Plasmodium vivax infection: a major determinant of severe anaemia in infancy

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Abstract

Background: Most malarious countries outside of Africa are co-endemic for Plasmodium falciparum and Plasmodium vivax. The comparative burden of anaemia in the community caused by these two species is incompletely characterized.

Methods: A three-stage, cross-sectional, community survey was used to determine the proportion of moderate or severe anaemia (haemoglobin <7 g/dL attributable to patent P. vivax, P. falciparum and mixed parasitaemia in Papua, Indonesia. Adjusted population-attributable fractions were calculated from multivariable logistic regression models. Eight hundred and twenty-five households were surveyed with a total of 5255 occupants, 3890 (74%) of whom were present and provided a blood sample. Plasmodium falciparum parasitaemia was present in 8.1% (n = 315) of participants, P. vivax in 6.4% (n = 250) and mixed infections in 1.9% (n = 72). Overall, P. falciparum was associated with a mean reduction in haemoglobin of 1.16 g/dL compared to those without patent parasitaemia [95% confidence interval (95% CI) 0.91, 1.41 g/dL]. The corresponding values for P. vivax and mixed infections were 0.66 g/dL (95% CI 0.35, 0.96) and 1.25 g/dL (0.71, 1.80), respectively. Overall, 16.7% (95% CI 8.52, 24.2%) of haemoglobin concentrations <7 g/dL in the community were estimated to be attributable to patent parasitaemia. The fractions for infants and 1–5 years old were 34.4% (95% CI –3.30, 58.3%) and 23.2% (95% CI 3.34, 39.0%), respectively. Plasmodium vivax was associated with a greater than threefold higher attributable fraction of anaemia in infants compared with P. falciparum [27.6% (95% CI −3.20, 49.2%) versus 7.94% (−5.87, 20.0%)].

Conclusion: Despite comparatively low-level endemicity, malaria is associated with a significant proportion of all cases of community anaemia in southern Papua. Contrary to its benign reputation, P. vivax is an important and preventable risk factor for anaemia during infancy—a probable consequence of relapsing disease prior to the development of immunity.

Keywords: Malaria, Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Anaemia, Indonesia

Background

Anaemia is a common manifestation of Plasmodium infection [1, 2]. Its impact is most apparent in the hospital setting where it accounts for a substantial proportion of malaria morbidity and, to a lesser extent, mortality [3–6].

The burden of malarial anaemia outside of healthcare facilities is less well understood and its contribution to ‘indirect’ malaria morbidity and mortality is largely unknown [7]. There are two major explanations for this uncertainty. Firstly, the adverse effects of mild or moderate anaemia per se are not clearly understood. Haemoglobin concentrations below 7 g/dL probably confer an increased risk of poor pregnancy outcomes such as haemorrhagic shock [5], low birth weight [8, 9] and poor neurocognitive development [10, 11] but other, less tangible, effects such as decreased resilience to infectious
Setting provides an opportunity to establish the com-
direct malaria-attributable mortality rate [19]. Such a
tropical African nations [one to four infective bites per
It has a lower entomological inoculation rate than most
America, is co-endemic for
P. falciparum

P. vivax

P. farauti and Anopheles punctulatus. The annual incidence
of clinical or asymptomatic malaria is approximately
876 episodes per 1000 people, 46 % due to P. falciparum
and 39 % due to P. vivax [28]. Given its equatorial loca-
tion, the climate in Timika does not change significantly
throughout the year and malaria transmission is peren-
nial. At the time of the survey, patients with uncompli-
cated malaria were generally treated with chloroquine
plus sulfadoxine-pyrimethamine, or chloroquine alone.
High rates of resistance to these antimalarials are present
in both P. falciparum and P. vivax isolates in the region
and therefore both regimes were abandoned in favour of
artemisinin-based combination therapy shortly after this
survey was conducted. A 14-day course of primaquine
was encouraged for those with vivax malaria, however
administration was unsupervised.

