria or other infections *if they are non-anemic* at baseline.
  iii. The results of this research may ultimately benefit the children of Ghana (and other West African countries) by informing policy on the appropriate use of iron in children in high malaria burden areas at risk of iron deficiency anemia.
- Discuss why risks to subjects reasonable in relation to anticipated benefits
  i. There is relative equipoise for subjects in the control and intervention groups for many reasons. Subjects with iron deficiency anemia may benefit from the powdered iron supplement and subjects without iron deficiency may benefit from being in the control group. Risks are similarly equal among the two groups.
  ii. All subjects will receive treated bed nets which will mitigate the risk of contracting malaria.

6.4 Importance of the Knowledge to be Gained

- Discuss importance of knowledge to be gained
    i. Currently there is great confusion regarding the safety of providing iron to children at high risk of iron deficiency anemia in a high burden malaria area. If a child with severe iron deficiency anemia remains untreated, death may ensue. With moderate or even mild iron deficiency anemia, there is documentation of adverse developmental consequences including delayed motor and cognitive development that may not be reversible.
    ii. The decision to withhold iron supplements (UNICEF/WHO guidance) was based primarily on a single study from Zanzibar in a highly malaria endemic region, where bed nets were neither provided nor widely used. In that study it was the children without anemia who fared the worst.
    iii. The knowledge to be gained from this study may help inform public policy on this important issue.
- Discuss why risks to subjects are reasonable in relation to importance of knowledge that may be expected to result
    i. In most developing countries it is not possible to screen children for iron deficiency anemia. The currently available tools for screening are insensitive and often more costly than the intervention. Thus, it is recommended that when the prevalence of anemia is greater than 40%, blanket fortification or supplementation is warranted.
    ii. As previously noted, it is the children who do NOT have iron deficiency anemia that seem to be at higher risk of adverse outcomes if they are given iron and concomitantly have malaria. But this observation is primarily from a single study.
    iii. In the current study, because of the design, there is a risk of giving iron or not giving iron depending on the individual circumstances of the child. However, the form of iron (microencapsulated iron) and the delivery system (as a powder and added to food), may protect all children from adverse effects of iron, even if they have malaria.
    iv. The information that hopefully will be obtained from the results of this study has the potential to be very important for forming public policy regarding the use of iron in a high malaria burden area.

6.5 Data and Safety Monitoring Plan

- General description of a monitoring plan that will establish as the overall framework for data and safety monitoring. Describe entity that will be responsible for monitoring and the process by which Adverse Events will be reported to the Institutional Review Board, the funding I/C, the NIH Office of Biotechnology Activities (OBA), and the Food and Drug Administration (FDA) in accordance with Investigational New Drug (IND) or Investigational Device Exemption (IDE) regulations.
    i. Field workers will describe any possible adverse events to the field supervisors. At weekly meetings with the study site coordinator, potential adverse events will be reviewed. A written summary of this weekly meeting will be shared with the co-investigator who will decide if the information should be shared with the PI. Any adverse events reported to the PI will be reported to the IRBs in Ghana and Canada.
- Options for monitoring trials include, but not limited to: PD/PI (required); Institutional Review Board (required); Independent individual/safety officer; Designated medical monitor; Internal Committee or Board with explicit guidelines; Data and Safety Monitoring Board (required by NIH

for multi-site clinical trials involving interventions that entail potential risk to participants, and generally for Phase III clinical trials)

i. A detailed Data and Safety Monitoring Plan will be submitted to IRBs in both Toronto and Ghana and (subsequently) the funding IC for approval prior to the accrual of human subjects.

6.6 ClinicalTrials.gov Requirements

- NIH encourages registration of ALL trials in ClinicalTrials.gov whether required under the law or not.
  - i This trial will be registered at ClinicalTrials.gov

7. INCLUSION OF WOMEN AND MINORITIES

This project is specifically directed to children in the age range 6 – 35 months, thus, women will not be recruited to be included as subjects. In most cases, however, the mothers of the children will contacted as the surrogate to provide consent for their children to be included in the study. All ethnic, racial and minority groups will be included in the recruitment and the study.

