

Plasmodium vivax and Mixed Infections Are Associated with Severe Malaria in Children: A Prospective Cohort Study from Papua New Guinea

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Funding: The morbidity surveillance was established as a component of field site preparation for malaria vaccine trials funded by grant from USAID and AusAID. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Academic Editor: Stephen Rogerson, Royal Melbourne Hospital, Australia

Citation: Genton B, D'Acremont V, Rare L, Baea K, Reeder JC, et al. (2008) *Plasmodium vivax* and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. PLoS Med 5(6): e127. doi:10.1371/journal.pmed.0050127

Received: December 7, 2007

Accepted: May 2, 2008

Published: June 17, 2008

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Abbreviations: df, degree of freedom; PNG, Papua New Guinea; SM, severe malaria; SP, sulfadoxine-pyrimethamine; UM, uncomplicated malaria; WBC, white blood cells

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ABSTRACT

Background

Severe malaria (SM) is classically associated with *Plasmodium falciparum* infection. Little information is available on the contribution of *P. vivax* to severe disease. There are some epidemiological indications that *P. vivax* or mixed infections protect against complications and deaths. A large morbidity surveillance conducted in an area where the four species coexist allowed us to estimate rates of SM among patients infected with one or several species.

Methods and Findings

This was a prospective cohort study conducted within the framework of the Malaria Vaccine Epidemiology and Evaluation Project. All presumptive malaria cases presenting at two rural health facilities over an 8-y period were investigated with history taking, clinical examination, and laboratory assessment. Case definition of SM was based on the World Health Organization (WHO) criteria adapted for the setting (i.e., clinical diagnosis of malaria associated with asexual blood stage parasitaemia and recent history of fits, or coma, or respiratory distress, or anaemia [haemoglobin < 5 g/dl]). Out of 17,201 presumptive malaria cases, 9,537 (55%) had a confirmed *Plasmodium* parasitaemia. Among those, 6.2% (95% confidence interval [CI] 5.7%–6.8%) fulfilled the case definition of SM, most of them in children <5 y. In this age group, the proportion of SM was 11.7% (10.4%–13.2%) for *P. falciparum*, 8.8% (7.1%–10.7%) for *P. vivax*, and 17.3% (11.7%–24.2%) for mixed *P. falciparum* and *P. vivax* infections. *P. vivax* SM presented more often with respiratory distress than did *P. falciparum* (60% versus 41%, $p = 0.002$), but less often with anaemia (19% versus 41%, $p = 0.0001$).

Conclusion

P. vivax mono-infections as well as mixed *Plasmodium* infections are associated with SM. There is no indication that mixed infections protected against SM. Interventions targeted toward *P. falciparum* only might be insufficient to eliminate the overall malaria burden, and especially severe disease, in areas where *P. falciparum* and *P. vivax* coexist.

The Editors' Summary of this article follows the references.

Introduction

Most of the research and published literature on malaria focuses on *P. falciparum* and much less on *P. vivax* [1,2]. This focus is due to the very high burden of mortality attributed to the *falciparum* species in Africa [3]. However, there is growing evidence that *P. vivax* is responsible for a significant burden of disease worldwide accounting for half of all malaria cases in Asia and Latin America [4]. As the term “benign tertian malaria” implies, *vivax* malaria is usually an uncomplicated disease that runs a benign course and is rarely fatal. This clinical paradigm has been challenged recently [5] by numerous reports of symptoms and signs of severe disease, and even deaths due to *P. vivax* mono-infections [6–9]. However, most of the published literature consists of case reports or small descriptive clinical series lacking denominators. The relative contribution of *P. vivax* versus *P. falciparum* to severe morbidity has not been properly assessed, except for one study in Thailand that found very little severe disease and no death due to *P. vivax* [10]. This Thai study suggested, rather, a protecting effect of *vivax*, as had a study from Vanuatu [11,12]. It is only recently that severe disease due to *P. vivax* has received more attention with a recent report from a hospital in northeastern Indonesian New Guinea (Papua) where they found 36 severe malaria (SM) cases among 1,135 *P. vivax* infected patients (3.2%) [13].

The malaria epidemiology in Papua New Guinea (PNG) offers the opportunity to investigate in the same population the respective contribution of the different *Plasmodium* species in terms of morbidity, and severe disease in particular. Indeed, it is one of the few countries where all four *Plasmodium* species coexist at sufficient prevalence to allow proper comparisons. The aim of the present clinico-epidemiological study was to assess the proportion of cases presenting at health facilities with severe manifestations of malaria and associated *P. vivax* parasitaemia, to compare this proportion to that of *P. falciparum* in the same setting, and to investigate the potential of mixed *P. falciparum* and *P. vivax* infection to protect against severe disease.

Methods

Study Area and Population

The Wosera lies in the East Sepik Province of PNG. Transmission of malaria is perennial, but the distribution of rainfall shows some seasonality with 65% falling during the so-called wet season occurring from October to April. Malariometric surveys conducted in the area showed an overall *Plasmodium* prevalence rate of 60% in the early 1990s decreasing to 35% by 2002. The reduction was from 38% to 22% for *P. falciparum*, 20% to 10% for *P. vivax*, 16% to 4% for *P. malariae* [14,15], and was probably related to a gradual increase in the use of insecticide treated nets and a change in treatment policy.