Cross-sectional survey methods
Households for this survey were chosen by cluster ran-
dom sampling. First, the four largest of the 12 sub-dis-
tricts in Mimika were chosen purposively. Second, the
number of clusters required in each sub-district was
apportioned according to the relative populations of the
sub-districts. In most cases, clusters constituted discrete
villages, however, in Mimika Baru the very large popula-
tion size dictated that villages within this sub-district be
sub-divided into census blocks. Once mapped, clusters
and 25 houses within each cluster were chosen randomly
according to WHO recommendations [29]. Household
members were defined as people who lived under one
roof, ate from one kitchen and who had resided in the
study area for at least 6 months. There were no exclusion
criteria. Sociodemographic information, self-reported
pregnancy status and history of fever were recorded for
all household members using a standardized question-
naire. For household members that were not present at
the time of the survey, this information was collected
from the head of the household. Those present at the
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using a uniscale. Axillary temperature was recorded
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of blood taken for blood film examination and haemo-
globin measurement. Patients with microscopically con-

confirmed malaria were treated according to the Indonesian
Ministry of Health Guidelines. Those with anaemia were
given iron supplementation according to local protocols.
The survey was carried out between July and December
2005.

Methods
Study site
The geography, climate and demographics of Mimika
District and its capital city, Timika, have been described
elsewhere [19, 22, 28]. A census in 2004 estimated the
local population to be 130,000. Malaria in the region is
restricted to lowland areas where it is associated with
three mosquito vectors: Anopheles koliensis, Anopheles
farauti and Anopheles punctulatus. The annual incidence
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Laboratory methods
Blood films were read locally by certified microscopists with at least 10 years experience. A thick smear was considered negative on initial review if no parasites were seen in 100 high power fields. A thin smear was also examined to confirm parasite species and used for quantification if parasitaemia was greater than 200 per 200 WBC. Upon cross-checking 200 high power fields were checked before slides were considered negative. Parasitaemias were calculated assuming a white cell count of 7300 cells/µL. All positive films and 10 % of the negative slides were crosschecked at the National Institute of Health Research and Development reference laboratory in Jakarta. Results that differed were reviewed by the two lead microscopists for final assessment. Haemoglobin concentrations were determined using a calibrated portable Hemacue® machine.

Statistical analyses
All analyses were done in STATA® version 10.1 (Stata-Corp, College Station, TX, USA). The primary outcomes in this study were: the absolute haemoglobin concentration, the odds of moderate or severe anaemia and the population-attributable fraction of moderate or severe anaemia associated with infection by the different Plasmodium species. For the purposes of this study, a haemoglobin concentration of 7 g/dL was chosen a priori as an appropriate distinction between mild and moderate or severe anaemia (as recommended by Snow and colleagues [7]).

Tests for trend were done using the ptrend module for STATA. Univariable linear regression of continuous haemoglobin data was performed for the following exposures: Plasmodium species (P. falciparum, P. vivax and mixed P. falciparum/P. vivax species infections), age group (<1 year, 1 to <5 years, 5 to <15 years, ≥15 years), self-reported ethnicity (non-Papuan, Highland Papuan, Lowland Papuan), pregnancy status, weight for age/gender/ethnicity (≥survey mean, <survey mean) and household income per person (≥75th centile, 25th–75th centile, <25th centile). Weight for age/gender/ethnicity was established by creating a nomogram from the survey data.

Multivariable linear and logistic regression analyses of the effect of Plasmodium parasitaemia on absolute haemoglobin and odds of moderate or severe anaemia were done for each of the age groups as well as for the study population as a whole. To account for the study design, multivariable models were adjusted for the categorical variable ‘subdistrict’ and the variance–covariance matrices of both univariable and multivariable models were adjusted for within-household correlation (giving robust standard errors). Given the effect of menstruation on haemoglobin concentrations, the hypothesis was that gender would modify the relationship between age and anaemia. The interaction between age and a composite variable incorporating gender and pregnancy status was found to be statistically significant and therefore was included in multivariable models of the whole study population.

