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Anemia among Children Exposed to Polyparasitism in Coastal Kenya

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Abstract. Anemia represents a substantial problem for children living in areas with limited resources and significant parasite burden. We performed a cross-sectional study of 254 Kenyan preschool- and early school-age children in a setting endemic for multiple chronic parasitic infections to explore mechanisms of their anemia. Complete venous blood cell counts revealed a high prevalence of local childhood anemia (79%). Evaluating the potential links between low hemoglobin and socioeconomic factors, nutritional status, hemoglobinopathy, and/or parasite infection, we identified age < 9 years (odds ratio [OR]: 12.0, 95% confidence interval [CI]: 4.4, 33) and the presence of asymptomatic malaria infection (OR: 6.8, 95% CI: 2.1, 22) as the strongest independent correlates of having anemia. A total of 130/155 (84%) of anemic children with iron studies had evidence of iron-deficiency anemia (IDA), 16% had non-IDA; 50/52 of additionally tested anemic children met soluble transferrin-receptor (sTfR) criteria for combined anemia of inflammation (AI) with IDA. Children in the youngest age group had the greatest odds of iron deficiency (OR: 10.0, 95% CI: 3.9, 26). Although older children aged 9–11 years had less anemia, they had more detectable malaria, *Schistosoma* infection, hookworm, and proportionately more non-IDA. Anemia in this setting appears multifactorial such that chronic inflammation and iron deficiency need to be addressed together as part of integrated management of childhood anemia.

INTRODUCTION

Childhood anemia remains a significant global health problem that is estimated to affect over 25% of the world's population.[1] Because of its impact on growth, development, and fitness, anemia can have a major impact on individual human health as well as the social and economic success of families and communities in resource-limited countries.[2] Young children are the particularly vulnerable population, with 47% of preschool children currently living with anemia.[2] Morbidity associated with anemia derives from reduced oxygen supply to the tissues, which is most critical during pregnancy and early childhood, when metabolic demand is high. Early childhood iron deficiency and anemia have been particularly associated with impaired cognition and poor learning ability.[3,4] It is widely assumed that most of the cases of anemia are due to iron-deficiency anemia (IDA), which can result from poor dietary intake and be exacerbated by blood loss (e.g., menstruation in older girls and hookworm infection).[1] Non-iron deficiency anemia (NIDA) in malaria-risk zones is often attributed to hereditary hemoglobinopathies (sickle cell anemia, thalassemia), but increasing evidence indicates that a large burden of NIDA is associated with pro-inflammatory states caused by chronic infections.[5] Anemia of inflammation (AI), sometimes called anemia of chronic disease, appears to overlap with IDA on a frequent basis. The resulting anemia, having multiple etiologies, often requires micronutrient supplementation as well as treatment of the underlying infections to achieve successful therapy.[3,6,7] In practical terms, the measurement of two serum biomarkers, serum ferritin (SF) and soluble transferrin receptor (sTfR) has proven useful in determining whether IDA, AI, or a combination of the mechanisms is responsible for anemia in the presence of various chronic infections.[8]

Polyparasitism is a common condition that occurs in less-developed areas in which a person experiences disease from two or more concurrent, chronic infections with helminthic and/or protozoan parasites.[9,10] Polyparasitic diseases due to overlapping soil-transmitted helminth (STH, hookworm, ascariasis, and trichuriasis) infections have a well-documented association with anemia and impaired growth.[7,11] As a result, current international World Health Organization (WHO)-endorsed efforts include early treatment of 2- to 5-year-old children with the antihelminthic drugs albendazole or mebendazole against STH to prevent anemia-related morbidity.[11–13] Cross-sectional studies have also suggested synergism between *Schistosoma* and *Plasmodium* infections in risk for anemia.[14] With more sensitive testing, there is greater evidence that *Schistosoma* infection begins in very early childhood.[15–17] While children under 5 years are not yet included in WHO preventive chemotherapy protocols,[18] anti-schistosomal treatment with praziquantel for preschool children could be easily synergized with the ongoing STH early childhood strategies for the prevention of anemia.[18] The aim of this study was to use more in-depth laboratory testing to identify the most likely mechanisms for anemia among children in a resource-limited area endemic for multiple chronic parasitic infections, with the goal of informing local and regional choices for management of early childhood anemias.

