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RESEARCH ARTICLE

Burden and Subnational Disparities in Micronutrient Deficiencies Among Children Aged 1–60 Months in Northern Ghana: A Biomarker-Based Comparative Study

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Abstract

Background

National and regional micronutrient surveys are indispensable for tracking population nutrition, but may conceal substantial heterogeneity between neighbouring districts, with direct implications for how nutrition programmes are geographically targeted. Using a harmonised, multi-biomarker panel, we compared the prevalence and co-occurrence of anaemia, iron deficiency, vitamin A deficiency, zinc deficiency and indicative iodine insufficiency among children aged one to sixty months in two administratively distinct districts of northern Ghana, Bongo and East Mamprusi District, that share broadly similar rural, agro-ecological and socioeconomic profiles.

Methods

A community-based cross-sectional survey enrolled 403 children aged 1–60 months using a three-stage sampling design combining purposive district selection, stratified random selection of communities, and household-level enrolment. Haemoglobin, serum ferritin, soluble transferrin receptor (sTfR), Retinol-Binding Protein 4 (RBP4), serum zinc and thyroglobulin (Tg), C-reactive protein (CRP) and malaria were measured. Ferritin and vitamin A biomarkers were adjusted for sub-clinical inflammation using the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) regression method. A multiple micronutrient deficiency burden score (0–4) was derived for each child from iron deficiency, vitamin A deficiency, zinc deficiency and indicative iodine insufficiency, and co-occurrence of deficiency pairs was examined. Differences in continuous outcomes between districts were tested using Welch t-tests, and differences in proportions using Pearson chi-square tests, with a Bonferroni correction applied within each set of group comparisons (sex, age, district). Multivariable logistic regression, adjusting for district, age, sex, inflammation (CRP), malaria and dietary diversity, was used to identify independent determinants of each deficiency outcome and of concurrent (≥2) deficiencies.

Results

Anaemia affected 91.1% of children (95% CI 88.3 – 93.9; 90.2% restricted to children aged 6–59 months), functional iron deficiency (elevated sTfR) 53.3%, BRINDA-adjusted iron deficiency 25.3%, Iron Deficiency Anaemia (IDA) 23.3%, and BRINDA-adjusted Vitamin A Deficiency (VAD) 10.7% far below the anaemia burden, indicating that iron deficiency alone cannot account for the very high anaemia prevalence. Indicative iodine insufficiency, provisionally defined by serum thyroglobulin >40 μg/L, affected 54.8% of children. Multiple concurrent deficiencies (≥2 of iron, vitamin A, zinc and indicative iodine insufficiency) affected 25.6% of children and ≥3 deficiencies affected 3.2%; indicative iodine insufficiency and iron deficiency were the most frequently co-occurring pair (15.1%). Children in Bongo District had significantly lower mean haemoglobin (9.17 vs 9.57 g/dL, $p = 0.007$) and lower adjusted RBP4 (1.00 vs 1.11 μmol/L, $p < 0.001$), and more than twice the prevalence of VAD (15.3% vs 6.0%, $p = 0.002$; adjusted OR 3.23, 95% CI 1.55–6.73), than children in East Mamprusi District district; the RBP4 and VAD differences remained significant after Bonferroni correction for multiple district-level comparisons, whereas the haemoglobin difference did not.

Conclusions

A high burden of childhood micronutrient deficiency coexisted with substantial heterogeneity between two socioeconomically similar districts, with Bongo District bearing a disproportionately higher burden of vitamin A deficiency, anaemia and indicative iodine insufficiency than East Mamprusi District. The marked discordance between anaemia, iron-status biomarkers and vitamin A status further illustrates the limitations of single indicators for characterising micronutrient vulnerability. While national surveys remain essential for monitoring population nutrition, these findings suggest that strategically targeted subnational biomarker surveillance can identify pockets of vulnerability that national and regional averages conceal, supporting a precision public health

nutrition approach in which universal actions (addressing the shared high burden of anaemia and functional iron deficiency) are complemented by district-specific targeted actions (such as intensified vitamin A programming in Bongo District) rather than uniform, region-wide approaches alone.

Key words

Micronutrient, Anaemia, Iron, Zinc, Vitamin A, Iodine, Haemoglobin, ferritin, thyroglobulin, multiple micronutrient deficiencies, precision public health nutrition

Introduction

Nationally representative surveys are indispensable for tracking population nutrition status and guiding policy, but national and even first-level regional estimates may conceal substantial within-region heterogeneity. This presents a particular challenge for micronutrient programming, because supplementation coverage, food fortification compliance, infection burden, dietary patterns and access to nutrition and health services can vary considerably across relatively small geographic areas. As countries move towards more geographically targeted nutrition interventions, understanding whether micronutrient burdens vary meaningfully below the regional level, even between neighbouring, apparently similar populations, has become increasingly important for the efficient allocation of scarce programme resources, and fine-scale geospatial and small-area estimation approaches are increasingly used to reveal such heterogeneity in other domains of maternal and child nutrition (1–3). Ghana provides a useful case study of this broader, global-health problem: national and regional averages can obscure major subnational heterogeneity in micronutrient deficiencies even between districts that share similar agro-ecological and socioeconomic conditions, a pattern documented elsewhere in sub-Saharan Africa for anaemia specifically (1).

Micronutrient malnutrition, often termed ‘hidden hunger’, refers to deficiencies of essential vitamins or minerals that persist even where overall energy intake appears adequate. Among children aged 6–59 months, deficiencies of iron, zinc, iodine and vitamin A are of particular concern because this period coincides with the most rapid phase of physical growth, brain development and immune maturation, so that nutritional insults occurring within the first 1,000 days and the subsequent period through age five can have lasting, often irreversible, consequences for growth and cognitive development (4–6).

Anaemia, frequently used as a proxy indicator of iron deficiency though caused by multiple aetiologies including malaria, helminthiasis, inflammation and inherited haemoglobinopathies, continues to affect a substantial proportion of preschool children worldwide. Global Burden of Disease (GBD) estimates attribute a pooled anaemia prevalence among children under five of approximately 61% under the revised World Health Organization (WHO) 2024 diagnostic criteria (7), with the African region bearing among the highest regional burden of childhood anaemia and iron deficiency of any WHO region (8). A systematic review and meta-analysis of iron deficiency and iron deficiency anaemia among children under five reported pooled global prevalences of 17.95% and 16.42% respectively, concentrated disproportionately in Asia and Africa (9), while a separate meta-analysis restricted to school-age children found pooled community-setting iron deficiency anaemia prevalence rising from 9.4% globally to 21.9% in sub-Saharan Africa and 29.7% in low-income countries specifically (10).

Vitamin A and zinc deficiencies compound this burden. WHO's global vitamin A deficiency database estimated that approximately one-third of preschool-age children worldwide were biochemically vitamin A deficient in the mid-2000s, with the WHO Africa Region accounting for roughly 56 million of the estimated global caseload (11). Zinc deficiency, assessed indirectly in the absence of widespread biochemical surveys, was estimated to place approximately 17% of the world's population at risk of inadequate intake, correlating positively with childhood stunting prevalence (12). Iodine deficiency, historically assessed through median urinary iodine concentration in school-age children, was estimated in an early global assessment to affect more than 1.9 billion people worldwide, including approximately 285 million school-age children, though with substantial regional variation (13).

In Ghana, the principal national source of biomarker-based micronutrient data remains the 2017 Ghana Micronutrient Survey (GMS), a nationally representative cross-sectional survey of preschool children and non-pregnant women of reproductive age implemented by GroundWork, the University of Ghana, the Ghana Health Service and international partners (6,14). The GMS found that 35.6% of Ghanaian children aged 6–59 months were anaemic, 21.5% were iron deficient (inflammation-adjusted ferritin), 12.2% had iron deficiency anaemia, and 20.8% were vitamin A deficient. A more recent scoping review of Ghanaian micronutrient intervention strategies corroborated the continuing moderate-to-severe public health significance of these deficiencies nationally, while highlighting the comparative scarcity of recent, nationally representative serum-zinc data (15). The most recent Ghana Demographic and Health Survey (16) found that national anaemia prevalence among children 6–59 months had risen to approximately 49%, a marked increase relative to the 2017 GMS estimate (14) A time-trend analysis spanning the 2003–2022 GDHS rounds found that, although anaemia prevalence had declined over the longer term, absolute and relative inequalities across region, household wealth, maternal education and residence had widened rather than narrowed, with the gap between the highest- and lowest-prevalence regions growing from 21.2 to 34.0 percentage points over the study period, and anaemia consistently highest in the Northern and North East regions and lowest in Ahafo and Greater Accra (17). A climate-zone-stratified re-analysis of Ghanaian survey data similarly found that iron deficiency was significantly associated with child anaemia in the Northern Belt (comprising the Northern, Upper West and Upper East regions) but not in the Southern Belt, where inflammation rather than iron deficiency per se was the stronger correlate, leading the authors to argue that anaemia-control programming in Ghana should be region-specific rather than uniform (18).

District-disaggregated, biomarker-based evidence for Ghana remains considerably scarcer than regional-level survey data and is concentrated overwhelmingly in the Northern Region. A 2025 pilot

study conducted in the neighbouring Tolon and Kumbungu districts reassessed anaemia, micronutrient status and inflammation biomarkers among preschool children and found an anaemia prevalence of 36%, iron deficiency of 57%, zinc deficiency of 67%, and vitamin A deficiency of 19% figures that substantially exceeded the corresponding 2017 national GMS estimates and illustrated how national averages can mask a markedly higher burden in specific northern districts (19). A separate community-based cross-sectional study in rural North East region of Ghana likewise found a high district-level burden, with anaemia and undernutrition rates well above national figures, and identified household food expenditure, maternal education, feeding practices and healthcare utilisation as key correlates (20). At the sub-national level, iodine status has also shown marked geographic heterogeneity: median urinary iodine concentration among school-age children in the Ashanti Region was found to be adequate (21), whereas toddlers in northern Ghana showed iodine deficiency in more than a quarter of cases, with a majority of dietary iodine traced to iodised salt used in commercially produced bouillon cubes rather than direct household salt purchase, a pathway not captured by conventional salt-coverage surveys (22,23)

Despite this accumulating evidence, no published, biomarker-based study has directly compared the Bongo District of the Upper East Region with the East Mamprusi District District of the North East Region two adjoining, predominantly rural districts that share broadly similar agro-ecological, socioeconomic and epidemiological characteristics, including high poverty rates, limited dietary diversity, poor sanitation coverage and a high burden of malaria and diarrhoeal illness, all recognised correlates of childhood micronutrient deficiency (14,22). Both regions have consistently ranked among Ghana's most nutritionally vulnerable in successive GDHS rounds, with stunting and anaemia prevalence exceeding national averages (14,17). Whether these two adjoining districts nonetheless differ meaningfully from one another in the prevalence and severity of specific micronutrient deficiencies, and in the sociodemographic and clinical correlates of those deficiencies, is not

established in the literature and has direct implications for whether nutrition programming in this part of Ghana can be designed uniformly across district boundaries or must instead be tailored at the community level.

This study aimed to determine and compare the prevalence, co-occurrence and underlying biomarker concentrations of anaemia, iron deficiency, iron deficiency anaemia, vitamin A deficiency, zinc deficiency and indicative iodine insufficiency among children under five years in the Bongo and East Mamprusi District districts of Ghana, and thereby to examine whether two adjoining, socioeconomically similar districts nonetheless differ meaningfully in the burden of specific micronutrient deficiencies, a question of relevance to whether nutrition surveillance and programming below the regional level can safely rely on broader area-level averages.

Materials and Methods

Study Design and Setting

A community-based cross-sectional survey design was used to assess micronutrient deficiency profiles and infectious disease burden among children aged 1–60 months in two districts of northern Ghana. The cross-sectional design permits simultaneous measurement of exposure and outcome variables at a defined point in time, enabling rapid estimation of disease prevalence and of cross-sectional associations at the population level (24), and is well suited to generating district-level baseline data on child nutrition and health indicators to inform policy and programming. This survey constitutes Phase I of a broader research programme whose subsequent phases may include a community-based nutrition and hygiene support intervention trial.