Adjusted population fractions of moderate-to-severe anaemia attributable to patent parasitaemia were calculated using the alogit module for STATA [30]. The attributable fractions cannot be summed as the model assumes a mutually exclusive scenario where each risk factor is deemed to be the first to be eliminated [31]. Outputs can therefore be interpreted as the proportion of moderate or severe anaemia that could be prevented by addressing the particular factor of interest in isolation. Patients with Plasmodium malariae infections were excluded from all regression models due to small numbers.

Results
Parasitaemia
In total, 5255 individuals resided in the 825 households surveyed of whom 3890 (74 %) were present and consented to providing a finger-prick sample of blood (Fig. 1). Those who either declined to provide a sample or were not present at the time of survey were an average of 4 years older (24.7 vs 20.6 years) and more likely to be male (71.2 vs 48.2 %) than their counterparts who provided blood samples. Patent parasitaemia was detected in 17.0 % of the participants, with P. falciparum present in 8.1 % (n = 315), P. vivax in 6.4 % (n = 250) and mixed infections in 1.9 % (n = 72) (Table 1). A history of fever in the preceding 24 h was present in 33.3 % (105/315) of those with P. falciparum, 29.2 % (73/250) with P. vivax and 34.7 % (25/72) with mixed infections. More infants (<1 year) and children between the ages of 1 and under 5 years were infected with P. vivax compared to P. falciparum [12 vs five infants (p = 0.09) and 62 vs 50 children (1 to <5 years (p = 0.3)), respectively] whereas the opposite was observed for all other age groups. After infancy, there was a statistically significant trend to decreasing prevalence of P. vivax parasitaemia with increasing age (p = 0.003 for trend) but no such trend for P. falciparum (p = 0.13). Unlike in highly endemic regions, there was no age-associated decrease in the likelihood of having concomitant fever with parasitaemia for either species (p = 0.55 for P. vivax and p = 0.92 for P. falciparum). Overall, a higher proportion of Highland and Lowland Papuans were parasitized (21.2 and 17.3 %, respectively) than non-Papuans (13.1 %; p < 0.001 and p = 0.01, respectively).
Anaemia

The mean haemoglobin in the sample as a whole was 11.0 g/dL (95% reference range 6.1–15.9 g/dL) (Table 2) with 5.7% of individuals having a concentration less than 7 g/dL (222/3890). Presence of patent parasitaemia shifted the haemoglobin distribution curve markedly to the left and was associated with a bimodal pattern of haemoglobin concentrations with peaks at 9 and 10.5 g/dL (Fig. 2). Indigenous Papuans and females had significantly lower haemoglobin concentrations than their counterparts in univariable analyses while mean haemoglobin increased with increasing age up to 15 years. No correlation existed between haemoglobin and loge parasite density for P. falciparum [Pearson’s correlation coefficient \( r = -0.08, n = 290, 95\% CI -0.19, 0.04, p = 0.2 \)] whereas for P. vivax there was a weak negative
Table 2 Haemoglobin by presence of parasitaemia (regardless of presence of symptoms) and demographic characteristics

| Plasmodium species | N    | Mean Hb | Std dev | Coef (95 % CI) |
|-------------------|------|---------|---------|---------------|
| Negative          | 3229 | 11.2    | 2.41    | 0             |
| *P. falciparum*   | 315  | 9.8     | 2.43    | −1.38 (−1.67, −1.09) |
| *P. vivax*        | 250  | 10.2    | 2.58    | −0.99 (−1.33, −0.65) |
| *P. malariae*     | 24   | 8.9     | 2.42    | −0.18 (−2.52, −1.17) |
| Mixed infection   | 72   | 9.4     | 2.65    | −0.18 (−2.52, −1.17) |

Gender and pregnancy status

|                | N    | Mean Hb | Std dev | Coef (95 % CI) |
|----------------|------|---------|---------|---------------|
| Male           | 1874 | 11.4    | 2.70    | 0.85 (0.69, 1.00) |
| Female non-pregnant | 1929 | 10.6    | 2.18    | 0             |
| Female pregnant| 87   | 10.1    | 1.86    | −0.47 (−0.88, −0.07) |

