*Am. J. Trop. Med. Hyg.*, 102(4), 2020, pp. 851–856
doi:10.4269/ajtmh.19-0828


# Associations among Soil-Transmitted Helminths, G6PD Deficiency and Asymptomatic Malaria Parasitemia, and Anemia in Schoolchildren from a Conflict Zone of Northeast Myanmar

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*Abstract.* In tropical areas of developing countries, the interactions among parasitic diseases such as soil-transmitted helminths (STHs) and malaria, and glucose-6-phosphate dehydrogenase deficiency (G6PDd), are complex. Here, we investigated their interactions and impact on anemia in school students residing in a conflict zone of northeast Myanmar. A cross-sectional survey was conducted between July and December 2015 in two schools located along the China–Myanmar border. Stool samples from the schoolchildren were analyzed for STH infections, whereas finger-prick blood samples were analyzed for G6PDd, hemoglobin concentrations, and *Plasmodium* infections. Among 988 enrolled children, *Plasmodium vivax*, *Plasmodium falciparum*, hookworm, *Ascaris lumbricoides*, and *Trichuris trichiura* infections occurred in 3.3%, 0.8%, 31.5%, 1.2%, and 0.3%, respectively. Glucose-6-phosphate dehydrogenase deficiency was present in 16.9% of the children, and there was a very high prevalence of anemia (73%). Anthropometric measures performed on all children showed that 50% of the children were stunted and 25% wasted. Moderate to severe anemia was associated with STH infections, stunting, and wasting. In addition, children had increasing odds of anemia with increasing burden of infections. This study revealed a high prevalence of G6PDd, STHs, and anemia in schools located in a conflict zone. In areas where malnutrition and STH infections are rampant, testing for both glucose-6-phosphate dehydrogenase and anemia should be considered before treating vivax malaria with 8-aminoquinolines.

## INTRODUCTION

Malaria and helminthic infections are among the most significant public health problems affecting the children of the tropical and the subtropical world.[1] Despite intensive control efforts, malaria still infects more than 200 million people annually, and about half of the world's population remains at risk of contracting malaria.[2] In addition, it is estimated that more than a third of the world's population is infected with soil-transmitted helminths (STHs), the most common of which are roundworms (*Ascaris lumbricoides*), hookworms, and whipworms (*Trichuris trichiura*).[3,4] Although STHs are rarely the direct cause of death, they are associated with high morbidity and contribute to almost five million disability-adjusted life years. Preschool- and school-aged children are the highest risk group and harbor the greatest burden of STHs. As a result, they experience growth stunting and reduced physical fitness, which have long-lasting adverse consequences. Distribution of malaria and helminthiasis overlap geographically, and coinfections with both parasites occur frequently.[5,6] Such coinfections often have additive or synergistic adverse impacts, with the most significant consequence being severe anemia.[7,8] Children with coinfections often have compromised cognitive and physical development, leading to reduced learning, reduced school achievements, and increased susceptibility to other infections.[9,10]

In Southeast Asia, almost 200 million people live in extreme poverty, with nearly half of them infected with STHs.[11] Malaria is also highly endemic in this region, with Myanmar accounting for nearly 70% of the region's cases. Unlike Africa, a large proportion of the malaria in the region is due to *Plasmodium vivax*, for which the standard of care is treatment with chloroquine and primaquine. Vivax malaria is resilient to control measures, and radical cure of vivax malaria requires administration of primaquine, a drug that can cause acute hemolytic anemia in subjects with glucose-6-phosphate dehydrogenase deficiency (G6PDd).[12] Glucose-6-phosphate dehydrogenase deficiency is a common X-linked enzyme deficiency causing anemia in humans, affecting more than 400 million people worldwide.[13] There is a high prevalence of G6PDd among the ethnic groups and hill tribes living along the international borders of the Greater Mekong Subregion (GMS).[14,15] Unfortunately, because of resource constraints, glucose-6-phosphate dehydrogenase (G6PD) testing is not performed before treatment of vivax malaria with primaquine, a potential contributory factor to the high rates of anemia in this population.

