

## Febrile status, malarial parasitaemia and gastro-intestinal helminthiases in schoolchildren resident at different altitudes, in south-western Cameroon

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In the many areas where human malaria and helminthiases are co-endemic, schoolchildren often harbour the heaviest infections and suffer much of the associated morbidity, especially when co-infected. In one such area, the Buea district, in south-western Cameroon, two cross-sectional surveys, together covering 263 apparently healthy schoolchildren aged 4–12 years, were recently conducted. The prevalences of fever, malarial parasitaemia and intestinal helminth infections, the seroprevalences of anti-*Plasmodium falciparum* IgG and IgE and anti-glycosylphosphatidylinositol (anti-GPI) IgG, plasma concentrations of total IgE, and the incidence of anaemia were all investigated.

The mean (S.D.) age of the study children was 7.56 (1.82) years. Overall, 156 (59.3%) of the children were found parasitaemic, with a geometric mean parasitaemia of 565 parasites/μl. Parasitaemia and fever were significantly associated ( $P=0.042$ ). The children who lived at low altitude, attending schools that lay 400–650 m above sea level, had significantly higher parasitaemias than their high-altitude counterparts ( $P<0.01$ ). At low altitude, the children attending government schools had significantly higher parasitaemias than their mission-school counterparts ( $P=0.010$ ). Of the 31 children (11.9%) found anaemic, 22 (70.4%) had mild anaemia and none had severe anaemia. A significant negative correlation ( $r=-0.224$ ;  $P=0.005$ ) was observed between haemoglobin concentration and level of parasitaemia. Infection with *Plasmodium* appeared to reduce erythrocyte counts ( $P=0.045$ ), a condition that was exacerbated by co-infection with helminths ( $P=0.035$ ). Plasma concentrations of total IgE were higher in the children found to be excreting helminth eggs than in those who appeared helminth-free, while levels of anti-*P. falciparum* IgE were higher in the children with low-grade parasitaemias than in those with more intense parasitaemias. Levels of anti-GPI IgG increased with age and were relatively high in the children who lived at low altitude and in those who were aparasitaemic.

The survey results confirm that asymptomatic malarial parasitaemia frequently co-exists with helminth infections in schoolchildren and indicate links with fever, altitude and school type. Immunoglobulin E may play a role in immune protection against helminthiasis whereas anti-GPI antibodies may be important in the development of antimalarial immunity in such children. In Cameroon, as in other areas with endemic malaria, control programmes to reduce the prevalences of infections with intestinal helminths and malarial parasites in schoolchildren, which may effectively reduce the incidence of anaemia, are clearly needed.

Malaria remains one of the most intractable public-health problems, especially in the

children who live in the endemic regions of sub-Saharan Africa. About 20% of all childhood deaths in Africa are associated with the disease (WHO, 2003). Malaria contributes significantly to anaemia (Adiel-Tegman and

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DeMaeyer, 1985) — which in its severe form increases the risk of death (Stoltzfus, 1997; Brabin *et al.*, 2001) — and adversely affects school performance (Fernando *et al.*, 2003). The vast majority of the symptomatic infections take the form of acute, uncomplicated, febrile episodes (Fernando *et al.*, 2003), although asymptomatic malarial parasitaemia is often common among schoolchildren (Al Serouri *et al.*, 2000).

In many tropical countries, particularly those in sub-Saharan Africa, anaemia, malaria and multiple helminth infections co-exist and are inter-related (Stoltzfus *et al.*, 1997). As with malaria, much of the burden posed by intestinal helminths and much of the associated morbidity is borne by children of school age (Crawley, 2004). Intestinal helminth infections have been associated with nutritional deficiency and impaired physical development, cognitive function and learning ability (Nokes and Bundy, 1994; Al Serouri *et al.*, 2000).

In spite of the high prevalence of malaria in children, research in endemic regions has focused mainly on the disease during early childhood (Cornet and Bundy, 1994; Ekvall *et al.*, 2001; Njama-Meya *et al.*, 2002), so that relatively little is known about malaria in older children, who are known to have some degree of naturally-acquired immunity to the infection. Although there are some reports of asymptomatic malarial infection in the schoolchildren who live near Mount Cameroon, in Cameroon (Nkuo-Akenji *et al.*, 2002; Adio *et al.*, 2004; Kimbi *et al.*, 2004), it is unclear if such infection has any impact on anaemia or the children's susceptibility to helminth or other infections. It seems possible that *Plasmodium falciparum*-specific glycosylphosphatidylinositol (GPI), which induces tumour necrosis factor (a pro-inflammatory cytokine), interleukin 1, intercellular adhesion molecule 1, nitric oxide synthase and nitric oxide, as well as humoral responses, may affect the development not only of cerebral malaria but also that of other infections (Tachado *et al.*, 1996; De Souza *et al.*, 2002; Boutlis *et al.*,

2003; Keenihan *et al.*, 2003; Suguitan *et al.*, 2004; Perraut *et al.*, 2005; Naik *et al.*, 2006).