8. TARGETED/PLANNED ENROLMENT TABLE

Not applicable

9. INCLUSION OF CHILDREN

This project is specifically directed to children in the age range 6 – 35 months. This age range was chosen because it is considered to be both the most vulnerable period of life for the infant and young child, and also a 'window of opportunity'. It is vulnerable because growth is faster during this period than at any other time in the life of the child, thus the need for nutrients is higher during this time. If individual nutrients are missing from the child's diet during this time period, the consequences can be long-lasting. For example, there is documentation that young children diagnosed with iron deficiency anemia during the first year of life were at risk of retarded development in certain key areas, including some emotional development and educational attainment. This age range is also specifically pertinent to the questions posed in the RFA, since it refers to the recent study conducted in Pemba, which included only infants and young children.

Both investigators (Owusu and Zlotkin) have experience in performing research in infants and young children. Zlotkin specifically is a pediatrician. His clinical and research focus is on children. His laboratory is at the Hospital for Sick Children in Toronto, and the IRB is located at the Hospital for Sick Children. The Hospital for Sick Children is the largest children's hospital in Canada, and possibly in North America, and has one of the largest Research Institutes associated with it. The IRB at the Hospital and Research Institute is thus extremely well-suited to evaluate the ethical aspects of research involving children. Field researchers at the Kintampo Health Research Centre (KHRC) have experience working with children and their parents, and phlebotomists at KHRC are experienced in taking blood samples from children. Further, there are sufficient numbers of children in the catchment area of KHRC to meet the sampling needs of the study. The fertility rate in Ghana is 3.78 children born/women (2008 estimate) (https://www.cia.gov/library/publications/the-world-factbook/geos/gh.html).

10. VERTEBRATE ANIMALS

Not applicable

11. SELECT AGENT RESEARCH

Not applicable

12. MULTIPLE PD/PI LEADERSHIP PLAN

This project will be a joint effort between the University of Toronto, the Research Institute at Hospital for Sick Children, and the Ghanaian Ministry of Health, through the Kintampo Health Research Centre (KHRC). The PI from the University of Toronto, Department of Paediatrics, Nutritional Sciences and Public Health Sciences has extensive experience in research involving children, specifically research on iron and anemia in developing countries, including Ghana. The co-investigator is the Director of the KHRC. He has extensive research experience dealing with malaria, including iron and malaria, in Ghana. Drs. Zlotkin and Owusu have worked successfully together in the past, thus one of the advantages of this collaboration is the trust between the two institutions and individuals running the study.

As has been the case with past studies involving the two institutions, there will be a clear *a priori* communications plan. For the weeks preceding the start of the study, there will be an on-site presence of the PI and/or his delegate. There will be weekly conference calls for the first 4-6 weeks of the study, and bi-weekly calls thereafter. There will be routine e-mail communication between the study co-coordinator in Toronto and her equivalent in Kintampo. All important decisions about the protocol and procedures will have been made before the protocol is submitted to the NIH. Ongoing major decisions will be made jointly by the PI and co-investigator, as has been the case in past studies. Any potential differences in opinion will be worked out through discussion and compromise. Publications will be jointly authored; with Dr. Owusu as the primary author on those publications pertaining to malaria, and Dr. Zlotkin as the primary author on those pertaining to anemia.

IRB reviews will be completed in both Ghana and Canada. In Canada, Dr. Zlotkin will be responsible for shepherding the review, while Dr. Owusu will be responsible in Ghana. The budget allocation (Ghana vs. Canada) is clearly shown in the budget component of this application.

13. CONSORTIUM/CONTRACTUAL ARRANGEMENTS

An agreement including programmatic, fiscal and administrative arrangements will be developed and signed by both organizations (the Kintampo Health Research Centre and the Research Institute at the Hospital for Sick Children). Similar agreements have been used in past collaborative projects between the two organizations.

14. LETTERS OF SUPPORT

See the attached letter of support from the consortium.

15. RESOURCE/DATA SHARING PLAN

Findings from the proposed research will be shared according to the applicable NIH policy for foreign institutions.