MATERIALS AND METHODS

Ethical oversight and informed consent. This study was conducted in Jego village in Lunga Lunga Constituency, Kwale County, Kenya, in 2012 as part of a community-wide survey of parasitic infections.[10,19] All resident children aged 3–11 years were eligible for enrollment in this sub-study of anemia. For these participating children, written informed consent was obtained from their parents, and all children over the age of 7 years also provided their individual assent to participate. The study protocol was approved and supervised by the Ethical Review Committee of the Kenya Medical Research Institute (protocol non-SSC 087) and the Institutional

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Review Board of Case Western Reserve University (protocol 11-07-42).

Study population enrollment and screening. After consent, demographic information was obtained by household questionnaire, and parasitic infection status was determined by centralized testing for malaria, hookworm, filariasis (*Wuchereria bancrofti* infection), and *Schistosoma haematobium* infection (see Diagnosis of parasite infections, below).[10,19] At the household level, information was obtained on global positioning system (GPS) location, type of construction, inventory of indicator possessions (for estimation of socioeconomic status[10]), and the demography of the household residents. Each consenting subject was assigned a unique identification number. By census, there were 868 children aged 3–11 reside in Jego village in 2012. A total of 307 children in this target age range were enrolled in this study of polyparasitism, and for this nested anemia sub-study, 276 (mean age: 7.3 years, range: 3–11 years) had blood drawn for anemia testing, and of those, 254 completed all phases of examinations and automated blood count testing. Subject anthropometrics (height, weight, mid-arm circumference, triceps skinfold thickness) were obtained by trained qualified technicians, and those ambulatory children older than 5 years were tested for physical fitness in the 20-m shuttle-run test.[19]

Diagnosis of parasite infections. Laboratory testing included point-of-care testing for malaria (ICT Diagnostics, Sydney, Australia) and filaria (Binax, Portland, ME), standardized urine filtration for *S. haematobium*[20] and stool Kato-Katz testing[21] for intestinal worms, including hookworm. Anti-*Schistosoma* IgG4 and anti-filarial IgG4, markers of active or very recent infection by these helminth parasites, were detected and quantified by specimen-sparing multiplex flow bead immunoassay as previously described.[22]

Testing for anemia. Venous whole blood (4–5 mL) was collected from each subject by venipuncture. The sample was divided in two microtainers, 0.5 mL in EDTA microtainer for complete blood count and reticulocyte measurement, 0.5 mL in EDTA microtainer for hemoglobin electrophoresis, and the remaining 3–4 mL whole blood and serum were separated and frozen for transport to the United States for serum iron, iron-binding, haptoglobin, ferritin, IgG4 antibody, and sTfR measurements. For all participants, hemoglobin was initially measured on site by point-of-care testing using a Hemocue spectrophotometer (Hemocue, Angelholm, Sweden), followed later the same day by automated complete blood cell counts (Beckman Coulter, Brea, CA), and a microscopic reticulocyte count performed at Msambweni Hospital.

Additional laboratory testing was performed at University Hospitals Laboratory Services (Cleveland, OH) for serum iron, total iron-binding capacity, haptoglobin, and ferritin, and hemoglobin electrophoresis was performed on stored red cell pellets to quantify subject's levels of hemoglobins A, A2, S, C, and F. To correctly classify the status of subjects with features of AI,[8] sTfR levels were assayed for those children who had intermediate SF levels (30–100 μg/L). sTfR was assayed using a commercial transferrin receptor enzyme-linked immunosorbent assay (Eagle Biosciences, Nashua NH).

Statistical analysis. Demographic data collected in the field were double entered in hand-held devices (Dell Axim, Round Rock, TX) using Visual CE 10 (Cambridge, MA) and a paper form. Data were then transferred in duplicate into ACCESS 2007 (Microsoft, Seattle, WA) and the databases compared for errors. Parasitology, anthropometric data, and anemia-related laboratory test results were similarly entered to complete the database.

Our primary outcome, anemia, was defined as Hb < 11.5 g/dL in accordance with WHO guidelines for children < 12 years.[23] In analysis of potential moderating factors, *S. haematobium* infection was defined as either having *S. haematobium* eggs on urine filtration, having a positive anti-*Schistosoma* IgG4 serum antibody test, or having both. Filarial infection was defined as having either a positive circulating antigen test (Binax), or a positive anti-*Brugia* IgG4 serum antibody test, or both. IDA was defined as the presence of anemia (Hb < 11.5 g/dL) and a SF < 30 ng/mL, or a total iron-binding capacity > 400 μg/dL and transferrin saturation < 20%. AI, also called anemia of chronic disease, was defined as the presence of anemia, SF > 100 ng/dL, or for those with SF 30–100 ng/dL, sTfR to log (ferritin) ratio of less than one.[8] Finally, mixed AI with accompanying iron deficiency was defined as the presence of anemia, a SF of 30–100 ng/dL, and a sTfR/log SF ratio > 2.[8]