The effect that infection with intestinal or other helminths may have on malaria also remains a matter of controversy, with the results of previous studies indicating the possibility of increased or no susceptibility to other infections, including malaria, in children infected with helminths (Brooker *et al.*, 1999; Shapiro *et al.*, 2005). In one recent study, infection with *Schistosoma haematobium* was shown to promote Th2 polarized immune responses to *Helicobacter pylori*, thus decreasing the risk of gastric atrophy (Du *et al.*, 2006). *Ascaris* antigens can induce Th1 and Th2 responses (Bethony *et al.*, 2005), and helminths may cause their human hosts to produce high levels of specific or non-specific IgE and IgG, which may or may not offer protection against the same or different parasitic infections (Bethony *et al.*, 2005; Richter *et al.*, 2005).

The aim of the present study, based in south-western Cameroon, was to explore the prevalences and intensities of fever, malarial infection, helminth infection and anaemia in apparently healthy schoolchildren, and attempt to relate them to each other and to other haematological and immunological characteristics of the children.

## SUBJECTS AND METHODS

### Study Area

This study was conducted in Buea, an urban town located along the windward slopes of Mount Cameroon and the provincial headquarters of the South West province. There are two main seasons in the area, the dry (November–March) and the rainy (March–November), with a mean annual rainfall of 2625 mm, a mean daily temperature of about 20°C and constantly high relative humidity (75%–80%). Human malaria is meso-endemic during the dry season but becomes hyper-endemic in the rainy season,

with incidence peaking in July–October (Minang *et al.*, 2004). The prevalence of malaria parasitaemia in the low-altitude areas ranges from 30% in the dry season to 65% in the rainy (Nkuo-Akenji *et al.*, 2002). The transmission of *P. falciparum*, which causes almost all (>95%) of the local cases of malaria, is intense and perennial, with *Anopheles gambiae* s.s. the dominant vector (Wanji *et al.*, 2003).

### Subjects

Overall, 263 children aged 4–12 years were enrolled into the study, with 132 of the children coming from three government primary schools and the rest from three mission primary schools. Four of the schools (all three government schools and one of the mission schools) were located at altitudes of 400–650 m above sea level ('low altitude') whereas the other two schools (both mission) were situated at 900–1000 m above sea level ('high altitude').

Before the surveys began, their objectives, methods and possible benefits were explained to the staff and, via letters and consent forms subsequently sent out with the pupils, to the parents/guardians of the children in the study schools. A school was only investigated with the approval of its headteacher and a child was only enrolled if his or her caregivers gave their written informed consent. Ethical clearance was obtained from the Institutional Review Board of the University of Buea, Cameroon, and authorization to conduct the surveys was obtained from the Ministry of National Education's provincial delegate for South West province. After the blood and stool samples had been collected, any child found to have malarial and/or helminth infection was given the appropriate treatment

### Sample Collection and Processing

As part of a larger study on the pathogenesis of severe malaria in children, the mission schools were sampled in a cross-sectional

survey carried out in the rainy season (December) of 2004 and the government schools were investigated, in a similar survey, in the dry season (March–April) of 2005.

The gender, age and area of residence of each child enrolled during these surveys were recorded. The child's axillary temperature was then measured using a digital thermometer, with fever defined as an axillary temperature of  $\geq 37^{\circ}\text{C}$ . A blood sample (3–5 ml) was then collected from each child, by venepuncture, into a heparinized Vacutainer® (BD Vacutainer Systems, Plymouth, U.K.). A drop of this blood was used to make a thick smear, other subsamples were used to determine the haemoglobin concentration and for leucocyte and erythrocyte counts, and the rest of the sample was centrifuged so that the supernatant plasma could be separated off and stored in a freezer, at  $-86^{\circ}\text{C}$ , until it could be checked for antibodies and assayed for total IgE (see below). A single stool sample was collected from each child, transported on ice to the research laboratory, and there rapidly used to make Kato–Katz smears (see below).

### PARASITOLOGY

The thick bloodsmears were air-dried, Giemsa-stained and then checked for malarial parasites by light microscopy, at  $\times 1000$ . A smear was only considered negative if no malarial parasites were seen in 50 fields. With each positive smear, the level of parasitaemia was estimated by counting the parasites against at least 200 leucocytes and then using the corresponding leucocyte count to calculate the number of parasites/ $\mu\text{l}$  blood (Rooth *et al.*, 1991). A parasitaemia with  $\leq 5000$  parasites/ $\mu\text{l}$  was considered low-grade whereas all the more intense parasitaemias were categorized as high-grade.

Stool samples were used to prepare Kato–Katz smears, which were examined within 1 h of staining (WHO, 1996). All helminth

eggs observed were counted so that intensities of infection with each intestinal helminth could be expressed as eggs/g faeces.

#### HAEMATOLOGY

Haemoglobin concentrations were determined using a HemoCue haemoglobinometer (HemoCue, Ängelholm, Sweden) while erythrocyte and leucocyte counts were made in a haemocytometer. Children with <7.0, 7.0–10.0 and >10.0–11.0 g haemoglobin/dl were considered to have severe, moderate and mild anaemia, respectively.

