Cross-sectional characterization of malaria in Sanma and Shefa Provinces, Republic of Vanuatu: malaria control implications

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SUMMARY

Endemic malaria still exists in the Republic of Vanuatu, an 80-island archipelago that sits astride the southeast margin of the Southeast Asian-Melanesian malaria band (Buxton Line 170° E, 20° S). The annual parasite incidence has decreased dramatically over the past decade, which has been attributed to an intensive insecticide-treated bednet distribution program and implementation of a revised Plasmodium falciparum treatment policy that employs combination chloroquine + sulfadoxine/pyrimethamine as a first-line therapy. Standard malariometric surveys were conducted at 10 locations in 2 provinces, screening 2351 adults and children towards the end of the peak transmission season. Spleen rates were consistent with mesoendemic malaria. Examination of blood slides revealed a mean slide-positive rate of 22% (range 4% to 33%). P. falciparum predominated, accounting for 73% of infections, followed by P. vivax (25%). Among 396 individuals with P. falciparum, the gametocyte rate was 54%, with 37% presenting gametocytes alone without asexual stages. Only 8% and 4% of persons with asexual stage P. falciparum and P. vivax parasitaemia, respectively, were symptomatic. These data suggest that malaria transmission has increased in some locations in Vanuatu over the past decade and this report underscores the importance of appropriate bednet use and vector control in this setting as well as the impact of adding sulfadoxine/pyrimethamine and removing primaquine from the national malaria treatment formulary.

Introduction

Malaria, caused by Plasmodium falciparum, Plasmodium vivax and Plasmodium malariae, remains endemic in the Republic of Vanuatu, an 80-island archipelago crossed by the southeast margin of the Southeast Asian-Melanesian malaria band (Buxton Line 170°E, 20°S). Anopheles farauti sensu stricto is the only known vector species in the country. Seasonal malaria transmission peaks from January through July and nadirs from August through December, corresponding with the hot/wet and cooler/dry seasons, respectively. Transmission intensity declines from the northernmost to southernmost islands of the archipelago (1).

The Malaria and other Vector Borne Diseases Control Unit (VBDCU), located in the capital city of Port Vila on Efate Island, is responsible for malaria control throughout Vanuatu. Each of 6 provinces has a malaria control supervisor and centrally based malaria laboratories that provide results to village health centres submitting smears from ill patients. These data are compiled and reported as annual parasite incidence (API) per 1000 population. The VBDCU is also responsible for assessing and revising malaria treatment and control policies for

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countrywide implementation and outbreak response.

Malaria control strategies have been modified over the last 2 decades in response to environment concerns with insecticide use, emergence of parasite resistance to antimalarials and primaquine toxicity. Chloroquine (CQ)-resistant *P. falciparum* was first described in Vanuatu in the early 1980s (2,3). The DDT indoor residual spraying program was abandoned around the same time. Between 1988 and 1992, malariometric survey slide-positive rates averaged about 12% (1). The Ministry of Health developed new treatment guidelines for uncomplicated malaria in 1991, based on confirmatory microscopic diagnoses to direct specific therapies, CQ (10 mg/kg po qd x 3 days) for *P. vivax* and CQ (10 mg/kg po qd x 3 days) + sulfadoxine/pyrimethamine (SP) (1.25 mg/kg P component) for *P. falciparum* (4). At the same time, primaquine (PQ) was abandoned based on case reports of PQ-associated haemolysis and reports of severe glucose-6-phosphate dehydrogenase (G6PD) deficiency rates ranging between 0 and 39% (mean 7%) at several locations throughout the archipelago (1,4-6). The 1995 strategic malaria control plan targeted an API of less than 10 by the year 2000 (7). During the 1990s, the Ministry of Health achieved near 100% permethrin-impregnated bednet distribution on this 180,000-strong island nation (8). The provincial malaria laboratories also promote early diagnosis through utilization of their microscopy program to guide therapeutic decisions. Between 1988 and 2000, the malaria API declined from 184 to 34 (Figure 1).

To assess the current state of malaria control activities and provide a background for future investigations, we conducted cross-sectional surveys to estimate the prevalence of *P. falciparum* and *P. vivax* infections among populations at specific locations in the Sanma and Shefa Provinces of Vanuatu through mass blood screening of healthy and ill individuals. Secondary objectives included estimating malaria endemicity, naturally acquired immunity and G6PD deficiency rates in populations at specified locations.

**Materials and Methods**

We conducted all surveys in response to the written request of the Republic of Vanuatu Ministry of Health. Standard methodologies for malariometric surveys were employed (9). During March 2002, cross-sectional surveys were conducted at 3 villages on Malo Island, 3 villages in southern Espiritu Santo and 1 village at Big Bay Espiritu Santo in the Sanma Province and 3 villages on Epi Island in the Shefa Province (Figure 2). Following approval from the village leaders one to two days in advance, the survey team opened
Figure 2. Malarialometric survey locations, Republic of Vanuatu, March 2002.
and maintained makeshift screening clinics at village meeting places or schools from early morning through early evening hours to ensure that working adults also had opportunity for screening. Technicians visited homes when specifically requested by a relative.