In initial exploratory analysis, bivariate χ^2 testing was used to evaluate the association between categorical variables and the presence or absence of anemia, and Student's *t* test was used for significance testing in group-wise comparisons of continuous data. Data not normally distributed were analyzed using the Mann–Whitney *U* test. Differences among multiple age groups for continuous outcomes were evaluated using analysis of variance (ANOVA) with post hoc pairwise comparisons using Tukey's method, or the Kruskal–Wallis test, as appropriate. Next, multiply-adjusted OR for anemia outcomes were estimated using logit-distributed generalized linear estimating equation (GEE) models that accounted for intra-class correlation at the household level. For these multivariable models, backward selection of demographic, exposure, and infection variables was used, and significance for inclusion was set at the 0.05 level. All analyses were performed using SPSS v.22 software (IBM, Armonk, NY). *P* values less than 0.05 were considered significant.

RESULTS

Study population. Demographic analysis of the participating 3- to 11-year-old subjects from Jego Village is shown in Table 1. Compared with the overall 3- to 11-year-old 2012 census population (*N* = 868), the 307 children whose parents agreed to have them participate in our survey of polyparasitism[10] were somewhat older (mean age: 7.3 versus 6.6 years old) with underrepresentation of children under age 4. Among the 307 polyparasitism study subjects who either did (*N* = 254) or did not (*N* = 53) participate in the anemia sub-study reported here; there were no significant between-group differences in terms of average age, sex distribution, number of household residents, infection prevalence, stunting, wasting, or anemia as detected on screening point-of-care testing (Table 1). Because of cost constraints, just the first 254/307 enrolled subjects received full laboratory testing. Later, of the 254 subjects initially tested for anemia by laboratory-based complete blood counts, the iron status, serum ferritin, and hemoglobin electrophoresis were measured for 191 individuals (a random selection of 155/201 anemic children and 36/53 non-anemic children) and sTfR was determined only for the 73 individuals who had SF values in the 30–100 ng/dL range.

TABLE 1
Demographic characteristics of participants and eligible nonparticipants aged 3–11 years for the anemia sub-study performed in Jego Village, 2012

Characteristic	Village census, $N = 868$	Enrolled in 2012 polyparasitism survey, $N = 307$	Included in anemia sub-study, $N = 254$	Not included in anemia sub-study, $N = 53$
Mean age ± SD (range)	6.6 ± 2.5 (3–11)	7.3 ± 2.6 (3–11)	7.3 ± 2.3 (3–11)	7.1 ± 3.5 (3–11)
M:F ratio	0.97	0.99	0.94	1.30
Median residents per house (range)	8 persons (2–19)	8 persons (3–19)	8 persons (3–19)	10 persons (3–17)
Median number of children < 17 year residing per house (range)	3 children (1–11)	5 children (1–11)	5 children (1–11)	6 children (1–11)
Stunted* (%)	–	117 (38)	92 (36)	25 (47)
Wasted† (%)	–	25 (8)	21 (8)	4 (8)
Anemic‡ (%)	–	173 (56)	145 (57)	28 (53)
Malaria (%)	–	87 (28)	73 (29)	12 (22)
Filaria (%)	–	4 (1)	2 (1)	2 (4)
Hookworm (%)	–	45 (15)	38 (17)	6 (11)
Schistosoma haematobium egg positive (%)§	–	20 (7)	15 (6)	5 (9)

F = female; M = male; SD = standard deviation.
*Height for age z score (HAZ) < −2.
†Body mass index (BMI) for age z score (BAZ) < −2.
‡Hemoglobin < 11.5 g/dL by point-of-care testing (Hemocue cassette).
§During the initial screening surveys, only egg filtration was performed to obtain the comparative results presented here. Later prevalence values for the anemia study sub-group (Table 2) included more sensitive diagnostic results from serological testing for anti-*Schistosoma* antibodies.