#### IMMUNOLOGY

The plasma samples were tested for anti-malarial antibodies (IgE and IgG), anti-GPI IgG and total IgE, in ELISA.

*Plasmodium falciparum*-specific IgG and IgE were measured using an extract of (schizont-enriched) blood-stage parasites (F32) as the capture antigen (Perlmann *et al.*, 1989). The IgG antibodies were measured using goat anti-human-IgG (Fc-specific; Sigma) whereas the total and malaria-specific IgE were measured using affinity-purified rabbit IgG specific for human IgE (5 µg/ml; MIAB Antibodies, Uppsala, Sweden). From checkerboard titrations, it was determined that the optimal concentrations for coating plates with the crude blood-stage antigens were 10 µg/ml for the specific IgG and 5 µg/ml for the specific IgE. Each test plasma was screened at a 1:200 dilution for IgG and a 1:100 dilution for IgE, with phosphate-buffered saline (PBS) containing 0.5% (v/v) Tween 20 (PBS–Tween) as the diluent. For the ELISA, the microtitre plates (Corstar, Cambridge, MA) were incubated overnight at 4°C with the crude antigens in carbonate buffer (50 µl/well) and then blocked, for 3 h at 37°C, with PBS containing 0.5% bovine serum albumin (BSA). Duplicate wells were subsequently incubated, for 1 h at 37°C, with 50 µl diluted test plasma/well, washed and then probed with the relevant isotype-specific anti-serum

conjugated to alkaline phosphatase (ALP). Since the detection of IgE antibodies in the presence of excess IgG antibodies requires prolonged interaction times (Elghazali *et al.*, 1997), incubation with the sample was at room temperature overnight. Total IgE was assayed using biotinylated rabbit anti-human-IgE and then ALP-conjugated streptavidin (Mabtech, Stockholm), as described in detail by Perlmann *et al.* (1994). After incubation with the ALP conjugates, paranitrophenol phosphate (Sigma) was added as substrate before the optical density at 405 nm (OD) of each well was read on an ELISA plate reader (Titertek Multiscan; Flow Laboratories, Rockville, MD).

For the measurement of anti-GPI IgG, a stock solution of GPI was prepared in water-saturated 1-butanol (0.1 µg/ml) and then diluted in methanol to give 2 ng GPI/50 µl methanol. ELISA plates were coated with this solution, at 50 µl/well, dried at 37°C, washed with PBS–Tween, and blocked with 0.5% (w/v) BSA in PBS–Tween. Plasma samples, diluted 1:100 in PBS–Tween, were then incubated in the wells for 1 h at 37°C, which were then washed and probed, again for 1 h at 37°C, with the second antibody — anti-human-IgG conjugated to horseradish peroxidase (R&D Systems, Minneapolis, MN). Enhanced K-Blue® TMB (Neogen, Lexington, KY) was used as substrate, with OD read at 450 nm.

In each assay, test samples giving OD that exceeded the corresponding mean value plus two S.D. for 25 non-immune European donors were considered seropositive (for anti-*P. falciparum* IgG or IgE, anti-GPI IgG, or total IgE).

#### Statistical Analyses

Analyses were performed using version 10 of the SPSS for Windows software package (SPSS Inc., Chicago, IL). Levels of parasitaemia and OD were log-transformed before analysis. The significances of differences in prevalence were explored in Pearson's  $\chi^2$  tests whereas the differences

in group means were assessed using Student's *t*-tests and analyses of variance (ANOVA). The levels of correlation between variables were determined by calculating Pearson's correlation coefficients (*r*). A difference or correlation giving a *P*-value of  $\leq 0.05$  was considered statistically significant.

## RESULTS

Overall, 127 boys and 136 girls aged 4–12 years, with a mean (S.D.) age of 7.56 (1.82) years, were enrolled into the study. Their body temperatures, malarial and helminth infections, haemoglobin concentrations and blood-cell counts are summarized in Table 1.

### Malarial Parasitaemias

The prevalence of parasitaemia in the study children was 59.3% (156/263), with a geometric mean level of parasitaemia of 565 parasites/ $\mu$ l blood (range=37–649,600). Levels of parasitaemia did not appear to vary significantly with age or gender. The parasitaemic children had significantly lower haemoglobin concentrations ( $P=0.034$ ) and erythrocyte counts ( $P=0.045$ ) than the aparasitaemic (Table 2).

### Helminth Infections

Forty-four of the 209 (22.1%) children who provided stool samples were found to be infected with *Ascaris* (19 children) or *Trichuris* (16 children) or both of these parasites (nine children). The geometric mean counts did not vary significantly with gender, age or altitude (Table 3). Compared with their apparently helminth-free counterparts, the children found to have helminth infections had significantly lower leucocyte counts ( $P=0.006$ ) but similar erythrocyte counts and haemoglobin concentrations ( $P>0.05$  for each). Although 29 (65.3%) of the helminth-positive children

were also parasitaemic, there was no significant association between parasitaemia and helminth infection (odds ratio=1.35;  $P>0.05$ ). As shown in Table 2, the children who were co-infected with *Plasmodium* and at least one type of intestinal helminth had significantly lower erythrocyte counts than the children who appeared uninfected or the children infected with helminths only or *Plasmodium* only. Although counts of *T. trichuria* eggs (recorded for 18 children) increased with the age of the child ( $r=0.466$ ;  $P=0.05$ ), they showed no correlation with the *Ascaris* egg counts.

