

Multidrug-Resistant *Plasmodium vivax* Associated with Severe and Fatal Malaria: A Prospective Study in Papua, Indonesia

Emiliana Tjitra¹, Nicholas M. Anstey², Paulus Sugiarto³, Noah Warikar^{4,5}, Enny Kenangalem^{4,6}, Muhammad Karyana¹, Daniel A. Lampah^{4,6}, Ric N. Price^{2,7*}

1 National Institute of Health Research and Development, Ministry of Health, Jakarta, Indonesia, **2** International Health Division, Menzies School of Health Research and Charles Darwin University, Darwin, Northern Territory, Australia, **3** Mitra Masyarakat Hospital, Timika, Papua, Indonesia, **4** Menzies School of Health Research-National Institute of Health Research and Development Malaria Research Program, Timika, Indonesia, **5** International SOS, Tembagapura, Papua, Indonesia, **6** District Health Authority, Timika, Papua, Indonesia, **7** Centre for Vaccinology and Tropical Medicine, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford, United Kingdom

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Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio; *Pv*, *Plasmodium vivax*; *Pf*, *Plasmodium falciparum*; RSMM, Rumah Sakit Mitra Masyarakat; RDS, respiratory distress syndrome; SMA, severe malarial anaemia

* To whom correspondence should be addressed. E-mail: rnp@menzies.edu.au

ABSTRACT

Background

Multidrug-resistant *Plasmodium vivax* (*Pv*) is widespread in eastern Indonesia, and emerging elsewhere in Asia-Pacific and South America, but is generally regarded as a benign disease. The aim of the study was to review the spectrum of disease associated with malaria due to *Pv* and *P. falciparum* (*Pf*) in patients presenting to a hospital in Timika, southern Papua, Indonesia.

Methods and Findings

Data were prospectively collected from all patients attending the outpatient and inpatient departments of the only hospital in the region using systematic data forms and hospital computerised records. Between January 2004 and December 2007, clinical malaria was present in 16% (60,226/373,450) of hospital outpatients and 32% (12,171/37,800) of inpatients. Among patients admitted with slide-confirmed malaria, 64% of patients had *Pf*, 24% *Pv*, and 10.5% mixed infections. The proportion of malarial admissions attributable to *Pv* rose to 47% (415/887) in children under 1 y of age. Severe disease was present in 2,634 (22%) inpatients with malaria, with the risk greater among *Pv* (23% [675/2,937]) infections compared to *Pf* (20% [1,570/7,817]; odds ratio [OR] = 1.19 [95% confidence interval (CI) 1.08–1.32], $p = 0.001$), and greatest in patients with mixed infections (31% [389/1,273]); overall $p < 0.0001$. Severe anaemia (haemoglobin < 5 g/dl) was the major complication associated with *Pv*, accounting for 87% (589/675) of severe disease compared to 73% (1,144/1,570) of severe manifestations with *Pf* ($p < 0.001$). Pure *Pv* infection was also present in 78 patients with respiratory distress and 42 patients with coma. In total 242 (2.0%) patients with malaria died during admission: 2.2% (167/7,722) with *Pf*, 1.6% (46/2,916) with *Pv*, and 2.3% (29/1260) with mixed infections ($p = 0.126$).

Conclusions

In this region with established high-grade chloroquine resistance to both *Pv* and *Pf*, *Pv* is associated with severe and fatal malaria particularly in young children. The epidemiology of *P. vivax* needs to be re-examined elsewhere where chloroquine resistance is increasing.

The Editors' Summary of this article follows the references.

Introduction

The burden of malaria in Asia has been under appreciated, despite recent evidence suggesting that the continent contributes almost 40% of the world's malaria [1]. In sub-Saharan Africa the overwhelming majority of malaria-associated morbidity and mortality occurs with *P. falciparum* infections. In Asia, *P. vivax* often accounts for 50% of the malaria prevalence, and yet the morbidity associated with this infection and its spectrum of disease are largely ignored. Although *P. vivax* is widely regarded as benign, its propensity to recur is increasingly recognized by clinicians in endemic areas to result in appreciable disease, particularly in young children [2,3]. There have been increasing numbers of case reports describing severe manifestations of vivax malaria in recent years [3–5], however in the absence of a denominator, the true incidence of severe vivax malaria is unknown.

The public health importance of *P. vivax* has been magnified by the spread of resistance to chloroquine and sulfadoxine-pyrimethamine. While initially considered sporadic in Indonesia and Papua New Guinea (PNG), clinical studies show a high prevalence of *P. vivax* chloroquine resistance across Indonesia, with a rising prevalence throughout South and Southeast Asia [3,6] and recently in South America [7]. Few studies have quantified the relative burden of both *P. vivax* and *P. falciparum* in areas of mixed endemicity, with no prospective studies in regions with high-grade *P. vivax* chloroquine resistance.

Almost half of Indonesia's population of 250 million live in malaria endemic areas with 15 million people seeking treatment for clinical malaria each year. Papua province not only has the highest prevalence of malaria in Indonesia but also the highest prevalence of multidrug resistance to both *P. vivax* and *P. falciparum*. In this region, day 28 treatment failure with chloroquine and chloroquine plus sulfadoxine-pyrimethamine ranges from 65% to 95% for both *P. vivax* and *P. falciparum* [8–10]. To define the relative burden of vivax and falciparum malaria in this region we initiated a comprehensive malaria surveillance network. We report a prospective study of all patients with malaria attending the only hospital in Timika, southern Papua, over a 4-y period.

Methods

Study Site

The study was carried out at Rumah Sakit Mitra Masyarakat (RSMM) hospital, Timika, in the southern lowlands of Papua, Indonesia. The hospital has 110 beds with a high-dependency unit and an emergency department open 24 h a day. The outpatient department reviews approximately 300 patients per day, 6 d per week. RSMM is the only hospital in the district, servicing a population of approximately 150,000 people spread over an area of 21,522 km². The area is largely forested with both coastal and mountainous areas. Malaria transmission is restricted to the lowland area where it is associated with three mosquito vectors: *Anopheles koliensis*, *An. Farauti*, and *An. punctulatus* [11,12]. The annual incidence of malaria in the region is 885 per 1,000 person years, divided 62:38 between *P. falciparum* and *P. vivax* infections (unpublished data). In 2005 a household survey rate found 7.4% of respondents to be positive for *P. falciparum*, 6.4% for *P. vivax*, and 1.9% mixed infection with both species (unpublished

data). Due to economic migration the ethnic origin of the local population is diverse, with highland Papuans, lowland Papuans, and non-Papuan migrants all resident in the region. Hospital policy dictates that all patients presenting with history of fever and all pregnant women irrespective of symptoms should have a blood film examination for malaria.