Along the Myanmar–China border, there is high prevalence of all these etiological factors contributing to anemia in children. In addition, the situation was further exacerbated, as multiple civil wars in the last decade have led to the establishment of camps for internally displaced persons (IDPs). The IDP population relied heavily on international nongovernmental organizations (NGOs) for humanitarian supports. Therefore, this study aimed to investigate the complex interactions among G6PDd, *Plasmodium* infection, STHs, malnutrition, and risk of anemia, and its impact on growth in schoolchildren residing in the conflict zone of northeast Myanmar. We hypothesized that G6PDd individuals with STHs and malaria would be at a significantly higher risk of anemia and its adverse consequences, compared with G6PD normal subjects.

## MATERIALS AND METHODS

**Study area and population.** The study was conducted in a remote border area of northeast Myanmar, with a large

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population of IDPs who were fleeing military conflicts. Residents were living in overcrowded temporary shelters with poor sanitary conditions. A cross-sectional survey was conducted between July and December 2015 in two primary -middle schools: one in the Laiza township and the other serving the IDP settlement. The IDP settlement had a population size of approximately 9,000 in 2012, comprised mainly of the Kachin ethnic group. Malaria transmission is perennial, with a peak occurring during the rainy season between May and July.[16] *Plasmodium vivax* is the predominant parasite in this area and accounts for > 85% of malaria incidence.[17] Healthcare infrastructure and public health preventive measures are poor, and there have been no school-based deworming programs in these areas in the last few years.

**Ethical considerations.** The study protocol was approved by the Institutional Review Boards of Kunming Medical University, the Kachin Bureau of Heath, and the Pennsylvania State University. Before recruitment, sensitization meetings were held at these schools. The purpose and procedures of the study were fully explained, and parents, students, and teachers had the opportunity to ask questions. School students willing to participate provided written informed assent, and written informed consent was obtained from the teachers and parents (or guardians).

**Sample collection procedures.** The same research staff conducted the surveys at both schools and followed the same study procedures. After obtaining informed consent, anthropometric measurements were performed. Heights were measured by making the student stand barefoot, upright against a wall. Weight of the student was measured using a digital scale after the students removed shoes and heavy clothes. Weight and height information was used to calculate the body mass index (BMI) (weight [kg]/height squared [$m^2$]). Anthropometric indices—*z* scores of height-for-age (HAZ), weight-for-age (WAZ), and BMI-for-age (BMIZ)—were calculated using AnthroPlus software.[18] Children were classified as stunted, wasted, or underweight if their HAZ, WAZ, and BMIZ were less than 2 SDs from the references.

Children were provided with sterile, leak-proof, wide-neck, plastic stool containers and instructed to put a teaspoon of stool into the containers and bring their stool samples the next morning. About 10 μL of finger-prick blood was collected in ethylenediaminetetra-acetic acid (EDTA) anticoagulated tubes to measure hemoglobin (Hb) and G6PD levels. Thick and thin blood films were prepared for malaria microscopy and dried blood spots on filter paper for polymerase chain reaction (PCR) detection of *Plasmodium* parasites.

**Detection and quantification of helminthes.** The Kato–Katz thick smear technique was used for quantitative determination of helminth ova.[19] Water- or urine-contaminated stools were rejected. Stool samples were processed within 12 hours of collection, and each slide was allowed to clear for 30 minutes, and then examined at ×100 total magnification within 1 hour of preparation to avoid missing hookworm eggs. To ensure consistency of the result and as a form of quality control, every stool sample was analyzed three times sequentially by three trained laboratory technicians. Each slide was counted for eggs in the 41.7 mg of stool, and the average of three slide counts multiplied by 24 was used to calculate the number of eggs per gram of feces.