### Fever

Although 73 (27.8%) of the children were found to be febrile, only 36 (50%) of the febrile children checked for malarial infection were found to be parasitaemic. There was, however, still a significant association ( $\chi^2=4.149$ ;  $P=0.042$ ) between fever and parasitaemia. The prevalence of fever did not appear to be affected by age, gender, helminth infection or altitude. The child with the most intense parasitaemia observed during either survey (649,600 parasites/ $\mu$ l blood) had an axial temperature of 38.9°C.

### Haematological Indices

Infection with *Plasmodium* reduced erythrocyte counts, a condition that was exacerbated by co-infection with helminths (Table 2). Fever was also associated with relatively low erythrocyte counts ( $P=0.043$ ). The aparasitaemic children who were found infected with helminths had higher leucocyte counts than the parasitaemic children who appeared helminth-free ( $P=0.01$ ).

Haemoglobin concentrations were found to be negatively correlated with level of malarial parasitaemia ( $r=-0.224$ ;  $P=0.005$ ) but positively correlated with age ( $r=0.151$ ;  $P=0.026$ ). Although anaemia was recorded in 31 (11.9%) of the 260 study subjects who had their haemoglobin concentrations determined, 22 (70.4%) of the anaemic

TABLE 1. *The body temperatures of the study children and the results of the haematological and parasitological investigations*

| Variable                           | All subjects     |                                |  | Rainy-season subjects |                       |  | Dry-season subjects |                       |                |
|------------------------------------|------------------|--------------------------------|--|-----------------------|-----------------------|--|---------------------|-----------------------|----------------|
|                                    | No. investigated | Mean value, (s.d.) and [range] |  | No. investigated      | Mean value and (s.d.) |  | No. investigated    | Mean value and (s.d.) | P <sup>*</sup> |
| Axillary temperature (°C)          | 263              | 37.17 (0.41) [36.1–39.8]       |  | 132                   | 37.24 (0.38)          |  | 131                 | 37.10 (0.43)          | 0.006          |
| Haemoglobin (g/dl)                 | 260              | 12.22 (1.26) [7.3–16.0]        |  | 129                   | 12.35 (1.34)          |  | 131                 | 12.10 (1.17)          | 0.11           |
| Leucocyte count (cells/ $\mu$ l)   | 256              | 6780 (2667) [2200–27,400]      |  | 126                   | 7057 (2342)           |  | 130                 | 6512 (2933)           | 0.102          |
| Erythrocyte count (cells/nl)       | 258              | 3670 (930) [900–9160]          |  | 127                   | 3670 (1110)           |  | 131                 | 3680 (730)            | 0.993          |
| Parasitaemia (parasites/ $\mu$ l)* | 156              | 565 [37–649,600]               |  | 71                    | 804                   |  | 85                  | 422                   | 0.01           |
| EGG COUNT (eggs/g faeces)*         |                  |                                |  |                       |                       |  |                     |                       |                |
| <i>Ascaris</i>                     | 28               | 793 [24–12,672]                |  | 16                    | 477                   |  | 12                  | 1567                  | 0.066          |
| <i>Trichuris</i>                   | 25               | 78 [24–768]                    |  | 13                    | 73                    |  | 12                  | 84                    | 0.755          |

\*The mean values for parasitaemia and eggs counts are geometric.

<sup>†</sup>For the comparison between dry- and rainy-season subjects.

TABLE 2. Comparison of the haematological indices in the primary schoolchildren of different Plasmodium- and helminth-infection status

| Variable                         | Mean value, (s.d.) and [no. of subjects investigated], for subjects: |                                      |                                     |
|----------------------------------|----------------------------------------------------------------------|--------------------------------------|-------------------------------------|
|                                  | Co-infected                                                          | Infected with <i>Plasmodium</i> only | Infected with helminths only        |
| ERYTHROCYTE COUNT (cells/nl)     |                                                                      |                                      |                                     |
| Rainy-season subjects            | 2870 (490)[16]                                                       | 3550 (1200)[54]                      | 3850 (910)[7]                       |
| Dry-season subjects              | 3720 (770)[13]                                                       | 3660 (760)[72]                       | 3760 (520)[8]                       |
| All subjects                     | 3250 (760) <sup>†</sup> [29]                                         | 3610 (970) <sup>†</sup> [126]        | 3800 (700)[15]                      |
| LEUCOCYTE COUNT (cells/ $\mu$ l) |                                                                      |                                      |                                     |
| Rainy-season subjects            | 7246.67 (2859.29)[15]                                                | 6853.70 (2561.38)[54]                | 7385.71 (1880.10)[7]                |
| Dry-season subjects              | 6641.67 (1959.80)[12]                                                | 6625.00 (2240.08)[72]                | 10,012.50 (8040.87)[8] <sup>‡</sup> |
| All subjects                     | 6977.78 (2474.07)[27]                                                | 6723.02 (2375.88)[126]               | 8786.67 (5973.50) <sup>§</sup> [15] |
| HAEMOGLOBIN (g/dl)               |                                                                      |                                      |                                     |
| Rainy-season subjects            | 12.12 (1.15)[16]                                                     | 12.06 (1.40)[55]                     | 12.96 (1.10)[7]                     |
| Dry-season subjects              | 11.92 (1.44)[13]                                                     | 12.11 (1.18)[72]                     | 11.91 (0.94)[8]                     |
| All subjects                     | 12.03 (1.26)[29]                                                     | 12.09 (1.28) <sup>†</sup> [127]      | 12.40 (1.12)[15]                    |