Results of hematological testing. Automated complete blood counts performed on anticoagulated venous blood revealed that 201/254 (79%) of study children were anemic (Hb < 11.5 g/dL) and 20 (8%) had severe anemia (Hb < 9 g/dL). Thirty (12%) had hemoglobin S (HbS) trait detected at levels of 23–36% on electrophoresis, and of these, 23 (77%) were anemic, but only 2/30 (7%) of this group with HbS trait had low haptoglobin levels (< 26 mg/dL) suggestive of active hemolysis. No homozygous HbS cases were found, and no child had hemoglobin C detected. Among the 31 subjects who were detected with low haptoglobin levels, 26 (81%) were positive on malaria rapid diagnostic testing (RDT), suggesting active subclinical *Plasmodium* infection as the probable cause for hemolysis. Of note, when reticulocyte levels were scored for those children having hemoglobin levels ≤ 10 g/dL, none had greater than 2% reticulocytosis, suggesting chronic ongoing suppression of erythrocytogenesis.

Twenty-six (10%) of all tested children had erythrocytosis (elevated total red blood cells [RBCs]) with microcytosis (low MCV) suggestive of thalassemia minor, and of these, 16/26 (61%) had mild anemia. There were no cases of severe anemia among this group. No child had red cell macrocytosis or hyperchomasia (elevated MCV or MCHC, respectively).

Prevalence of parasitic infections. Figure 1 shows the prevalence of the most common parasitic infections and polyparasitism (two or more parasitic infections) for the different age groups within the study cohort. Malaria was the most prevalent infection overall (29%), followed by *S. haematobium* (28%), *W. bancrofti* (25%), and hookworm (15%). (N.B.: These prevalence values for *S. haematobium* and for *W. bancrofti* are higher than those reported in Table 1 because they include additional infected subjects detected by supplemental post-survey serological testing [see Methods]) Polyparasitism was found in 28% of the study children. Prevalence of each infection and polyparasitism increased significantly with age. Among the 3- to 5- , 6- to 8-, and 9- to 11-year age groups, there was a progressive increase in *S. haematobium* prevalence by age (χ^2 for trend, 9.07, $P = 0.003$) and a progressive increase in filarial infection (χ^2 for trend, 7.61, $P = 0.006$) and polyparasitism (χ^2 for trend, 9.07, $P = 0.003$). Malaria and hookworm were more common among children over the age of 5 years; however, the observed age group differences were not statistically significant for these parasites.

Association between anemia and age, sex, infection, and/or household factors. Table 2 shows the associations between anemia and individual subject characteristics in terms of demographics, nutritional status, and the presence of chronic parasitic infections. Odds of anemia and of severe anemia were significantly higher among children under 9 years of age and among those having positive malaria tests. In univariate analysis, we noted reduced odds of any anemia among those with filarial infection or with RBC parameters suggestive of thalassemia trait. In subsequent multiply-adjusted logistic regression models, a younger age (≤ 8 years) and the presence of subclinical malaria (by RDT) remained significant independent predictors of anemia. The multiply-adjusted odds ratio (aOR) for young age was 12 (95% CI: 4.4, 33) and the aOR for malaria was 6.8 (95% CI: 2.1, 22).

Anemia, iron status, and inflammatory markers by age group. Table 3 details the key automated blood count (CBC) data, along with average serum iron and ferritin studies, haptoglobin levels, and sTfR levels for three different age groups

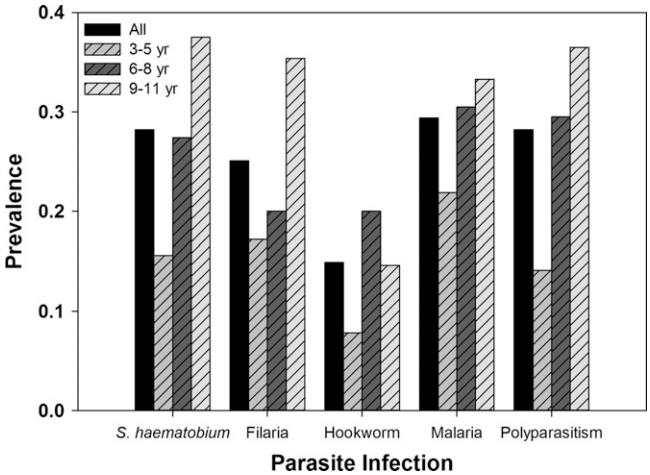

FIGURE 1. Parasite infection prevalence by age group. The vertical bars indicate the prevalence of different parasitic infections detected in the study cohort. Values as shown for the group at large (all children) and for those in the 3- to 5-year, 6- to 8-year, and 9- to 11-year subgroups.