The study was conducted in the Bongo District of the Upper East Region and the East Mamprusi District District of the North East Region of Ghana. Both districts are characterised by predominantly rural, farming communities with high poverty rates, limited dietary diversity, poor sanitation coverage, and a high burden of infectious disease, including malaria and diarrhoeal illness conditions that create fertile ground for childhood malnutrition and micronutrient deficiency (14,22). The Bongo District lies in the Upper East Region, one of Ghana's poorest and most nutritionally vulnerable regions, where stunting and anaemia prevalence consistently exceed national averages (14). The East Mamprusi District District, in the North East Region, shares broadly similar ecological, socioeconomic and epidemiological characteristics, providing a meaningful comparative sub-study population. District health directorates in both districts provided operational support for community entry and participant recruitment.

Biochemical analyses of blood samples for micronutrient concentrations were performed at the Kintampo Health Research Centre (KHRC) Laboratory, Kintampo. Full blood counts and Giemsa-

stained malaria microscopy were performed at the Bongo District Hospital Laboratory and at the ProCare Medical Laboratory and Diagnostic Centre, Nalerigu. These facilities were selected on the basis of accreditation status, equipment availability, staff expertise, geographic proximity to study communities, and established quality management systems, and operated in compliance with Ghana Health Service standard operating procedures and international laboratory quality standards.

Study Population and Eligibility

The study population comprised children from one to sixty months resident in the Bongo and East Mamprusi District districts, spanning the entire age five category in line with WHO and UNICEF child nutrition surveillance frameworks (25). This age band was chosen because the first 1,000 days of life, together with the subsequent period through age five, represent a critical developmental window during which nutritional insults exert lasting effects on physical and cognitive growth (4,5). Socio-demographic, dietary and healthcare-seeking information was collected from the children's primary caregivers, predominantly mothers, who were required to be the child's biological parent or legal guardian and resident in the study community.

Children were eligible if they were from one to sixty months, resident in the selected communities at the time of the survey, and had a verifiable age (birth certificate, child welfare clinic card, or health facility birth record); if their caregiver could provide written informed consent; and if the child was not acutely ill to an extent precluding safe venepuncture. Children were excluded if aged above 60 months; if they were non-resident visitors or temporary settlers; if currently enrolled in another research study or nutrition intervention programme; if receiving long-term pharmacotherapy for a chronic illness that could confound biochemical results; if their caregiver declined consent; or if an adequate blood sample could not be obtained after two venepuncture attempts.

Sample size determination

The minimum required sample size was calculated using the Cochran's formula for estimating population proportions, $n = z^2 \times p(1 - p) / e^2$ (26), where z corresponds to the desired confidence level (1.96 for 95% confidence), p is the estimated prevalence of the primary outcome, and e is the acceptable margin of error. Using the national estimate of anaemia prevalence among children under five ($p = 0.49$) (16) as the primary outcome and a 5% margin of error, a minimum sample size of 384 children was obtained. An additional 4% was added to allow for non-response, haemolysis, incomplete questionnaires, and laboratory attrition, bringing the adjusted minimum to approximately 400 children, distributed equally between the two districts. The final analytic sample comprised 403 children providing adequate power ($\alpha = 0.05$) to detect between-district differences in the prevalence of the principal nutritional outcomes, consistent with sample sizes used in comparable district-level nutrition surveys in sub-Saharan Africa (12)

Sampling procedure

A multistage sampling strategy was used, reflecting the hierarchical structure of the study population across the two districts. In the first stage, the Bongo and East Mamprusi District districts were purposively selected from among the districts of northern Ghana on the basis of documented high prevalence of childhood malnutrition and infectious disease, geographic representation of two distinct administrative regions, accessibility for field operations, and the presence of functional health facilities capable of supporting laboratory analysis (16). In the second stage, communities within each district were treated as primary sampling units (clusters); a list of all communities was obtained from district health directorates and Ghana Statistical Service sub-district registers, communities were stratified by residence type (rural versus peri-urban), and four communities per district (eight communities in total) were selected by simple random sampling without replacement, excluding

communities with fewer than 40 households to ensure adequate sample density. This stratification yielded a final sample distribution of 87.6% rural and 12.4% peri-urban participants. In the third stage, households within each selected community were identified through enumeration lists compiled with community health volunteers and local sub-chiefs; enumerators visited households in a pre-specified direction until the required number per community was reached, and all eligible children within a visited household were invited to participate at a common ground for data collection. Community sensitisation meetings, involving chiefs, elders, unit committee members and community health workers, preceded data collection in each cluster. As contained in Participant Recruitment Flow Chart *(Appendix)*, a total of 621 households were enumerated, 545 children screened, 485 confirmed eligible and consented, 82 excluded for not meeting eligibility criteria (including Age-ineligible or no age record, non-resident, declined consent, acutely ill or unable to take blood sample), resulting in the recruitment of 403 children.

Data collection

Data collection spanned three domains: socio-demographic and dietary information obtained via interviewer-administered questionnaires and venous blood sample collection for micronutrient and inflammation biomarkers, Full Blood Count (FBC) and for malaria assessments. Data collection was from 2nd to 10th June, 2025 in the Bongo district and 19th to 24th June, 2025 in the East Mamprusi District district. All data were collected by trained research assistants under direct supervision of the principal investigator, following two days of standardised training in questionnaire administration, aseptic blood sampling, and research ethics.

Socio-Demographic, Dietary and Infection Questionnaire

A pre-tested, structured, interviewer-administered questionnaire, developed and piloted specifically for this study, captured child and household socio-demographic characteristics (age, sex, household size), feeding practices and dietary diversity (using WHO Infant and Young Child Feeding indicators (27), recent infection history (diarrhoea, fever, malaria, respiratory illness in the preceding two weeks), medication and supplementation history, and water, sanitation and hygiene practices. Questionnaires were administered in the local languages (Gurune/Frafra in Bongo District; Mampruli in East Mamprusi District District) by trained bilingual research assistants.

A 24-hour dietary recall across eight defined food groups was collected for all children in the sample: breastmilk; grains, roots and tubers; legumes, nuts and seeds; dairy products; flesh foods (organ meat, fish); eggs; vitamin-A-rich fruits and vegetables; and other fruits and vegetables (27); (28). The Dietary Diversity Score (DDS) was calculated for every child as the number of food groups (0–8) consumed in the preceding 24 hours and used descriptively across the full 1–60 month sample. The formal WHO/UNICEF Minimum Dietary Diversity (MDD) indicator, defined as consumption of five or more of the eight food groups, was applied only among children aged 6–23 months, the age band

for which the indicator is designed and validated; it was not applied to, or labelled as inadequate diversity for, children outside this age range, for whom the DDS is reported as a descriptive food-group count only.

Blood Sample Collection

Venous blood was drawn by registered medical laboratory scientists and experienced phlebotomists following WHO phlebotomy guidelines (29) and Clinical and Laboratory Standards Institute (CLSI) procedures for the collection, transport and processing of blood specimens (30). Blood was collected into EDTA tubes (for haemoglobin and full blood count) and plain (serum) tubes, with trace-element-free tubes used for zinc analysis where available, in accordance with International Zinc Nutrition Consultative Group (IZiNCG) guidance (31). EDTA tubes were inverted eight to ten times immediately after collection; serum tubes were allowed to clot for 20–30 minutes at room temperature before centrifugation at 2,000–3,000 × g for 10 minutes within two hours of collection. Haemolysed, lipaemic or inadequately labelled specimens were excluded from analysis. Serum aliquots were frozen at −20°C within four hours of collection and transported under cold-chain conditions to the KHRC Laboratory for analysis, with samples for retinol-binding protein analysis protected from light throughout (32).

Laboratory Analyses and Biomarker Definitions

Haemoglobin concentration was measured on EDTA whole blood using the HemoCue® Hb 301 system, a method validated against the International Committee for Standardization in Haematology reference method and recommended by WHO for field-based anaemia surveys (33). Anaemia was defined as haemoglobin <11.0 g/dL for children aged 1–60 months, with mild (10.0–10.9 g/dL), moderate (7.0–9.9 g/dL) and severe (<7.0 g/dL) sub-classifications (34). Full blood counts were

performed on an automated haematology analyser with daily calibration against manufacturer-supplied control materials (30).

Serum ferritin, soluble transferrin receptor (sTfR), Retinol-Binding Protein 4 (RBP4), and C-Reactive Protein (CRP) were measured by sandwich enzyme-linked immunosorbent assay (ELISA) using an established combined protocol (35). Iron deficiency was defined as serum ferritin <12 µg/L (34), and functional iron deficiency (iron-deficient erythropoiesis) as sTfR > 8.3 mg/L, a cut-off validated for populations in sub-Saharan Africa (36). Vitamin A status was assessed using RBP4 as a validated, field-feasible proxy for serum retinol (32,37), with vitamin A deficiency (VAD) defined as RBP4 < 0.70 µmol/L and marginal vitamin A status as RBP4 0.70–1.05 µmol/L, equivalent to the serum retinol cut-offs recommended by WHO for population vitamin A surveys. Serum zinc was measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), and zinc deficiency defined as serum zinc < 9.9 µmol/L (65 µg/dL) for a non-fasting morning sample in children aged 0–9 years, following IZiNCG reference limits (31,38). Systemic inflammation was defined as CRP > 5 mg/L (39). Because serum ferritin, RBP4 and zinc are acute-phase reactants whose concentrations are altered independently of true micronutrient status during infection and inflammation; a substantial concern in this malaria-endemic setting all three biomarkers were adjusted for CRP-defined inflammation using the regression-based correction developed by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project (39–41). Reported deficiency prevalences use BRINDA-adjusted values for ferritin and RBP4 unless otherwise specified, and unadjusted-versus-adjusted comparisons are reported explicitly (Table 4).

Population iodine status was assessed using serum thyroglobulin (Tg) concentration, measured by ELISA following the same general protocol used for ferritin and RBP4. In the absence of an internationally validated paediatric reference range for serum or plasma Tg equivalent to the urinary

iodine concentration (UIC) thresholds established for iodine status assessment (42,43), Tg concentrations above 40 μg/L were interpreted, provisionally and consistent with the approach adopted for this study population, as indicative of iodine insufficiency. This provisional threshold, and the more general scarcity of validated serum/plasma iodine biomarker reference ranges for children under five, is discussed further as a methodological limitation.

Malaria parasitaemia was assessed using both Giemsa microscopy and a Histidine-Rich Protein 2 (HRP2)-based rapid diagnostic assay for Plasmodium falciparum. Quality assurance measures applied across all laboratories included duplicate ELISA analysis with a within-assay coefficient of variation requirement of <10%, inclusion of certified reference materials and quality control samples in every analytical batch, exclusion of haemolysed or lipaemic specimens, and blind re-analysis of 10% of samples.

Statistical Analysis

Data were cleaned using range and consistency checks and all issues of missing data were traced to the raw data and resolved. Statistical analyses performed in IBM SPSS Statistics (version 26.0), with statistical significance set at $p < 0.05$. Descriptive statistics summarised sample characteristics, with continuous variables expressed as means and standard deviations or medians and interquartile ranges depending on distribution and categorical variables as frequencies and percentages. Prevalence estimates for micronutrient deficiencies were calculated with 95% confidence intervals using the Wilson score method.

Associations between categorical variables (micronutrient status by sex, age and district) were tested using Pearson chi-square tests, and associations between continuous variables using Pearson or Spearman correlation coefficients as appropriate. Differences in continuous outcomes between districts, sexes, and malaria-status groups were tested using independent-samples Welch t-tests (or Mann Whitney U tests for non-normally distributed variables), and differences in proportions using Pearson chi-square tests. All biomarker-based analyses were conducted with and without BRINDA inflammation adjustment to assess sensitivity to inflammation correction.

