

THE POPULATION STRUCTURE OF *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* DURING AN EPIDEMIC OF MALARIA IN THE EASTERN HIGHLANDS OF PAPUA NEW GUINEA

IVO MUELLER, JAPALIS KAIOK, JOHN C. REEDER, AND ALFRED CORTÉS

PNG Institute of Medical Research, Goroka, Papua New Guinea; PNG Institute of Medical Research, Madang, Papua New Guinea; Health Services, Obura Valley, Evangelical Brotherhood Church, Goroka, Papua New Guinea; Tropical Health Program, Australian Centre of Tropical Health and Nutrition, School of Population Health, University of Queensland, Herston, Queensland, Australia

Abstract. Although most of the Papua New Guinea highlands are too high for stable malaria transmission, local epidemics are a regular feature of the region. Few detailed descriptions of such epidemics are available, however. We describe the investigation of a malaria epidemic in the Obura Valley, Eastern Highlands Province, Papua New Guinea. Of the 244 samples examined by microscopy, 6.6% were positive for *Plasmodium falciparum* only, 9.4% were positive for *Plasmodium vivax* only, and 1.2% were mixed infections. *MSP2* and *MSP3 α* genotyping and *AMA1* sequencing were used to determine the genetic variation present in a sample of *P. falciparum* and *P. vivax* infections. The *P. vivax* infections were found to be genetically highly diverse. In contrast, all *P. falciparum* samples were of a single genotype. This striking difference in genetic diversity suggests endemic, low-level local transmission for *P. vivax* but an outside introduction of *P. falciparum* as the most likely source of the epidemic.

INTRODUCTION

Although many of the 2 million inhabitants of the Papua New Guinea (PNG) highlands live at altitudes that preclude stable transmission of malaria parasites, malaria is a common feature of deep intermontane valleys and highlands fringe areas.^{1,2} At altitudes below 1,300 m, malaria tends to be endemic, whereas above 1,800 m, temperatures are normally too low for malaria transmission to occur. At intermediate altitudes, malaria transmission is unstable, often seasonal, and prone to epidemics.^{3–6}

Although large-scale epidemics are relatively rare and linked to major climatic events, such as the “El niño” phenomenon,^{7,8} local epidemics are commonly observed^{2,4,9} and reported to health authorities. Although small in scale, these epidemics are associated with heavy morbidity and often mortality.¹⁰ Epidemics that were studied in detail were caused mainly by *Plasmodium falciparum* infections, whereas *Plasmodium vivax* dominated in the interepidemic periods,² similar to the epidemic patterns observed in remote Amerindian populations in the Amazon.¹¹ Earlier studies linked the occurrence of local epidemics either to seasonal patterns of rainfall¹² or to vertical migration of populations in deep intermontane valleys.²

Such localized epidemics represent a major challenge to the control of malaria in the Highlands region of PNG, where the bulk of the population lives at altitudes of 1,600–2,000 m. To devise rational measures for control and possibly prevention of such epidemics in the highlands area, a better knowledge of the patterns underlying these local epidemics is needed.

Genotyping methods developed for *P. falciparum* and *P. vivax* now allow detailed description of the genetic diversity of parasites present during malaria epidemics and can provide important information on the origin and dynamic of the epidemic. Populations of *P. falciparum* are known to be genetically diverse, even at low levels of endemicity.^{13–15} Major epidemics associated with abnormal weather and the extension of malaria transmission into neighboring nonmalarious areas also have been found to be genetically diverse.¹⁶ Small epidemics of *P. falciparum* in remote areas may consist of a single strain,^{17,18} however, indicating a single introduction as the cause for the epidemics.

In the context of the Asia-Pacific region, a shortcoming of past analyses of genetic diversity in epidemic situations is that they have failed to consider the interplay of *P. falciparum* with *P. vivax*. We report for the first time data on the genetic structure of the population of *P. falciparum* and *P. vivax* parasites, found during an epidemic of malaria in the Obura valley, Eastern Highlands Province, PNG.

MATERIALS AND METHODS

Survey population. The epidemic described here occurred in March–July 2001, in 3 villages (Himarata, Tisara, and Numisaira) in the vicinity of Obura, in the upper Lamari valley, Eastern Highlands Province. All these villages are situated in the mountains at 1,600–1,800 m but have coffee gardens along the Lamari River at 1,200–1,400 m. During the coffee season (January–August), people move regularly between village and coffee garden and often sleep in the gardens in makeshift houses. The coffee season coincides with the second half of the rainy season and the start of the dry season, when the number of anopheline mosquitoes found in the highlands is the highest,¹² and locally transmitted malaria cases are most frequent.¹⁹

Malaria cases presenting at Obura Health Center tend to rise in January every year with the beginning of the coffee season, but a sharp increase in the number of cases starting in March/April alerted the local health authorities, and special malaria clinics were conducted in local villages. Because the local health center does not have a microscopist, no blood slides were taken, but in some clinics presumptive cases were tested for *P. falciparum* using the ParasightF test (Becton & Dickinson, Sparks, MD).