TABLE 2
Associations of anemia or severe anemia with personal and environmental factors

Factor	Any anemia*		Severe anemia†	
	Unadjusted bivariable OR (95% CI)	Multiply adjusted OR (95% CI)‡	Unadjusted bivariable OR (95% CI)	Multiply adjusted OR (95% CI)‡
Male sex	1.69 (0.9, 3.1)		2.6 (0.97, 7)	
Age < 9 years	**7.53 (3.4, 16.8)**	**12.0 (4.4, 33)**	2.1 (0.82, 5.6)	**3.0 (1.04, 8.7)**
Sickle hemoglobin trait	0.70 (0.27, 1.8)	–	0.63 (0.14, 2.8)	–
Thalassemia minor	**0.38 (0.16, 0.89)**	0.23 (0.05, 1.1)	–	–
Schistosoma infection§	0.74 (0.38,1.4)	–	0.61 (0.2, 1.9)	–
Filarial infection‖	**0.38 (0.2, 0.73)**	–	0.73 (0.23, 2.3)	–
Hookworm infection¶	0.84 (0.37, 1.9)	–	1.00 (0.28, 3.6)	–
Malaria infection**	**3.4 (4.5, 8.0)**	**6.8 (2.1, 21.9)**	**2.6 (1.04, 6.6)**	2.5 (0.9, 6.8)
Polyparasitism	0.66 (0.35, 1.3)	–	1.1 (0.41, 3.0)	–
Stunting††	**1.96 (1.01, 3.8)**	–	2.2 (0.86, 5.4)	–
Wasting‡‡	1.17 (0.38, 3.6)	6.0 (0.57, 63)	0.56 (0.1, 4.4)	–
Low SES§§	0.98 (0.52, 1.8)	–	1.2 (0.47, 3.0)	–
Crowding¶¶	0.86 (0.47, 1.6)	–	1.8 (0.69, 4.5)	2.3 (0.84, 6.1)
Large family‖‖	1.13 (0.61, 2.1)	–	0.95 (0.37, 2.4)	–

95% CI = 95% confidence interval; OR = odds ratio; SES = socioeconomic standing. Significant associations ($P < 0.05$) are shown in bold face.
*Hemoglobin < 11.5 g/dL blood.
†Hemoglobin < 9 g/dL.
‡Multiply-adjusted OR estimated by multivariable logistic regression modeling using backward selection of predictor variables from the list of factors above.
§Egg-positive on urine filtration or anti-SWAP IgG4 positive.
‖Antigen positive on Binax rapid diagnostic testing, or anti-BMA IgG4 positive.
¶Egg positive on Kato-Katz stool testing.
**Antigen positive on ICT rapid diagnostic testing.
††Height for age z score (HAZ) < −2.
‡‡Body-mass index for age z score < −2.
§§Lower middle or lowest SES quartile based on asset score.
‖‖More than eight persons in household.
¶¶More than five children per family.

in our study, that is, 3- to 5-year olds, 6- to 8-year olds, and 9- to 11-year olds. The results indicate significantly lower hemoglobin levels among the younger two age groups than among the 9- to 11-year olds ($P < 0.001$). Figure 2 shows a scatterplot of hemoglobin values by age for the overall study group. There were significantly lower MCV values among younger children, and significantly lower iron levels and iron saturation findings among the youngest 3- to 5-year-old age group (Table 3). By contrast, haptoglobin levels were significantly greater among 3- to 5-year olds than among 6- to 8-year olds, who in turn, had higher average haptoglobin levels than the 9- to 11-year olds ($P < 0.02$). A significantly higher proportion of 3- to 5-year olds and 6- to 8-year olds were classified as iron deficient and anemic (87% and 89%, respectively) as compared with the 9- to 11-year-old group (69%, $P < 0.05$). When we used GEE multivariable modeling to identify the contribution of individual and combined infections on risk for IDA among those fully tested ($N = 190$), male sex, younger age, multiple infection (polyparasitism) and nutritional wasting (low body mass index [BMI] z score) were associated with IDA in the best fit model, whereas the presence of filaria was associated with lower odds of anemia (i.e., a protective

TABLE 3
Anemia and anemia biomarkers by age group among the Jego village children