Because each of these three comparisons (Tables 5-7) involved multiple simultaneous tests, a Bonferroni correction was applied within each table (sex: 12 tests, adjusted $\alpha = 0.0042$; age: 12 tests, adjusted $\alpha = 0.0042$; district: 14 tests, adjusted $\alpha = 0.0036$); results that no longer reach significance after this correction are flagged in the corresponding results text.

To identify independent determinants of each deficiency outcome (anaemia, vitamin A deficiency, iron deficiency, iodine insufficiency, and zinc deficiency) and account for potential confounding, five separate multivariable logistic regression models were fitted, each adjusting simultaneously for district, child age (months), sex, inflammation (CRP $\geq$ 5.0 mg/L), malaria (Giemsa microscopy), and

dietary diversity score. Adjusted odds ratios (aOR) with 95% confidence intervals are reported for each predictor (Table 8). Because the zinc deficiency model exhibited quasi-complete separation (no malaria-positive child was zinc deficient), Firth's penalized-likelihood logistic regression was used for this outcome to obtain stable, finite estimates. Variance Inflation Factors (VIF) were calculated for all predictors in the regression models to assess multicollinearity; all VIFs were below 1.3, indicating no meaningful multicollinearity among covariates.

To characterise the co-occurrence of deficiencies, a multiple micronutrient deficiency burden score (range 0–4) was derived for each child as the count of iron deficiency, vitamin A deficiency, zinc deficiency and indicative iodine insufficiency present (BRINDA-adjusted definitions as above; anaemia was analysed separately as an outcome rather than included in the burden score), and its distribution (0, 1, ≥2, ≥3 deficiencies) was compared by district using Pearson chi-square (Table 9). Pairwise co-occurrence of the four deficiencies was tabulated descriptively (Table 10). A sixth multivariable logistic regression model, adjusting for the same covariates as the five models above, was fitted for the binary outcome of ≥2 concurrent deficiencies. As a sensitivity analysis, and because the applicability of the WHO <11.0 g/dL anaemia threshold and the WHO/UNICEF minimum dietary diversity (MDD) indicator to infants below six months is not established, anaemia prevalence and dietary diversity were additionally examined restricted to children aged 6–59 months (for anaemia) and 6–23 months (the age band for which the MDD indicator is designed), alongside the primary 1–60 month estimates.

The study used a two-stage cluster sampling design, with eight communities (four per district) selected as primary sampling units. All biomarker-based analyses were conducted with and without BRINDA inflammation adjustment to assess sensitivity to inflammation correction.

A post-hoc power analysis was conducted for the district-level comparisons of the five principal deficiency outcomes, using a two-proportion test with the observed prevalences, group sizes (n=202 Bongo; n=201 East Mamprusi District) and $\alpha = 0.05$ (Supplementary Table S1).

Ethical Considerations

The study protocol was reviewed and approved by the Committee on Human Research, Publications and Ethics (CHRPE) of the Kwame Nkrumah University of Science and Technology (KNUST), School of Medical Sciences, and the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana (Reference number *CHRPE/AP/181/24*), with additional site-level approvals from the district health directorates of Bongo and East Mamprusi District of the Ghana Health Service *(GHS/UER/ORD/RES/001030)*. Written informed consent was obtained from all caregivers prior to any study procedure; for non-literate participants, the study information sheet was read and explained in the relevant local language by a bilingual research assistant, and thumb-print consent was recorded in the presence of a witness. Participant confidentiality was maintained through unique identification codes, with no personally identifying information recorded on laboratory or analytical data collection instruments. Children with severe anaemia, a positive malaria test, or clinical signs of acute illness at enrolment were referred immediately to the nearest health facility, and caregivers received verbal and written feedback of their child's hematological and micronutrient biomarker results upon completion of laboratory analysis.

Results

Table 1 presents information on the Sociodemographic, dietary and clinical characteristics of 403 children from one to sixty months involved in the study. The mean age was 22.4 months (SD 16.0). There were slightly more females 210 (52.1%) than male 193 (47.9%) children. Children aged 24-60 months were the majority recording 39.0%. Recruitment was almost evenly split between Bongo district and East Mamprusi District district, recording 202 (50.1%) and 201 (49.9%) respectively. Mothers of the children were averagely 27.5 years, with two or three children, in a household of about seven persons. Dietary diversity was low across the sample: the mean DDS was 3.4, and more than two-thirds of children (68.2%) consumed fewer than four of the eight food groups (DDS < 4), signaling widespread suboptimal feeding practices across the sample as a whole. Restricted to the age band for which the WHO/UNICEF Minimum Dietary Diversity indicator is designed (6–23 months, n=184), 40.2% met minimum dietary diversity (DDS ≥ 5), compared with 31.8% if the indicator were, inappropriately, applied to the full 1–60 month sample; the age-restricted 40.2% estimate is considered the more valid figure for comparison with other MDD-based surveys. Nearly one in five children (19.4%) showed evidence of acute inflammation (CRP ≥ 5 mg/L), a finding with direct implications for the interpretation of iron and vitamin A biomarkers reported, while malaria prevalence differed considerably by diagnostic method (6.5% by microscopy versus 13.6% by HRP2 antigen RDT), consistent with the higher sensitivity of antigen-based testing relative to microscopy, particularly for detecting low-density parasitaemia.

Table 1: **Sociodemographic, Dietary and Malaria Assessment Characteristics of Respondents**

Characteristic	n	%	M (SD)
Demographic characteristics			
Age (months)	–	–	22.4 (16.0)
Sex			
Male	210	52.1	–
Female	193	47.9	–
Age group (months)			
1 - 5	52	12.9	–
6 -11	83	20.6	–
12 - 23	111	27.5	–
24 - 60	157	39.0	–
Study District			
Bongo District	202	50.1	–
East Mamprusi District District	201	49.9	–
Mother's characteristics			
Mother's age (years)	–	–	27.5 (6.8)
Number of children	–	–	2.6 (1.7)
Household characteristics			
Household size (persons)	–	–	7.2 (2.8)
Dietary and clinical characteristics			
Dietary diversity score (DDS)	–	–	3.4 (1.8)
Low dietary diversity (DDS < 4)	275	68.2	–
Acute inflammation (CRP ≥ 5 mg/L)	78	19.4	–
Malaria-positive (Giemsa microscopy)	26	6.5	–
Malaria-positive (HRP2 antigen RDT)	55	13.6	–

Continuous variables reported as M (SD); Percentages based on N = 403; DDS = Dietary Diversity Score

Table 2 describes the statistics of micronutrient status and related characteristics of participants. On average, the biomarker panel revealed a sample with biologically compromised iron, vitamin A, and inflammation status. The recorded mean haemoglobin (9.37 g/dL, SD 1.48) was below the 11.0 g/dL WHO threshold for anaemia. Also, the distribution minimum (5.20 g/dL) indicates that some children presented with severe anaemia. Ferritin and CRP were both noticeably right-skewed, with means exceeding medians by a wide margin (unadjusted ferritin M = 26.06 versus Median = 19.88 µg/L; CRP M = 2.81 versus Median = 0.74 mg/L) and maximum values many multiples of the median, reflecting the influence of acute-phase inflammatory responses in a subset of children. Applying the BRINDA correction shifted the central tendency of both ferritin (lower, 23.91 versus 26.06 µg/L) and RBP4 (higher, 1.05 versus 1.00 µmol/L) in the directions expected for an inflammation-affected sample, consistent with correction for acute-phase suppression of RBP4 and elevation of ferritin. HRP2 antigen concentrations were extremely right-skewed (median 0.00, maximum 118.84 µg/L), as expected for a marker that is at or near zero for most uninfected children and markedly elevated only in a small infected subgroup. Serum zinc and thyroglobulin; a proxy for indicative iodine insufficiency were both above averages at 18.37 µmol/L and 49.93 µg/L compared to their thresholds of 9.9 µmol/L and 40.0 µg/L respectively; meaning the average child was zinc sufficient and, by the indicative thyroglobulin criterion, iodine insufficient.

Table 2: **Descriptive Statistics of Micronutrients and Inflammation Indicators**

Biomarker (unit)	n	M	SD	Median	IQR	Min	Max
Haemoglobin (g/dL)	403	9.37	1.48	9.30	8.60–10.05	5.20	16.90
Serum ferritin (unadj.) (µg/L)	403	26.06	20.24	19.88	12.36–31.92	5.45	97.88
Serum ferritin (BRINDA-adj.) (µg/L)	403	23.91	18.05	18.47	11.98–31.19	5.05	98.22
sTfR (mg/L)	403	10.25	6.04	8.75	6.28–12.01	3.37	39.54
CRP (mg/L)	403	2.81	6.72	0.74	0.35–2.98	0.00	97.87
RBP4 (unadj.) (µmol/L)	403	1.00	0.28	1.01	0.80–1.16	0.43	1.92
RBP4 (BRINDA-adj.) (µmol/L)	403	1.05	0.28	1.05	0.88–1.20	0.45	1.91
Serum zinc (µmol/L)	403	18.37	8.31	17.40	13.55–21.75	2.20	69.80
Thyroglobulin (Tg) (µg/L)	403	49.93	28.92	42.43	27.45–63.08	7.97	162.04
HRP2 antigen (µg/L)	403	1.40	10.68	0.00	0.00–0.00	0.00	118.84

M = mean; SD = standard deviation; IQR = interquartile range (Q1–Q3). BRINDA-adjusted values derived per Namaste et al. (2017). HRP2 antigen concentration

Table 3 presents findings on prevalence of micronutrient deficiencies. The results indicate a high burden of micronutrient deficiency in the two districts. Almost every child (91.1%) had any form of anaemia, with the majority (70.0%) falling in the moderate category and severe anaemia present in 3.0% of children a pattern indicating that anaemia in this setting is predominantly moderate rather than mild, and therefore of substantial public health concern. Because the applicability of the WHO <11.0 g/dL threshold to infants below six months is not firmly established, a sensitivity analysis restricting the sample to children aged 6–59 months (n=346, thereby also permitting more direct comparison with GDHS/GMS and most global estimates, which use this age band) found an anaemia prevalence of 90.2% (95% CI 86.6–92.9), closely similar to the 91.1% observed across the full 1–60 month sample; the very high anaemia burden documented here is therefore not an artefact of including younger infants.

Iron status indicators told a more nuanced story: only 25.3% of children met the ferritin-based threshold for iron deficiency, whereas 53.3% showed functional iron deficiency by elevated sTfR, a marker less affected by inflammation; this divergence suggests that ferritin-based estimates alone likely understate the true burden of iron deficiency in this inflamed population, and that sTfR provides a more sensitive index of iron-restricted erythropoiesis here. Iron-deficiency anaemia affected roughly one in four children (23.3%), indicating that iron deficiency is a major, but not sole, contributor to the very high anaemia prevalence. Indicative iodine insufficiency (Tg greater than 40 µg/L) was also widespread (54.8%), and vitamin A status was frequently marginal or sub-optimal (51.4% combined), with 10.7% meeting the threshold for frank deficiency. Zinc deficiency was comparatively less prevalent (8.9%). As in Table 1, the discrepancy between malaria prevalence by microscopy (6.5%) and by HRP2 antigen RDT (13.6%) again underscores the diagnostic sensitivity gap between the two methods and has implications for how residual malaria-related inflammation is

accounted for in the biomarker adjustments presented next. Also, it's worth noting that, 19.4% of the children suffered inflammation (CRP $\geq$ 5.0 mg/L)