Health Services

The morbidity surveillance was conducted in two health facilities, Kunjigini Health Subcentre in the South Wosera and Kaugia Health Subcentre in the North Wosera, within the framework of the Malaria Vaccine Epidemiology and Evaluation Project [16]. Both health facilities are staffed with nurses and have beds to admit severe cases for a short period of time. Limited laboratory tests are available (light microscopy for

malaria, haemoglobin photometer, and urine dipstick). The local referral centre is Maprik Hospital, a 110-bed government hospital. Recourse to health services is usual in case of fever, especially in children, but referral is very uncommon, due to transport problems and lack of qualified staff in the hospital. First-line treatment policy for uncomplicated malaria (UM) was a 3-d regimen of oral amodiaquine in children < 20 kg, or chloroquine in those > 20 kg; treatment for severe or resistant malaria included a 5-d regimen of intramuscular quinine combined with a single dose of oral sulfadoxine-pyrimethamine (SP). In 2000, the first-line treatment changed to a combination of amodiaquine or chloroquine (3 d) plus SP (single dose) for UM, and the second-line to artesunate (7 d) plus SP (single dose on day 3). The 28-d failure rates with the regimens used in the area during the study were ~20% for *P. falciparum* and ~0% for *P. vivax* [17,18].

Morbidity Surveillance

Patients were prospectively recruited if they were diagnosed on clinical grounds only as having malaria (presumptive diagnosis). The diagnosis was based on a history of fever without obvious symptoms or signs of another disease. These presumptive malaria cases were further investigated by a research nurse. After recording demographical information, closed questions were asked on the characteristics of the morbid episode (history of fever, headache, cough, dyspnea, vomiting, diarrhoea, abdominal pain, and convulsions). A standardized physical examination was then performed (axillary temperature, level of consciousness, respiratory rate, chest indrawing, and Hackett's grading of spleen). A blood sample was taken for parasitological examination by microscopy and haemoglobin measurement (for details see Genton et al. [19]).

Laboratory Tests

Parasitology. Blood films were stained with 4% Giemsa and examined for 100 microscopic thick film fields prior to being declared negative. In positive films, parasite species were identified and densities were recorded as the number of parasites per 200 white blood cells (WBC). This procedure was followed for each species. Densities were converted to parasites per microliter of blood, assuming 8,000 WBC per microliter. A routine quality control procedure was performed [20]. Briefly, 10% of randomly selected slides and all those that had a density between 1 and 5 parasites per 200 WBC (for any of the four species) were reread, in a blinded fashion, by a supervisor microscopist. When the results on a batch of ~1,000 slides did not reach 75% agreement on positivity/negativity, species, and density (including a margin of error that increased with increasing density), the entire batch was reread and the same quality control applied again.

Haemoglobin concentration. Haemoglobin level was determined using the HemoCue photometer (Ängelholm).

Ethical approval and informed consent

The morbidity surveillance in the Wosera area received ethical approval from the Medical Research Advisory Committee (MRAC) within the framework of the larger Malaria Vaccine Epidemiology and Evaluation Project [16]. Patients or patients' guardians were asked for oral informed consent by the research nurse. This mode of consent was

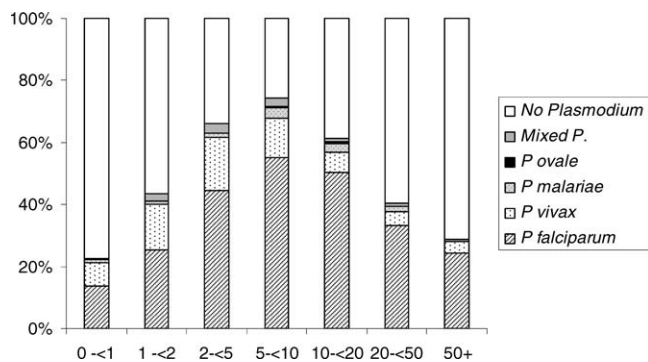


Figure 1. Age-Specific Prevalence of Parasitaemia among Presumptive Malaria Cases

doi:10.1371/journal.pmed.0050127.g001

approved by the MRAC, and considered appropriate for this setting where many people cannot read and write. Moreover, the procedures performed (systematic history taking, clinical examination, and diagnostic test for malaria) provided patients with a laboratory-confirmed diagnosis of malaria and consequently with better management of the illness.

Data Analysis

Case definitions. A UM case was defined as a presumptive malaria patient (see above) associated with documented *Plasmodium* asexual blood-stage parasitaemia by light microscopy.