\*Significantly lower than the corresponding values for the children with *Plasmodium* only ( $P=0.035$ ), the children with helminths only ( $P=0.025$ ) or the uninfected children ( $P=0.002$ ).

<sup>†</sup>Significantly lower than the corresponding value for the uninfected children ( $P=0.045$ ).

<sup>‡</sup>Significantly higher than the corresponding values for the co-infected children ( $P=0.039$ ), the children with *Plasmodium* only ( $P=0.006$ ) or the uninfected children ( $P<0.001$ ).

<sup>§</sup>Significantly higher than the corresponding values for the children with *Plasmodium* only ( $P=0.011$ ) or the uninfected children ( $P=0.006$ ).

<sup>†</sup>Significantly lower than the corresponding value for the uninfected children ( $P=0.034$ ).

TABLE 3. The prevalences and intensities of malarial parasitaemia and geohelminth infection, the prevalences of fever, haemoglobin concentration and leucocyte and erythrocyte counts in schoolchildren in the Mount Cameroon area, split by altitude of residence and (for the low-altitude children) by season

| Variable                                   | Low altitude (400–650 m) |              |                       |              |                     |              | High altitude (900–1000 m) |              |     |        |
|--------------------------------------------|--------------------------|--------------|-----------------------|--------------|---------------------|--------------|----------------------------|--------------|-----|--------|
|                                            | All subjects             |              | Rainy-season subjects |              | Dry-season subjects |              | Dry-season subjects†       |              |     |        |
|                                            | No.                      | Value        | No.                   | Value        | No.                 | Value        | No.                        | Value        | No. | P*     |
| Prevalence of parasitaemia (%)             | 184                      | 63.6         | 129                   | 55           | 55                  | 83.6         | 76                         | 51.3         | 76  | <0.001 |
| Geometric mean parasitaemia (parasites/µl) | 117                      | 741          | 71                    | 804          | 46                  | 653          | 39                         | 252          | 39  | 0.49   |
| MEAN VALUE AND (S.D.) FOR                  |                          |              |                       |              |                     |              |                            |              |     |        |
| Haemoglobin (g/dl)                         | 184                      | 12.21 (1.29) | 129                   | 12.34 (1.34) | 55                  | 11.86 (1.12) | 76                         | 12.27 (1.12) | 76  | 0.019  |
| Leucocyte count (cells/µl)                 | 181                      | 7192 (2397)  | 126                   | 7056 (2341)  | 55                  | 7504 (2513)  | 75                         | 5784 (3020)  | 75  | 0.249  |
| Erythrocyte count (cells/nl)               | 182                      | 3640 (970)   | 127                   | 3670 (1110)  | 55                  | 3600 (590)   | 76                         | 3730 (810)   | 76  | 0.652  |
| Prevalence of fever (%)                    | 187                      | 31           | 132                   | 25.1         | 55                  | 5.9          | 76                         | 19.7         | 76  | 0.036  |
| PREVALENCE OF GEOHELMINTH INFECTION        |                          |              |                       |              |                     |              |                            |              |     |        |
| <i>Ascaris</i> and/or <i>Trichuris</i>     | 147                      | 22.4         | 109                   | 15.6         | 38                  | 6.8          | 62                         | 17.7         | 62  | 0.529  |
| <i>Ascaris</i>                             | 147                      | 14.3         | 109                   | 10.9         | 38                  | 3.4          | 62                         | 11.3         | 62  | 0.518  |
| <i>Trichuris</i>                           | 147                      | 13.6         | 109                   | 8.8          | 38                  | 4.8          | 62                         | 8.1          | 62  | 0.597  |
| GEOMETRIC MEAN EGG COUNT AND (S.D.)        |                          |              |                       |              |                     |              |                            |              |     |        |
| (eggs/g faeces)                            |                          |              |                       |              |                     |              |                            |              |     |        |
| <i>Ascaris</i>                             | 21                       | 579 (2041)   | 16                    | 477 (1031)   | 5                   | 1083 (3349)  | 7                          | 2039 (4329)  | 7   | 0.366  |
| <i>Trichuris</i>                           | 20                       | 83 (217)     | 13                    | 73 (212)     | 7                   | 105 (239)    | 5                          | 61 (13)      | 5   | 0.534  |

\*For differences in variables between the rainy and dry seasons at low altitude.

†No permission was received to sample the high-altitude schools during the rainy season.

\*For differences in variables, for all subjects, at low and high altitudes.