**Analysis of blood samples to determine the Hb concentration.** Approximately 100 μL of fingertip blood was collected from each consenting student into labeled EDTA tubes. Hemoglobin concentration was obtained immediately using a TEK-II Mini automated hematology analyzer (Jiangxi Tekang Technology Co. Ltd, Nanchang, China) following the manufacturer's instructions. Anemia was defined using age- and gender-specific WHO thresholds. Specifically, children aged 5–11 years with Hb levels (g/L) ≥ 115, 110–114, 80–109, and < 80 were diagnosed as non-anemic, mildly anemic, moderately anemic, and severely anemic, respectively. These anemia categories were set at ≥ 120, 110–119, 80–109, and < 80 for children aged 12–14 years.[20]

***Plasmodium* detection by microscopy and nested PCR.** Thick and thin blood films were prepared and stained with 10% Giemsa and examined under a light microscope by two experienced microscopists. Parasite density was counted against 200 white blood cells (WBCs) (or 500 WBCs when the initial number of parasites was < 99) on the thick blood films.[21] All dried blood spots on filter paper were analyzed for *Plasmodium* DNA using nested PCR, as previously reported.[22] In brief, a 2-mm-diameter blood spot was punched out of each filter paper sample and washed with 30 μL of distilled water at 50°C for 3 minutes. After removal of water, PCR mixture was added directly to the sample, and nested PCR was performed targeting the small subunit rRNA genes.[23] PCR products were run in 1.5% agarose gels to determine the presence of parasite DNA and parasite species. *Plasmodium* infection was defined as positive by PCR analysis.

**Glucose-6-phosphate dehydrogenase test.** Glucose-6-phosphate dehydrogenase enzyme activity was measured using a fluorescence spot test kit (Micky Co. Ltd., Guangzhou, China) that was used to screen G6PDd in newborns in China.[24] Samples producing fluorescence within 10 minutes of incubation were considered to have normal G6PD activity. Otherwise, they were considered as G6PD deficient. Each sample was tested twice. Each batch of tests included a G6PD normal and G6PDd (< 40% of normal activity) control.

**Statistical analysis.** Two group comparisons were performed using the chi-square test, and where appropriate, the Fisher's Exact test was performed for categorical variables. The Student's *t*-test was used for continuous variables, when they were normally distributed, or the Mann–Whitney U test, when assumptions of normality were not met. Odds ratios (ORs) were used to quantify the magnitude of association between the factors of interest and outcomes. The Cochran–Armitage test for trend was used to test whether there are increasing or decreasing linear trends in the proportions across levels. To adjust for the effects of confounding, and to test for interactions, logistic regression models were fit with anemia as the outcome variable. Because G6PDd is gender linked and duration of infection or exposure to past infections increases with age, gender and age were used as covariates in these models. Parameter estimates were exponentiated to compute adjusted ORs (aORs). *P*-values were interpreted in a two-tailed fashion, and a $P < 0.05$ was considered statistically significant.

## RESULTS

**Demographic characteristics of the study population.** From July to December 2015, two schools with a total of 1,210 students were approached to participate in the study. Of these, 988 (82%) provided blood and stool samples and constituted the final study population. Students were aged 6 to 15 years, with the age distribution biased toward the younger ages (Table 1). The population was highly malnourished, with

approximately half the enrolled students stunted (< –2 SD HAZ) and 25% wasted (< –2 SD WAZ). Prevalence of anemia was extremely high, with 73% having moderate to severe anemia (Table 1). The qualitative G6PD fluorescence spot test identified 16.9% (167/988) individuals as G6PDd.

**Prevalence of *Plasmodium* and STH infections.** Light microscopic examination of the 988 blood smears detected only 18 (1.8%) *P. vivax* infections. Parasite density was low, with a median of 104 (Q1–Q3: 32–148) parasites/μL. By contrast, nested PCR detected 41 (4.1%) *Plasmodium*-positive samples, including 33 *P. vivax* and 8 *Plasmodium falciparum*. It is noteworthy that none of the participants with *Plasmodium* infections had any symptoms of malaria, and thus were considered asymptomatic parasite carriers (or chronic infections).

Overall, 32% of all the school students were infected with at least one helminth species of hookworms, *A. lumbricoides*, or *T. trichiura*. Hookworm infection was the most common, with a prevalence of 31.5%, followed by *A. lumbricoides*, with a prevalence of 1.2%, and *T. trichiura* had the lowest prevalence of 0.3%. Infection intensities were high with a median (interquartile range) of 232 (96–632), 704 (74–1,746), and 72 (8–168) eggs/g of stool for hookworms, *A. lumbricoides*, and *T. trichiura*, respectively. Although most children had a single helminth species infection, seven had mixed STHs and 15 had a coinfection of STHs with *Plasmodium*. The prevalence of malaria parasite infections in the STH-positive children was 4.7% (15/319) and was not significantly different from that in STH-negative children (3.9% [26/669]; OR = 1.22 [95% CI: 0.64–2.34]).