Study Procedures

Since January 2003, prospective demographic and malar-iometric data have been collected routinely from hospital records, including active surveillance of all patients with malaria parasitemia attending either the outpatient or inpatients departments. The first year of the study constituted a pilot phase during which the surveillance systems were set up, expanded, and quality assured. The current study includes all data recorded by the prospective surveillance programme between January 2004 and December 2007.

For all outpatients, the hospital number, date of presentation, age, sex, and the species of *Plasmodium*, were recorded on the computerized hospital records system (Q-Pro). Since there is a low threshold for admitting sick patients to hospital it can be assumed that all patients with severe disease are admitted. Other reasons for admission to hospital include general malaise and an inability to tolerate oral medication. Further details were collected from hospitalised patients by a medically trained member of an onsite research team who reviewed all patients admitted for more than 12 h with malaria. In these patients a standard report form was completed documenting admission details including the duration of stay, demographic details, pregnancy status, outcome, and significant complications including admission to the intensive care and death. Standard care according to hospital guidelines was provided by the attending physician. Oxygen saturations were recorded using a pulse oximeter. Venous blood samples were collected in 94% of all inpatients with malaria, and the full blood count measured by coulter counter (JT Coulter). Routine biochemistry was available when clinically indicated. Assessment of coma (Glasgow coma score ≤ 10 or Blantyre coma scale ≤ 2), respiratory distress, and anaemia (haemoglobin < 5 g/dl) were routinely performed in all patients with malaria according to World Health Organization (WHO) guidelines [13]. Respiratory distress was defined by an oxygen saturation less than 94% or an age-stratified increased rapid respiratory rate (> 32 /min in adults, > 40 in children 5–14 y, > 50 in children aged 2 mo to 5 y, and > 60 in babies less than 2 mo) [13,14]. The remaining WHO criteria for severity are predominantly biochemical, and, since they were not routinely documented in all inpatients, were biased towards active case detection in more severely ill patients enrolled in severe malaria studies. Since their prevalence could not be reliably determined, the definition of severe disease was restricted to coma, severe anaemia, and respiratory distress.

Malaria diagnosis was confirmed by microscopy of Giemsa-stained blood films. Slides were considered negative after review of 100 high-power fields. The RSMM microscopy laboratory participates in an ongoing quality assurance process. In 2004 the hospital microscopy service was assessed for quality control, and a random sample of 1,200 positive slides reread by an independent expert microscopist of more than 10 y experience blind to the original microscopist. Slides were available in 90% (1,083/1,200) of cases with concordance

between the readings of 90% (979/1,083). In 1.7% (18/1,083) of slides, the second reading was negative, and in 4% (38/922) of cases slides reported as mono-infection were in fact found to be mixed infections.

In view of the high number of malarial infections in non-immune patients, local protocols recommend that all patients with patent parasitaemia are given antimalarial therapy. At the start of the study local treatment guidelines advocated intravenous quinine for severe malaria, chloroquine plus sulfadoxine-pyrimethamine for uncomplicated *P. falciparum*, and chloroquine monotherapy for non-*falciparum* uncomplicated malaria. However an assessment of these treatment regimens in 2004 demonstrated day 28 failure rates of 65% for *P. vivax* and 48% for *P. falciparum* infections [10]. In May 2005 protocols for the treatment of severe malaria were revised to intravenous artesunate [14], and in March 2006 the first-line treatment of uncomplicated falciparum and vivax malaria was changed to dihydroartemisinin-piperaquine [15].

Statistical Analysis

Data recorded in the hospital administrative system were collated monthly and summaries exported to Excel spreadsheets. Active surveillance data on inpatients were entered on to an Excel spreadsheet, which was cross-validated monthly. Data were analysed using SPSS for Windows (version 15, SPSS). The Mann-Whitney U test or Kruskal-Wallis method were used for nonparametric comparisons, and Student's *t*-test or one-way analysis of variance for parametric comparisons. For categorical variables percentages and corresponding 95% confidence intervals (95% CIs) were calculated using Wilson's method. Proportions were examined using χ^2 with Yates' correction or by Fisher's exact test.

A multiple logistic regression model was used to determine adjusted odds ratios (AORs) for risk factors for adverse outcomes; variables were entered into the equation and the model constructed using the Wald statistic with $p < 0.05$ the cutoff for significance.

Ethical Considerations The study was approved by the Ethics Committee of the National Institute of Health Research and Development, Indonesian Ministry of Health (Jakarta, Indonesia) and the Ethics Committee of Menzies School of Health Research (Darwin, Australia). Since patients underwent no additional interventions above routine medical care, individual consent was not sought, unless the patient was enrolled in associated studies.

Results

Outpatients

Between January 2004 and December 2007, a total of 373,450 patients attended the hospital outpatients department of whom 63,404 (17%) were treated for malaria. Slide confirmation was made in 95% (60,226) of cases (Table 1). Overall 52% (31,566/60,226) of outpatients with confirmed malaria were males, and this slight predominance was apparent across all age groups and species of infection. The age-stratified prevalence of symptomatic malaria amongst all outpatients varied between species, with *P. vivax* peaking in children aged 1–4 y (9.0% [4,912/54,660]) compared to *P. falciparum*, which peaked at 5–14 y of age (19% [7,353/38,887]) (Figure 1A). Children under 5 y of age accounted for 41% (6,611/16,113) of *P. vivax* infections compared to 22% (8,631/

39,434) of *P. falciparum* infections (odds ratio [OR] = 1.87 [95% CI 1.83–1.92], $p < 0.0001$).