Ethnicity

|               | N    | Mean Hb | Std dev | Coef (95 % CI) |
|---------------|------|---------|---------|---------------|
| Non-Papuan    | 1823 | 11.9    | 2.27    | 0             |
| Highland Papuan | 1044 | 10.0    | 2.48    | −1.82 (−2.06, −1.58) |
| Lowland Papuan| 1023 | 10.5    | 2.32    | −1.35 (−1.58, −1.12) |

Age (years)

|       | N    | Mean Hb | Std dev | Coef (95 % CI) |
|-------|------|---------|---------|---------------|
| <1    | 177  | 9.4     | 1.64    | −2.35 (−2.63, −2.07) |
| 1–5   | 642  | 9.8     | 1.98    | −1.91 (−2.09, −1.72) |
| 5–15  | 821  | 10.4    | 2.03    | −1.29 (−1.47, −1.10) |
| >15   | 2250 | 11.7    | 2.56    | 0             |

Weight for age/gender/ethnicity

|              | N    | Mean Hb | Std dev | Coef (95 % CI) |
|--------------|------|---------|---------|---------------|
| ≥Mean        | 1809 | 11.2    | 2.47    | 0             |
| <Mean        | 2081 | 10.8    | 2.47    | −0.42 (−0.59, −0.25) |

Household income per person

|                | N    | Mean Hb | Std dev | Coef (95 % CI) |
|----------------|------|---------|---------|---------------|
| >75th centile | 823  | 11.3    | 2.35    | 0.14 (−0.13, 0.40) |
| 25th–75th centile | 1776 | 11.2   | 2.51    | 0             |
| <25th centile | 974  | 10.7    | 2.43    | −0.46 (−0.74, −0.18) |
| All           | 3890 | 11.0    | 2.48    | 0             |

Hb haemoglobin, Std dev standard deviation, n number, coef linear regression coefficient, 95 % CI 95 % confidence interval

correlation ($r = −0.24$, $n = 248$, 95 % CI −0.35, −0.12, $p < 0.001$). Those with parasitaemia and fever within the last 24 h ($n = 205$) had similar mean haemoglobin concentrations as parasitaemic individuals without fever ($n = 456$) (mean haemoglobin 9.85 vs 9.93 g/dL, $p = 0.4$). Forty-two per cent (1629/3890) of the survey participants had a history of fever in the last month and these individuals had both a significantly lower mean haemoglobin concentration and an increased unadjusted odds ratio for moderate or severe anaemia compared to those without a history of fever (mean haemoglobin concentration, 10.6 vs 11.3 g/dL, $p < 0.001$, odds ratio for moderate or severe anaemia, 1.7, 95 % CI 1.3, 2.5, $p < 0.001$).

After adjusting for age, gender, ethnicity, pregnancy, and weight for age/gender/ethnicity, presence of *P. falciparum* parasitaemia was associated with an absolute reduction in haemoglobin of 1.16 g/dL (95 % CI 0.91, 1.41, $p < 0.001$) (Table 3). The corresponding values for *P. vivax* and mixed infections were 0.66 g/dL (95 % CI 0.35, 0.96) and 1.25 g/dL (0.71, 1.80), respectively ($p < 0.001$ for both).

Although numbers were small, *P. vivax* was associated with a larger mean reduction in haemoglobin of 1.93 g/dL (95 % CI 0.96, 2.89, $p < 0.001$) in infants. There was a smaller and non-statistically significant reduction for infants with *P. falciparum* (mean reduction = 0.66 g/dL, 95 % CI −0.86, 2.18, $p = 0.4$).

Table 4 shows adjusted odds ratios for having haemoglobin less than 7 g/dL. Individuals with mixed infections were most likely to have moderate or severe anaemia ([Adjusted Odds Ratio (AOR) = 3.18, 95 % CI 1.67, 6.07, $p < 0.001$] followed by patients with *P. falciparum* (AOR = 2.29, 95 % CI 1.52, 3.44, $p < 0.001$) and *P. vivax*, respectively (AOR = 1.93, 95 % CI 1.14, 3.25, $p = 0.01$). For infants with *P. vivax* the AOR was 47.7 (95 % CI 3.19, 712, $p = 0.005$) and for infants with *P. falciparum* it was 6.02 (95 % CI 0.54, 67.0, $p = 0.1$).