Outcome	3- to 5-year olds	6- to 8-year olds	9- to 11-year olds	All children
Number with CBC data	63	95	96	254
Hemoglobin in g/dL, mean ± SD	10.1 ± 0.9	10.4 ± 1.2	11.0 ± 1.1	10.5 ± 1.2§
Percent anemic	95	85	62	79†
Percent severely anemic	11	12	2	8*
MCV in fL mean (range)	73 (56–86)	75 (58–91)	77 (55–90)	75 (55–91)§
Percent microcytosis	83	74	66	73
MCHC in g/dL Mean (range)	30 (26–35)	30 (25–36)	31 (27–36)	31 (25–36)
Percent hypochromasia	63	63	53	59
Number with iron test data	57	75	58	190
Serum iron in μg/dL mean ± SD	59 ± 37	75 ± 42	85 ± 46	73 ± 43§
TIBC in μg/dL mean (range)	534 (202–859)	544 (348–842)	523 (314–1017)	535 (202–1017)
Average iron % saturation (range)	11 (3–27)	14 (4–39)	17 (2–34)	14 (2–39)§
Serum ferritin in μg/L, mean (range)	81 (4–977)	57 (5–329)	53 (0.5–206)	63 (0.5–977)
Haptoglobin in mg/dL, mean (range)	180 (7–445)	141 (7–455)	112 (7–627)	144 (7–627)§
Anemic children meeting IDA criteria (%)	47/54 (87)	58/65 (89)	25/36 (69)	130/155 (84)*
Number with sTfR test data	12	34	27	73
sTfR (mean ± SD)	19 ± 6	24 ± 10	18 ± 6	21 ± 9‡
sTfR/log (ferritin)	5.0 (3–8)	6.2 (3.2–14)	4.7 (2–8)	5.5 (2–14)‡
Anemic children meeting AI criteria (%)	10/10 (100)	25/26 (96)	15/16 (94)	50/52 (96)
Anemic children meeting both AI and IDA criteria (%)	10/10 (100)	25/26 (96)	15/16 (94)	50/52 (96)

AI = anemia of inflammation; CBC = automated complete blood count; IDA = iron-deficiency anemia; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; SD = standard deviation; sTfR = soluble transferrin receptor serum level; TIBC = total serum iron binding capacity.
Significant differences among age groups by χ^2 testing, *$P < 0.05$, †$P < 0.001$.
Significant difference among age groups by ANOVA, ‡$P < 0.03$, §$P < 0.005$.

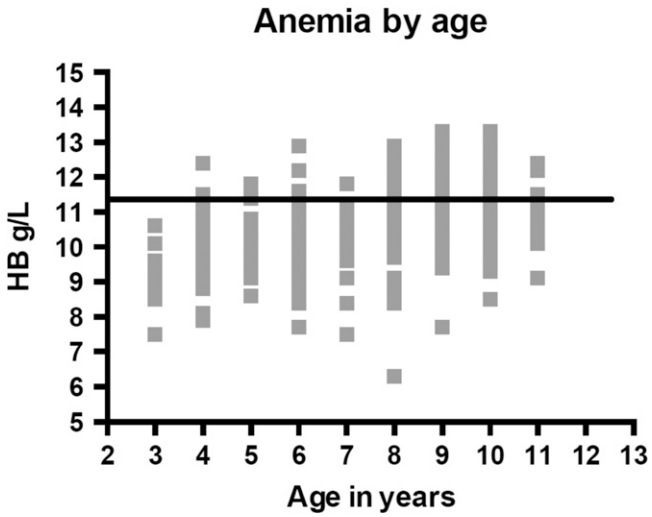

FIGURE 2. Scatterplot of hemoglobin values by age for the Jego children in the anemia study group (N = 254). The solid horizontal black line indicates a hemoglobin level of 11.5 g/dL, below which children under the age of 12 are considered anemic by World Health Organization (WHO) criteria.

effect; see Table 4). For non-IDA, malaria was the only significant correlate of anemia after adjustment for age and sex (Table 5).

Although children aged 9–11 years had less anemia, they had proportionately more non-IDA than younger children (31% versus 12% of anemia, respectively, $P < 0.01$). Average sTfR levels were elevated in all age groups, and highest in the 6- to 8-year-old group. Nearly all (96%) of anemic children who had sTfR testing done (N = 52) met the criteria[8] for combined anemia of inflammation with true iron-deficiency anemia (AI+IDA, Table 3). Figure 3 shows boxplots of the calculated sTfR/logSF ratio values for the sub-groups of parasite-infected (N = 51) versus uninfected (N = 22) children who received sTfR testing. These children, found to be infected with malaria, hookworm, *Schistosoma,* and/or filaria, had a higher mean sTfR/logSF value, 5.8 ± 2.3, compared with 4.8 ± 1.9 for the 22 tested children who did not have detectable infection (P = 0.06).