Inpatients

Of the 37,800 patients admitted to hospital during the study period, 12,171 (32%) had slide-confirmed malaria. The proportions of malaria attributable to each species were similar to that seen in outpatients, although mixed-species infections were significantly more common in inpatients (10.5% [1,273/12,171]) compared to outpatients (5.7% [3,403/60,226], OR = 1.95 [95% CI 1.82–2.09], $p < 0.001$) (Table 1). The subsequent analysis is restricted to the 12,027 inpatients infected with *P. falciparum*, *P. vivax*, or a mixture of both species. Infection with *P. vivax* accounted for 47% (415/887) of malarial admissions in children under 1 y of age. This proportion fell to 28% (817/2,913) in children aged 1 to 4 y and 20% (339/1,728) in children aged 5 to 14 y old, but did not change thereafter (Figure 1B).

The majority of patients admitted with malaria were of Papuan ethnicity (85% [10,250/12,017]), with adults significantly more likely to be non-Papuan (21% [1,362/6,483]) compared to children (7.3% [399/5,484], OR = 3.39 [95% CI 3.01–3.82], $p < 0.0001$). In total, 53% (6,310/12,027) of inpatients were female, with pregnancy identified in 31% (1,155/3,728) of adult women. The proportion of inpatients who were female was significantly higher in *P. vivax* infections (65% [1,917/2,937]) compared to *P. falciparum* infections (49% [3,833/7,817], OR = 2.0 [95% CI 1.8–2.1], $p < 0.0001$). The predominance of females in patients with *P. vivax* infection, became apparent in children over 1 y old and increased with age reaching 79% (95% CI 75–83) in young adults. In contrast, children less than 15 y with pure *P. falciparum* infections were more likely to be male than those with pure *P. vivax* infections (OR = 1.86 [95% CI 1.6–2.1], $p < 0.001$) (Figure 2).

Severe Malaria

Of the 12,027 patients admitted with malaria, 861 (7.2%) required admission to the high dependency unit and 22% (2,634) were reported to have severe disease. Although 60% (1,570/2,634) of severe malaria was due to *P. falciparum*, the risk was greater among patients with *P. vivax* infection (23% [675/2,937]) than in those with *P. falciparum* infection alone (20% [1,570/7,817], OR = 1.19 [95% CI 1.07–1.32], $p = 0.001$) (Figure 3, Table 2). The risk was even greater in patients with mixed infections (31% [389/1,273], OR = 1.67 [95% CI 1.46–1.90], $p < 0.0001$). The proportion of patients with severe disease decreased with age, and this relationship differed among species ($p < 0.001$) (Figure 4).

Severe malarial anaemia (SMA) (haemoglobin < 5 g/dl) was present in 87% (589/675) of inpatients with severe *P. vivax* infections compared to 73% (1,144/1,570) of inpatients with severe *P. falciparum* and 81% (314/389) of severe mixed infections (overall $p < 0.0001$). Children less than 5 y old were at greater risk of SMA (27% [1,017/3,776]), compared to children aged 5–14 y (20% [338/1,714]), and adults (10% [677/6,487]) ($p < 0.001$). Of the 240 infants (< 1 y old) with SMA, 53% (127) had *P. vivax* infection, 30% (73) *P. falciparum* infection, and 17% (40) had mixed infections ($p = 0.001$). After 1 y of age this proportion reversed, with falciparum malaria accounting for 60% (1,067/1,792) of SMA, vivax malaria for 25% (452), and mixed infections for 15% (273) ($p < 0.0001$).

Table 1. Number of Symptomatic Patients with Laboratory-Confirmed Malaria at RSMM Hospital (2004–2007)

Hospital Department	Patient Category	All Species	<i>P. falciparum</i>	<i>P. vivax</i>	Mixed Infections	<i>P. malariae</i>	<i>P. ovale</i>
Outpatient	Number of patients with slide confirmed malaria	60,226	39,434	16,113	3,403	1,239	37
	Percent of all outpatients ^a	16.1	10.6	4.3	0.9	0.3	0.001
	Percent of all patients with malaria	100	66	27	5.7	2.1	0.1
Inpatient	Number of patients with slide confirmed malaria	12,171	7,817	2,937	1,273	141	3
	Percent of all inpatients ^a	32	21	7.8	3.4	0.4	0.01
	Percent of all patients with malaria	100	64	24	10.5	1.2	0.02

^aDuring the study period, a total of 373,450 patients were assessed in the outpatient department, and 37,800 patients were admitted to hospital.
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Respiratory distress was also more prevalent in young children (<5 y old) with malaria (4.6% [172/3,776]) compared to older children and adults (3.4% [276/8,201], OR = 1.42 [95% CI 1.2–1.7], *p* = 0.0005). Although the risk of respiratory distress in children was similar between species, in adults it was greatest following *P. falciparum* infection, either alone or

mixed, compared to pure infection with pure *P. vivax* (OR = 3.06 [95% CI 1.7–5.7], *p* < 0.0001) (Table 2). In contrast, coma alone was more common in adults with malaria than in children (OR = 1.72 [95% CI 1.3–2.3], *p* < 0.0001) (Table 2).

Patients of Papuan origin were more likely to be severely anaemic (19% [1,951/10,250]) compared to 5.2% (92/1,767) of