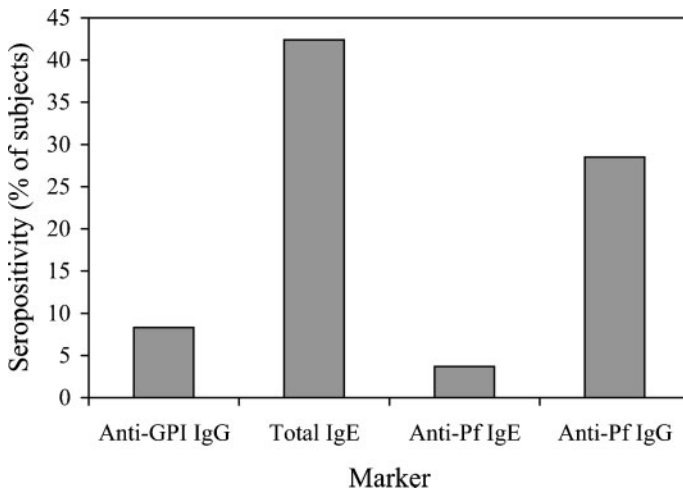


FIG. The seropositivities recorded, for *Plasmodium falciparum*-specific (Anti-PF) IgE and IgG, anti-glycosylphosphatidylinositol (Anti-GPI) IgG and elevated plasma concentrations of total IgE, among apparently healthy schoolchildren from south-western Cameroon.

individuals only had mild anaemia and none had severe anaemia.

### Effects of Altitude, School Type and Season

When compared with those at lower altitude, the children at high altitude had relatively low parasitaemias and leucocyte counts ( $P=0.0001$  for each). At low altitude, the children attending government schools had higher prevalences ( $\chi^2=13.62$ ;  $P<0.01$ ) and intensities ( $P=0.010$ ) of parasitaemia than those attending mission schools. At the end of the rains (December), at low altitude, the prevalence of parasitaemia was higher, the prevalence of fever was lower and haemoglobin concentrations were generally lower (but the prevalences of infection with *Trichuris* and/or *Ascaris* were similar) than at the beginning of the rains (March).

### *Plasmodium*-specific IgG and IgE, Anti-GPI IgG and Total IgE

The observed seropositivities, which are summarized in the Figure, all appeared independent of age, 'season' (December *v.* April), febrile status and school type (government *v.* mission). Children at low

altitude were, however, more likely to be seropositive for anti-*P. falciparum* IgE than their high-altitude counterparts ( $\chi^2=7.991$ ;  $P=0.005$ ), and the aparasitaemic children were more likely to be found positive for anti-GPI IgG than the parasitaemic ( $\chi^2=5.018$ ;  $P=0.025$ ).

The mean (S.D.) OD for anti-GPI IgG, total IgE, anti-*P. falciparum* IgE and anti-*P. falciparum* IgG were 0.05 (0.10) ( $N=205$ ), 0.16 (0.29) ( $N=71$ ), 0.025 (0.028) ( $N=213$ ) and 0.07 (0.05) ( $N=219$ ), respectively. The mean OD for anti-GPI were relatively low in children from low altitude ( $F=6.150$ ;  $P=0.014$ ) and children attending government schools ( $F=3.762$ ;  $P=0.05$ ) and were just significantly lower in the children sampled during April than in those checked in December, with means (S.D.) of 0.06 (0.08) and 0.04 (0.11), respectively ( $P=0.05$ ). The OD for anti-GPI were also negatively correlated with level of parasitaemia ( $r=-0.384$ ;  $P=0.0001$ ).

The children with helminth infection had significantly more total IgE in their plasma than either the uninfected children ( $P=0.012$ ) or those with malarial infection only ( $P=0.01$ ). The children with low-grade parasitaemias had higher levels of anti-*P. falciparum* IgE than those with high-grade

parasitaemias ( $P < 0.05$ ). Mean levels of *P. falciparum*-specific IgG were, however, apparently unaffected by the intensity or prevalence of parasitaemia, or by age, gender, altitude, type of school, or helminth-infection status.

## DISCUSSION

In general, the results of the present study tally with current understanding of the prevalence and intensity of malarial and helminth co-infections, their impact on anaemia, and the humoral immune responses to these infections. The study children were recruited from the classroom and were, therefore, clinically well on the day of sampling. Almost all (>96%) were aged  $\geq 5$  years and therefore, presumably, had some degree of antimalarial immunity as a result of repeated exposure to infective mosquito bites.