A SM case was defined using the standard criteria established by the World Health Organization (WHO), except that we also included species other than *P. falciparum*, and we did not take into account a parasite density $>250,000/\mu\text{l}$ since we expected lower densities in *P. vivax* than in *P. falciparum* infections [21]. Because clinical and laboratory facilities were limited in the setting we were working in, we could only assess a subset of criteria defining SM. Thus, our definition included a patient with presumptive malaria associated with documented *Plasmodium* asexual blood-stage parasitaemia, and with at least one of the following: history of more than one convulsion in the last 24 h; coma (patient unable to localize a painful stimulus); respiratory distress (high respiratory rate >50 for children 0 to <2 mo of age and >40 for others including adults) and chest indrawing or shortness of breath); and haemoglobin <5 g/dl. Since there is ongoing debate about whether it is appropriate or not to include patients with associated symptoms or signs (comorbidity), we also report on a more restricted definition excluding patients with associated cough and/or diarrhoea, two features that may or may not be related to malaria [22]. The latter symptoms were usually mild since, if significant, the patients would not have been diagnosed as presumptive malaria.

Analysis. We analysed the proportion of SM cases by species (*P. falciparum* monoinfection, *P. vivax* monoinfection, and mixed *P. falciparum* and *P. vivax* infections) among all malaria cases (UM and SM) recorded in the morbidity surveillance from 1997 to 2004. We stratified our analysis by age group, since we expected different effects in individuals with different level of pre-existing immunity. We focused most of the analyses in the 0- to 5-y age group, since in highly endemic areas of PNG *vivax* malaria and SM, in particular, are encountered mainly in young children [23,24].

The chi square test was used (i) to compare rates of SM between the groups of malaria cases infected with *P. falciparum*, *P. vivax*, or mixed species; (ii) to investigate seasonal variation of SM rates; and (iii) to compare the distribution of clinical and laboratory features defining SM between groups of SM patients infected with *P. falciparum*, *P. vivax*, or mixed species. The proportions of cases with each of these features among *P. falciparum*, *P. vivax*, or mixed species SM are illustrated by Venn diagrams (created using free software that draws three set area proportional graphs) [25]. A chi square for trend was used to investigate the pattern of SM over the 8-y period.

Parasite densities were compared between severe and uncomplicated malaria as well as between species using log-transformed data and *t*-tests.

Data management was performed using the Foxpro software and data analysis Stata version 6. Since only a subsample of our patients in the morbidity surveillance were also included in the demography surveillance database (different villages), no systematic follow-up was possible.

Results

A total of 73,620 patients were registered in the 8-y period of the morbidity surveillance from 1997 to 2004. Among those, 17,201 cases were diagnosed as presumptive malaria cases and investigated further by the research nurse. A total of 9,537 of those patients (55%) had asexual *Plasmodium* parasitaemia. Among those, 6,886 (72%) had pure *P. falciparum*, 1,946 (20%) *P. vivax*, 328 (3%) *P. malariae*, 27 (0.3%) *P. ovale*, and 350 (4%) mixed infections (314 *P. falciparum* and *P. vivax*; 21 *P. falciparum* and *P. malariae*; ten *P. vivax* and *P. malariae*; three *P. falciparum* and *P. ovale*; one *P. malariae* and *P. ovale*; and one *P. falciparum*, *P. vivax*, and *P. malariae*). The age-specific parasite prevalence and species distribution among all presumptive malaria cases is shown in Figure 1. The highest parasite prevalence was found in children aged 5 to <10 y (peak age 7 y). On the other hand, the highest proportion of *P. vivax* was seen in children 2 to <5 y (peak age 2 y), with up to 20% parasitaemic.

Among parasitaemic individuals with all data available (7,759), 6.2% (95% confidence interval [CI] 5.7%–6.8%) fulfilled the case definition of SM. Table 1 details the proportion of SM cases associated with *P. falciparum*, *P. vivax*, and mixed *P. falciparum* and *P. vivax* infections by age group using two different case definitions: standard and restricted (excluding patients with cough or diarrhoea). Children aged <2 y were more likely to have SM (standard definition) than those aged 2 to <5 y (odds ratio [OR] 2.2, 95% CI 1.8–2.7) and those aged 5 to <10 y (OR 6.4, 95% CI 4.7–8.6); for individuals infected with *P. vivax*, the ORs were of 2.8 (95% CI 1.8–4.4) and 7.5 (95% CI 3.5–15.7), respectively; for individuals infected with *P. falciparum*, the ORs were of 2.0 (95% CI 1.5–2.6) and 6.0 (95% CI 4.2–8.5); for individuals with mixed infections, the ORs were of 3.2 (95% CI 1.3–8.0) and 32.1 (95% CI 3.2–319, Fisher's exact <0.001). The decrease in risk of severe disease was linear with age for all patients with *P. falciparum*, *P. vivax*, or mixed infections (Figure 2). The decrease in risk was significantly more pronounced for mixed (OR 0.52/y, CI 0.38–0.72) compared to single infections (OR 0.72/y, 95% CI 0.68–0.76, likelihood ratio [LR] $\chi^2 = 4.7$, degree of freedom [df] = 1, $p = 0.03$), but similar for *P. falciparum* (OR

Table 1. Proportion of SM Cases Associated with *P. falciparum*, *P. vivax*, and Mixed *P. falciparum* and *P. vivax* Infections by Age Group Using Two Case Definitions: Standard and Restricted (Excluding Patients with Cough or Diarrhoea)