In early July, a team from the PNG Institute of Medical Research visited the area and conducted a cross-sectional survey in the most affected village, Himarata. The survey included 65 households with a total of 244 individuals present at the day of the survey, representing 70% of the village population. In each individual, a blood slide was made, the spleen was palpated, and axillary temperature was taken. Hemoglobin levels were measured using portable HemaCue Hb meters (HemoCue AB, Ängelholm, Sweden). Symptomatic individuals were tested with Optimal test kits (DiaMed, Cressier Swit-

zerland), and those found positive were treated. A questionnaire on malaria episodes in the 2 weeks before the survey, antimalarial use, and recent travels was administered to each person or to the guardian in case of small children. Three Centers for Disease Control (CDC) light traps (2 indoor, 1 outdoor) were set in the village, and an extensive search for anopheline larvae was conducted in the vicinity of the village. No traps could be set in the garden areas because they are several hours' walk away from the village. Blood slides were examined under microscope for 100 thick film fields under oil immersion before being declared negative. The parasite species in positive films were identified, and densities were recorded as number of parasites/200 white blood cells. Densities were converted to parasites/ μl , assuming 8,000 white blood cells/ μl .

Genetic analysis. All *P. falciparum* microscopy-positive samples were tested by polymerase chain reaction (PCR) with *P. falciparum*-specific primers (for *PfMSP2* gene), and *P. vivax* microscopy-positive samples with parasite densities $>400/\mu\text{l}$ were tested by PCR with *P. falciparum*-specific and *P. vivax*-specific (for *PvMSP3 α* gene) primers. This procedure was chosen to ensure that no *P. falciparum* sample in an undetected mixed infection was missed. DNA was prepared from Isocode Dipsticks (Schleicher & Schuell, Dassel, Germany) when available (6 *P. falciparum*-positive samples, 7 *P. vivax*-positive samples). For the other samples, DNA was extracted from the Giemsa-stained slides using a slight modification of a previously described method.²⁰ Briefly the thick film on the Giemsa-stained slide was scratched first with a disposable pipette tip in 150 μl of buffer containing 10 mM of tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 8.0, 2 mM of ethylenediaminetetraacetic acid (EDTA), 10 mg/ml of dithiothreitol (DTT), 0.5 mg/ml of proteinase K, and 0.1% sodium dodecyl sulfate (SDS). This solution was transferred into a 1.5-ml reaction tube and incubated overnight at 37°C. DNA was extracted 3 times with phenol-chloroform, ethanol precipitated and resuspended in 20 μl of double distilled water (ddH₂O), of which 1–5 μl was used for PCR analysis. A total of 11 positive *P. falciparum* and 11 positive *P. vivax* samples could be used for further genetic analyses. Agreement between microscopy and PCR diagnosis was good for the 13 samples for which Dipsticks were available (only 1 sample, *P. falciparum* positive by microscopy, could not be PCR amplified). Many of the low-level infections ($<1,500/\mu\text{l}$) in which DNA was available only from the blood slide did not yield a PCR product, however. The results presented here might contain a bias toward high-density infections.

All PCR-positive samples were genotyped using restriction fragment length polymorphism (RFLP). For *P. falciparum*, *MSP2* RFLP was selected as an indicator of strain diversity because it is a highly polymorphic gene for which at least 38 different genotypes exist in malaria endemic areas of PNG,²¹ most of which can be distinguished easily with a well-established PCR-RFLP method.²¹ *MSP2* genotyping was done using nested PCR as previously described²¹ and *Hinf I* digestion. For *P. vivax*, *MSP3 α* RFLP was selected also because this gene is highly polymorphic and a robust PCR-RFLP method is available.²² *MSP3 α* nested PCR and *AluI* and *HhaI* digestions were performed as described,²² then products were analyzed on 10% polyacrylamide gels. The highly polymorphic domain I of *PfAMA1*^{23,24} of 5 *P. falciparum*-positive samples also was amplified by nested PCR

and sequenced, as will be described at a later time (Cortés et al., in preparation).