Table 3
Prevalence of Micronutrient Deficiencies, Inflammation and Malaria

Indicator	n	N	%	95% CI	Diagnostic criterion
Anaemia (haemoglobin)					
Any anaemia	367	403	91.1	[87.9, 93.5]	HGB < 11.0 g/dL
Mild anaemia	73	403	18.1	[14.7, 22.2]	HGB 10.0–10.9 g/dL
Moderate anaemia	282	403	70.0	[65.3, 74.2]	HGB 7.0–9.9 g/dL
Severe anaemia	12	403	3.0	[1.7, 5.1]	HGB < 7.0 g/dL
Vitamin A status (BRINDA-adjusted RBP4)					
Vitamin A deficiency	43	403	10.7	[8.0, 14.1]	adj. RBP4 < 0.70 µmol/L
Marginal vitamin A status	164	403	40.7	[36.0, 45.6]	adj. RBP4 0.70–1.05 µmol/L
Sub-optimal vitamin A status	207	403	51.4	[46.5, 56.2]	adj. RBP4 < 1.05 µmol/L
Zinc (serum zinc)					
Zinc deficiency	36	403	8.9	[6.5, 12.1]	Serum zinc < 9.9 µmol/L
Iodine (thyroglobulin)					
Indicative iodine insufficiency †	221	403	54.8	[50.0, 59.6]	Tg > 40 µg/L
Iron status (BRINDA-adjusted ferritin / sTfR)					
Iron deficiency	102	403	25.3	[21.3, 29.8]	adj. ferritin < 12 µg/L
Functional iron deficiency	215	403	53.3	[48.5, 58.2]	sTfR > 8.3 mg/L
Iron-deficiency anaemia (IDA)	94	403	23.3	[19.5, 27.7]	adj. ferritin < 12 & HGB < 11.0
Inflammation					
Acute inflammation	78	403	19.4	[15.8, 23.5]	CRP ≥ 5.0 mg/L
Malaria Test					
Malaria (Giemsa microscopy)	26	403	6.5	[4.4, 9.3]	Giemsa-stained film positive
Malaria (HRP2 antigen RDT)	55	403	13.6	[10.6, 17.3]	HRP2 antigen positive (HRP2_CAT)

Note. Prevalences reported as n/N (%) with 95% Confidence Intervals. VAD = vitamin A deficiency; IDA = Iron Deficiency Anaemia; sTfR = soluble transferrin receptor. BRINDA inflammation-adjusted values used for RBP4 and ferritin cut-offs. † Indicative iodine insufficiency prevalence is based on a provisional serum thyroglobulin threshold (Tg > 40 µg/L) and should not be equated with internationally validated urinary-iodine-based iodine deficiency estimates (see Methods)

Table 4 compares unadjusted and BRINDA inflammation-adjusted values for iron and ferritin most susceptible to acute-phase effects. Despite substantial inflammation prevalence in the sample (19.4% with CRP 5 mg/L) and regression coefficients confirming that CRP was associated with both RBP4 ($\beta1$ = -0.0563, 95% CI -0.0749 to -0.0376, $p < 0.001$) and ferritin ($\beta1$ = 0.0759, 95% CI 0.0286 to 0.1232, $p = 0.002$) in the expected directions, the net effect of BRINDA adjustment on point prevalence estimates was modest and not statistically significant.

Vitamin A deficiency prevalence shifted only marginally downward 11.2% to 10.7% and iron deficiency also shifted marginally upward; 24.8% to 25.3%. This suggests that, while inflammation measurably distorts individual biomarker concentrations in this sample, its net impact on population-level deficiency classification is small, likely because adjustment simultaneously reclassifies some children into and out of the deficient category, which largely cancels out at the aggregate level.

Adjusted values computed as: adjusted biomarker = unadjusted biomarker × exp [$\beta1$ × (mean reference ln (CRP+0.01) − observed ln (CRP+0.01))]. McNemar's test was used to test whether the paired change in deficiency classification (unadjusted vs. adjusted) was statistically significant; neither shift reached significance (RBP4 $p = 0.79$; ferritin $p = 0.69$). Using a stricter reference group changed adjusted iron deficiency only marginally, 24.1% vs. 25.3%, and left VAD unchanged at 10.7%, confirming robustness.

Table 4
Unadjusted vs. BRINDA-Adjusted Micronutrient Values and Deficiency Prevalence

Biomarker	Unadjusted	BRINDA-adjusted	Case change	% change
RBP4 (vitamin A)				
Mean (SD) µmol/L	1.00 (0.28)	1.05 (0.28)	–	–
Deficiency n (%)	45 (11.2%)	43 (10.7%)	−2	−0.5 pp
Ferritin (iron)				
Mean (SD) µg/L	26.06 (20.24)	23.91 (18.05)	–	–
Deficiency n (%)	100 (24.8%)	102 (25.3%)	+2	+0.5 pp

BRINDA regression: ln(biomarker) ~ ln (CRP + 0.01), n = 403; reference group CRP < 1.0 mg/L, n = 237, mean ln (CRP) = -1.1035. β1 = -0.0563 (RBP4), β1 = 0.0759 (ferritin).

RBP4: ln(RBP4) = -0.0460 + (-0.0563 × ln(CRP + 0.01)); SE(β1) = 0.0095; 95% CI -0.0749 to -0.0376; p < 0.001; R^2 = 0.081.

Ferritin: ln(Ferritin) = 3.0085 + (0.0759 × ln(CRP + 0.01)); SE(β1) = 0.0241; 95% CI 0.0286 to 0.1232; p = 0.002; R^2 = 0.024.

Table 5 presents the findings of micronutrient status by child's gender. BRINDA-adjusted ferritin was significantly higher in girls than boys (26.20 versus 21.80 µg/L, p = 0.015, d = -0.245), a small-to-moderate effect), and dietary diversity was significantly higher in boys than girls (3.66 versus 3.16, p = 0.006, d = 0.274). Anaemia, Vitamin A, functional Iron deficiency (sTfR) iodine and zinc status, were statistically not significantly different, though varied numerically.

No other continuous biomarkers (haemoglobin, RBP4, sTfR, zinc, thyroglobulin) differed significantly by sex, nor did any of the deficiency or infection prevalences (anaemia, vitamin A deficiency, zinc deficiency, indicative iodine insufficiency, iron deficiency, or malaria by either

diagnostic method), all p greater than 0.05. Taken together, these results indicate that sex was not a strong driver of micronutrient status in this sample, aside from a modest female disadvantage in iron stores that did not translate into a significant difference in overall iron deficiency or anaemia prevalence, and a modest male advantage in dietary diversity that likewise did not correspond to significant differences in deficiency outcomes.

Table 5: **Micronutrient Deficiency Differences by Child Gender**

Variable	Male (n=210)	Female (n=193)	Test statistic	p	Effect size
Continuous biomarkers (Welch t-test)					
Haemoglobin (g/dL)	9.27 (1.48)	9.49 (1.47)	t (398.7) = -1.47	0.143	d=-0.146
RBP4 adj. (µmol/L)	1.07 (0.27)	1.04 (0.29)	t (393.2) = 1.04	0.299	d=0.104
Ferritin adj. (µg/L)	21.80 (16.89)	26.20 (19.01)	t (385.4) = -2.45	0.015	d=-0.245
sTfR (mg/L)	10.75 (6.05)	9.70 (6.01)	t (398.5) = 1.75	0.080	d=0.175
Zinc (µmol/L)	17.89 (8.85)	18.91 (7.67)	t (399.6) = -1.24	0.215	d=-0.123
Thyroglobulin (µg/L)	47.37 (26.56)	52.71 (31.12)	t (379.0) = -1.85	0.065	d=-0.186
Dietary diversity score (DDS)	3.66 (1.81)	3.16 (1.82)	t (397.5) = 2.74	0.006	d=0.274
Deficiency prevalences (chi-square)					
Anaemia	91.4%	90.7%	χ^2 (1) = 0.07	0.791	–
VAD (BRINDA-adj.)	9.5%	11.9%	χ^2 (1) = 0.60	0.437	–
Zinc deficiency	10.5%	7.3%	χ^2 (1) = 1.28	0.257	–
Indicative iodine insufficiency	56.2%	53.4%	χ^2 (1) = 0.32	0.569	–
Iron deficiency (BRINDA-adj.)	29.0%	21.2%	χ^2 (1) = 3.24	0.072	–

Continuous variables: Welch independent-samples t-test with Cohen's d.

Categorical variables: Pearson chi-square (df =1). BRINDA-adjusted values used for RBP4 and ferritin. Bonferroni-corrected significance threshold for the 12 comparisons in this table: $\alpha = 0.0042$; no comparison remained significant after correction.

Table 6 presents the findings of micronutrient status by child's age group. Age was a much stronger correlate of micronutrient status than sex. With a Bonferroni correction applied across the 12 comparisons in this table (adjusted $\alpha = 0.0042$), haemoglobin ($H(3) = 22.18$, $p < 0.001$), zinc ($H(3) = 16.16$, $p = 0.001$), dietary diversity ($H(3) = 110.17$, $p < 0.001$), and iodine insufficiency ($\chi^2(3) = 25.67$, $p < 0.001$) all remained significant; the ferritin ($p = 0.045$), thyroglobulin ($p = 0.005$) and anaemia ($p = 0.025$) age differences did not survive correction and should be interpreted as suggestive only. Haemoglobin varied significantly across the four age bands ($H(3) = 22.18$, $p < 0.001$), with the lowest mean in the youngest group (under 6 months: 8.98 g/dL) and the highest in the oldest (24–60 months: 9.66 g/dL), consistent with the well-documented physiological base in haemoglobin during early infancy followed by recovery as complementary feeding becomes established. Dietary diversity increased sharply and monotonically with age (from 1.06 in children under 6 months to 4.08 in children 24–60 months, $H(3) = 110.17$, $p < 0.001$), as expected given that dietary diversity is definitionally tied to the introduction of complementary foods. Zinc, thyroglobulin, and BRINDA-adjusted ferritin all declined nominally with age (all $p \leq 0.045$, before correction), suggesting that the youngest children carry higher circulating concentrations of these markers, potentially reflecting a combination of physiological maturation and greater inflammation-related elevation of ferritin among infants. sTfR and RBP4 did not differ significantly across age groups ($p = 0.059$ and 0.561, respectively).

At the level of deficiency prevalence, anaemia ($p = 0.025$, not significant after Bonferroni correction) and indicative iodine insufficiency ($p < 0.001$, remains significant after correction) varied significantly by age, whereas vitamin A deficiency, zinc deficiency, iron deficiency, and malaria did not reach significance. Notably, indicative iodine insufficiency declined steadily and substantially with age (from 75.0% under 6 months to 40.1% at 24–60 months) (Table 6; Fig. 1).

Table 6: **Micronutrient Deficiency Differences by Age**

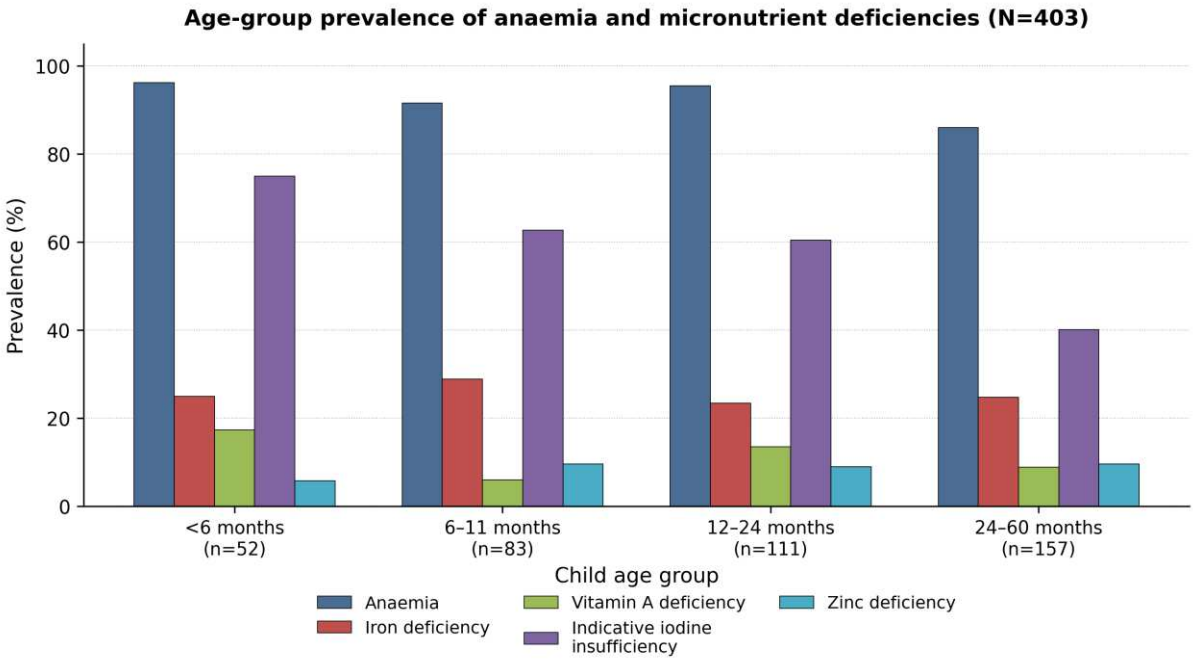
Micro Nutrient/DDC	<6 months (n=52)	6-11 months (n=83)	12-24 months (n=111)	24-60 months (n=157)	Test	P value
Haemoglobin	8.98 (1.73)	9.41 (1.72)	9.13 (0.97)	9.66 (1.50)	H (3) = 22.18	< 0.001
RBP4 adj.	1.01 (0.28)	1.08 (0.25)	1.05 (0.31)	1.05 (0.28)	H (3) = 2.05	0.561
Ferritin adj.	33.00 (25.89)	24.20 (17.62)	20.15 (13.52)	23.39 (17.04)	H (3) = 8.03	0.045
sTfR	9.50 (6.28)	10.26 (4.48)	11.07 (6.74)	9.91 (6.16)	H (3) = 7.45	0.059
Zinc	19.43 (7.41)	21.07 (11.06)	17.60 (8.37)	17.15 (6.35)	H (3) = 16.16	0.001
Thyroglobulin	62.58 (35.98)	51.04 (26.76)	47.59 (24.20)	46.80 (29.59)	H (3) = 12.78	0.005
DDC	1.06 (0.42)	3.05 (1.85)	3.86 (1.93)	4.08 (1.27)	H (3)=110.17	< 0.001