Definition	Category	Age Group in Years										Total							
		0 to < 2		2 to < 5		5 to < 10		10 to < 15		≥ 15		95% CI	n	Percentage	95% CI	n	Percentage	95% CI	
		n	Percentage	95% CI	n	Percentage	95% CI	n	Percentage	95% CI	n								Percentage
Patients with parasitaemia and available data		1,019	—	—	2,423	—	—	2,280	—	—	786	—	—	1,251	—	—	7,759	—	—
Patients with <i>P. falciparum</i>		588	—	—	1,635	—	—	1,681	—	—	636	—	—	1,044	—	—	5,584	—	—
Patients with <i>P. vivax</i>		356	—	—	622	—	—	410	—	—	86	—	—	139	—	—	1,613	—	—
Patients with <i>P. mixed (Pf and Pv)</i>		45	—	—	97	—	—	80	—	—	14	—	—	16	—	—	252	—	—
Standard ^a	SM	173	17	15–19	207	9	7–10	71	3	2–4	10	1	1–2	22	2	1–3	483	6	6–7
	<i>P. falciparum</i> SM	104	18	15–21	157	10	8–11	58	3	3–4	7	1	0–2	16	2	1–2	342	6	6–7
	<i>P. vivax</i> SM	51	14	11–18	35	6	4–8	9	2	1–4	1	1	0–7	4	3	1–7	100	6	5–7
	<i>P. mixed</i> SM	13	29	18–43	11	11	6–19	1	1	0–7	1	7	0–34	0	0	0–23	26	10	7–15
Restricted ^b	SM	61	6	5–8	140	6	5–7	48	2	2–3	8	1	0–2	11	1	0–2	268	3	3–4
	<i>P. falciparum</i> SM	37	6	5–9	111	7	6–8	41	2	2–3	5	1	0–2	8	1	0–2	202	4	3–4
	<i>P. vivax</i> SM	15	4	3–7	21	3	2–5	4	1	0–3	1	1	0–7	1	1	0–4	42	3	2–4
	<i>P. mixed</i> SM	8	18	9–32	6	6	3–13	1	1	0–7	1	7	0–34	0	0	0–23	16	6	4–10

^aPresumptive malaria associated with a documented asexual blood stage parasitaemia and: coma; or fits; or respiratory distress (high respiratory rate >50 for children 0 to < 2 mo and > 40 for others and shortness of breath or chest indrawing); or anaemia (haemoglobin < 5).

^bPresumptive malaria associated with a documented asexual blood stage parasitaemia and: no cough; and no diarrhoea; and: no cough; or fits; or respiratory distress (high respiratory rate > 50 for children 0 to < 2 months and > 40 for others and shortness of breath or chest indrawing); or anaemia (haemoglobin < 5).

P. Plasmodium; Pf, P. falciparum; Pv, P. vivax.
doi:10.1371/journal.pmed.0050127.t001

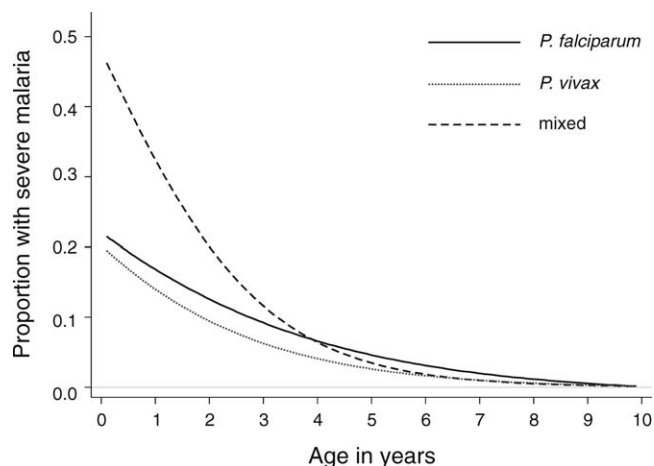


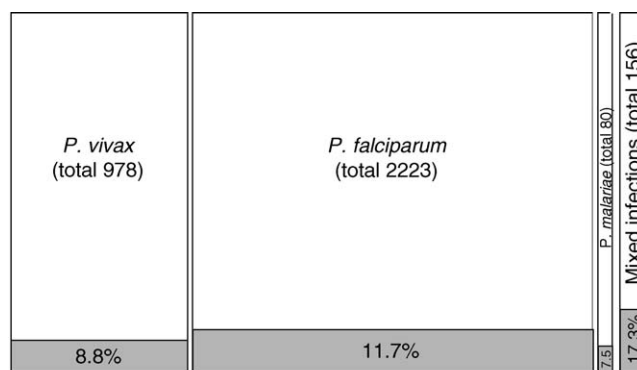
Figure 2. Age-Specific Risk of Severe Disease with *P. falciparum*, *P. vivax*, and Mixed Infections in Children < 10 y
doi:10.1371/journal.pmed.0050127.g002

0.73/y, 95% CI 0.68–0.74) and *P. vivax* single infections (OR 0.65/y, CI 0.56–0.77, likelihood ratio [LR] $\chi^2 = 2.5$, $df = 1$, $p = 0.11$).