Statistical analysis. Because hemoglobin levels are known to be age and gender dependent, nonlinear regression was used to obtain residual scores, which are corrected for these effects. Parasite densities were (natural) log-transformed. Differences in categorical variables were tested using chi-square and Fisher exact test; differences in continuous variables were tested via *t*-test and analysis of variance. A Poisson model was used to test for clustering of cases within families. All statistical analyses were done using SPLUS (Insightful Corp, Seattle, WA 2001) and STATA statistical software (Stata Corp, College Station, TX 1999).

RESULTS

Of the 244 blood slides taken in Himarata, 42 (17.2%) were positive by microscopy (Table 1); 16 (38.1%) were *P. falciparum*, 23 (54.8%) were *P. vivax*, and 3 (7.1%) were mixed infections. Although present in the New Guinea Highlands²⁵ (I. Mueller, unpublished data), neither *Plasmodium malariae* nor *Plasmodium ovale* was found. There was no significant association of prevalence of malarial infection with gender (chi-square₍₁₎ = 0.27, *P* > 0.5). Children <10 years old tended to be infected more frequently than adolescents and adults with *P. vivax* (15% versus 8%, chi-square₍₁₎ = 2.7, *P* = 0.1) but not with *P. falciparum* (6% versus 8%, chi-square₍₁₎ = 0.3, *P* > 0.5), indicating a certain degree of immunity to *P. vivax* but not to *P. falciparum* infections. No clustering of malaria cases within families was observed (Poisson model: Deviance = 66.1, d.f. = 65, dispersion estimated at 1.03). The parasite densities of the positive slides were relatively low: 11, <200 parasites/ μl ; 7, 200–499 parasites/ μl ; 2, 500–999 parasites/ μl ; 12, 1,000–4,999 parasites/ μl ; and 10, >5,000 parasites/ μl . There were no significant differences in geometric mean densities between species ($t_{(38)} = 0.09$, *P* > 0.5) or among age groups ($F_{(3,38)} = 0.8$, *P* = 0.5). Mixed infections had higher densities than single infections (3,088 parasites/ μl versus 853 parasites/ μl), but because of the low number of mixed infections, this difference did not reach significance ($t_{(41)} = 1.08$, *P* = 0.29).

In 2 malaria clinics held in the 2 other affected villages 2 weeks before the cross-sectional survey, 18 (47.4%) of 38 presumptive malaria cases tested positive for *P. falciparum* by ParasightF test. There were no significant differences in the proportion of *P. falciparum* cases in different age groups (Fisher exact, *P* > 0.5).

Of the positive cases, 26 of 42 had presented with fever ($>37.5^\circ\text{C}$, 4 cases), reported having had fever in last 3 days (15 cases), or reported having had a clinical episode consistent with a malaria episode in the last 2 weeks (7 cases). There

TABLE 1
Prevalence of different *Plasmodium* infections according to age groups

Age (years)	No. sampled	No. positive (%)	No. <i>P. falciparum</i> (%)	No. <i>P. vivax</i> (%)
<2	15	1 (6.7)	0 (0.0)	1 (6.7)
2–4	28	5 (17.9)	1 (3.6)	4 (14.3)
5–9	35	11 (31.4)	4 (11.4)	7 (20.0)
10–19	41	6 (14.6)	4 (9.8)	3 (7.3)
≥ 20	125	19 (15.2)	10 (8.0)	11 (8.8)
Total	244	42 (17.2)	19 (7.8)	26 (10.6)

were no significant differences between *P. falciparum* and *P. vivax* cases in the reporting of fevers (Fisher exact test, $P > 0.5$). The symptomatic cases had significantly higher geometric mean densities than asymptomatic cases (1,549/ μ l, IC [651,3681] versus 410/ μ l, IC [189,888], $t_{(39)} = 2.2$, $P = 0.03$).

Hemoglobin levels were influenced strongly by the type of malaria infection (Figure 1) ($F_{(3,229)} = 29.07$, $P < 0.001$) and decreased with increasing (ln) parasite density (-0.55 g/dl, $F_{(1,39)} = 15.1$, $P < 0.001$). Corrected for age and gender difference, the level of hemoglobin was reduced by 3.1 g/dl (confidence interval, -3.9 , -2.4) in *P. falciparum* cases, 1.9 g/dl (confidence interval, -2.5 , -1.2) in *P. vivax* cases, and 1.1 g/dl (confidence interval, -1.9 , -0.2) in people with negative slides but a history of malaria treatment in the 2 weeks before the survey. Only 5 enlarged spleens (average spleen size 2.3) were detected, 1 with concurrent *P. falciparum* infection, 2 with *P. vivax* infection, and another 2 with microscopically negative blood slides. Six (31.6%) of the *P. falciparum*-positive but only 2 (8.7%) of the *P. vivax*-positive cases reported having received antimalarial treatment (chloroquine plus sulfadoxine/pyrimethamine [Fansidar]) 2 weeks prior (Fischer exact, $P = 0.11$). None of the people with positive slides had traveled outside the village in the month before the survey. One woman had traveled to the highly endemic lowlands, however, and on her return she presented with malaria at the local health center, where she received treatment.