Population-attributable fractions of anaemia due to malaria

Patent parasitaemia due to any species of *Plasmodium* was responsible for 16.7 % (95 % CI 8.52, 24.2 %) of cases of moderate or severe anaemia in this study (Table 5). The corresponding values for *P. falciparum*, *P. vivax* and mixed infections were 8.35 % (95 % CI 3.25, 13.2 %), 4.94 % (95 % CI 0.18, 9.48 %) and 3.41 % (95 % CI 0.08, 5.95 %), respectively. The attributable fraction was greatest in infants (34.4 %, 95 % CI −3.30, 58.3 %) and decreased with increasing age thenceforth. *Plasmodium vivax* was responsible for greater than three times the proportion of moderate or severe anaemia in infants compared with *P. falciparum* (27.6 % (95 % CI −3.20, 49.2 %) versus 7.94 % (−5.87, 20.0 %)) although the precision of these estimates was poor. Figure 3 indicates that in general, a greater proportion of moderate or severe anaemia (haemoglobin <7 g/dL) is attributable to malaria than mild anaemia (haemoglobin <11 g/dL).

Discussion

Despite comparatively low-level endemicity, patent parasitaemia in southern Papua is associated with 17 % of all haemoglobin concentrations under 7 g/dL. In infants and young children, the corresponding proportions rise to 34 and 23 %, respectively. Although *P. vivax* is less prevalent than *P. falciparum* overall, this study has shown that in Mimika District it is the commoner species in children under 5 years of age and that it is associated with a higher population-attributable fraction of anaemia in infants. This study also suggests that mixed species infections are associated with a greater reduction in haemoglobin than *P. falciparum* or *P. vivax* infections alone.
In this survey, 17% of individuals were parasitaemic and approximately one-third of these were febrile. Presence of fever did not confer a greater risk of anaemia. Although parasitaemia was strongly associated with anaemia, the population attributable fractions presented are still likely to represent under-estimates of the true total effect of malaria on haemoglobin concentrations in the community. Full haematological recovery takes several weeks following acute malaria [32] suggesting that many aparasitaemic individuals may have been experiencing the haematological after-effects of recent malaria infection. Nearly a half of the survey participants had a history of fever in the preceding month and these individuals had significantly lower haemoglobin concentrations than those without a history of fever. Since 35% of fevers in the community are estimated to be due to

Table 3  Multiple linear regression showing the effect of *Plasmodium* parasitaemia on mean haemoglobin concentration (g/dL)

| Malaria                  | <1 year | 1–5 years | 5–15 years | >15 years | All |
|--------------------------|---------|-----------|------------|-----------|-----|
| Negative                 | 0       | 0         | 0          | 0         | 0   |
| All species              | −1.35 (−2.24, −0.46) | 0.003 | −1.27 (−1.65, −0.89) | <0.001 | −1.08 (−1.45, −0.70) | <0.001 | −1.08 (−1.36, −0.80) | <0.001 | −0.97 (−1.18, −0.77) | <0.001 |
| *P. falciparum*          | −0.66 (−2.18, 0.86) | 0.4   | −1.60 (−2.07, −1.12) | <0.001 | −1.40 (−1.90, −0.89) | <0.001 | −1.27 (−1.60, −0.94) | <0.001 | −1.16 (−1.41, −0.91) | <0.001 |
| *P. vivax*               | −1.93 (−2.89, −0.96) | <0.001 | −0.83 (−1.33, −0.33) | 0.001 | −0.38 (−0.91, 0.14) | 0.2    | −0.78 (−1.23, −0.32) | 0.001 | −0.66 (−0.96, −0.35) | <0.001 |
| Mixed                    | 1.60 (1.01,2.19) | <0.001 | −1.87 (−2.88, −0.85) | <0.001 | −1.82 (−2.39, −1.26) | <0.001 | −1.14 (−2.04, −0.25) | 0.01  | −1.25 (−1.80, −0.71) | <0.001 |