Figure 3 shows the proportion of SM in children < 5 y infected with any species (except *P. ovale* because cases were too few) or mixed infections. Children < 5 y infected with both *P. falciparum* and *P. vivax* infections (mixed) were more likely to present with SM than those who were infected with *P. falciparum* only (17% versus 12%, OR 1.5, 95% CI 1.0–2.4), or *P. vivax* only (17% versus 9%, respectively, OR 2.1, 95% CI 1.3–3.5). In children < 2 y, almost one-third (29%) with mixed infections had SM. In this age group, the proportion of SM among *P. vivax* infections was close to that among *P. falciparum* infections (14% versus 18%, respectively, for the standard definition, 4% versus 6% for the restricted definition) (see Table 1).

The proportion of SM cases (< 5 y) presenting with each type of clinical manifestation or laboratory feature defining SM is shown schematically in Figure 4 by Venn diagrams for the group of SM infected with *P. vivax*, *P. falciparum*, or mixed species. *P. vivax* SM and mixed SM presented more often with respiratory distress than did *P. falciparum* SM (61% versus 41%, $\chi^2 = 9.85$, $p = 0.002$ and 67% versus 41%, $\chi^2 = 5.88$, $p = 0.015$, respectively), but less often with anaemia; the latter being only significant for the comparison between *P. vivax* and *P. falciparum* (19% versus 41%, $\chi^2 = 14.57$, $p = 0.0001$ and 29% versus 41%, $\chi^2 = 1.36$, $p = 0.2$). Neurological manifestations tended to be less frequent in mixed SM than in *P. falciparum* or *P. vivax* SM (8% versus 23% and 26%, respectively), but none of the differences were significant, probably due to the small numbers. Comparing the rate of each neurological sign between *P. vivax* and *P. falciparum* SM, fits were as frequent in both species (22% for both, $\chi^2 = 0$, $p = 0.96$); as well as coma (4% versus 2%, respectively, $\chi^2 = 1.25$, $p = 0.26$).

More generally, among all children < 5 y with parasitaemia, the prevalence of anaemia was 5.1% in patients infected with *P. falciparum*, 1.6% in those with *P. vivax*, and 5.3% in those with mixed infections; respiratory distress was found in 5.5% of *P. falciparum* patients, 5.1% of *P. vivax* patients, and 10.3% of those with mixed infections; and repeated convulsions



There was no case of *P. ovale* severe malaria

Figure 3. Mosaic Plot of SM by Species in Children < 5 y

Horizontal, species; vertical, proportion of SM.

doi:10.1371/journal.pmed.0050127.g003

were encountered in 2.5% and coma in 0.2% of *P. falciparum* patients, in 2% and 0.3%, respectively, of *P. vivax* individuals, and in 1.1% and 0%, respectively, of those with mixed infections.

To investigate whether the increased risk of SM in children infected with two species was due to a higher overall parasite burden, we compared densities in mixed infections versus those in mono-infections. As shown in Figure 5 (restricted to children < 5 y), total parasite densities were indeed significantly higher in children with mixed infections than in those with *P. falciparum* or *P. vivax* mono-infections, whether all cases ($t = -2.41$, $df = 2,968$, $p = 0.02$ and $t = -11.57$, $df = 1,372$, $p < 0.01$, respectively) or the severe ones only ($t = -2.38$, $df = 283$, $p = 0.02$ and $t = -5.39$, $df = 108$, $p < 0.01$, respectively) were considered. Densities tended to be lower in the severe *P. falciparum* and *P. vivax* cases than in the uncomplicated ones; the reverse was true in cases with mixed infections. In the latter, the average proportion of *P. falciparum* density/total parasite density was 83% in the UM and 87% in SM.

Age was the prominent risk factor for presenting with SM, but the season and year of attendance also had a significant effect. Malaria (parasitaemic) patients attending during the wet season were more likely to have SM than those attending during the dry season (OR 1.3, 95% CI 1.1–1.6), and this effect was significant for *P. vivax* (OR 1.9, 95% CI 1.2–2.9), but not for *P. falciparum* (OR 1.1, 95% CI 0.9–1.4), or mixed infections (OR 0.8, 0.4–1.9). Over the years, there was a significant decrease in the rate of SM (OR 0.8, 95% CI 0.7–0.8), with a clear step observed from 2000 onward, probably as a consequence of the new treatment policy that was introduced in that year (adding SP to amodiaquine or chloroquine).