All 11 *P. falciparum* samples genotyped showed the same *MSP2* allele, the *WOS6* allele²¹ of the FC27 *MSP2* allelic family (Figure 2A and data not shown for 1 sample analyzed later). All the bands that define the *WOS6* allele have a size of < 200 bp. The bands > 200 bp observed in certain samples are nonspecific PCR products that were present before digestion (data not shown). The *WOS6* allele is an uncommon *MSP2* allele elsewhere in PNG (allelic frequency, ≤ 0.03 ^{21,26}). The single-strain origin of *P. falciparum* infections was supported further by sequencing the most highly polymorphic region of *AMA1* (domain I) in 5 randomly selected *P. falciparum*-positive samples. The sequence was identical in all 5 samples analyzed and showed a haplotype that is found at a

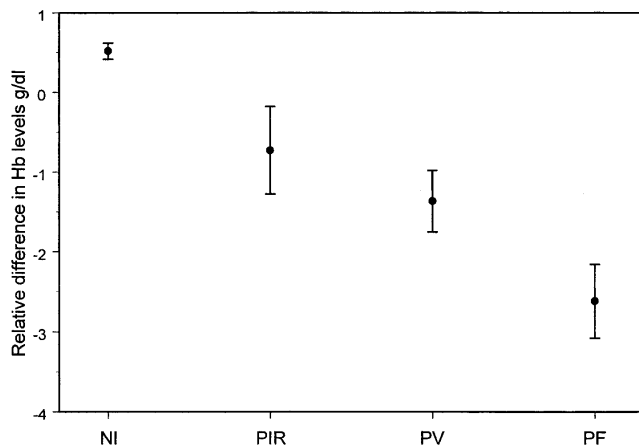


FIGURE 1. Relative differences in hemoglobin (Hb) levels according to infection status. Hb values corrected for age and gender effects using nonlinear regression. Bars indicate standard errors. NI, not infected; PIR, previous infection reported (2 weeks before survey); PV, *P. vivax* infection; PF, *P. falciparum* infection.