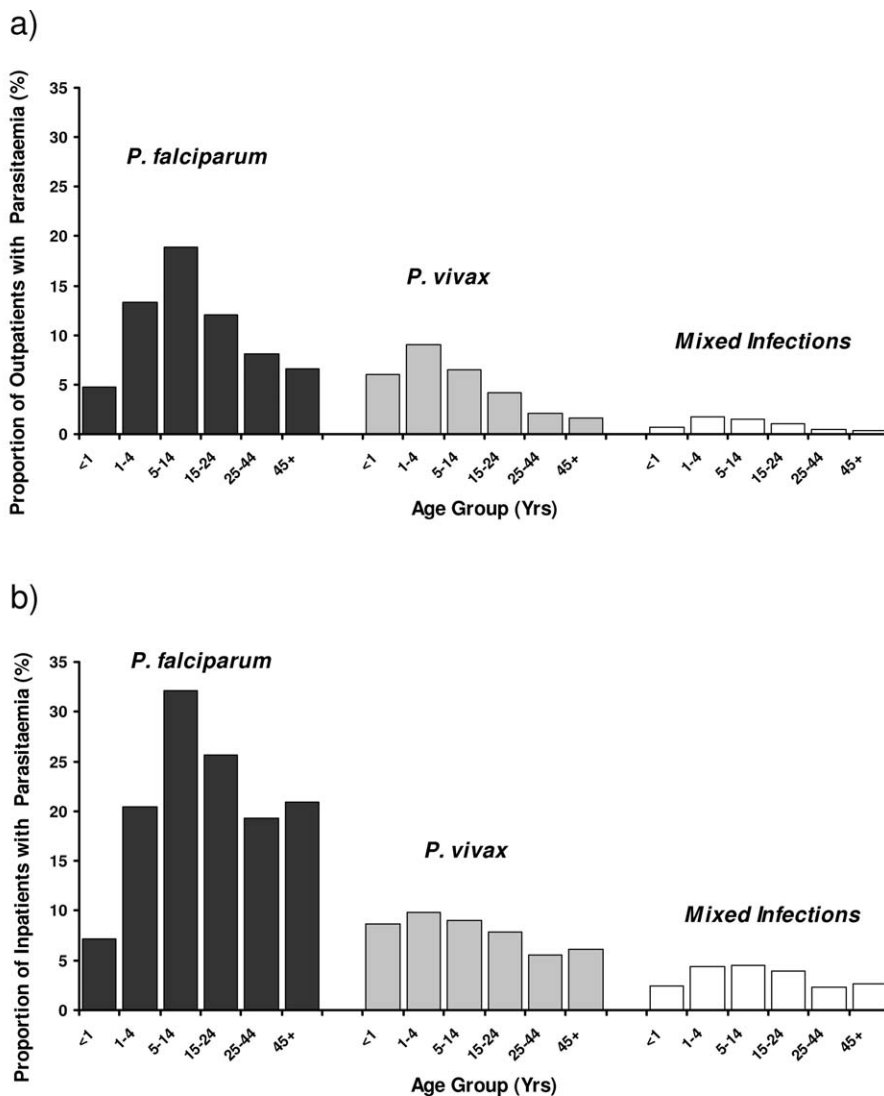


Figure 1. Age Stratified Proportions of Hospital Patients with Malaria

Bars represent proportion of all patients attending outpatients (A) or admitted to hospital (B) who were parasitaemic.
doi:10.1371/journal.pmed.0050128.g001

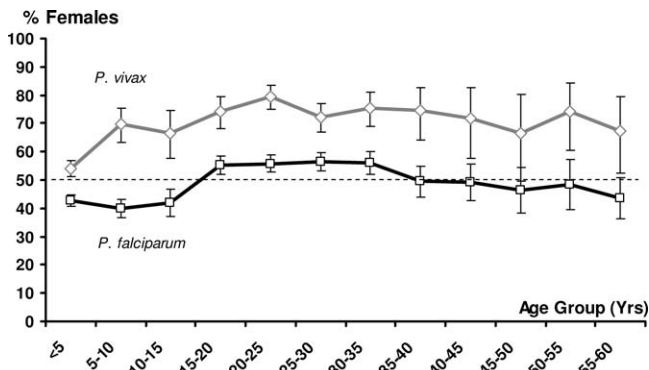


Figure 2. Age-Stratified Percentage of Females in Patients Hospitalised with *P. falciparum* (Squares) and *P. vivax* Infection (Diamonds) Error bars represent 95% CI, and dotted line denotes males and females present in equal proportions. doi:10.1371/journal.pmed.0050128.g002

non-Papuans (OR = 4.3 [95% CI 3.4–5.3], $p < 0.0001$). Conversely the risk for malaria associated with coma was greatest in non-Papuans: 4.7% (83/1,767) compared to 2.5% (253/10,250) in Papuans (OR = 2.0 [95% CI 1.5–2.5], $p < 0.0001$).

There were 78 patients with respiratory distress and pure *P. vivax* infection, of whom 30 (38%) also had other markers of severity (including 26 patients with severe anaemia) and a further nine (14%) patients were cotreated for pneumonia. Forty-two patients with pure *P. vivax* infections were admitted with coma, of whom nine (21%) had other markers of severity.

Mortality

Information on death during hospital admission was available in 98.9% (11,898/12,027) of patients. A total of 242

(2.0%) inpatients with malaria died, accounting for 15% (242/1,608) of all-cause inpatient mortality over the same period. Malaria accounted for 19% (134/719) of deaths within 48 h and 12% (108/889) of deaths thereafter. The case-fatality rate in patients infected with pure *P. vivax* was 1.6% (46/2,916), comparable to that in patients with *P. falciparum* either alone (2.2% [167/7,722]) or mixed (2.3% [291/1,260]); overall $p = 0.126$. Malaria patients admitted with severe disease had a risk of death of 6.7% (173/2,599) compared to 0.7% (69/9,299) in patients without severe disease (OR = 9.5 [95% CI 7.1–12.8]; $p < 0.0001$). The mortality in vivax malaria was 4.1% (27/666) in patients with one or more criteria compared to 0.8% (19/2,250) in patients with no severe criteria (OR = 5.0 [95% CI 2.6–9.4]; $p < 0.001$). The three severe criteria, either alone or in combination, identified 75% (125/167) of deaths associated with *P. falciparum* and 72% (21/29) with mixed infections, but had significantly lower sensitivity for predicting *P. vivax* associated deaths (59% [27/46]); $p = 0.05$.

The mortality rate associated with severe anaemia alone was low (1.6% [30/1,884]). In *P. vivax* infections, the presence of severe anaemia in combination with respiratory distress without coma significantly increased the risk of death with *P. vivax* (OR = 65 [95% CI 10–520], $p < 0.0001$), although this was not apparent in *P. falciparum* infections (Figure 5). Overall mortality was significantly higher in adults compared to children (OR = 1.58 [95% CI 1.2–2.1], $p = 0.0009$), non-Papuans compared to Papuans (OR = 1.60 [95% CI 1.2–2.2], $p = 0.0038$), and males compared to females (OR = 1.56 [95% CI 1.2–2.0], $p = 0.0009$). In a multivariate model including all infecting species, only markers of severity and older age were independently associated with death (Table 3).