Survey activities included collection of demographic information and child spleen examinations. Each participant, or his/her parent if a minor (<18 years of age), provided information about age, gender and current residence. Interviewers assessed symptomatology through the open-ended question, “Are you feeling well?”. A negative response prompted details. Illness was defined as more than 2 of the following: fever, chills, headache, myalgia, arthralgia, nausea or vomiting, diarrhoea and abdominal pain. Physicians conducted spleen examinations on children between 2 and 9 years of age, and spleen size was graded according to Hackett’s criteria (9).

Microscopists prepared thick and thin blood smears from fingerstick specimens for staining with Giemsa. They examined thick smears under a 1000X oil-immersion lens, recording number of asexual stage parasites, gametocytes and white blood cells and reporting parasite densities as the number of asexual stage parasites and/or gametocytes per 200 white blood cells. A minimum of 200 microscopic fields were examined before declaring a smear negative. All positive smears were identified to species by examination of the thick smear or thin smear, if necessary. Technicians reported results for all blood smears within 24 hours, and all persons with parasitaemia received treatment per Ministry of Health guidelines. Slide-positive rates were calculated as percentage of slides containing asexual and/or sexual stage plasmodial species.

Glucose-6-phosphate dehydrogenase deficiency testing was conducted at most sites whenever possible. At the same time as finger stick blood was collected for microscopy, 50 µl was also collected into a heparinized microcapillary tube and expressed onto Whatman® No 1 filter paper for immediate testing by a qualitative fluorometric technique (Sigma Diagnostic G6PD Kit 203A). Only a sample of persons presenting for malaria screening could be tested for G6PD deficiency. Because of the time-sensitive nature of the assay, a dedicated technician conducted serial batch testing in blocks of 20 consecutively presenting individuals. During batch processing and interpretation, additional individuals presenting for screening provided fingerstick blood specimens for malaria microscopy only. When the laboratory technician was ready for the next batch, staff reinitiated collection of blood for G6PD deficiency testing with the next person on cue.

Data obtained during these surveys were analyzed and described using standard descriptive statistics, ie means and rates. Confidence intervals for relative risk (RR) were calculated using Taylor series, and for odds ratio (OR) by Cornfield or exact limits when appropriate.

Results

Demographic data at each location are summarized in Table 1. Community participation was generally excellent. The apparently lower participation on Malo Island reflects that only 3 of the 45 villages could be sampled during the amount of time available. Children and women generally presented for screening in the morning hours, while adult males presented later in the day. In Narango and Pongovia, the number of individuals screened exceeded the published population, the excess attributed in part to participation by people from surrounding communities as well as possible increases in population since the 1999 census. The overall male:female ratio of participants was 1:1 with children accounting for two-thirds of the individuals screened. Sample gender and age distributions were representative of known population demographics at all locations except Nandiuti, Ebenezer and Lamen Bay, where screening was conducted at primary schools during morning hours. In those locations, the only adults screened included teachers and support staff.

The overall slide-positive rate (SPR) was 22%, ranging between 4% and 33% by village (Table 1). With the exception of Matantas Village at Big Bay Santo, where all 3 individuals with parasitaemia among 70 screened had *P. vivax, P. falciparum* predominated (73%, range 52%-94%) followed by *P. vivax* (25%, range 7%-43%) and mixed *P. falciparum*/*P. vivax* (2%, range 0%-5%). Age-specific SPRs, all locations combined, were 23% (59/259), 30% (159/
TABLE 1

DEMOGRAPHIC CHARACTERISTICS AND MALARIA SLIDE-POSITIVE RATES AMONG 2351 INDIVIDUALS AT 10 LOCATIONS IN THE SANMA AND SHEFA PROVINCES, REPUBLIC OF VANUATU, MARCH 2002

<table>
<thead>
<tr>
<th>Village</th>
<th>Population</th>
<th>Number screened (%)</th>
<th>Gender distribution of those screened Age distribution of those screened %</th>
<th>SPR %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Malo Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avunatari</td>
<td>-</td>
<td>637</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Nanuca</td>
<td>-</td>
<td>219</td>
<td>44</td>
<td>56</td>
</tr>
<tr>
<td>Nandiuti</td>
<td>-</td>
<td>146</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>4195</td>
<td>1002 (24)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>South Santo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Narango</td>
<td>235</td>
<td>245 (104)</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>Sarete</td>
<td>194</td>
<td>130 (67)</td>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>Ebenezer</td>
<td>139</td>
<td>139 (100)</td>
<td>49</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>568</td>
<td>514 (90)</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>Big Bay Santo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matantas</td>
<td>126</td>
<td>70 (56)</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>Epi Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamen Bay</td>
<td>259</td>
<td>237 (92)</td>
<td>47</td>
<td>53</td>
</tr>
<tr>
<td>Pongovia</td>
<td>213</td>
<td>333 (156)</td>
<td>46</td>
<td>54</td>
</tr>
<tr>
<td>Yevali</td>
<td>-</td>
<td>195</td>
<td>43</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td>765</td>
<td>45 (55)</td>
<td>55</td>
<td>35</td>
</tr>
<tr>
<td>Overall Total</td>
<td>-</td>
<td>2351</td>
<td>49</td>
<td>51</td>
</tr>
</tbody>
</table>