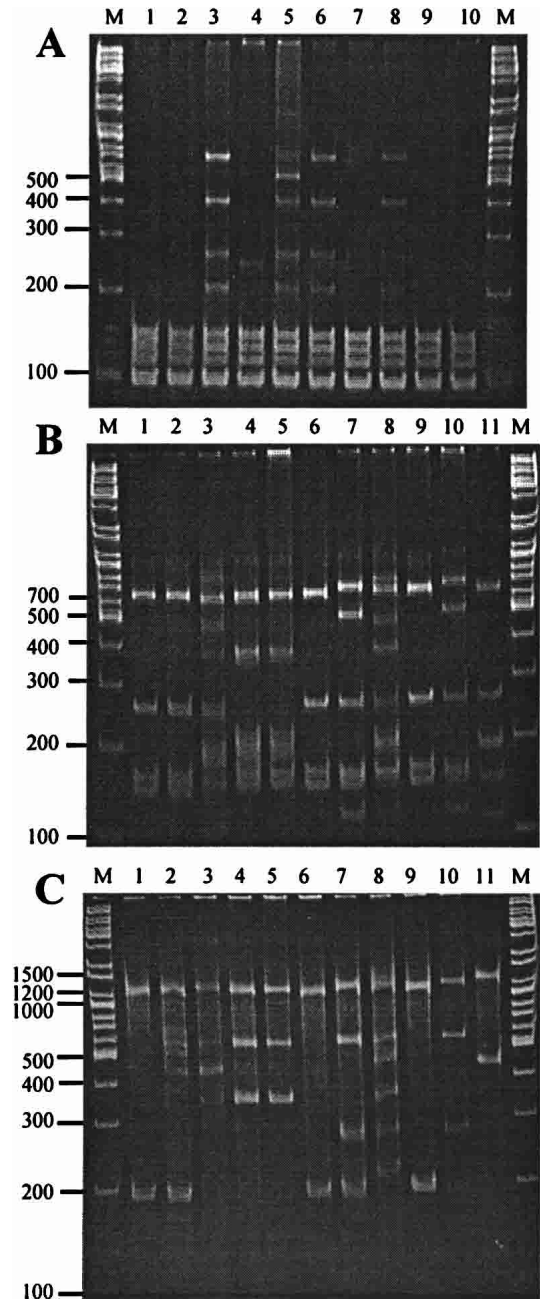


FIGURE 2. Restriction fragment length polymorphism analysis of *PfMSP2* and *PvMSP3 α* genes in some Himarata malaria-positive samples. *HinfI* digestion of *PfMSP2* nested polymerase chain reaction (PCR) products (A), *AluI* digestion of *PvMSP3 α* nested PCR products (B), and *HhaI* digestion of *PvMSP3 α* nested PCR products (C). The 11 *MSP3 α* samples are the same and equally numbered in panels B and C. M, DNA size marker.

low frequency in another region of PNG (Cortés et al., in preparation). These data strongly suggest that a single strain was responsible for the *P. falciparum* malaria cases observed during the epidemic.

In contrast, the 11 *P. vivax* isolates analyzed revealed at least 6 distinct *MSP3 α* alleles, when taken together the results of the *AluI* and the *HhaI* digestions (Figure 2B and C). Multiplicity of infection can be determined by comparing the size of the undigested PCR products (data not shown) with

the sum of the size of the digestion fragments. Two of the samples examined contained multiple *P. vivax* strains with different *MSP3 α* alleles (Figure 2B and C, lanes 3 and 8), providing further evidence of the diversity of *P. vivax* strains in this population.

The entomologic surveys showed no presence of *Anophele* mosquitoes. The 3 light traps used on 2 consecutive nights caught only *Culicines*, and no obvious *Anophele* breeding site was found in the vicinity of the village.

DISCUSSION

We have presented the first molecular analysis of a localized, mixed *P. falciparum*/*P. vivax* epidemic. The main feature of this epidemic was that it consisted of multiple *P. vivax* strains but only a single *P. falciparum* strain. This feature suggests endemic, albeit low-level, local transmission for *P. vivax* but an outside introduction of an epidemic strain of *P. falciparum* into the area.

The genotyping methods used to determine the parasite population structure are based on highly diverse genes (*MSP2* and *MSP3 α*), which have proved informative in many previous genetic studies.^{21,22} The sequencing of the highly variable *AMA1* gene^{23,24} in some samples further increased the power to identify different strains of *P. falciparum*. It is remarkable that only a single *P. falciparum* strain was detected in all tested samples.

Although the formal possibility remains that there is >1 *P. falciparum* strain, sharing identical *MSP2* and *AMA1* alleles, in practice this is unlikely considering the high diversity of alleles for these 2 genes and the low frequency of the alleles of the Obura strain elsewhere in PNG.^{21,26} It is also possible that more genotypes would have been found if more samples had been successfully PCR-amplified for the genetic analysis or if samples from an earlier time during the epidemic could have been investigated. If other undetected strains were present, however, it is unlikely that they were major contributors to the epidemic.

Malaria epidemics caused by a single strain of *P. falciparum* have been observed before in isolated populations in Africa¹⁷ and South America,¹⁸ where recent introductions were the likely cause of the epidemics. Epidemics that are caused by an extension of malaria transmission from an endemic into usually nonmalarious areas during periods of abnormal climate are likely to be triggered by multiple independent introductions from a genetically diverse neighboring population. These are often large-scale epidemics and expected to be genetically diverse, as was found for an epidemic in Mauritania.¹⁶

In endemic transmission settings, a remarkably high genetic diversity of *P. falciparum* parasites has been observed even in low transmission areas,^{13–15} although lower endemicity usually is correlated with lower genetic diversity compared with medium and high endemicity settings.²⁷ In areas with strongly seasonal transmission, such as the Sudanese Sahel, a diverse *P. falciparum* population is maintained by regularly observed chronic infections that last for 9 months and span the entire dry season.²⁸ The high diversity observed in Sudanese populations²⁹ is in striking contrast to our findings in Obura. A recent introduction is a more likely source of the *P. falciparum* strain in the Obura epidemic, rather than the carry-over of an infection from the previous transmission season.