Population-Based Risk of Severe Disease

On the basis of an estimated annual total of 45,525 clinical episodes of *P. vivax* in the study area (unpublished data), the

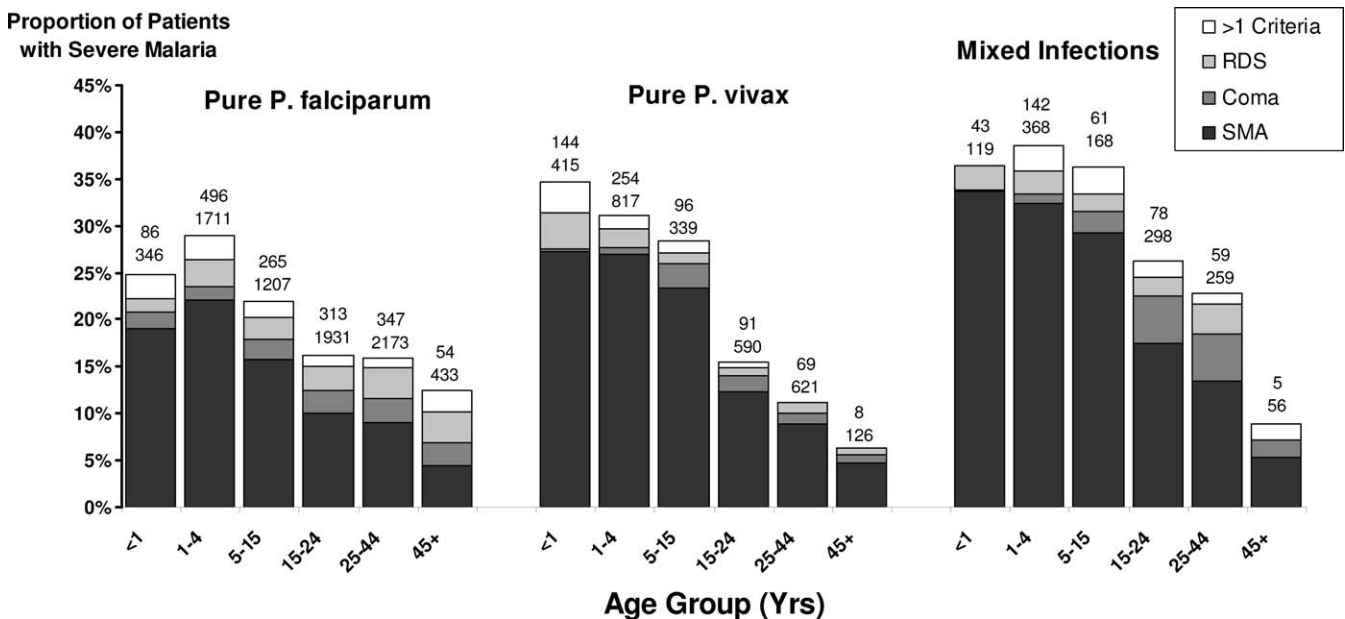


Figure 3. The Prevalence of Severe Disease in Patients Hospitalised with Malaria Stratified by Species of Infection and Age Group Severe disease defined by respiratory distress (RDS), coma, and SMA. For each age group the upper number above each column represents the total number of patients with severe disease (numerator), and the lower number is the total number of patients with the species of infection (denominator). Data on age were unavailable in 50 patients. doi:10.1371/journal.pmed.0050128.g003

Table 2. Prevalence of Severe Malaria Stratified by Species of Infection for Patients Admitted with Clinical Malaria to RSMM Hospital (2004–2007)

Patients	Marker of Disease Severity	Pure <i>P. falciparum</i>		Pure <i>P. vivax</i>		Mixed Infections		Total	
		n	Percentage	n	Percentage	n	Percentage	n	Percentage
Children	All	3,264	—	1,571	—	655	—	5,490	—
	Coma alone	55	1.7	16	1.0	8	1.2	79	1.4
	Severe anaemia alone	635	20	412	26	208	32	1,255	23
	Respiratory distress alone	82	2.5	36	2.3	15	2.3	133	2.4
	More than one criteria	75	2.3	30	1.9	15	2.3	120	2.2
Adults	All	4,537	—	1,337	—	613	—	6,487	—
	Coma alone	112	2.5	18	1.3	29	4.7	159	2.5
	Severe anaemia alone	410	9.0	134	10	90	14.7	634	9.8
	Respiratory distress alone	136	3.0	13	1.0	14	2.3	163	2.5
	More than one criterion	56	1.2	3	0.2	9	1.5	68	1.0
Total		7,801	—	2,908	—	1,268	—	11,977	—

Data restricted to 11,977 of patients (2,611 with severe disease) who had age recorded. doi:10.1371/journal.pmed.0050128.t002

risk of severe disease was estimated to be one in 270 clinical infections and that for death as one in 3,959. The corresponding risks among the 72,721 clinical episodes of *P. falciparum* were one in 185 and one in 1,742 respectively. These estimates assume all severe malaria cases and deaths are admitted to hospital, and are thus conservative.

Discussion

Our study in southern Papua, where high levels of resistance have emerged to *P. vivax* as well as *P. falciparum*, demonstrates that both species are associated with significant morbidity and mortality. In this region, malaria accounted for 16% of the all hospital outpatient consultations and 32% of admissions, a quarter of which were attributable to *P. vivax*. Severe disease was present in 22% of hospitalised patients, with a greater risk among patients infected with *P. vivax* than in those with *P. falciparum* (OR = 1.19). Inpatient case fatality rates of 1.6% of patients with *P. vivax* did not differ

significantly from the 2.2% mortality among patients with pure *P. falciparum* infection. Importantly, whereas mixed infections have been associated previously with a lower risk of severe disease [16,17] and anaemia [18], in our study they were at significantly greater risk than either species alone.

Outside of Africa *P. vivax* infection is a prominent cause of clinical malaria [3,19]. Although widely considered benign, studies from Asia and the Pacific have demonstrated that vivax malaria accounts for a substantial proportion of hospitalised malaria [16,20–24.]; however, when commented upon, the rates of infection associated with severe disease were reportedly less than 1%, with virtually no deaths. The dominant paradigm of *P. vivax* being a benign infection has been challenged recently by retrospective studies from northern Papua and Pakistan, which document hospitalisation, severe disease, and death in patients presenting with vivax malaria [25,26]. Population-based studies in Venezuela have also demonstrated a rising trend in deaths associated with vivax malaria, particularly in children [27]. Our prospective study supports these findings, adding further weight to the body of evidence highlighting *P. vivax* as a major cause of severe malaria, particularly in settings with established or emerging chloroquine resistance. The predominance of uncomplicated *P. vivax* infection in early life has been highlighted by others, and postulated to be due to a faster acquisition of immunity in *P. vivax* compared to *P. falciparum* [17,28]. However, in contrast to a recent study from an area of PNG with more stable endemicity [28], we observed a significant burden of disease in adults with *P. vivax*, with severe disease occurring into the fifth decade of life. This is likely to be attributable to the diverse origin of the local Timika population, a consequence of economic migration, resulting in large numbers of pauci-immune individuals being exposed to malaria for the first time in later life.