The results show a high prevalence of asymptomatic malarial infection in schoolchildren, an observation made previously in the study area (Kimbi *et al.*, 2004) and in southern Cameroon (Deloron *et al.*, 1999). The high prevalence parallels the intense perennial transmission of *Plasmodium* in the study region (Wanji *et al.*, 2003). The intensity of parasitaemia (but not the prevalence) varied significantly with altitude, the children from the low-altitude schools generally having more intense parasitaemias than their high-altitude counterparts. Again, similar observations have been made elsewhere (Ellman *et al.*, 1998; Maxwell *et al.*, 2003; Clarke *et al.*, 2004). The lowlands tend to be warmer and flatter than the highlands, leading to more breeding sites for, and faster breeding by, the vectors, and thus larger vector populations than those seen at higher altitude. In addition, in Buea, there has been much road building and house building at low altitude over the last few years and this has added to the areas of standing water in which *An. gambiae* s.s. breeds. It is worth noting, however, that the

distances between the high-altitude schools and low-altitude schools investigated in the present study are sufficiently short for many of the 'high-altitude' children to participate regularly in socio-economic activities at low altitude. Such movements probably reduce the apparent effects of altitude on malarial infection, since they bring children who live at high altitude into the 'high-risk' lowlands.

The observation of higher levels of parasitaemia among the non-fee-paying children attending government schools than among the fee-paying schoolchildren attending mission schools (made, at low altitude, in the present study) has also been reported previously in South West province (Kimbi *et al.*, 2004). The children attending the government schools tend to come from relatively poor homes, where poor hygiene and lack of personal-protection methods against mosquito bites tend to lead to relatively high risks of malarial infection. In addition, the infrastructure in the government schools is relatively poor (many pupils spend the greater part of the school day playing out of class, without supervision), with teachers who are not as committed to their pupils as the teachers in the mission schools, and little attention given to hygiene or health education.

Worryingly, 28% of the schoolchildren were febrile when they were recruited in their classes. One child had an axial temperature as high as 38.9°C and a very intense parasitaemia (649,600 parasites/ $\mu$ l) yet was still in school 'learning'. It is unclear if the febrile children were sent to school by caregivers who knew of their fever or by caregivers who were unaware of the fever (and who were, perhaps, incapable of detecting fever in their dependents). In general, a significant proportion of febrile illness in children resident in malaria-endemic areas is associated with malarial infection and, as in the present study, fever and parasitaemia are often found to be significantly associated. At least some of the children with asymptomatic parasitaemia may progress to symptomatic disease,

which can adversely affect school performance, especially after repeated episodes (Fernando *et al.*, 2003).

About 21% of the children investigated in the present study were found to be infected with intestinal helminths, although, surprisingly, only two species of such helminths were observed. Only 11% of the pregnant Nigerian women investigated by Egwunyenga *et al.* (2001) were found to be excreting helminth eggs but higher prevalences of geohelminth infection have been recorded elsewhere in Cameroon (38.3%; Adio *et al.*, 2004) and in Uganda (55.9%; Kabatereine *et al.*, 2001). Over much of Africa, in fact, soil-transmitted helminths are hyper-endemic, especially among children (Odutan, 1974; Obiamiwe, 1977; Nwosu, 1981; Udonsi, 1984; Holland *et al.*, 1989; Obiamiwe and Nmorsi, 1991). The high risk of geohelminth infection in children has been associated with their particularly poor hygiene and recreational or other activities that regularly bring them close to areas contaminated with human faeces. Differences in prevalence may reflect variation in the faecal pollution of soil and water supplies, around homes and schools, that results from poor sanitation and improper sewage disposal. Exposure to helminth infection may also vary markedly within small areas, as the result of environmental variables such as distance to the transmission site, and over relatively short times (Klump and Webbe, 1987; Kloos *et al.*, 1997).

Booth *et al.* (2004a, b) highlighted the importance of considering the joint impact of *Plasmodium*–helminth co-infection when addressing the health of children in malaria-endemic areas. There are, however, conflicting reports on how helminth infection affects malarial infection and vice versa. In the present study, the geohelminth-positive children tended to be parasitaemic but there was no statistically significant association between helminth status and parasitaemia. A similar observation was made, in Uganda, by Shapiro *et al.* (2005). In Thailand,

however, a positive and statistically significant association between geohelminth and malarial infection has been reported (Nacher *et al.*, 2002a), although those infected with geohelminths appeared to be protected against severe malaria (Nacher *et al.*, 2002b).

The low prevalence of anaemia observed in the present study (11.9%) corroborates previous reports on the schoolchildren of South West province (Adio *et al.*, 2004). The anaemia observed was predominantly of mild form (70.4%), again consistent with the results of earlier studies in the same area (Nkwo-Akenji *et al.*, 2002). Since the prevalence of anaemia declines with age (Cornet and Bundy, 1994), schoolchildren tend to have less anaemia than infants, and ancylostomiasis — which is an important risk factor for iron-deficiency anaemia in schoolchildren (Akenzua *et al.*, 1985) — has not been found in Buea. Although malaria plays a key role in the aetiology of anaemia in endemic countries (Mackintosh *et al.*, 2004), poor nutritional status (Dallman *et al.*, 1980; Tatala *et al.*, 1998), micronutrient deficiencies (Fleming and Werblinska, 1982), HIV infection (Van Eijk *et al.*, 2002) and haemoglobinopathies (Fleming and Werblinska, 1982; Greenwood *et al.*, 1987) may all make important additional contributions.