The single strain composition of the *P. falciparum* cases in the Obura epidemics is in stark contrast to the high genetic diversity in *P. vivax* and suggests a different transmission pattern for the 2 parasite species in the Obura valley. Because of its long-lasting liver stage, which allows *P. vivax* to stay in a host population for prolonged periods, even if transmission is interrupted and the primary infections have been treated successfully, a higher genetic diversity in low-endemicity settings might be expected for the *P. vivax* population when compared with *P. falciparum*. The situation found in Obura is likely to represent endemic *P. vivax* transmission combined with a recently introduced strain of *P. falciparum*.

The epidemiology of malaria in the Obura valley is linked strongly to vertical migration associated with the picking of coffee in the lower lying coffee gardens. In the villages up the hill, anopheline mosquitoes are rare or absent and temperatures are usually too low for effective transmission. In the gardens, which lie 400–500 m below and are 2–3°C warmer, transmission is possible. Because PNG anophelines are zoophilic,³⁰ vector populations are sustained easily during the dry (noncoffee) season by feeding on wild animals, especially wild pigs. When people move to the coffee gardens to start picking in January, transmission of *P. vivax* could ensue through people experiencing relapses of infections acquired during the previous coffee season. Because money from coffee sales is readily available during this time, people also travel more often to the malarious coastal areas, potentially leading to an increase of imported cases. This rise in locally transmitted *P. vivax* and imported *P. falciparum* and *P. vivax* cases may account for the increase in people presenting with fevers at the local health center. If such an imported *P. falciparum* case is not treated properly during the coffee season and carries gametocytes down to the gardens, a *P. falciparum* epidemic, such as the one described in this article, can be started.

Although it has been shown that an introduced *P. falciparum* strain can persist in a local population for >1 year,¹⁷ this is unlikely to happen in Obura. During the noncoffee season, when people live only in their village above 1,600 m, transmission is almost totally interrupted, and any occurring symptomatic cases can be handled easily by the local health system.

In summary, the malaria situation in the Obura valley is characterized by 2 main factors: seasonal vertical migration that limits transmission to the coffee season and relative isolation that limits the number of introductory events during the transmission season. This differentiates the Obura epidemic from the one observed in Cabo Verde,¹⁷ which was caused by the reintroduction of *P. falciparum* into an island, where malaria had disappeared owing to control activities, but the vectors were still present. The situation in isolated parts of the Amazon described by Laserson et al.^{11,18} is more similar. There, *P. vivax* malaria is endemic, but *P. falciparum* was important only during intermittent epidemics, with isolation, small size of the populations, and possibly seasonal climate being the reasons for the limited transmission of *P. falciparum*.¹²

In light of the different transmission patterns, it is surprising that there was so little difference in morbidity, apart from lower hemoglobin levels, between the *P. falciparum* cases and the *P. vivax* cases in our study. Several reasons may account for this. Earlier on in the epidemic, many more symptomatic patients were reported as being seen and treated at the local

health center, many of whom tested positive for *P. falciparum*, using the ParasightF test. Among those, the death of a pregnant woman occurred. Because the tested samples were taken near the end of the epidemic, several months after the first cases were seen, some people may have been exposed to this particular strain and may have developed a certain degree of clinical immunity. Alternatively, because >30% of the *P. falciparum* cases in the survey reported having received anti-malarial treatment in the previous 2 weeks, heightened awareness late in the epidemic, leading to prompt treatment, may have reduced the amount of morbidity seen. The high number of *P. falciparum* cases having been recently treated by drugs also might indicate a certain degree of drug resistance of the strain responsible for the epidemic.

It also could be speculated that the co-occurrence of *P. vivax* in the Obura region might have helped to reduce the morbidity resulting from *P. falciparum*. Although the overall evidence for such a cross-protective effect is inconclusive, a study in PNG has shown that a prior infection with *P. vivax* reduced the likelihood of a later clinical *P. falciparum* episode but not the risk of a *P. falciparum* infection.³¹

Good diagnosis and prompt treatment of all malaria cases, but especially of imported *P. falciparum*, is the key for the prevention of future epidemics in the Obura valley. Distribution of insecticide-impregnated bed nets might support these efforts by reducing local transmission. To be effective nets, however, they must be combined with education highlighting the need to use them every time the family goes into the coffee gardens. Residual house spraying is not a suitable option, for either epidemic control or prevention, because the garden houses, where most malaria transmission takes place, are makeshift and widely dispersed, both of which make spraying difficult and ineffective.

The situation in Obura is typical of local epidemics in the PNG highlands, where vertical migration into lower lying valleys is part of the local subsistence system. The epidemics in Enga province described by Sharp^{2,6} follow that pattern, although migration is less linked to coffee but rather to the harvesting of sago or pandanus. The described epidemic pattern also might be common in isolated populations, however, from other parts of the world, especially in areas where transmission intensity is low and seasonal.