Kruskal–Wallis H-test (continuous variables reported as mean (SD).

Indicator	< 6 months	6-11 months	12-24 months	24-60 months	Test	p
Anaemia	96.2%	91.6%	95.5%	86.0%	χ^2 (3) = 9.34	0.025
VAD (BRINDA)	17.3%	6.0%	13.5%	8.9%	χ^2 (3) = 5.73	0.126
Zinc deficiency	5.8%	9.6%	9.0%	9.6%	χ^2 (3) = 0.77	0.858
Indicative iodine insufficiency	75.0%	62.7%	60.4%	40.1%	χ^2 (3) = 25.67	< 0.001
Iron deficiency	25.0%	28.9%	23.4%	24.8%	χ^2 (3) = 0.80	0.849

Note. Chi-square test of independence (df=3)

Fig. 1 Age-Group Prevalence of Anaemia and Micronutrient Deficiencies



Note. Prevalence of anaemia, BRINDA-adjusted iron deficiency, BRINDA-adjusted vitamin A deficiency, indicative iodine insufficiency (Tg >40 µg/L) and zinc deficiency by age group, corresponding to the values reported in Table 6. Indicative iodine insufficiency and, to a lesser extent, vitamin A deficiency and haemoglobin show the clearest age gradients, declining from infancy toward the pre-school years, consistent with a critical window of vulnerability during infancy and the complementary-feeding period.

Table 7 compares the prevalences of micronutrient, malaria and DDS differences in the Bongo and East Mamprusi District districts. The micronutrient profiles of the two districts differed in several dimensions, though effect sizes (d) were consistently small. A Bonferroni correction was applied across the 14 comparisons in this table (adjusted $\alpha = 0.0036$); findings that do not survive this correction are noted explicitly below.

Bongo recorded a nominally lower mean haemoglobin than in East Mamprusi District (9.17 ± 1.57 vs 9.57 ± 1.36 g/dL; t (393.9) = −2.73, p = 0.007, d = −0.27), a difference that did not survive Bonferroni correction (adjusted $\alpha = 0.0036$), and anaemia prevalence was correspondingly higher in Bongo, although this difference reached only borderline significance before correction (93.6% vs 88.6%; χ^2 (1) = 3.11, p = 0.078).

Vitamin A status was poorer in Bongo on both measures, and both differences remained significant after Bonferroni correction: mean RBP4 was lower (1.00 ± 0.29 vs 1.11 ± 0.26 μmol/L; $t(393.7) = -3.84$, $p < 0.001$, $d = -0.38$), and the prevalence of vitamin A deficiency was more than twice as high (15.3% vs 6.0%; χ^2 (1) = 9.29, $p = 0.002$; adjusted OR 3.23, 95% CI 1.55–6.73, from the multivariable model in Table 8).

The mean Thyroglobulin (μg/L), an indicative proxy for iodine status (see Methods), was nominally higher in Bongo compared to the East Mamprusi District district (53.12 ± 30.63 vs 46.72 ± 26.80 μg/L; t (394.6) = 2.23, $p = 0.026$, $d = 0.22$), a difference that did not survive Bonferroni correction. The large and overlapping standard deviations suggest there's a lot of person-to-person variability, and the two groups' distributions overlap heavily even though their averages differ. There was no significant difference in the (indicative) prevalence of iodine insufficiency between the two districts (58.9% vs 50.7%; $p = 0.100$).

The remaining measures did not differ significantly between districts. Ferritin, soluble transferrin receptor, and zinc concentrations were comparable, as were the prevalences of zinc deficiency, iron deficiency, and malaria by both microscopy (Giemsa) and antigen (HRP2) testing (all $p > 0.05$). Dietary diversity score was modestly lower in Bongo (3.25 ± 1.81 vs 3.59 ± 1.83), a difference of borderline significance ($p = 0.059$).

Particularly, Bongo's higher anaemia is not accompanied by a corresponding difference in iron status. Bongo had numerically higher ferritin and lower BRINDA-adjusted iron deficiency (22.3% vs 28.4%, though non-significant), yet lower haemoglobin and more vitamin A deficiency. This dissociation suggests the anaemia in Bongo is more strongly associated with vitamin A deficiency (and possibly other non-iron factors) than with iron depletion, a pattern relevant for tailoring intervention by district. The overall pattern is that Bongo carries a somewhat heavier micronutrient burden with significantly lower haemoglobin and vitamin A, higher vitamin A deficiency, and poorer indicative iodine status

while East Mamprusi District is comparable or slightly better on most markers. Also, it's worth noting that, both districts show a severe shared burden of anaemia above 90% and indicative iodine insufficiency of roughly 50 to 60%, underscoring a common public-health concern regardless of district.

Table 7: Micronutrient and Deficiency Differences by Study District

Variable	Bongo (n=202)	EM (n=201)	Test statistic	P value	Effect size
Continuous biomarkers (Welch t-test)					
Haemoglobin (g/dL)	9.17 (1.57)	9.57 (1.36)	t (393.9) = -2.73	0.007	d=-0.272
RBP4 adj. (µmol/L)	1.00 (0.29)	1.11 (0.26)	t (393.7) = -3.84	< 0.001	d=-0.382
Ferritin adj. (µg/L)	25.57 (18.52)	22.24 (17.44)	t (399.8) = 1.86	0.064	d=0.185
sTfR (mg/L)	10.45 (5.91)	10.05 (6.19)	t (399.9) = 0.66	0.508	d=0.066
Zinc (µmol/L)	17.78 (7.68)	18.97 (8.88)	t (392.2) = -1.45	0.149	d=-0.144
Thyroglobulin (µg/L)	53.12 (30.63)	46.72 (26.80)	t (394.6) = 2.23	0.026	d=0.223
DDS	3.25 (1.81)	3.59 (1.83)	t (400.9) = -1.90	0.059	d=-0.189
Deficiency prevalences (chi-square)					
Anaemia	93.6%	88.6%	χ^2 (1) = 3.11	0.078	–
VAD (BRINDA-adj.)	15.3%	6.0%	χ^2 (1) = 9.29	0.002	–
Zinc deficiency	9.9%	8.0%	χ^2 (1) = 0.47	0.495	–
Indicative iodine insufficiency†	58.9%	50.7%	χ^2 (1) = 2.71	0.100	–
Iron deficiency (BRINDA-adj.)	22.3%	28.4%	χ^2 (1) = 1.97	0.160	–
Malaria (Giemsa)	7.4%	5.5%	χ^2 (1) = 0.64	0.425	–
Malaria (HRP2)	14.9%	12.4%	χ^2 (1) = 0.50	0.480	–

Note. Continuous variables: Welch independent-samples t-test with Cohen's d.
Categorical variables: Pearson chi-square (df=1). BRINDA-adjusted values used for RBP4 and ferritin. Bonferroni-corrected significance threshold for the 14 comparisons in this table: α = 0.0036. Only the RBP4 and vitamin A deficiency district differences remained significant after correction. Indicative iodine insufficiency estimates are based on a provisional, non-internationally-validated serum thyroglobulin threshold and are indicative only (see Methods)

Table 8 presents the results of five multivariable logistic regression models, one for each principal deficiency outcome, each adjusting simultaneously for district, child age, sex, inflammation (CRP ≥ 5.0 mg/L), malaria (Giemsa microscopy), and dietary diversity score. After adjustment for these covariates, residence in Bongo District remained independently associated with higher odds of anaemia (aOR 2.70, 95% CI 1.27–5.75, p = 0.010), vitamin A deficiency (aOR 3.23, 95% CI 1.55–6.73, p = 0.002), and iodine insufficiency (aOR 1.67, 95% CI 1.09–2.55, p = 0.019), but not with iron deficiency (aOR 0.73, 95% CI 0.46–1.17, p = 0.195) or zinc deficiency (aOR 1.38, 95% CI 0.68–2.80, p = 0.377). Older age was independently associated with lower odds of anaemia (aOR 0.96 per month, p < 0.001) and iodine insufficiency (aOR 0.97 per month, p < 0.001). Inflammation (CRP ≥ 5.0 mg/L) was independently associated with more than double the odds of vitamin A deficiency (aOR 2.35, 95% CI 1.14 – 4.85, p = 0.020), consistent with the expected acute-phase suppression of circulating RBP4, even after BRINDA adjustment. Sex, malaria (by microscopy), and dietary diversity score were not independently associated with any of the five outcomes after adjustment. All variance inflation factors were below 1.3 across the five models (Methods), indicating no meaningful multicollinearity.

Table 8: Multivariable Logistic Regression for Micronutrient Deficiency Outcomes

Predictor	Anaemia	Vitamin A deficiency	Iron deficiency	Indicative iodine insufficiency	Zinc deficiency†
District (Bongo=1)	2.70 (1.27–5.75) *	3.23 (1.55–6.73) **	0.73 (0.46–1.17)	1.67 (1.09–2.55) *	1.38 (0.68–2.80)
Age (months)	0.96 (0.93–0.98) ***	0.98 (0.95–1.00)	1.00 (0.99–1.02)	0.97 (0.96–0.98) ***	1.00 (0.97–1.02)
Sex (Female=1)	0.98 (0.48–2.00)	1.34 (0.69–2.61)	0.68 (0.42–1.08)	0.90 (0.59–1.36)	0.73 (0.36–1.49)
Inflammation (CRP≥5)	0.75 (0.32–1.77)	2.35 (1.14–4.85) *	1.32 (0.75–2.31)	1.44 (0.85–2.44)	1.82 (0.84–3.94)
Malaria (microscopy+)	0.63 (0.17–2.34)	0.45 (0.10–2.10)	0.52 (0.17–1.56)	0.95 (0.41–2.20)	0.16 (0.01–2.67)
DDS (per unit)	1.21 (0.96–1.53)	1.15 (0.94–1.40)	0.95 (0.82–1.09)	0.99 (0.88–1.13)	1.09 (0.89–1.35)

*Values are adjusted odds ratios (95% confidence interval); *p<0.05, **p<0.01, ***p<0.001. n = 403 for all models.*

Table 9 presents the distribution of the multiple micronutrient deficiency burden score (0–4 deficiencies per child, combining iron deficiency, vitamin A deficiency, zinc deficiency and indicative iodine insufficiency; anaemia was kept separate as an outcome rather than a specific micronutrient deficiency). Overall, 117 children (29.0%) had none of the four deficiencies, 183 (45.4%) had exactly one, 103 (25.6%) had two or more, and 13 (3.2%) had three or more; the proportion with ≥2 concurrent deficiencies did not differ significantly by district (χ^2 (2) = 3.70, p = 0.157).