**Associations of G6PDd with growth and anemia.** Glucose-6-phosphate dehydrogenase deficiency was more prevalent in males (19.1%, 98/514) than in females (14.6%, 69/474), although the difference was not statistically significant (Table 1). Glucose-6-phosphate dehydrogenase deficiency prevalence did not differ across the age-groups. Anthropometric measures such as height and weight, and malnutrition indicators such as stunting and wasting did not differ by G6PD status. Similarly, the distribution of anemia levels in the G6PDd and G6PD normal were not different ($P$ = 0.18, Cochran–Armitage test for trend) (Table 1).

**Associations of STHs, asymptomatic *Plasmodium* infections, and G6PDd with anemia.** Helminthiasis, *Plasmodium* infections, and G6PDd are potential contributory factors to anemia in children. Considering moderate to severe anemia to be of clinical importance, anemia was significantly associated with increasing odds of stunting, wasting, and being underweight (Table 2).

*Independent effects of STHs and asymptomatic* Plasmodium *infections.* Although STH infections were significantly associated with moderate to severe anemia (aOR = 1.44, 95% CI: 1.05–1.98; $P < 0.05$), this was not apparent for asymptomatic *Plasmodium* infections (aOR = 1.19, 95% CI: 0.57–2.46).

*Effects of coinfections of STHs and asymptomatic* Plasmodium *infections.* When examining coinfections with number of infections as none, either *Plasmodium* or STHs, and both asymptomatic *Plasmodium* and STH infections, the risk of anemia increased with increasing numbers of infections (OR = 1.36 and 1.65, respectively, $P < 0.05$, test for trend) (Table 2).

*Effect modification of STHs by G6PD status.* A stratified analysis by G6PD status showed no difference in the ORs for the association between STHs and anemia for the G6PDd versus G6PD normal children, with aOR = 1.91 and 1.35, respectively ($P$ = 0.41 for interaction between STHs and G6PDd)

TABLE 1
Population characteristics of schools on the Myanmar–China border grouped by G6PD status

| Feature | All children, *N* (%) | G6PD deficient, *N* (%) | G6PD normal, *N* (%) | Odds ratio (95% CI) |
|---|---|---|---|---|
| All | 988 (100%) | 167 (100%) | 821 (100%) | – |
| School locations | | | | |
| Internally displaced person camp | 718 (73%) | 115 (69%) | 603 (73%) | 1 |
| Laiza town | 270 (27%) | 52 (31%) | 218 (27%) | 0.72 (0.52–1.01) |
| Gender | | | | |
| Male | 514 (52%) | 98 (59%) | 416(51%) | 1 |
| Female | 474 (48%) | 69 (41%) | 405 (49%) | 1.25 (0.87–1.80) |
| Age (years) | | | | |
| 6–7 | 249 (25%) | 34 (20%) | 215 (26%) | 1* |
| 8–9 | 256 (26%) | 48 (29%) | 208 (25%) | 1.46 (0.90–2.36) |
| 10–11 | 183 (18%) | 36 (21%) | 147 (18%) | 1.55 (0.93–1.59) |
| 12–13 | 195 (20%) | 31 (19%) | 164 (20%) | 1.20 (0.71–2.03) |
| 14–15 | 105 (11%) | 18 (11%) | 87 (11%) | 1.31 (0.70–2.44) |
| Age (years) μ ± SD | 9.80 ± 2.67 | 9.87 ± 2.60 | 9.78 ± 2.70 | n.s. |
| Hemoglobin μ ± SD | 9.76 ± 2.61 | 9.96 ± 2.50 | 9.72 ± 2.60 | n.s. |
| Height (meters) μ ± SD | 1.25 ± 0.16 | 1.25 ± 0.16 | 1.24 ± 0.16 | n.s. |
| Weight (kg) μ ± SD | 25.5 ± 9.80 | 25.3 ± 9.60 | 25.5 ± 9.90 | n.s. |
| BMI (kg/m$^2$) μ ± SD | 15.9 ± 2.8 | 15.9 ± 3.0 | 16.0 ± 2.7 | n.s. |
| Malnutrition Indicators | | | | |
| Weight-for-age < –2 SD | 228 (25%) | 35 (21%) | 193 (24%) | 0.86 (0.57–1.29) |
| Height-for-age < –2 SD | 492 (50%) | 408 (50%) | 84 (50%) | 1.02 (0.73–1.43) |
| BMI-for-age < –2 SD | 126 (13%) | 27 (16%) | 99 (12%) | 1.41 (0.89–2.23) |
| Anemia | | | | |
| None | 199 (20%) | 40 (24%) | 159 (19%) | 1* |
| Mild | 69 (7%) | 13 (8%) | 56 (7%) | 0.92 (0.46–1.85) |
| Moderate | 494 (50%) | 78 (47%) | 416 (51%) | 0.74 (0.49–1.14) |
| Severe | 226 (23%) | 36 (21%) | 190 (23%) | 0.75(0.46–1.24) |
| Malaria | 41 (4%) | 9 (5%) | 32 (4%) | 1.40 (0.66–3.0) |
| Soil-transmitted helminths | 319 (32%) | 56 (39%) | 263 (32%) | 1.07 (0.75–1.52) |