Acknowledgments: The authors thank Johnson Juni and Marta Melombo for assistance with the genetic analysis work; Rex Ivivi for microscopy; the MSCU Goroka, the staff at Obura Health Center for help during sample collection; and Jerome Whitfield for logistical support.

This work received approval by the Papua New Guinea Medical Research Advisory Council.

Financial support: This work was supported by the World Health Organization through the Regional Office for the Western Pacific and the Global Roll Back Malaria Initiative. I. Mueller is supported by the Swiss National Science Foundation, the Rudolf Geigy Foundation, and P. Mueller AG.

Authors' addresses: Ivo Mueller and John C. Reeder, PNG Institute of Medical Research, P.O. Box 60, Goroka, Papua New Guinea. Japalis Kaiok, Health Services, Obura Valley, Evangelical Brotherhood Church, Goroka, Papua New Guinea. Alfred Cortés, PNG Institute of Medical Research, P.O. Box Madang, Papua New Guinea.

Reprint requests: Ivo Mueller, PNG Institute of Medical Research, P.O. Box 60, Goroka, EHP Papua New Guinea, Telephone: 675-732-2800, Fax: 675-732-1998, E-mail: ivo@pngimr.org.pg

REFERENCES

- Hii J, Dyke T, Dagoro H, Sanders RC, 1997. Health impact assessments of malaria and Ross River virus infection in the Southern Highlands Province of Papua New Guinea. *PNG Med J* 40: 14-25.
- Sharp PT, 1982. Highlands malaria: malaria in Enga Province of Papua New Guinea. *PNG Med J* 25: 253-260.
- Black RH, 1954. A malaria survey of the people living on the Minj River in the Highlands of New Guinea. *Med J Aust* 2: 782-787.
- Peters W, Christian SH, Jameson JL, 1958. Malaria in the highlands of Papua and New Guinea. *Med J Aust* 2: 409-416.
- Peters W, Christian SH, 1960. Studies on the epidemiology of malaria in New Guinea: IV. unstable highlands malaria: the clinical picture. *Trans R Soc Trop Med Hyg* 54: 529-536.
- Sharp PT, 1980. *Malaria in Enga Province: An Epidemiological Study of Malaria in a Highlands Province of Papua New Guinea*. Thesis, University of Sydney.
- Bangs MJ, Subianto DB, 1999. El Nino and associated outbreaks of severe malaria in highland populations in Irian Jaya, Indonesia: a review and epidemiological perspective. *Southeast Asian J Trop Med Public Health* 30: 608-619.
- Flew S, 1999. *Human Health, Nutrition and Heavy Metals: Report of a Survey from the Fly River, Western Province, Papua New Guinea*. Ok Tedi Mining Ltd, Tabubil, Papua, New Guinea.
- Spencer TET, Spencer M, Jameson MT, Tommerup JWH, 1956. Special patrol report: malaria in the Mount Hagen area. *PNG Med J* 1: 110-113.
- Temu P, 1978. *The Lum Health Survey Report*. Lutheran Hospital Mambisanda, Wapanamanda, Enga Province, PNG.
- Laserson KF, Wypij D, Petralanda I, Spielman A, Maguire JH, 1999. Differential perpetuation of malaria species among amazonian Yanomami amerindians. *Am J Trop Med Hyg* 60: 767-773.
- Peters W, Christian SH, 1960. Studies on the epidemiology of malaria in New Guinea: Part V. unstable highlands malaria: the entomological picture. *Trans R Soc Trop Med Hyg* 54: 537-542.
- Babiker HA, Lines J, Hill WG, Walliker D, 1997. Population structure of *Plasmodium falciparum* in villages with different malaria endemicity in East Africa. *Am J Trop Med Hyg* 56: 141-147.
- Bendixen M, Msangeni HA, Pedersen BV, Shayo D, Bodker R, 2001. Diversity of *Plasmodium falciparum* populations and complexity of infections in relations to transmission intensity and host age: a study from the Usambara Mountains, Tanzania. *Trans R Soc Trop Med Hyg* 95: 143-148.
- Paul REL, Brockman A, Price RN, Luxemburger C, White NJ, Looareesuwan S, Nosten F, 1999. Genetic analysis of *Plasmodium falciparum* infections on the north-western border of Thailand. *Trans R Soc Trop Med Hyg* 93: 587-593.
- Jordan S, Jelinek T, Aida AO, Peyrel-Hoffmann G, Heuschkel C, el Vally AO, Christophel EM, 2001. Population structure of *Plasmodium falciparum* isolates during an epidemic in southern Mauritania. *Trop Med Int Health* 6: 761-766.
- Arez AP, Snounou G, Pinto J, Sousa CA, Modiano D, Ribeiro H, Franco AS, Alves J, do Rosario VE, 1999. A clonal *Plasmodium falciparum* population in an isolated outbreak of malaria in the Republic of Cabo Verde. *Parasitology* 188: 347-355.
- Laserson KF, Petralanda I, Almera R, Barker RH Jr, Spielman A, Maguire JH, Wirth DF, 1999. Genetic characterization of an epidemic of *Plasmodium falciparum* malaria among Yanomami Amerindians. *J Infect Dis* 180: 2081-2085.
- Peters W, Christian SH, 1960. Studies on the epidemiology of malaria in New Guinea: Part IV. unstable Highland Malaria—the clinical picture. *Trans R Soc Trop Med Hyg* 54: 529-536.
- Reeder JC, Reickmann KH, Genton B, Lorry K, Wines B, Cowman AF, 1996. Point mutations in the dihydrofolate reductase and dihydropteroate synthetase genes and in-vitro susceptibility to pyrimethamine and cycloguanil of *Plasmodium falciparum* isolates from Papua New Guinea. *Am J Trop Med Hyg* 55: 209-213.
- Felger I, Tavul L, Kabintik S, Marshall V, Genton B, Alpers M, Beck HP, 1994. *Plasmodium falciparum*: extensive polymor-