_Coef coefficient, 95% CI 95% confidence interval_

* Models also include gender, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan) and weight for age/gender/ethnicity (<mean, ≥mean)

* Models also include gender, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan) and weight for age/gender/ethnicity (<mean, ≥mean) and pregnancy status

* Model also includes age (as a continuous variable) by gender/pregnancy status, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan) and weight for age/ gender/ethnicity (<mean, ≥mean)
malaria, parasitaemia is likely to have been responsible for a sizeable, but unmeasured, proportion of all reduced haemoglobin concentrations in the aparasitaemic group.

The estimated adjusted population-attributable fractions of moderate or severe anaemia in infants were based on small numbers. Nevertheless, the finding that *P. vivax* accounts for a greater fraction of anaemia than *P. falciparum* is in agreement with results from the local hospital [1, 19, 33] as well as a previous cross-sectional survey in Papua New Guinea [34]. At the hospital, *P. vivax* is the most common cause of malaria-related admission in the first year of life and produces an equal or greater reduction in haemoglobin than *P. falciparum* [1, 19, 33]. Others have also observed that morbidity from vivax malaria is maximal at a much younger age than falciparum malaria [35–38], a phenomenon that Maitland and colleagues speculate is due to greater ease of transmission and more rapid acquisition of immunity [39]. In keeping with this hypothesis, it is the authors’ view that both the greater prevalence of vivax malaria and the severity of the associated anaemia in infancy observed in this study are not chance findings but are related to multiple relapses causing repetitive insults to the haematological system and inducing early development of immunity. Two sources of evidence from this study support this hypothesis. First, there was a statistically significant reduction in the prevalence of *P. vivax* parasitaemia with age, whereas there was no such reduction for *P. falciparum*. Second, infants with *P. vivax* parasitaemia in this study had had significantly more episodes of fever in the last month than infants with *P. falciparum* (median 1 vs 0 episodes, *p* = 0.007). The subsequent decline in the fraction of anaemia attributable to either species of *Plasmodium* with increasing age is likely to relate to three main factors: the acquisition of some degree of immunity, especially in the case of vivax malaria, the increasing importance of alternative causes of anaemia, such as intestinal helminthiasis and chronic infections, and lastly the increasing likelihood that lack of parasitaemia represents a state of remission or a period between primary infections rather than a state of complete malaria naivety.

Table 4  Adjusted odds ratios for having a haemoglobin concentration less than 7 g/dL

| Parasite negative | <1 year<sup>a</sup> | 1–5 years<sup>a</sup> | 5–15 years<sup>a</sup> | >15 years<sup>b</sup> | All<sup>c</sup> |
|-------------------|---------------------|----------------------|----------------------|----------------------|---------------------|
|                   | AOR (95 % CI) p      | AOR (95 % CI) p      | AOR (95 % CI) p      | AOR (95 % CI) p      | AOR (95 % CI) p      |
| Parasite negative | 1                   | 1                    | 1                    | 1                    | 1                    |
| Any species       | 12.7 (2.36, 68.8) 0.003 | 2.26 (1.12, 4.59) 0.02 | 2.47 (1.22, 5.01) 0.01 | 2.01 (1.20, 3.35) 0.008 | 2.25 (1.61, 3.15) <0.001 |
| P. falciparum     | 6.02 (0.54, 67.0) 0.1 | 2.64 (1.11, 6.26) 0.03 | 3.02 (1.23, 7.42) 0.02 | 1.08 (0.95, 3.44) 0.07 | 2.29 (1.52, 3.44) <0.001 |
| P. vivax          | 47.7 (3.19, 712) 0.005 | 1.37 (0.48, 3.77) 0.5 | 1.84 (0.62, 5.49) 0.3 | 2.12 (0.93, 4.82) 0.07 | 1.93 (1.14, 3.25) 0.01 |
| Mixed species     | –                   | 4.46 (1.49, 13.3) 0.008 | 2.11 (0.46, 9.62) 0.3 | 2.87 (0.64, 12.9) 0.2 | 3.18 (1.67, 6.07) <0.001 |