Malarial parasitaemia was found to be associated with relatively low haemoglobin concentrations in the present study, confirming the much older observations on young children in Papua New Guinea (Spencer, 1966) and The Gambia (McGregor *et al.*, 1966). Among infants, the haemoglobin deficit associated with parasitaemia tends to decrease with age (McGregor *et al.*, 1966; Spencer, 1966). In older children, however, this age-related trend is less obvious (Spencer, 1966; Stoltzfus, 1997; present study), probably because of the partial immunity to malarial infection that is acquired rapidly in areas of very high transmission (Snow *et al.*, 1999). *Plasmodium* infection is thought to lower

haemoglobin levels by inducing haemolysis. Erythrocyte destruction may occur as a result of the direct action of the intra-erythrocytic malarial parasites and/or the result of parasite-induced immune responses that lead to the lysis of both parasitised and non-parasitised erythrocytes (Verhoef *et al.*, 2002). Chronic loss of blood from the soil-transmitted helminths (Migesana *et al.*, 1984) appears to cause lower erythrocyte counts in parasitaemic individuals who are co-infected with such helminths than in the aparasitaemic but helminth-infected or in the parasitaemic but helminth-free (Table 2).

Parasite infections often trigger eosinophilia (Rothenburg and Hoogan, 2006) and the elevated leucocyte counts associated with helminth infestation in the present study are probably the result of such a response. The relatively low leucocyte counts at high altitude (Table 3) may be explained by the relatively intense parasitaemias at low altitude, with leucocytosis (perhaps, again, predominantly eosinophilia) increasing with the intensity of malarial infection.

The present study provides further evidence for an active role for IgE in human immunity to helminth infection and for parasite-specific IgE in immunity to *P. falciparum* malaria. Nematode-infected children tend to have elevated plasma concentrations of total IgE (Pritchard *et al.*, 1992; Pritchard and Walsh, 1995; Pettit *et al.*, 2005; present study). In the present study, a child at low altitude, where malaria transmission was most intense, was more likely to be seropositive for anti-*P. falciparum* IgE than a child at high altitude but a child with high-grade parasitaemia had lower levels of such parasite-specific IgE than the other parasitaemics, indicating a protective role for such antibodies.

As, in humans, antibody responses to helminth infection may not confer protection against the current infection or re-infection, it has been suggested that anti-helminth immunity may not be based

on the host's humoral immune system (King *et al.*, 2005). There is, however, much evidence to indicate that IgG and, particularly, IgE responses are involved in the killing of helminth larvae. Eosinophils and IgE may act together to kill the larvae, via antibody-dependent cell-mediated cytotoxicity (Quinnell *et al.*, 2004). Recent studies have demonstrated either a Th2 (Falcone *et al.*, 2004; Lertanekawattana *et al.*, 2005) or a mixed Th1/Th2 response to helminth infection in humans (Quinnell *et al.*, 2004). In populations resident in helminth-endemic area, levels of total IgE are thought to be influenced by genetic factors in the population. A capacity for good IgE responses could thus have been vital in our ancestor's 'wormy and malarious' past. The relative lack of variation seen in anti-*P. falciparum* IgG in the present study (in terms of OD or seropositivities) probably reflects the young age of the subjects studied.

Glycosylphosphatidylinositol (GPI), the anchor molecule for some membrane proteins of *Plasmodium*, has been implicated in the induction of immunopathology during malarial infections. Neutralization of GPI, by antibodies, may thus reduce the severity of clinical attacks of malaria (Tachado *et al.*, 1996). Adults who have clinical resistance to malaria have been found to have high levels of persistent anti-GPI antibodies (Naik *et al.*, 2006) and such antibodies have been associated with protection against cerebral malaria (Perraut *et al.*, 2005). In the present study, levels of anti-GPI IgG were negatively correlated with malarial parasitaemia, an observation that may explain why the aparasitaemic children were the most likely to be seropositive for anti-GPI and why the children who lived at low altitude, were sampled in April and/or attended government schools had relatively high levels of IgG that reacted with GPI. Although these trends lend support to a protective role for anti-GPI, the results of several earlier studies do not seem to show such a role. Anti-GPI antibodies were found not to prevent placental malarial infections or

malaria-associated poor birth outcomes (Suguitan *et al.*, 2004), for example, or to confer resistance to mild or severe malaria (De Souza *et al.*, 2002). It has been suggested that host age, independent of the cumulative exposure to malaria, represents a key determinant of the qualitative and quantitative nature of the IgG response to *P. falciparum* GPI (Keenihan *et al.*, 2003). Anti-GPI antibodies have been shown to be predominantly of the IgG<sub>3</sub> subclass, with a lesser contribution from IgG<sub>1</sub> (Boutlis *et al.*, 2003). Further prospective case-control studies are required to elucidate the role of anti-GPI antibodies in protection against symptomatic malaria.

Although asymptomatic malaria parasitaemia and helminths have previously been shown to co-exist in schoolchildren from south-western Cameroon (Adio *et al.*, 2004), the results of the present study stress the importance of fever, altitude and school type when designing intervention strategies aimed at controlling helminthiasis and malaria in such children. The present results also confirm a possible protective role for IgE antibodies against helminths and indicate that anti-GPI antibodies may be important in the development of anti-malarial immunity in these children — findings that have implications in the development of malaria vaccines.

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