Table 9: Multiple Micronutrient Deficiency Burden Score by District

Number of deficiencies	Overall (N=403)	Bongo (n=202)	East Mamprusi District (n=201)
0	117 (29.0%)	50 (24.8%)	67 (33.3%)
1	183 (45.4%)	96 (47.5%)	87 (43.3%)
≥2	103 (25.6%)	56 (27.7%)	47 (23.4%)
≥3	13 (3.2%)	7 (3.5%)	6 (3.0%)
Mean (SD) burden score	1.00 (0.83)	1.06 (0.85)	0.93 (0.81)

Note. Burden score = count of iron deficiency (BRINDA-adjusted ferritin <12 µg/L), vitamin A deficiency (BRINDA-adjusted RBP4 <0.70 µmol/L), zinc deficiency (serum zinc <9.9 µmol/L) and indicative iodine insufficiency (Tg >40 µg/L) present in a given child (range 0–4). Anaemia is not included in the burden score. χ^2 test for district differences in the 0/1/≥2 distribution: $\chi^2(2) = 3.70$, $p = 0.157$.

Table 10 presents the pairwise co-occurrence of these four deficiencies; indicative iodine insufficiency co-occurred with iron deficiency more often than any other pair (15.1% of children), while co-occurrence involving zinc deficiency was comparatively uncommon.

Table 10: Pairwise Co-occurrence of Micronutrient Deficiencies (N=403)

Deficiency pair	n (of N=403)	%
Iron deficiency + indicative iodine insufficiency	61	15.1
Vitamin A deficiency + indicative iodine insufficiency	29	7.2
Zinc deficiency + indicative iodine insufficiency	16	4.0
Iron deficiency + vitamin A deficiency	12	3.0
Iron deficiency + zinc deficiency	6	1.5
Vitamin A deficiency + zinc deficiency	5	1.2

Note. Percentages are of the full sample (N=403) and are not mutually exclusive; a child can appear in more than one pair. Definitions as in Table 9.

A post-hoc power analysis for the five district-level comparisons is presented in Supplementary Table S1 rather than in the main results, in keeping with the emphasis below on effect sizes, precision and confidence intervals rather than on achieved power as the primary basis for interpretation. Only the vitamin A deficiency comparison had adequate power (87.3%) to detect the observed effect at conventional (80%) thresholds; the anaemia (42.9%), indicative iodine insufficiency (38.1%), iron deficiency (29.1%), and particularly the zinc deficiency (10.3%) comparisons were underpowered given the observed effect sizes and available sample size (Supplementary Table S1). This does not invalidate the significant findings reported, whose 95% confidence intervals (Tables 7-8) exclude the null despite low power and therefore likely reflect true, and possibly larger, effects but it means that the absence of a significant district difference

for iron and zinc deficiency, whose confidence intervals remain wide, should not be interpreted as evidence of no true difference; substantially larger samples (up to approximately 3,500 children per district for zinc deficiency) would be required to reliably detect differences of the observed magnitude.

Supplementary Material

Supplementary Table S1: Post-Hoc Power Analysis for District-Level Comparisons

Outcome	Bongo %	E. Mamprusi %	Effect size (h)	Achieved power	n/group needed for 80% power
Vitamin A deficiency	15.3	6.0	0.309	87.3%	165
Anaemia	93.6	88.6	0.177	42.9%	499
Iodine insufficiency†	58.9	50.7	0.165	38.1%	577
Iron deficiency	22.3	28.4	−0.140	29.1%	796
Zinc deficiency	9.9	8.0	0.067	10.3%	3,534

Effect size h = Cohen's effect size for proportions. Power computed using a two-proportion (normal approximation) test at α = 0.05, two-sided, with observed group sizes (n=202 Bongo; n=201 East Mamprusi District). †Iodine insufficiency is an indicative estimate only (see Methods).

Discussion

This study provides a district-level, multi-biomarker comparison of micronutrient status between the Bongo District of the Upper East Region and the East Mamprusi District District of the North East Region, two adjoining, broadly similar districts of northern Ghana. Two findings stand out and are developed below, alongside their implications for nutrition surveillance and programming more broadly. First, the overall burden of micronutrient vulnerability recorded in this sample when a harmonised panel spanning anaemia, iron, vitamin A, zinc and indicative iodine status is considered together, rather than any single indicator in isolation, is substantial, with one in four children carrying two or more concurrent deficiencies. Second, despite sharing a broadly similar rural, agro-ecological and socioeconomic setting, the two districts differed significantly on several biomarkers, most strikingly in the prevalence of vitamin A deficiency, which was more than twice as high in Bongo District as in East Mamprusi District District; national and even regional Ghanaian averages would have concealed this difference entirely. Comparisons drawn below to national Ghanaian and global estimates should be read with the caveat that surveys differ in child age ranges, biomarker definitions and cut-offs, survey period, and whether inflammation adjustment was applied, all of which can materially affect estimated prevalence; these comparisons are therefore illustrative of scale rather than strictly like-for-like.

Exceptional overall micronutrient vulnerability

The 91.1% anaemia prevalence recorded here substantially exceeds both the pooled global estimate of approximately 61% under the revised WHO 2024 criteria (7) and the most recent Ghana Demographic and Health Survey estimate of approximately 49% nationally (16), as well as the 2017 Ghana Micronutrient Survey (GMS) estimate of 35.6% (6). This gradient, global, to

national, to the present two-district sample mirrors a pattern already well documented in the Ghanaian literature, national averages substantially understate the burden observed in specific northern districts (19). It is also consistent with the finding, from a trend analysis spanning the 2003–2022 GDHS rounds, that regional inequalities in anaemia prevalence have widened rather than narrowed over the past two decades, with the gap between the highest and lowest prevalence Ghanaian regions increasing from 21.2 to 34.0 percentage points, and with the Northern and North East regions consistently ranking among the most affected nationally (17). The present findings extend this evidence by showing that the burden in Bongo and East Mamprusi District districts is not merely elevated relative to the national average but is comparable to, or exceeds, the most severe sub-national estimates previously reported for the neighbouring Tolon and Kumbungu districts, where anaemia prevalence was 36% (19) and for rural North East Ghana more broadly, where anaemia affected approximately 60% of young children (20). This very high burden was not attributable to the inclusion of infants below six months, for whom the applicability of the standard anaemia threshold is less certain: a sensitivity analysis restricted to children aged 6–59 months, directly comparable with the age range used in GDHS and GMS, yielded an almost identical anaemia prevalence (90.2%).

BRINDA-adjusted iron deficiency (25.3%) and IDA (23.3%) in this sample were likewise higher than the 2017 GMS national estimates of 21.5% and 12.2% respectively (6), and higher than global pooled estimates of 17.95% for iron deficiency and 16.42% for IDA among children under five (9). Functional iron deficiency, defined by elevated sTfR (53.3%), was substantially more prevalent than ferritin-defined iron deficiency in this sample, a divergence consistent with sTfR's greater sensitivity to early, tissue-level iron-deficient erythropoiesis relative to the depletion of iron stores captured by ferritin (36,44), and a reminder that reliance on a single iron biomarker can

materially under- or overstate the true burden depending on which stage of iron depletion it captures.

BRINDA-adjusted VAD in the pooled two-district sample (10.7%) was lower than the 2017 GMS national estimate of 20.8% (6) and below global and sub-Saharan African pooled estimates, which have ranged from approximately 19.5% to 33% depending on survey methodology and period (11). However, this reassuring pooled figure conceals substantial within-sample heterogeneity: VAD in Bongo District alone (15.3%) approached the national estimate, while East Mamprusi District District (6.0%) was considerably below it. This finding illustrates, at a finer geographic resolution than has previously been reported for northern Ghana, the same phenomenon documented at the regional level by previous authors that a single point estimate, whether national or even single-district, can obscure clinically and programmatically important variation among neighbouring populations (18,19)

Zinc deficiency in this sample (8.9%) was markedly lower than the serum-zinc-based estimate of 67% reported in the Tolon and Kumbungu districts (19) and below the 20–83% range typically reported across serum-zinc surveys in sub-Saharan Africa (12).

Iodine insufficiency (54.8% by thyroglobulin criteria) was marginally below the historical national salt-iodisation coverage target of 55% and considerably higher than the adequate urinary iodine status documented among school-age children in the Ashanti Region (median urinary iodine concentration 201.85 μg/L (21), though broadly consistent with the more persistent iodine insufficiency documented among toddlers in other northern Ghanaian communities (23). Because this study measured serum thyroglobulin rather than the urinary iodine concentration used in most published Ghanaian and global surveys, and because no internationally validated paediatric reference range for serum thyroglobulin currently exists (43,45), this iodine finding should be

interpreted with the important methodological caveat that the two biomarkers are not directly comparable.

Considered individually, none of these prevalences alone conveys the full picture of vulnerability in this population. When iron deficiency, vitamin A deficiency, zinc deficiency and indicative iodine insufficiency were combined into a simple multiple micronutrient deficiency burden score (0–4 deficiencies per child), 29.0% of children had none of these four deficiencies, 45.4% had exactly one, 25.6% had two or more, and 3.2% had three or more; the mean burden was modestly higher in Bongo District (1.06) than in East Mamprusi District District (0.93), though the district difference in the proportion with ≥2 concurrent deficiencies did not reach significance ($\chi^2(2) = 3.70$, $p = 0.157$). Indicative iodine insufficiency co-occurred with iron deficiency more often than any other pair (15.1% of children), followed by indicative iodine insufficiency with vitamin A deficiency (7.2%); iron deficiency with vitamin A deficiency, and zinc deficiency with either indicator, were comparatively uncommon (1.2–4.0%). In a multivariable model adjusting for district, age, sex, inflammation, malaria and dietary diversity, having two or more concurrent deficiencies was independently associated with younger age (aOR 0.98 per month, 95% CI 0.96–1.00, $p = 0.018$) and with inflammation (aOR 2.02, 95% CI 1.17–3.51, $p = 0.012$), with a similar-magnitude but non-significant association with Bongo residence (aOR 1.53, 95% CI 0.94–2.47, $p = 0.084$). These findings reinforce the conclusion, developed further below, that reliance on any single micronutrient indicator understates the true scale of nutritional vulnerability in this population, consistent with recent global pooled estimates that over half of preschool-aged children worldwide are deficient in at least one micronutrient and that coexisting deficiencies are common (46), and that integrated, multi-micronutrient responses are more evidence-based than single-nutrient programming.

Anaemia is not synonymous with iron deficiency

One of the more internationally relevant findings of this study is the marked discordance between anaemia and the biomarker-defined measures of iron status. If 91.1% of children were anaemic but only 25.3% were BRINDA-adjusted ferritin-defined iron deficient, 53.3% had elevated sTfR, and 23.3% met criteria for iron-deficiency anaemia specifically, then iron deficiency alone cannot account for the extraordinary anaemia burden observed in this sample: anaemia, iron deficiency and iron-deficiency anaemia are related but distinct constructs, and programmes that treat anaemia as a proxy for iron deficiency risk both overestimating the iron-specific burden and overlooking its other drivers. Plausible, non-mutually-exclusive contributors to this discordance, none of which this study directly measured or can confirm, include residual sub-clinical inflammation and infection not fully captured by a single CRP measurement, vitamin A status (given the known interaction between vitamin A and iron metabolism and erythropoiesis), inherited haemoglobinopathies such as sickle cell trait and thalassaemia, which are common in West Africa and were not screened for in this study, and other micronutrient deficiencies not assessed here, such as folate and vitamin B12. These should be regarded as hypotheses requiring direct investigation rather than established explanations. The practical implication is that anaemia-control programming premised primarily on iron supplementation and fortification is unlikely, by itself, to resolve the anaemia burden documented in this setting, and that anaemia surveillance in similar high-burden settings would benefit from routine multi-biomarker assessment rather than haemoglobin alone.