Discussion

The present study shows that in a rural area of PNG where all four species of *Plasmodium* coexist, *P. vivax* is responsible for 21% of all SM cases, *P. falciparum* for 71%, and mixed *P. vivax* and *P. falciparum* infections for 5%. A feature of this clinico-epidemiological report is the simultaneous presence of *P. falciparum* as an internal control for *P. vivax*, so that the relative proportions of severe disease can be determined, regardless of its definition. The proportion of patients < 5 y

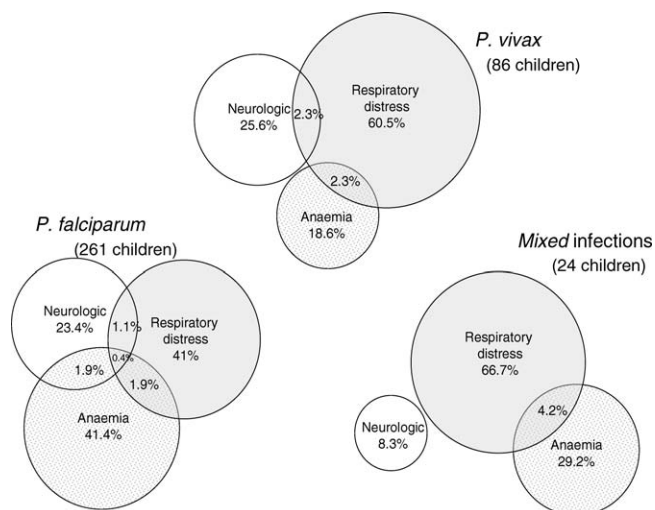


Figure 4. Proportion of SM Cases with Each of the Main Defining Clinical or Laboratory Features among Children <5 y Infected with *P. vivax*, *P. falciparum*, and Mixed Species (Venn Diagram)
doi:10.1371/journal.pmed.0050127.g004

who presented with SM among all *P. vivax* infected children was as high as 9%. The corresponding value for *P. falciparum* was 12%. Since the evaluators and the defining criteria for uncomplicated and severe malaria were strictly the same, we can be confident that the relative values (*P. vivax* versus *P. falciparum*) are correct. Moreover, the absolute value for the proportion of severe cases among all *P. falciparum*-infected children (12%) was lower than those found in numerous other studies of clinical malaria, the main reason being that our study took place in a peripheral health facility and not in a referral hospital as most, if not all, other studies [26–28]. It is clear that this absolute value depends entirely on the criteria used to define SM and on the population from which the cases are selected. Since we were working in a setting that did not allow us to measure certain parameters included in the WHO definition, such as glycemia or creatinemia, we probably slightly underestimated the true proportion of severe malaria caused by both species. Whichever definition is used, the relative contribution of *P. vivax* to the overall burden of SM morbidity is considerable in this region, and especially in children <2 y, where the proportion of *P. vivax* parasitaemic children presenting with severe disease reached a worrying rate of 14% (versus 18% for *P. falciparum*). These data on severe *vivax* malaria are in line with those reported in a joint paper from a hospital in Papua, Indonesia, also published in this issue of the journal [28]. In that setting, severe disease was present in 23% of the patients with malaria, with rates similar for *P. falciparum* and *P. vivax* (22%), but significantly higher in patients with mixed infections (34%). These two observational studies, although both originating from the same region of the world, add to the growing realization that *P. vivax* is not benign. They are the first studies, to our knowledge, to incorporate denominators that allow assessing the relative magnitude of the problem when compared to that of *P. falciparum*.

Although all types of malaria complications observed with *P. falciparum* were also encountered with *P. vivax*, their distribution was different: severe cases of *P. vivax* were less

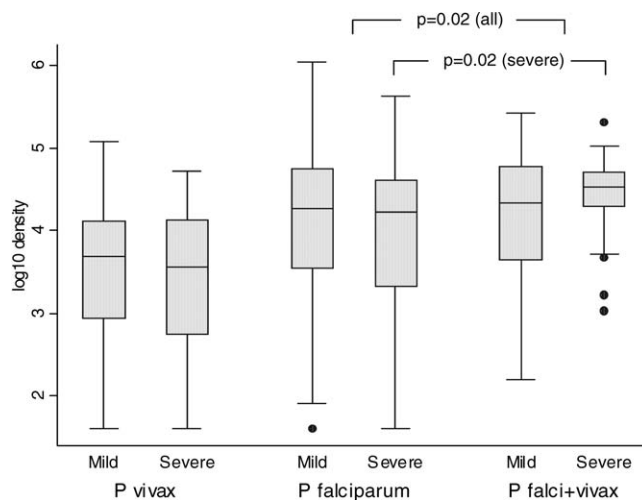


Figure 5. Total Parasite Densities in Uncomplicated and SM by Species (Children <5 y)

Line is median, boxes are 25th and 75th percentile, and whiskers are upper and lower adjacent values.

doi:10.1371/journal.pmed.0050127.g005

likely than those of *P. falciparum* to present with anaemia, but more likely to suffer from respiratory distress. These observations are understandable since we know that *P. vivax* malaria patients have lower densities than *P. falciparum* patients, and therefore the likelihood of having profound anaemia is less, even if other immunological factors may contribute to red cell destruction. The proportion of severe *P. vivax* cases with anaemia was much less in our study (19%) than in Tjitra's (86%) [28]. Their hypothesis of *P. vivax* resistance contributing largely to the high prevalence of anaemia in their setting is plausible since we had 0% *P. vivax* resistance in the Wosera during the study period [17,18] versus 65%–95% in theirs [29,30]. Our finding of increased risk of respiratory distress in *vivax* patients when compared to *falciparum* patients is in line with recent data from Anstey et al. who showed a more severe alveolar-capillary dysfunction in *vivax* than *falciparum* malaria [31]. These observations are consistent with a greater inflammatory response to a given parasite burden in *P. vivax* relative to that in *P. falciparum*, and suggest that *P. vivax* infected erythrocytes may sequester within the pulmonary microvasculature [31]. Severe *vivax* malaria is thus an emerging recognized entity, and the long-standing belief that *P. vivax* is a nonsequestering parasite might need to be revisited [31,32].