Whereas the ratio of males to females was approximately equal in patients with *P. falciparum*, there was a consistent predominance of females in patients with *P. vivax* malaria, which was most pronounced in adults. The reasons for this are unclear. Since the symptoms of uncomplicated vivax and falciparum malaria are similar [29], treatment seeking or referral biases are unlikely. The effect was maximal after

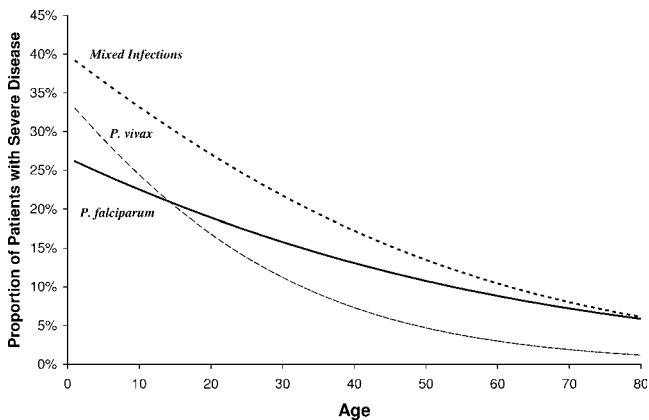


Figure 4. Age-Specific Risk of Severe Disease in Patients Admitted with *P. falciparum* (Bold Line), *P. vivax* (Hashed Line), and Mixed (Dotted Line) Infections

Lines represent predicted values from a logistic regression model in which age was entered as a linear effect, along with an interactive term for age and species.

doi:10.1371/journal.pmed.0050128.g004

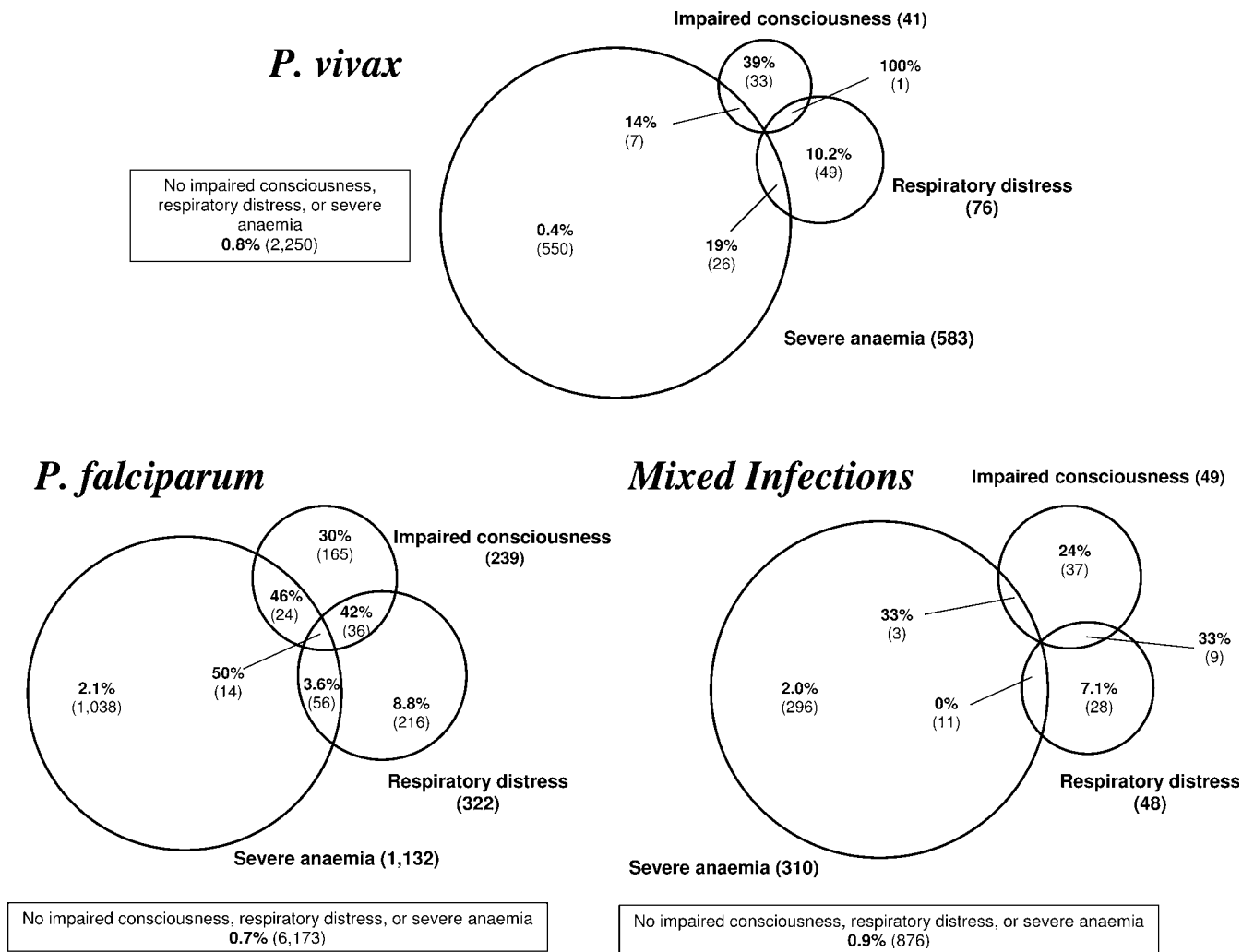


Figure 5. Prevalence, Overlap, and Mortality for Criteria of Severe Malaria Associated with Infection of *P. falciparum*, *P. vivax*, and Mixed Infections. Total numbers are given in parentheses, and mortality is given as a percentage. The sizes of the circles represent the relative proportions of patients with malaria with different severe manifestations of disease for each species. Data on mortality are missing in 129 (1.1%) patients, 95 with *P. falciparum*, 21 with *P. vivax*, and 13 with mixed infections. doi:10.1371/journal.pmed.0050128.g005

adolescence and may reflect the lower starting haemoglobin in women compared to men, and therefore a greater propensity to severe anaemia in response to *P. vivax*. While sex hormones including dehydroepiandrosterone (DHEAS) have been linked to reduced post-pubertal risk in *P. falciparum* infection [30], it is also possible that female sex hormones confer less protection against *P. vivax* malaria. However, neither of these explanations would adequately account for the emerging gender difference in early childhood.