National and regional averages conceal local heterogeneity

The most outstanding finding of this study is the significant divergence between Bongo and East Mamprusi District districts in vitamin A status, haemoglobin, and adjusted RBP4 and

thyroglobulin concentrations, despite the two districts sharing a broadly similar rural and comparable overall poverty profile. Several non-mutually-exclusive explanations merit consideration.

First, differential coverage or timing of vitamin A supplementation and food fortification programmes between the two districts, which this study did not directly measure, could plausibly account for differences in vitamin A status; the wider Ghanaian literature has already noted that market-level compliance with mandatory vegetable oil and wheat flour fortification varies considerably even within a single country (6).

Furthermore, subtle differences in local dietary patterns for example in the consumption of vitamin A-rich foods such as red palm oil, dark green leafy vegetables, or orange-fleshed staples, which were not itemised in the dietary diversity score used here could differentiate the two districts even where overall household income and general food security appear similar.

Also, the climate-zone-stratified analysis of national Ghanaian data has previously shown that the relationship between iron deficiency, inflammation and anaemia can differ systematically even within a single agro-ecological belt (18); an analogous, more localised heterogeneity in the drivers of vitamin A and iron status may exist between Bongo and East Mamprusi District districts, consistent with the qualitative literature describing distinct community-level beliefs and practices around anaemia and child feeding across different Ghanaian communities (20). The absence of a significant district difference in zinc deficiency, iron deficiency, or IDA prevalence, set against the presence of a significant difference in vitamin A status and haemoglobin, further suggests that whatever distinguishes the two districts operates on some but not all micronutrient pathways, rather than reflecting a uniform difference in overall nutritional adequacy or general household deprivation between the two populations. This pattern reinforces the conclusion drawn from the

wider Ghanaian and sub-Saharan African literature that anaemia and micronutrient deficiency in this setting are multi-causal, and that community or district specific rather than uniform regional programming is required (18).

Implications for precision public health nutrition

Taken together, these findings support a precision public health nutrition approach to micronutrient surveillance and programming in northern Ghana, in which national surveillance is complemented by subnational biomarker surveillance, investigation of district-specific determinants, and tailored intervention packages that combine universal and district-specific actions (Fig. 2).

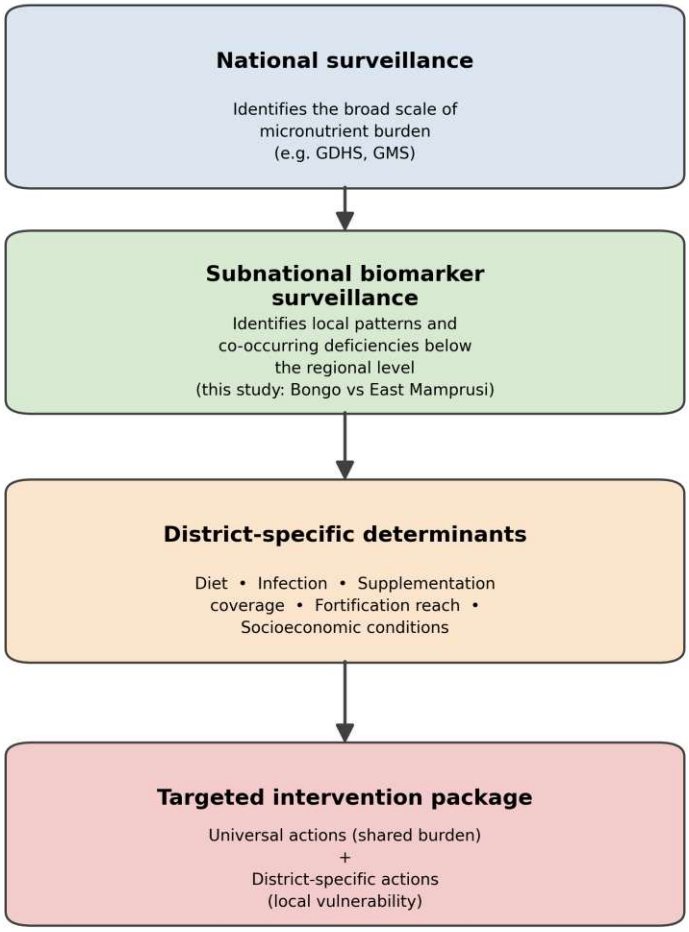


Fig. 2 *A precision public health nutrition framework for translating national surveillance into targeted district-level action, illustrated by the present study: national surveillance identifies the broad scale of the problem; subnational biomarker surveillance, as conducted here, identifies local patterns and coexisting deficiencies that national averages conceal; investigation of district-specific determinants explains why these patterns arise; and this evidence base informs a tailored intervention package combining universal and district-specific actions.*

Consistent with the age-stratified pattern already observed within this sample and with prior Ghanaian and international evidence, a comparable age gradient in anaemia risk has been documented in national Ghanaian survey data (17) and is consistent with the greater physiological iron and iodine demands of infancy and early complementary feeding, the potential for maternal micronutrient status to influence early infant status via breast milk, and the cumulative benefit of dietary diversification as children age (4).

The absence of a significant association between malaria positivity and any deficiency outcome, despite the well-established role of malaria as a driver of anaemia and inflammation in Ghana (6), is most plausibly explained by the comparatively low malaria positivity rate in this sample (6.5%), which limited statistical power to detect an effect; the bivariate association between malaria positivity and elevated ferritin nonetheless confirms the expected acute-phase relationship and reinforces the rationale for applying BRINDA inflammation adjustment when interpreting ferritin- and RBP4-based prevalence estimates in this and similar settings (39–41)

The substantial discrepancy between malaria prevalence by microscopy (6.5%) and by HRP2 antigen RDT (13.6%) merits further comment: microscopy and RDT results were significantly discordant (McNemar's $\chi^2 = 12.0$, $p < 0.001$), with far more children testing HRP2-positive than microscopy-positive, and RDT sensitivity relative to microscopy of only 53.8%. Because HRP2 antigen can persist in the circulation for two to four weeks after successful parasite clearance, this pattern is most consistent with the RDT detecting residual antigenaemia from recent, already-

treated infections rather than exclusively current parasitaemia, which limits its utility as a covariate for current inflammatory or nutritional status. For this reason, the more specific microscopy result was used as the primary malaria covariate in the multivariable models reported in Table 8; a sensitivity analysis substituting the HRP2 RDT result for microscopy did not materially change the conclusions for anaemia (aOR for RDT-positive malaria 1.36, 95% CI 0.44–4.21, p = 0.598) or vitamin A deficiency (aOR 0.40, 95% CI 0.13–1.29, p = 0.126). Malaria positivity did not differ significantly by district for either diagnostic method (microscopy: 7.4% Bongo vs 5.5% East Mamprusi District; RDT: 14.9% vs 12.4%; both p > 0.4), nor markedly by age group (microscopy 3.8%–9.6% across age bands; RDT 11.5%–14.5%), suggesting that the observed district differences in vitamin A and haemoglobin are unlikely to be confounded by differential malaria burden between districts.

Taken together, the age gradient in indicative iodine insufficiency and anaemia risk, the marked district-level divergence in vitamin A status between two ostensibly similar populations, and the substantial proportion of children carrying multiple concurrent deficiencies, point toward the value of a precision public health nutrition approach: not precision nutrition at the individual or genomic level, but the use of geographically and age-disaggregated biomarker data to target population-level interventions more efficiently, an approach with growing precedent in other domains of maternal and child nutrition surveillance in Africa (45). In this framing, national surveillance identifies the broad scale of the problem, strategically targeted subnational biomarker assessment identifies local patterns and coexisting deficiencies, and district-specific determinants of diet, infection, supplementation and fortification coverage inform a tailored intervention package. Practically, this distinction separates universal actions from targeted ones. The shared, high burden of anaemia and functional iron deficiency across both districts, together with the substantial multiple-deficiency burden documented here, supports universal actions continued anaemia and

iron-deficiency control, and integrated multi-micronutrient supplementation or fortification operating across both districts. The substantially higher vitamin A deficiency and indicative iodine insufficiency burden in Bongo District, by contrast, supports targeted actions: investigation of, and where warranted strengthening of, district-specific vitamin A supplementation coverage, dietary diversification promotion, and fortification reach in Bongo District specifically, alongside further validation of the indicative iodine findings. The youngest children, in whom indicative iodine insufficiency and low haemoglobin were most pronounced, represent a further age-based target for intensified intervention during infancy and the complementary-feeding period, a critical window during which nutritional insults are least reversible.

Strengths and Limitations

This study's principal strengths are its direct, biomarker-based comparison of two northern Ghanaian districts using a harmonised protocol and laboratory pipeline, its systematic application of BRINDA inflammation adjustment to ferritin and RBP4 (39–41), and its use of multiple complementary iron indicators (ferritin and sTfR) that together capture different stages of iron depletion (44).

Several limitations should be considered when interpreting these findings. First, the cross-sectional design precludes causal inference; longitudinal or intervention designs would be required to establish temporal and causal relationships (24) and the purposive selection of the two districts could limit generalisability to northern Ghana or Ghana as a whole. Also, iodine status was assessed using serum thyroglobulin rather than the urinary iodine concentration used in the great majority of comparable Ghanaian and global surveys, and applied a provisional, study-specific cut-off in the absence of a validated paediatric serum/plasma thyroglobulin reference range (43,45); the iodine findings reported here should therefore be regarded as indicative rather than directly comparable to prior urinary-iodine-based Ghanaian estimates, and future work should consider paired urinary iodine and thyroglobulin assessment to validate this approach in this population. Furthermore, dietary assessment relied on a summary dietary diversity score rather than quantitative or food-specific intake data, limiting the ability to identify the specific dietary drivers of the observed district-level differences in vitamin A status. Moreover, information on vitamin A supplementation history, food fortification exposure, and intestinal parasitism, all

plausible confounders or mediators of micronutrient status, was not incorporated. Again, purposive district selection and caregiver-reported dietary/infection recall were a potential source of selection or recall biases.

Also, the post-hoc power analysis (Supplementary Table S1) indicated that this study, although adequately powered for its primary pooled-sample anaemia estimate, was underpowered for several of the district-level comparisons that motivated its design, particularly for zinc deficiency (achieved power 10.3%) and, to a lesser extent, iron deficiency (29.1%), anaemia (42.9%) and iodine insufficiency (38.1%) at the district level; non-significant district differences for these outcomes should accordingly be interpreted as inconclusive rather than as evidence of no true difference. The sample size calculation was based on the pooled national anaemia prevalence rather than on the study's primary comparative aim, which likely explains this shortfall.

Furthermore, this study did not screen for inherited haemoglobinopathies (such as sickle cell disease and thalassaemia), which are prevalent in West Africa and can independently cause or exacerbate anaemia irrespective of iron status; population-based estimates suggest that sickle cell trait alone affects up to a quarter of individuals in parts of Ghana, and haemoglobinopathy status was therefore not available to adjust for in this analysis. This may account for some of the haemoglobin variation not explained by iron or vitamin A status. Again, although sociodemographic data on maternal age and household size were collected, this study did not incorporate a household wealth index, maternal educational attainment beyond a binary indicator, or household food security status, each an established correlate of childhood micronutrient status; these variables should be prioritised in future rounds of this research programme.

Conclusions

This study demonstrates that a high burden of childhood micronutrient deficiency can coexist with substantial heterogeneity between neighbouring, socioeconomically similar populations: despite sharing a broadly similar rural, agro-ecological and socioeconomic profile, Bongo and East Mamprusi District districts differed significantly in haemoglobin, adjusted RBP4, thyroglobulin and vitamin A deficiency prevalence, with Bongo District bearing more than twice the burden of vitamin A deficiency, and a higher burden of anaemia and indicative iodine insufficiency, than East Mamprusi District District. The marked discordance between anaemia, iron-status biomarkers (ferritin and sTfR) and iron-deficiency anaemia, and the finding that one in four children carried two or more concurrent micronutrient deficiencies, further illustrate the limitations of relying on any single indicator to characterise micronutrient vulnerability in this population.