Contrary to the study in Thailand that reported lower frequencies of SM in dual *P. vivax* and *P. falciparum* infections [10,33], we found that patients with mixed infections were more likely to present with SM than those infected with a single *Plasmodium* species. Our data are in line with case series from India [34], Malaysia [35], and Indonesia [28]. The mechanism for the higher proportion of SM among patients with mixed infections might be a higher overall parasite load. Indeed we found that severe cases with mixed infections had higher parasite densities than those with single *P. falciparum* and, even more so, *P. vivax* infection. This increased severity does not formally contradict the possible protective role of asymptomatic *P. vivax* infection against a subsequent *P. falciparum* morbid episode. In a study conducted in the same

area as the present one [36], asymptomatic individuals infected with *P. vivax*, *P. malariae*, or mixed infections were less likely to have a morbid episode with *P. falciparum* during follow-up. This may mean that indeed non-*falciparum* species could protect against the risk of morbid episode because of *P. falciparum*, but, once the disease is established, having mixed infection makes the patient sicker, owing to the higher parasite density, as found in our study. This hypothesis must be balanced against the observation that, also in our series, SM cases with single *P. falciparum* or *P. vivax* infection did not harbor higher parasite densities than uncomplicated cases. This has been repeatedly found in different settings. Parasite density has not been identified as a risk factor for death in one of the largest series of malaria cases in Africa [37].

Our study is limited by the fact that parasite species were identified by microscopy, and it is known that this method largely misses minority infections [15,38,39]. Thus, mixed infections may have been largely underestimated, and a more sensitive detection method may have led to different results. While this limitation may be valid, it is believed that, in general, the infections that make the patient sick are those of high density, and that these are the ones that are identified by microscopy, as in the present study; those with very low densities are unlikely to contribute much to the acute phase, and even less to severe manifestations.

Another potential limitation is the accuracy of malaria diagnosis. In a high transmission area, where the prior probability of a young child being parasitaemic is high, the diagnosis of clinical malaria is not straightforward. We know from comparable settings in Africa that sepsis is commonly misdiagnosed as malaria, or that pneumonia can be associated with malaria. Since no blood cultures and no chest radiographs were performed, we cannot be certain that these children did not have a disease other than malaria. However, this potential bias is inherent to all studies of SM, even those that include thorough clinical assessment and sophisticated laboratory procedures. In intervention studies, a parasite density threshold is advised to increase the specificity of the diagnosis. When using a *P. falciparum* density threshold that should have led to over 90% sensitivity and 90% specificity for a diagnosis of malaria in this area and age group (< 5 y) [40], we found that 74% of the *P. falciparum* UM and 71% of the *P. falciparum* SM were above 4,000 parasites/ μ l. When applying the same statistical methodology to *P. vivax* (I. Mueller, personal correspondence), 71% of the *P. vivax* UM and 66% of the *P. vivax* SM were above 1,000 parasites per microlitre. We can thus postulate that at least two-thirds of the cases were “true” malaria. Even if some of our cases actually had alternative/additional diagnoses, the finding of severe disease with an associated *P. vivax* parasitaemia cannot be disregarded, as in *P. falciparum* cases [41]. These children should still be managed as SM cases, whether illness is due to *P. falciparum*, to *P. vivax*, or to another cause indistinguishable from malaria [42,43]. This approach is supported by a recent retrospective analyses of hospital records in Jayapura, West Papua, Indonesia, which found risk of fatal outcomes among SM patients was comparable between those with *falciparum* versus *vivax* malaria [13]. Epidemiological studies such as the present one should be used as a notice for clinicians working in areas where *P. vivax* is present, and as a signal for researchers to further investigate the pathogenesis of *P. vivax*

malaria and the potential associated comorbidities, such as sepsis, in the same way as has been done for *P. falciparum* [44].

Why is *P. vivax* malaria only now recognized as a potential serious threat to life, although the parasite has been present for centuries? One of the reasons may be that SM has always been, and almost exclusively, associated with cerebral complications, and the latter are rare in *P. vivax*. The prejudice to only consider *P. falciparum* as potentially severe was therefore difficult to overcome. It is only in the 1990s that other clinical manifestations and laboratory parameters have been identified as indicators of poor outcome [26]. Anaemia and respiratory distress, for example, have been recently included in the case definition of SM, and these signs are encountered frequently in *P. vivax* malaria. There is therefore increased awareness to recognize manifestations that may be less apparent than coma, but still harbor poor prognosis. Also, the relatively recent establishment of large morbidity surveillance systems in different epidemiological settings has improved the ability of researchers to capture unexpected events or trends.

The finding that a significant proportion of SM morbidity is due to *P. vivax* calls for interventions that do not target *P. falciparum* parasites only, at least in areas where several *Plasmodium* species coexist. In areas with multiple species, new interventions should be assessed for their effectiveness on overall malaria morbidity, so that potential interactions, especially compensatory effects on other species, can be identified early enough, before widespread implementation.