AOR adjusted odds ratio, 95 % CI 95 % confidence interval

<sup>a</sup> Models also include gender, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan) and weight for age/gender/ethnicity (<mean, ≥mean)

<sup>b</sup> Models also include gender, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan) and weight for age/gender/ethnicity (<mean, ≥mean) and pregnancy status

<sup>c</sup> Model also includes age (as a continuous variable) by gender/pregnancy status, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan) and weight for age/gender/ethnicity (<mean, ≥mean)

Table 5  Adjusted population-attributable fractions of moderate or severe anaemia (haemoglobin concentration less than 7 g/dL) by presence or absence of *Plasmodium* parasitaemia

| Parasite negative | <1 year<sup>a</sup> | 1–5 years<sup>a</sup> | 5–15 years<sup>a</sup> | >15 years<sup>b</sup> | All<sup>c</sup> |
|-------------------|---------------------|----------------------|----------------------|----------------------|---------------------|
|                   | aPAF 95 % CI aPAF 95 % CI aPAF 95 % CI aPAF 95 % CI aPAF 95 % CI aPAF 95 % CI aPAF 95 % CI aPAF 95 % CI aPAF 95 % CI aPAF 95 % CI aPAF 95 % CI |
| Parasite negative | 1                   | 1                    | 1                    | 1                    | 1                    |
| Any species       | 3.44 (−3.30, 58.3) 23.2 3.34, 39.0 | 19.4 0.99, 34.5 | 12.1 1.80, 21.4 | 16.7 85.2, 24.2 |
| P. falciparum     | 7.94 (−5.87, 20.0) 10.5 −0.66, 20.5 | 13.7 0.04, 25.5 | 5.34 −1.69, 11.9 | 8.35 32.5, 13.2 |
| P. vivax          | 27.6 (−3.20, 49.2) 4.07 −6.21, 13.4 | 4.20 −4.90, 12.7 | 4.16 −2.14, 10.1 | 4.94 0.18, 9.48 |
| Mixed species     | –                   | 8.55 1.93, 14.7 | 1.99 −3.08, 6.82 | 1.80 −1.91, 5.37 | 3.41 0.08, 5.95 |

aPAF adjusted population-attributable fraction, 95 % CI 95 % confidence interval

<sup>a</sup> Models also include gender, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan) and weight for age/gender/ethnicity (<mean, ≥mean)

<sup>b</sup> Models also include gender, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan) and weight for age/gender/ethnicity (<mean, ≥mean) and pregnancy status

<sup>c</sup> Model also includes age (as a continuous variable) by gender/pregnancy status, ethnicity (non-Papuan, Highland Papuan, Lowland Papuan) and weight for age/gender/ethnicity (<mean, ≥mean)
This study showed that mixed species infections were associated with a greater drop in haemoglobin and a higher risk of moderate or severe anaemia than infections with *P. falciparum* or *P. vivax* alone. This finding is consistent with other work from Papua [1, 19] and Papua New Guinea [40] but in direct contradiction to research carried out in Thailand and elsewhere [39]. Concomitant infection with *P. vivax* in northern Thailand has been postulated to attenuate the risk of severe anaemia secondary to *P. falciparum* infection, possibly due to some degree of cross-species immunity [32, 39]. In Papua, where endemicity of both species is higher, mixed infection may reflect a greater likelihood of having had multiple recent malaria infections (likely due to *P. vivax*), driving deeper levels of anaemia.

This study has several limitations. Due to the cross-sectional design it was not possible to draw solid conclusions about the direction of the observed associations. Although anaemia is an established sequel of both falciparum and vivax malaria, there is evidence that iron deficiency anaemia reduces the risk of falciparum malaria [41] and conversely, that administering iron supplements to iron-replete individuals may slightly increase the risk [42, 43]. The comparative effect of this reverse causation is likely to be small since there were no special community-wide supplementation programmes at the time of the survey.