BMI = body mass index;G6PD = glucose-6-phosphate dehydrogenase; n.s. = not significant by *t*-test. μ ± SD = mean ± SD. Malaria is defined as asymptomatic *Plasmodium* infection detected by PCR.

* No significant trend (Cochran–Armitage test for trend).

TABLE 2
Relationship between G6PD status, malaria, STH infections, and anemia

| Feature | Anemia, *N* (%) | No anemia, *N* (%) | OR (95% CI) | Adjusted OR (95%)‡ |
|---|---|---|---|---|
| Malnutrition indicators | | | | |
| Weight-for-age < –2 SD | 179 (25%) | 49 (18%) | 1.48 (1.04–2.10)† | § |
| Height-for-age < –2 SD | 385 (53%) | 107 (40%) | 1.73 (1.30–2.30)*** | § |
| BMI-for-age < –2 SD | 106 (15%) | 20 (7%) | 2.14 (1.30–3.53)** | § |
| STHs– | 474 (66%) | 195 (73%) | 1 | 1 |
| STHs+ | 246 (34%) | 73 (27%) | 1.39 (1.02–1.89)† | 1.44 (1.05–1.98)† |
| Malaria– | 689 (96%) | 258 (96%) | 1 | 1 |
| Malaria+ | 31 (4.3%) | 10 (4%) | 1.16 (0.56–2.40) | 1.19 (0.57–2.46) |
| Infections | | | | |
| No infections | 455 (63%) | 188 (70%) | 1* | 1* |
| Malaria or STHs | 253 (35%) | 77 (29%) | 1.36 (1.0–1.84)† | 1.41(1.03–1.93)† |
| Malaria and STHs | 12 (2%) | 3 (1%) | 1.65 (0.46–5.92) | 1.69 (0.47–6.07) |
| G6PD deficient | | | | |
| STHs– | 71 (62%) | 40 (75%) | 1 | 1 |
| STHs+ | 43 (38%) | 13 (25%) | 1.86 (0.90–3.87) | 1.91 (0.90–4.0) |
| G6PD normal | | | | |
| STHs– | 403 (66%) | 155 (72%) | 1 | 1 |
| STHs+ | 203 (34%) | 60 (28%) | 1.30 (0.41–1.98) | 1.35 (0.95–1.91) |
| G6PD deficient | | | | |
| Malaria– | 52 (98%) | 106 (93%) | 1 | 1 |
| Malaria+ | 1 (2%) | 8 (7%) | 3.92 (0.48–32.2) | 4.14 (0.50–34.5) |
| G6PD normal | | | | |
| Malaria– | 583 (96%) | 206 (96%) | 1 | 1 |
| Malaria+ | 23 (4%) | 9 (4%) | 0.90 (0.41–1.98) | 0.93 (0.42–2.04) |
| G6PD deficient | | | | |
| No infections | 66 (58%) | 39 (74%) | 1 | 1 |
| Malaria or STHs | 45 (39%) | 14 (26%) | 1.9 (0.93–3.90) | 1.92 (0.92–4.00) |
| Malaria and STHs | 3 (3%) | 0 (0%) | 4.16 (0.21–82.60)‖ | ND |
| G6PD normal | | | | |
| No infections | 389 (64%) | 149 (69%) | 1 | 1 |
| Malaria or STHs | 208 (34%) | 63 (29%) | 1.26 (0.90–1.78) | 1.31 (0.92–1.85) |
| Malaria and STHs | 9 (2%) | 3 (2%) | 1.14 (0.31–4.30) | 1.17 (0.31–4.41) |