As in other settings a proportion of patients with malaria are likely to have been admitted with alternative diagnoses and incidental parasitaemia [31,32]. In the present study quantification of parasitaemia and details on associated comorbidities and microbiology were not routinely available. Community based surveys in this region have revealed asymptomatic *P. vivax* infection present in 4.5% of the population and 4.9% for *P. falciparum* (unpublished data). However, while all inpatients with fever have a blood film examination, this is not routine practice in ambulatory patients. Hence the overall estimates of patent *vivax* parasitaemia in hospitalised patients will be significantly higher than the 8%

we have reported. Incidental parasitaemia therefore is likely to contribute only a minority of the cases reported as clinical malaria.

The appreciable burden of respiratory distress associated with vivax malaria in our study suggests that it is more frequent than suggested by the isolated case reports in the literature. While such reports have previously described mostly nonfatal syndromes in pauci-immune adults caused by acute lung injury [5], the present study highlights *vivax*-associated respiratory distress in all age groups, particularly children, with a case-fatality rate of 10% rising to 19% if associated with severe anaemia. In African children with *P. falciparum*, respiratory distress is associated with metabolic acidosis and/or concurrent pneumonia [32,33]. However the aetiology of *P. vivax*-associated respiratory distress in Asia is unknown and will require detailed clinical studies to determine the relative contributions of acute lung injury, possible pulmonary parasite sequestration, acidosis, and coinfections [34].

Another limitation of the present study is the under-diagnosis by routine microscopy of coinfection with both *P.*

Table 3. Factors Associated with Mortality in Patients Hospitalised with *Pv* and/or *Pf* Infections

Category	Risk Factor	Percent Mortality Rate (n/Total n)	OR [95% CI]	AOR [95% CI]
Species of Infection	<i>Pf</i>	2.2 (167/7,722)	1	1
	<i>Pv</i>	1.6 (46/2,916)	0.73 [0.5–1.02], <i>p</i> = 0.065	1.13 [0.79–1.63], <i>p</i> = 0.510
	Mixed infections	2.3 (29/1,260)	1.07 [0.7–1.6], <i>p</i> = 0.83	0.98 [0.63–1.52], <i>p</i> = 0.924
Age	Overall		1.024 [1.026–1.032] <i>p</i> < 0.001 ^a	1.030 [1.021–1.039] <i>p</i> < 0.001 ^a
	< 1 y	1.8 (16/880)	—	—
	1–4 y	1.6 (45/2,873)	—	—
	5–14 y	1.4 (24/1,699)	—	—
	15–24 y	1.7 (45/2,782)	—	—
	24–44 y	2.7 (82/3,004)	—	—
	45+ y	4.7 (29/610)	—	—
Markers of Severity	None	0.7 (69/9,299)	1	1
	Coma alone	30 (71/235)	57 [40–85], <i>p</i> < 0.0001 ^a	57 [39–83], <i>p</i> < 0.001 ^a
	Severe anaemia alone	1.6 (30/1,884)	2.2 [1.4–3.4], <i>p</i> = 0.0005 ^a	2.8 [1.8–4.4], <i>p</i> < 0.001 ^a
	Respiratory distress alone	8.9 (26/293)	13.0 [7.9–21], <i>p</i> < 0.0001 ^a	14 [8.8–23], <i>p</i> < 0.001 ^a
	More than one criteria	25 (46/187)	44 [28–67], <i>p</i> < 0.0001 ^a	54 [35–83], <i>p</i> < 0.001 ^a
Sex	Females	1.6 (101/6,249)	1	1
	Males	2.5 (141/5,649)	1.6 [1.2–2.0], <i>p</i> = 0.0009 ^a	1.17 [0.88–1.6], <i>p</i> = 0.283
Ethnicity	Papuan	1.9 (190/10,135)	1	1
	Non-Papuan	3.0 (52/1,753)	1.6 [1.2–2.2], <i>p</i> = 0.0038 ^a	1.14 [0.79–1.6], <i>p</i> = 0.476

^aRisk factors that reached statistical significance at the 0.05 level
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falciparum and *P. vivax*, particularly when parasites are present at the early ring stage [35]. Reassuringly, in a separate study based on the RSMM laboratory microscopic diagnosis, less than 10% of patients reported to have pure *P. vivax* infections were found to have mixed infection on microscopic re-examination or HRP2 dipstick analysis for *P. falciparum* (Paracheck) (unpublished data). Our study was unable to address the contribution to pathology of each component of such coinfections.

Although further studies are needed to define the *P. vivax* attributable fractions for uncomplicated and severe malaria [32,36], our data highlight an unusually high burden of disease compared to studies at other sites where the background prevalence of vivax malaria was higher [16,20–24]. This raises two alternative possibilities: that the associated morbidity of *P. vivax* in Papua is excessive compared to other endemic regions or that the burden of disease elsewhere is simply underreported [3].

A particular feature of *P. vivax* in eastern Indonesia is the high level of chloroquine resistance, with day 28 failure rates following standard treatment exceeding 65% for *P. vivax* and 52% for *P. falciparum* [8,10]. The global emergence of chloroquine resistance in *P. falciparum* has been associated with a rise in falciparum-related malaria morbidity and mortality [37]. It is likely that chloroquine resistance makes a similar contribution to the high burden of uncomplicated and severe vivax malaria in Papua and other regions where resistance is now emerging [38,39]. Recurrent infections due to treatment failure and relapse from the liver stages result in up to 80% of patients having recurrent malaria within 4 wk [10,40], and provide a plausible explanation for our observations that almost 20% of patients hospitalised with *P. vivax* in Papua have severe anaemia. Previous studies have also highlighted an increased risk of severe disease in drug resistant *P. falciparum* infections associated with failure to eliminate the parasites early in infection [41]. In this context the substantial proportions with severe anaemia particularly following mixed

infections may represent the additive effects of repeated exposures to both *P. falciparum* and *P. vivax* malaria, from recrudescences, reinfections, and relapses, which together compound rather attenuate the risk of severe disease.