The population-attributable fractions were estimated using odds ratios as approximations of relative risk. Since moderate or severe anaemia was not a particularly rare outcome, this may have resulted in slight overestimation of the attributable fractions, particularly for 1–5 years olds who had a prevalence of moderate or severe anaemia of 9.4%.

Selection bias may have affected the population-attributable fractions due to their heavy reliance on the prevalence of parasitaemia in the sample. Those who did not provide a blood sample (mostly due to absence at the time of the survey) tended to be older males. Overall, there was relatively little effect of increasing age or gender on the odds of parasitaemia, however those who did not provide a blood sample could conceivably have been at greater risk of malaria acquisition due to behavioural or lifestyle factors. If this were true, the fraction of anaemia attributable to malaria may have been underestimated.

Several potentially important confounders could not be controlled for in analyses. Infestation with intestinal helminths has been shown to cause an additive reduction in haemoglobin concentrations in malaria co-infected children [44]. Although there is a great deal of geospatial overlap between malaria and intestinal helminths [45], the immunological relationship remains less clear [46, 47]. Two studies by Nacher and Spiegel, respectively, suggest that the presence of intestinal helminths increases the risk of falciparum malaria by a factor of between 1.5 and 2.2 [48, 49]. Even if this is the case, the results of this study for infants and children under 5 years are unlikely to be significantly confounded since intestinal helminth density does not typically peak until early adulthood.

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**Fig. 3** Proportion of participants with haemoglobin concentrations <11 and <7 g/dL who had parasitaemia (bar labels = absolute numbers)
and a previous study showed minimal impact of intestinal helminthiasis on haemoglobin concentrations before 30 months of age [50].

Haemoglobin and red cell abnormalities as a whole are protective against severe malarial anaemia [51–53] but their effect on the risk of uncomplicated Plasmodium infection is less clear and may differ between species [51, 54, 55]. Since these disorders are themselves risk factors for anaemia, differences in their distribution between participants with and without parasitaemia could potentially have confounded the models.

Incorporating weight for age/gender/ethnicity into the multivariable regression models should have accounted for at least some of the potential confounding caused by iron deficiency anaemia. Since iron deficiency is thought to be protective against Plasmodium infection, any residual confounding is likely to have biased the results towards the null. Finally, the effects of chronic disease and bacteremia could not be controlled for in this analysis. The former is unlikely to have been important in the younger age groups and bacteremia would be expected to be rare in the community setting.

Conclusions

Despite comparatively low-level Plasmodium endemicity, patent parasitaemia (whether symptomatic or not) is an important and preventable cause of anaemia in southern Papua. Young children bear the brunt of this burden but the haematological effects also extend into adulthood. Plasmodium vivax is an especially important cause of anaemia in infants, probably because it causes recurrent disease prior to the onset of immunity. Since infancy is a time of increased susceptibility to infectious diseases as well as rapid physical and neurological development, anaemia associated with vivax malaria may be an important and under-estimated contributor to indirect malaria mortality and developmental morbidity in regions where this species is prevalent.

Abbreviations

AOR: adjusted odds ratio; CI: confidence interval; OR: odds ratio; R: Pearson’s correlation coefficient.

Authors’ contributions

SY, ET, MK, NMA, and RNP designed the survey. EK, MK, LB, and JRP carried out the survey. JAS, NMD, and RNP carried out the analysis. JAS, RNP, and NMD wrote the first draft of the manuscript. EK, MK, LB, SY, JAS, ET, NMA, JRP, and RNP revised and commented on the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Ethical considerations

The cross-sectional survey was approved by the ethics committees of the National Institute of Health Research and Development, Indonesian Ministry of Health (Jakarta, Indonesia) and Menzies School of Health Research (Darwin, Australia). Written informed consent was obtained from all adult participants as well as the parents of all children.

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