BMI = body mass index; G6PD = glucose-6-phosphate dehydrogenase; OR = odds ratio; STHs = soil-transmitted helminths. ND, cannot estimate because of zero cell. Malaria is defined as asymptomatic *Plasmodium* infection detected by PCR.

* $P < 0.05$ test for trend.

† $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

‡ Adjusted for age and gender.

§ Because *Z* scores are age and gender normalized, no adjustment is needed. Reference categories are as follows: not wasted, not stunted, and BMI ≥ –2 SD.

‖ Logit odds computed by adding 0.5 to the zero cell.

(Table 2), suggesting that G6PDd did not modify the association between STHs and anemia.

*Effect modification of* Plasmodium *infection by G6PD status.* The G6PD status-specific aOR estimates for the association between *Plasmodium* infection and anemia were aOR = 4.14 versus 0.93 for G6PDd versus G6PD normal, respectively (Table 2). The effect modification was not statistically significant.

*Effect modification of STHs and* Plasmodium *coinfection by G6PD status.* There was no statistically significant effect modification by G6PD status for a single infection with STHs or *Plasmodium* versus having an STH and *Plasmodium* coinfection. Among G6PDd individuals, the ORs were 1.9 versus 4.16 (Table 2) for a single versus coinfections, respectively, compared with ORs of 1.26 and 1.14 for G6PD normal.

## DISCUSSION

Our study revealed an astoundingly high prevalence of malnutrition in schoolchildren in a war-inflicted zone of Myanmar, with almost half of the students having stunted growth and three-fourths having moderate to severe anemia. Anemia due to parasitic infections is complex, and the etiological basis may include a combination of chronic blood loss, hemolysis, and hematopoietic suppression. Anemia is especially common in populations living in conflict zones.[25] The prevalence of anemia at this study site was much higher than that reported in earlier studies among schoolchildren in other parts of Myanmar,[26,27] as well as in other countries in this region such as Nepal (37.9%), Thailand (31.0%), and Malaysia (26.2%).[28–31]

As found in other parts of the developing world, STH infections were highly prevalent in schoolchildren of this border region, with 32.8% being positive for at least one intestinal parasite. The prevalence of intestinal parasites was higher than those reported from neighboring GMS countries. For example, several surveys conducted over the past two decades in different rural areas of Thailand reported helminth infection rates ranging from 5.4% to 19.8%.[32–34] The higher infection rates in this study area may reflect the poor sanitary conditions in the newly established, crowded, IDP settlements.

Glucose-6-phosphate dehydrogenase deficiency is a genetically inherited enzymopathy that is widely distributed in malaria-endemic areas.[35] Although there is evidence that the prevalent G6PD Mahidol 487G > A mutation in the GMS reduces *P. vivax* parasitemia,[36] its impact alone and in combination with malaria and helminth infections on anemia has not been investigated in vivax-endemic areas where primaquine is used routinely. In African children presenting with severe malaria, G6PDd (moderate to severely deficient) was found to be associated with reduced Hb levels.[37] A study in Kenya reported a marginally significant increase in helminth infections

in G6PD heterozygous girls,[38] but on the other hand, a case–control study conducted in Senegal reported no association between G6PDd and risk of helminth infections.[39] Our study found significantly higher prevalence of anemia in STH-infected children than in those with no STH infections, but unlike the Kenyan study, there was no association between G6PDd and being infected with an STH. In addition, the association between STHs and anemia was not different for G6PDd versus G6PD normal children.