Zinc deficiency, iron deficiency and IDA showed no significant district-level differences, although the district-level comparisons for these outcomes were underpowered (Supplementary Table S1) and should not be read as evidence of no true difference. While national and regional surveys remain essential for monitoring population nutrition, these findings suggest that strategically targeted subnational biomarker surveillance can identify pockets of vulnerability that national and regional averages conceal, and could support more geographically responsive combinations of supplementation, fortification, dietary and infection-control interventions. Specifically, the shared high burden of anaemia and functional iron deficiency across both districts supports universal, district-wide anaemia and iron-deficiency control, whereas the substantially higher vitamin A deficiency and indicative iodine insufficiency burden in Bongo District supports investigation of,

and where warranted, intensified district-specific vitamin A supplementation, dietary diversification and fortification coverage. Further studies incorporating representative cluster designs that retain community-level identifiers, validated iodine biomarkers, and more detailed dietary and programme-exposure measurement are needed to establish the drivers of these local disparities.

List of abbreviations

BD - Bongo District
BRINDA - Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia
CHRPE - Committee on Human Research, Publications and Ethics
CLSI - Clinical and Laboratory Standards Institute
CRP - C-reactive protein
DDS - Dietary Diversity Score
ELISA - Enzyme-Linked Immunosorbent Assay
EMD - East Mamprusi District District
FBC - Full Blood Count
GBD - Global Burden of Disease
Hb - Haemoglobin
HRP2- Histidine-Rich Protein 2
IDA - Iron Deficiency Anaemia
IQR - Interquartile Range
IZiNCG - International Zinc Nutrition Consultative Group
KHRC - Kintampo Health Research Centre
M - Mean
RBP4 - Retinol-Binding Protein 4
RDT - Rapid Diagnostic Test
SD - Standard Deviation
SPSS - Statistical Package for Social Sciences
sTfR - Soluble Transferrin Receptor
Tg - Thyroglobulin
UIC - Urinary Iodine Concentration

VAD - Vitamin A Deficiency

WHO - World Health Organization

Declarations

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Committee on Human Research, Publications and Ethics (CHRPE) of the Kwame Nkrumah University of Science and Technology (KNUST), School of Medical Sciences, and the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana (Reference number *CHRPE/AP/181/24*), with additional site-level approvals from the district health directorates of Bongo and East Mamprusi District of the Ghana Health Service *(GHS/UER/ORD/RES/001030)*. For child participants, written informed consent was obtained from care givers.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. The authors will consider depositing a de-identified version of the dataset in a public repository

Competing interests

The authors affirm that they have no competing interests. Technical and logistical support received had no conditions that could affect the outcomes of this study.

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Authors' contributions

All authors contributed to the study's conceptualization, design, and interpretation of findings. D.A.N. conceived the study, designed the study, drafted the research proposal and sought for ethical approval, and led all data collection processes, performed statistical analysis, interpreted the results and drafted the manuscript. R.A.A., C.A. and M.A.T. supervised data collection and statistical analysis processes, coordinated and facilitated blood sample analyses, nutritional status assessments and reviewed the manuscript.

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Appendix I: Participant Recruitment Flow Chart

Bongo District and East Mamprusi District District

Four Communities Per District, With Exclusions Shown at Each Community

Bongo		**East Mamprusi District District**	
Community 1: Anafobiisi	**n**	**Community 1: Sumninboma No. 1**	**n**
Households enumerated	72	Households enumerated	87
Children screened	61	Children screened	81
Confirmed eligible & consented	56	Confirmed eligible & consented	73
Excluded		**Excluded**	
• *Age-ineligible / no age record*	4	• *Age-ineligible / no age record*	1
• *Non-resident / temporary settler*	0	• *Non-resident / temporary settler*	1
• *Caregiver declined consent*	1	• *Caregiver declined consent*	1
• *Acutely ill / other exclusion*	0	• *Acutely ill / other exclusion*	0
• *Inadequate sample ×2 attempts*	7	• *Inadequate sample ×2 attempts*	4
Total excluded in Anafobiisi	12	**Total excluded in Sumninboma No. 1**	7
Analysed, Anafobiisi	**44**	Analysed, Sumninboma No. 1	**66**
↓ Community 1 → Community 2 ↓			
Community 2: Balungu		**Community 2: Sakogu**	
Households enumerated	78	Households enumerated	89
Children screened	72	Children screened	83
Confirmed eligible & consented	67	Confirmed eligible & consented	64
Excluded		**Excluded**	
• *Age-ineligible / no age record*	0	• *Age-ineligible / no age record*	3
• *Non-resident / temporary settler*	0	• *Non-resident / temporary settler*	0
• *Caregiver declined consent*	0	• *Caregiver declined consent*	0
• *Acutely ill / other exclusion*	1	• *Acutely ill / other exclusion*	0
• *Inadequate sample ×2 attempts*	4	• *Inadequate sample ×2 attempts*	8
Total excluded in Balungu	5	Total excluded Sakogu	11
Analysed, Balungu	**62**	Analysed, Sakogu	**53**
↓ Community 2 → Community 3 ↓			
Community 3: Balungu doone		**Community 3: Dindani**	
Households enumerated	92	Households enumerated	59

Children screened	78	Children screened	54
Confirmed eligible & consented	71	Confirmed eligible & consented	49
Excluded		**Excluded**	
• *Age-ineligible / no age record*	3	• *Age-ineligible / no age record*	1
• *Non-resident / temporary settler*	1	• *Non-resident / temporary settler*	2
• *Caregiver declined consent*	2	• *Caregiver declined consent*	1
• *Acutely ill / other exclusion*	2	• *Acutely ill / other exclusion*	1
• *Inadequate sample ×2 attempts*	9	• *Inadequate sample ×2 attempts*	2
Total excluded in Balungu doone	17	Total excluded in Dindani	7
Analysed, Balungu doone	**54**	Analysed, Dindani	**42**
↓ **Community 3 → Community 4** ↓			
Community 4: Nabiisi		**Community 4: Naminboku**	
Households enumerated	77	Households enumerated	67
Children screened	58	Children screened	58
Confirmed eligible & consented	54	Confirmed eligible & consented	51
Excluded		**Excluded**	
• *Age-ineligible / no age record*	3	• *Age-ineligible / no age record*	2
• *Non-resident / temporary settler*	2	• *Non-resident / temporary settler*	1
• *Caregiver declined consent*	3	• *Caregiver declined consent*	1
• *Acutely ill / other exclusion*	1	• *Acutely ill / other exclusion*	1
• *Inadequate sample ×2 attempts*	3	• *Inadequate sample ×2 attempts*	6
Total excluded in Nabiisi	12	Total excluded in Naminboku	11
Analysed, Nabiisi	**42**	Analysed, Naminboku	**40**
↓ Sum across 4 communities ↓			
District total analysed (Bongo):	**202**	**District total analysed (East Mamprusi District):**	**201**
↓ Combine both districts ↓			
Combined analytic sample: N = 403			
(202 Bongo + 201 East Mamprusi District)			

Appendix II: STROBE Statement — Checklist of Items for Cross-Sectional Studies

Manuscript: Burden and Subnational Disparities in Micronutrient Deficiencies Among Children Aged 1–60 Months in Northern Ghana: A Biomarker-Based Comparative Study

Item	STROBE Recommendation	Status	Location in Manuscript
1a	Indicate the study's design with a commonly used term in the title or the abstract	**Reported**	Abstract, Methods ("community-based cross-sectional survey")
1b	Provide in the abstract an informative and balanced summary of what was done and what was found	**Reported**	Abstract (structured: Background/Methods/Results/Conclusions)
2	Explain the scientific background and rationale for the investigation being reported	**Reported**	Introduction, paras 1–6
3	State specific objectives, including any prespecified hypotheses	**Reported**	Introduction, final paragraph
4	Present key elements of study design early in the paper	**Reported**	Methods, "Study Design and Setting," para 1
5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	**Reported**	Methods, "Study Design and Setting" / "Sampling procedure"
6a	Give the eligibility criteria, and the sources and methods of selection of participants	**Reported**	"Study Population and Eligibility"; "Sampling procedure"
6b	(Cohort/case-control only – not applicable to this cross-sectional design)	**N/A**	—
7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers; give diagnostic criteria where applicable	**Reported**	"Laboratory Analyses and Biomarker Definitions"; "Socio-Demographic, Dietary and Infection Questionnaire"
8	For each variable of interest, give sources of data and details of	**Reported**	"Blood Sample Collection"; "Laboratory Analyses and Biomarker Definitions"

Item	STROBE Recommendation	Status	Location in Manuscript
	methods of assessment (measurement); describe comparability of assessment methods if more than one group		
9	Describe any efforts to address potential sources of bias	**Reported**	Discussion (HRP2-vs-microscopy discordance; BRINDA inflammation adjustment)
10	Explain how the study size was arrived at	**Reported**	"Sample size determination"
11	Explain how quantitative variables were handled in the analyses; describe which groupings were chosen and why	**Reported**	Tables 1, 3, 6 and "Laboratory Analyses and Biomarker Definitions" (age bands, deficiency cut-offs)
12a	Describe all statistical methods, including those used to control for confounding	**Reported**	"Statistical Analysis"
12b	Describe any methods used to examine subgroups and interactions	**Reported**	Tables 5–6 (sex, age subgroup comparisons)
12c	Explain how missing data were addressed	**Reported**	All missing data issues were traced to the raw data and resolved
12d	If applicable, describe analytical methods taking account of sampling strategy	**Reported**	"Sampling procedure"; "Statistical Analysis" (ICC/cluster-SE limitation noted)
12e	Describe any sensitivity analyses	**Reported**	Table 4 note (alternate BRINDA reference group); Discussion (HRP2-vs-microscopy sensitivity analysis)
13a	Report the numbers of individuals at each stage of the study — e.g., numbers potentially eligible, examined, confirmed eligible, included, completing follow-up, analysed	**Reported**	Captured in Sampling Procedure and Participant Flow Chart (Appendix I)
13b	Give reasons for non-participation at each stage	**Reported**	Contained in Sampling Procedure
13c	Consider use of a flow diagram	**Reported**	Participant Recruitment Flow Chart at Appendix I

Item	STROBE Recommendation	Status	Location in Manuscript
14a	Give characteristics of study participants and information on exposures and potential confounders	**Reported**	Table 1
14b	Indicate the number of participants with missing data for each variable of interest	**Reported**	All missing data issues were traced to the raw data and resolved
14c	(Cohort only – not applicable)	**N/A**	—
15	Report numbers of outcome events or summary measures	**Reported**	Table 3 (deficiency prevalences); Tables 5–7 (subgroup/district breakdowns)
16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% CI)	**Reported**	Table 7 (unadjusted district comparisons); Table 8 (adjusted odds ratios with 95% CI)
16b	Report category boundaries when continuous variables were categorized	**Reported**	Methods (anaemia severity bands, VAD/marginal RBP4 cut-points); Table 1/6 (age bands)
16c	If relevant, translate estimates of relative risk into absolute risk for a meaningful time period	**N/A**	Not applicable: cross-sectional prevalence study, no time-to-event estimates.
17	Report other analyses done — e.g., subgroup, interaction, sensitivity analyses	**Reported**	Tables 5–6 (subgroup); Table 4 note and Discussion (sensitivity); Supplementary Table S1 (post-hoc power)
18	Summarise key results with reference to study objectives	**Reported**	Discussion, opening paragraph; Conclusions
19	Discuss limitations, sources of potential bias or imprecision; discuss direction and magnitude of any potential bias	**Reported**	“Strengths and Limitations”
20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**Reported**	Discussion (throughout, with explicit Bonferroni/power caveats)
21	Discuss the generalisability (external validity) of the study results	**Reported**	Discussion (comparisons to national/global estimates)

Item	STROBE Recommendation	Status	Location in Manuscript
			Limitation reported generalization of findings
22	Give the source of funding and the role of the funders for the present study	**Reported**	"Funding" declaration