TABLE 4
Associations of demography, nutrition, and infection status with IDA

Predictor	aOR for IDA*	95% CI	*P* value
Age in years			
3–5	**10.038**	3.9, 26	**< 0.001**
6–8	**5.317**	2.5, 11.5	**< 0.001**
9–11	Reference	–	–
Sex			
Male	1.832	0.93, 3.6	0.079
Female	Reference	–	–
Filarial infection			
Present	**0.294**	0.11, 0.81	**0.018**
Absent	Reference	–	–
Polyparasitism			
Present	2.545	0.9, 7.2	0.079
Absent	Reference	–	–
Wasting			
Present	4.111	0.77, 22	0.097
Absent	Reference	–	–

CI = confidence interval; GEE = generalized linear estimating equation; IDA = iron-deficiency anemia; TIBC = total serum iron binding capacity. Significant associations ($P < 0.05$) are shown in bold face.
*Multiply-adjusted odds ratios estimated from the best-fit multivariable GEE logistic regression model using backward selection of predictor variables. IDA defined as Hb < 11.5, SF < 30, or TIBC > 400 and transferrin saturation < 20%. N = 190 children tested.

TABLE 5
Associations of demography and infection status with having non-IDA

Predictor	aOR for non-IDA*	95% CI	*P* value
Age in years			
3 to 5	0.81	0.27, 2.5	0.711
6 to 8	0.402	0.13, 1.2	0.114
9 to 11	(reference)		
Sex			
Male	0.763	0.3, 1.9	0.572
Female	(reference)		
Malaria			
Present	**2.991**	1.1, 7.8	**0.026**
Absent	(reference)		

CI = confidence interval; GEE = generalized linear estimating equation; IDA = iron-deficiency anemia. Significant associations ($P < 0.05$) are shown in bold face.
*Multiply-adjusted odds ratios estimated from the best-fit multivariable GEE logistic regression model using backward selection of predictor variables. Non-IDA was defined as having anemia in the absence of IDA criteria. N = 190.

DISCUSSION

The results of this cross-sectional study indicate a very high prevalence of anemia (79%) among younger preschool and early school-age children in an area of coastal Kenya that is endemic for multiple parasitic infections. These include malaria, hookworm, filariasis and urogenital schistosomiasis caused by *S. haematobium* infection.[10,14] In evaluating potential infectious and noninfectious causes of childhood anemia in our 3- to 11-year-old study group, we observed that malaria infection, chronic undernutrition (stunting), and younger age were each significant correlates of being anemic, whereas the presence of thalassemia trait or of filarial infection was

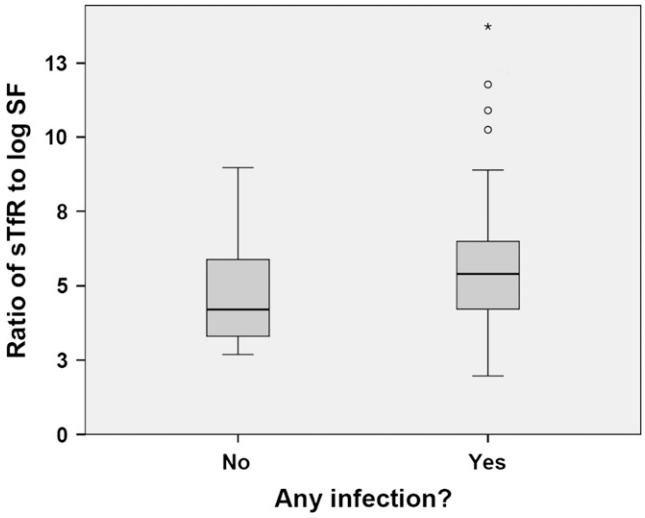

FIGURE 3. Boxplot comparing soluble transferrin receptor (sTfR) to log ferritin ratios (a biomarker of combined anemia of inflammation and iron-deficiency anemia)[8] for study children having infection(s) or without any detectable infection. The distributions of values for the ratio of sTfR level/log (serum ferritin) are compared for the subset of sTfR-tested children either having (N = 51) or not having (N = 22) parasitic infection detected at the time of study. P value for group-wise differences in values is 0.064 by Mann–Whitney U test.