We need to point out potential limitations of our study. It is possible that participation bias might be accounting for the high rates of STHs and anemia because parents or guardians who think their children have poor health encouraged their enrollment. This bias is unlikely because we had an extremely high participation rate, with 82% of all school students providing stool and blood samples as well as having anthropometric measures. A cross-sectional study in Papua Indonesia found that children with STH infections are nearly four times more likely to have asymptomatic vivax infections than those with no STH infections.[40] We failed to find this association. The endemic settings of the two studies may account for such a difference. The study in Indonesia was conducted in children aged < 5 years, and *P. vivax* infections were detected by microscopy, whereas our study was among older children, and *P. vivax* infections were mostly submicroscopic. It could also be stated that our study was underpowered to detect it because of a lower prevalence of asymptomatic *Plasmodium* infections. We think that this is unlikely. With our observed 4% prevalence of *Plasmodium* infections, 32% prevalence of STHs, and a sample size of 988, our study would have a greater than 95% power to detect the 4-fold risk detected in the Indonesian study, with a type-1 error of 5%.[40] It could be stated that children with moderate anemia might be reducing the magnitude of the association between STHs and anemia in our study. To avoid misclassification bias, we excluded moderate anemia and repeated the analysis contrasting only severe anemia with mild or no anemia. These comparisons did not alter our overall findings (G6PD versus anemia: aOR = 0.77; STHs versus anemia: aOR = 1.30; asymptomatic *Plasmodium* infection versus anemia: aOR = 1.08). Despite our large sample of nearly a 1,000 children, our study was underpowered to detect a statistically significant effect modification by G6PDd for the association between anemia and STHs with or without *Plasmodium* infection. However, the magnitudes of the ORs, the dose-response–like increase in risk of anemia, and the biological basis for this synergistic interaction all lead us to believe that our observation is not due to sampling variation or due to potential biases, but indeed a true phenomenon needs to be evaluated in larger studies.

Our study has several important public health implications. With malaria elimination efforts being ramped up in the GMS, several countries have already implemented strategies such as treating falciparum malaria with low-dose primaquine, in addition to artemisinin combination drugs, to kill gametocytes and thus prevent transmission of falciparum malaria. In consideration are strategies such as mass drug administration with primaquine to eliminate hypnozoite reservoirs and introduction of new hypnozoiticidal drugs such as tafenoquine. Randomized control trials have been conducted in G6PD normal subjects to ascertain the safety and effectiveness of primaquine and tafenoquine to prevent relapses of *vivax* malaria.[41–43] None of these trials have assessed the impacts of these treatments on children infected with STHs in vivax malaria–endemic areas with a high prevalence of anemia. Evidence suggests that a large proportion of the vivax infections in this region are due to relapses. Our data suggest an increasing risk of anemia with the number of infections. Although it is recognized that testing for G6PDd is essential before treatment of vivax malaria with 8-aminoquinolines, caution needs to be exercised in their use for impoverished populations with high prevalence of STHs and anemia. A case report of acute hemolytic anemia in a schoolchild as the result of unsupervised administration of primaquine in the study area, which required transfusion, further underlines the necessity of testing for G6PDd.[44]

The present study found that intestinal parasitic infections among schoolchildren are a serious public health problem in the conflict zone of northeast Myanmar bordering China. The weak public health system in this poverty-stricken region lacks the resources to deal with parasitic infections. The situation is worse in the recently established IDP settlements, where crowded living and poor sanitation conditions, together with malnutrition, increase the vulnerability of the population to infectious diseases. Although multiple international NGOs are present in this area, more humanitarian efforts are necessary from the international community to improve the health of the children. Implementation of integrated control strategies aimed at reducing anemia that include malaria control, anti-helminthic treatment, and micronutrient supplementation measures is clearly needed.

Received November 6, 2019. Accepted for publication January 5, 2020.

Published online February 10, 2020.

Acknowledgments: We thank the school communities for participation and the local bureau of health for coordination. This study was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health USA (U19AI089672). Z. Y. was supported by 31860604 and U1802286 from the National Science Foundation of China, and by 2018ZF0081 from Major Science and Technology Projects of Yunnan.

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