Studies on laboratory strains of *P. falciparum* suggest that there is marked variability in growth rates of isolates ex-vivo with chloroquine-resistant isolates growing faster than chloroquine-sensitive isolates [42]. We have recently described similar observations in field isolates of *P. vivax* [43]. Since *P. falciparum* isolates from patients with severe disease have greater multiplication rates ex-vivo compared to those from patients with uncomplicated disease [44], the possibility arises that the highly chloroquine resistant isolates found in Papua, may be more pathogenic to the host. When compounded by poor immunity, drug resistant parasites have a greater potential to result in more severe disease, although further studies are needed to confirm this.

In conclusion our study demonstrates a major burden of vivax malaria in southern Papua, similar to that observed in a recent retrospective hospital study in northern Papua [25] and a prospective community-based study in Papua New Guinea [38]. The clinical spectrum of disease associated with *P. vivax* in this region is greater than that reported elsewhere, with infants and those with mixed infections at greatest risk. Further studies are needed to confirm the underlying pathogenesis of severe disease, and the degree to which this is related to the emergence of multidrug resistant strains of *P. vivax*. The spread of drug resistant *P. vivax* to other parts of Indonesia, Southeast Asia, and South America [3] highlights an urgent need to re-examine the spectrum and burden of vivax malaria and for appropriately resourced control measures to be implemented against this emerging but neglected disease.

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Author contributions. ET, NMA, PS, and RNP designed the study. ET, NMA, and RNP analysed the data. NW, EK, MK, and DAL collected the data. ET, NMA, PS, NW, EK, MK, DAL, and RNP contributed to writing the manuscript.

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Editors' Summary

Background. Malaria, a parasitic disease transmitted to people by mosquitoes, is common throughout the tropical and subtropical areas of the world. In sub-Saharan Africa, infections with *Plasmodium falciparum* cause most of the malaria-associated illness and death. Elsewhere, another related parasite—*P. vivax*—is often the commonest cause of malaria. Both parasites are injected into the human blood stream when an infected mosquito bites a person. From there, the parasites travel to the liver, where they multiply for 8–9 d and mature into a form of the parasite known as merozoites. These merozoites are released from the liver and invade red blood cells where they multiply rapidly for a couple of days before bursting out and infecting more red blood cells. This cyclical accumulation of parasites in the blood causes a recurring flu-like illness characterized by fevers, headaches, chills, and sweating. Malaria can be treated with antimalarial drugs but, if left untreated, infections with *P. falciparum* can cause anemia (by destroying red blood cells) and can damage the brain and other vital organs (by blocking the capillaries that supply these organs with blood), complications that can be fatal.

Why Was This Study Done? Unlike falciparum malaria, vivax malaria is generally regarded as a benign or nonfatal disease even though there have been several reports recently of severe disease and deaths associated with vivax malaria. These reports do not indicate, however, whether *P. vivax* is responsible for a significant proportion of malarial deaths. Public health officials need to know this information because strains of *P. vivax* that are resistant to multiple antimalarial drugs are widespread in Indonesia and beginning to emerge elsewhere in Asia and South America. In this study, therefore, the researchers investigate the relative burden of vivax and falciparum malaria in Papua, Indonesia, a region where multidrug-resistant strains of both *P. falciparum* and *P. vivax* are common.

What Did the Researchers Do and Find? The researchers examined data collected from all the patients attending the outpatient and inpatient departments of a hospital that serves a large area in the southern lowlands of Papua, Indonesia between January 2004 and December 2007. Among those inpatients in whom malaria had been confirmed by finding parasites in blood samples, two-thirds were infected with *P. falciparum*, a quarter with *P. vivax*, and the rest with a mixture of parasites. Nearly one in four patients infected with *P. vivax* developed severe malaria compared with roughly one in five patients infected with *P. falciparum*. However, about one in three patients infected with both parasites developed severe disease. Whichever

parasite was responsible for the infection, the proportion of patients with severe disease was greatest among children below the age of five years. Severe anemia was the commonest complication associated with severe malaria caused by both *P. vivax* and *P. falciparum* (present in 87% and 73% of cases, respectively). Finally, one in 50 patients with malaria died; the risk of death was the same for patients infected with *P. falciparum*, *P. vivax*, or both parasites.

What Do These Findings Mean? These findings provide important information about the burden of malaria associated with *P. vivax* infection. They show that in a region where multidrug-resistant strains of both *P. falciparum* and *P. vivax* are common, *P. vivax* infection (as well as *P. falciparum* infection) is associated with severe and fatal malaria, particularly in young children. The findings also show that infection with a mixture of the two parasites is associated with a higher risk of severe disease than infection with either parasite alone. Most importantly, they show that similar proportions of patients infected with *P. falciparum*, *P. vivax*, or a mixture of parasites die. Further studies need to be done in other settings to confirm these findings and to learn more about the pattern of severe malaria associated with *P. vivax* (in particular, with multidrug-resistant strains). Nevertheless, these findings highlight the need to consider both *P. vivax* and *P. falciparum* when implementing measures designed to reduce the malaria burden in regions where these parasites coexist.

Additional Information. Please access these Web sites via the online version of this summary at <http://dx.doi.org/10.1371/journal.pmed.0050128>.

- A *PLoS Medicine* Research in Translation article by Stephen Rogerson further discusses this study and a related *PLoS Medicine* paper on vivax malaria in a community cohort from Papua New Guinea
- The MedlinePlus encyclopedia has a page on malaria (in English and Spanish)
- The US Centers for Disease Control and Prevention provides information on malaria (in English and Spanish)
- Vivaxmalaria provides information on topics related to *P. vivax*
- The Malaria Vaccine Initiative also provides a fact sheet on *P. vivax* malaria
- Information is available from the Roll Back Malaria Partnership on the global control of malaria