associated with reduced odds of anemia. After adjustment in multivariable models, young age and malaria remained significant, independent correlates of anemia in the study population. In biomarker studies performed among a subset of participants, we found that 84% of anemic children had findings compatible with iron deficiency (that is, low ferritin or elevated iron-binding capacity and saturation < 20%). However, 96% of anemic children with normal or moderately elevated ferritin met criteria for anemia of inflammation with true iron deficiency, having a significantly elevated sTfR relative to serum ferritin. Children of the oldest age group (9–11 years) had less anemia, but they had more detectable malaria, *Schistosoma* infection, hookworm, and filarial infection. Notably, their proportion of non-IDA (31%) was greater than that of younger age groups (12%). While this suggested potential age-related correction of iron stores, measures of chronic undernutrition (stunting), and socioeconomic standing were not strong correlates of anemia, and despite the positive trend for reduced levels of IDA, overall rates of anemia remained high (62%) in the 9- to 11-year age group. The proportional increase in non-IDA among the older age group may have been caused by a higher overall prevalence in AI in older children, related either to a greater severity of established infections with age (e.g., schistosomiasis, filariasis, hookworm), or to a higher prevalence of other (unmeasured) chronic infections.

These findings highlight the broad and complex spectrum of anemia in resource-limited areas. While malaria-related hemoglobinopathies (HbS and thalassemia trait) were minimally associated with anemia in our study group, iron status and inflammation appear to have played a more significant role in community prevalence of anemia in this age group. Although hookworm infection and schistosomiasis can cause chronic daily blood loss, likely contributing to relative iron deficiency,[24] clinical trials of deworming plus iron supplementation have shown mixed results in reversing anemia in children affected by these parasitic infections.[6,7] Whereas malaria can cause acute and chronic hemolytic anemia, this process is unlikely to result in reduced iron stores. What has become more evident over the last decade is that chronic inflammation caused by long-lasting parasitic infections appears to strongly reduce the body's ability to absorb dietary iron (via gut ferroportin) and to use existing body iron stores for erythropoiesis.[6,25] Infection-related AI has been strongly linked to the presence of subclinical infection, both for children with subclinical malaria in Cote d'Ivoire,[6] and for Philippine children having persistent or recurrent low-level *Schistosoma japonicum* infection after praziquantel therapy.[25,26] Our current study of the mechanisms of anemia associated with prevalence parasitic infections suggests that AI combined with true iron deficiency is the primary source of childhood anemia in this area of coastal Kenya.

Our study further highlights the limitations of standard tests for anemia and infection, as frequently used in field studies. A significant contrast was seen between the prevalence of anemia by cuvette assay of finger prick blood (56%, Table 1) and the prevalence of anemia detected by venous sampling and automated complete blood count (79%, Table 3). This suggests consistent overestimation of hemoglobin by the finger prick technique. Similarly, additional serological testing raised the estimate of *S. haematobium* prevalence among the early childhood study group from 7% (by egg detection alone, Table 1) to 28% (Figure 1), and the estimate of *W. bancrofti* from 4% (Table 1) to 25% (Figure 1). It is clear that, in the future, the links between infections, their associated inflammation, and consequent risk for AI will be most reliably established using only the most sensitive and specific diagnostics available.

There were strengths and limitations in our study. The breadth of individual laboratory testing provided a more detailed estimation of the etiology of anemia in the study subjects. However, we were unable to measure iron profiles and sTfR levels for all study patients due to budget limitations. Limited study size may have contributed to an inability to detect other significant factors related to anemia risk, such as schistosomiasis. For early life/low intensity infections, prevalence estimates based on egg detection are known to be significant underestimates of the true infection rate.[15,27] Adequately powered studies, applying sensitive antigen-detection tests for the diagnosis of *Schistosoma* infection, are needed to further investigate the likely association between the inflammation caused by schistosomiasis haematobia and anemia. While it is unlikely that children in this age group were HIV positive, we did not determine HIV status for study participants, however there is always a chance that some of these children were HIV infected, which could have allowed additional undetected opportunistic infections that could have contributed to AI and so obscure the results of our parasite-focused analysis. This was a cross-sectional study that did not include intervention follow-up as done in recent studies in Cote d'Ivoire[6,28]—further longitudinal studies of treatment impact on iron metabolism and anemia will be welcome to identify optimal treatment and prevention strategies for parasitic infection and anemia among preschool children in parasite-endemic areas.

Anemia represents a significant problem for children living in areas with limited resources and high parasite burden. Because early-life anemia can have significant consequences on cognitive development and childhood physical fitness, its multiple causes should be identified and treated appropriately. Successful treatment of anemia in this setting should rely on strategies based on this and other evidence of infection-related AI frequency. Our findings, combined with those of other recent studies,[6,25,28,29] suggest that both chronic infection and iron deficiency need to be addressed as part of integrated management of childhood anemia.[13]

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