- phism in merozoite surface antigen 2 in an area with endemic malaria in Papua New Guinea. *Exp Parasitol* 79: 106–116.
22. Bruce MC, Galinski MR, Barnwell JW, Snounou G, Day KP, 1999. Polymorphism at the merozoite surface protein-3 α locus of *Plasmodium vivax*: global and local diversity. *Am J Trop Med Hyg* 61: 518–525.
 23. Polley SD, Conway DJ, 2001. Strong diversifying selection on domains of the *Plasmodium falciparum* apical membrane antigen 1 gene. *Genetics* 158: 1505–1512.
 24. Escalante AA, Grebert HM, Chaiyaroj SC, Magris M, Biswas S, Nahlen BL, Lal AA, 2001. Polymorphism in the gene encoding the apical membrane antigen-1 (AMA-1) of *Plasmodium falciparum*: X. Asembo Bay Cohort Project. *Mol Biochem Parasitol* 113: 279–287.
 25. Purnomo, Solihin A, Gomez-Saladin E, Bangs MJ, 1999. Rare quadruple malaria infection in Irian Jaya Indonesia. *J Parasitol* 85: 574–579.
 26. Engelbrecht F, Felger I, Genton B, Alpers M, Beck HP, 1995. *Plasmodium falciparum*: malaria morbidity is associated with specific merozoite surface antigen 2 genotypes. *Exp Parasitol* 81: 90–96.
 27. Anderson TJC, Haubold B, Williams JT, Estrada-Franco JG, Richardson L, Mollinedo R, Bockarie M, Mokili J, Mharakurwa S, French N, Whitworth J, Velez ID, Brockham AH, Nosten F, Ferreira MU, Day KP, 2000. Microsatellite markers reveal a spectrum of population structures in the malaria parasite *Plasmodium falciparum*. *Mol Biol Evol* 17: 1467–1482.
 28. Hamad AA, el Hassan IM, el Khalifa AA, Ahmed GI, Abdelharim SA, Theander TG, Arnot DE, 2000. Chronic *Plasmodium falciparum* infections in an area of low intensity malaria transmission in the Sudan. *Parasitology* 120: 447–456.
 29. Babiker HA, Abdel-Muhsin AA, Hamad A, MacKinnon MJ, Hill WG, Walliker D, 2000. Population dynamics in an unstable malaria area of eastern Sudan. *Parasitology* 120: 105–111.
 30. Burkot TR, Dye C, Graves PM, 1989. An analysis of some factors determining the sporozoite rates, human blood indexes, and biting rates of members of the *Anopheles punctulatus* complex in Papua New Guinea. *Am J Trop Med Hyg* 40: 229–234.
 31. Smith T, Genton B, Baea K, Gibson N, Narara A, Alpers MP, 2001. Prospective risk of morbidity in relation to malaria infection in area of high endemicity of multiple species of *Plasmodium*. *Am J Trop Med Hyg* 64: 262–267.