Acknowledgments

We would like to thank the staff of Kunjingini and Wombisa health centres, who have been assessing these patients with dedication for over ten years, and the microscopists of the PNG IMR for their conscientiousness and accuracy in blood slide reading.

Author contributions. BG designed the study, performed clinical supervision and data checking, and wrote the manuscript. VDA was responsible for data cleaning and analysis. LR was responsible for the demography surveillance system and for the day-to-day activities in the Wosera. KB was the research nurse in charge and on-site microscopist. JCR was the PNG IMR director for the second part of the morbidity surveillance, in charge of overall activities. MPA was the former PNG IMR director; he designed the study and was in charge of overall activities for half of the study period. IM was in charge of data base management and coordinated the malaria activities on site for the second part of the study period.

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Editors' Summary

Background. Malaria is a parasitic infection that is transmitted to people by infected mosquitoes. Four different parasites cause malaria—*Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. Of these, *P. vivax* is the commonest and most widely distributed, whereas *P. falciparum* causes the most deaths. All these parasites enter their human host when an infected mosquito takes a blood meal. They then migrate to the liver where they replicate without causing any symptoms. Eight to nine days later, mature parasites are released from the liver cells and invade red blood cells. Here, they multiply rapidly before bursting out and infecting more red blood cells. The recurring flu-like symptoms of malaria are caused by this cyclical increase in parasitemia (parasites in the blood) and should be treated promptly with antimalarial drugs to prevent the development of potentially fatal complications. Infections with *P. falciparum* in particular can cause anemia by destroying the red blood cells and can damage vital organs (including the brain) by blocking the capillaries that supply them with blood.

Why Was This Study Done? It is generally believed that *P. vivax* malaria is rarely fatal. There is even some evidence that infection with *P. vivax* alone (monoinfection) or with other malaria parasites (mixed infection) provides protection against malarial complications. Recently, however, there have been reports of severe disease and deaths associated with infection by *P. vivax* alone. Most of these reports do not indicate what proportion of severe malaria cases are caused by *P. vivax* infections, but if *P. vivax* is responsible for a significant proportion of malarial deaths, efforts to prevent these deaths will need to target *P. vivax* as well as *P. falciparum*. In this study, therefore, the researchers estimate the proportion of cases of severe malaria among patients infected with one or several *Plasmodium* species in Papua New Guinea, a country where all four species coexist.

What Did the Researchers Do and Find? The researchers enrolled everyone attending two rural health facilities in the Wosera subdistrict of Papua New Guinea over an eight-year period with symptoms indicative of malaria but without symptoms of any other disease (presumptive malaria cases) into their prospective cohort study. They asked each patient about their symptoms, did a standard physical examination, looked for parasites in their blood, and measured their hemoglobin levels to see whether they were anemic. Out of 17,201 presumptive malaria cases, 483 had severe malaria (defined as parasitemia plus a recent history of fits, coma, breathing problems, or anemia). Most of the patients with severe malaria were less than five years old—children have little immunity to *Plasmodium* parasites. In this age group, 11.7% of

patients infected with *P. falciparum*, 8.8% of patients infected with *P. vivax*, and 17.3% of patients infected with both parasites had severe malaria. Patients with severe malaria caused by *P. vivax* presented with breathing difficulties more often than those infected with *P. falciparum*, whereas anemia was more common among patients with severe malaria caused by *P. falciparum* than by *P. vivax*.

What Do These Findings Mean? The researchers use these results and data on the numbers of infections with each parasite to calculate that, in this rural region of Papua New Guinea, *P. vivax* is responsible for one-fifth of severe malaria cases, *P. falciparum* is responsible for three-quarters of cases, and the rest involve mixed *P. falciparum*/*P. vivax* infections. Put another way, these findings suggest that about one in ten children under the age of five years infected with either *P. vivax* or *P. falciparum* may develop severe malaria. These findings provide no evidence, however, that mixed infections are protective. Because the diagnosis of severe malaria was not confirmed by outcome data (deaths or permanent disability), additional, more detailed studies are needed to confirm these results. Nevertheless, these findings (and those reported separately in a related article published at the same time in *PLoS Medicine*) suggest that a significant proportion of the illness associated with malaria may be caused by *P. vivax* infections. Thus, efforts to reduce or eliminate the malarial burden must target *P. vivax* as well as *P. falciparum* in regions where these species coexist.

Additional Information. Please access these Web sites via the online version of this summary at <http://dx.doi.org/10.1371/journal.pmed.0050127>.

- A *PLoS Medicine* Research in Translation article by Stephen Rogerson further discusses this study and a related paper on *vivax* malaria infection in patients attending a regional hospital in Papua, Indonesia
- 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)
- Information is available from the Roll Back Malaria Partnership on global control of malaria and on malaria in Papua New Guinea
- Vivaxmalaria provides information for the malaria research community on topics related to *Plasmodium vivax*
- The Malaria Vaccine Initiative also provides a fact sheet on *Plasmodium vivax* malaria