Population by 1999 census (where available)
Child = <18 years of age
SPR = slide-positive rate

538), 28% (166/601) and 15% (141/953) among individuals 0-4, 5-9, 10-14 and ≥15 years of age, respectively. Because we were interested in age-related morbidity associated with asexual stage parasitaemia, we analyzed the subset of slide-positive cases with asexual stage parasitaemia, excluding all gametocyte-only cases. Age-specific asexual stage parasite SPRs were 16% (42/259), 21% (114/538), 20% (122/601) and 10% (97/953) among individuals 0-4, 5-9, 10-14 and ≥15 years of age, respectively. Differences were not statistically significant between age groups 0-4, 5-9 and 10-14. However, the odds of having asexual parasitaemia at the time of screening were much higher in children under 15 years old than individuals ≥15: age 0-4 vs ≥15 OR 1.71 (95% CI 1.13-2.57); age 5-9 vs ≥15 OR 2.37 (95% CI 1.75-3.22); age 10-14 vs ≥15 OR 2.25 (95% CI 1.67-3.03) and age <15 vs ≥15 OR 2.19 (95% CI 1.70-2.83). Gametocyte rates for P. falciparum infections were high at all locations and 37% of individuals with P. falciparum had only sexual stages present in their peripheral blood...
samples based on microscopy (Table 2). Differences in gametocyte-only rates between individuals aged 0-4 (7%), 5-9 (9%), 10-14 (8%) and ≥15 (5%) were not statistically significant.

Spleen rates, measured at all locations except Epi Island, were consistent with mesoendemic malaria (Figure 3). Physical examinations did not reveal spleens larger than Hackett grade 2 and village splenic indices were 1.15 at Avunatari, 1.3 at Nauca, 1.1 at Nandiuti, 1.45 at Narango, 1.46 at Sarete, 1.0 at Ebenezer and 1.3 at Matantas. Illness rates among those screened were very low. Overall symptomatic case rates for individuals with *P. falciparum* and *P. vivax* asexual stage parasitaemia were 8% (20/249) and 4% (5/124), respectively. The differences in illness rates for all individuals with asexual *P. falciparum* parasitaemia, stratified by location (data not shown) and age, were not statistically significant. The relative risk for symptomatic *P. falciparum* among children (16/181) versus adults (4/68) was 1.5 (95% CI 0.52-4.34). The numbers of symptomatic *P. vivax* cases were even lower, only 5 among 108 children and 0 among 15 adults, precluding meaningful analysis. The mean qualitative G6PD deficiency rate among 360 persons screened at all sites was 10% (10.5% on Malo Island, 10% in South Santo, 7% at Lamen Bay, Epi and 12% at Pongovia, Epi). Among 36 individuals with abnormal G6PD activity, 7 (19%) had detectable parasitaemia compared to 77/324 (24%) with normal G6PD activity.

**Discussion**

In this cross-sectional malariometric survey, conducted during the peak transmission season, we confirmed the presence of mesoendemic malaria on Espiritu Santo, Malo and Epi Islands. The unexpectedly high prevalence of parasitaemia (22%) and low symptomatic case rates observed (4-8%) suggests that the passive case detection (PCD) currently employed to estimate API in Vanuatu, which relies on microscopically confirmed diagnoses in ill patients at health care centres, significantly underestimates the true incidence of malaria in Vanuatu. Reductions in reported API after 1991 (Figure 1) have been attributed to expansion of the countrywide insecticide-treated bednet (ITN) program together with new treatment guidelines in response to emerging chloroquine resistance in *P. falciparum* (1-3). Assuming an 8% symptomatic case rate, as observed in this study, and based on the reported API of 34 (year 2000 symptomatic, slide-confirmed case data), we extrapolated a potential API as high as 425/1000 population. Although the true API is most likely not this high, the calculation is provided to emphasize the observation that official PCD statistics generally only reflect symptomatic cases presenting for medical attention. Although good indicators of the burden of disease in the community, PCD statistics greatly underestimate the burden.

**TABLE 2**

*P. falciparum* and *P. vivax* gametocyte rates identified by mass blood surveys at Malo, Santo and Epi Islands, Republic of Vanuatu, March 2002

<table>
<thead>
<tr>
<th>Location</th>
<th><em>Plasmodium falciparum</em></th>
<th></th>
<th></th>
<th><em>Plasmodium vivax</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>GR %</td>
<td>GOR %</td>
<td>Number</td>
<td>GR %</td>
</tr>
<tr>
<td>Malo</td>
<td>149</td>
<td>56</td>
<td>36</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>Santo</td>
<td>107</td>
<td>60</td>
<td>43</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Epi</td>
<td>140</td>
<td>48</td>
<td>34</td>
<td>57</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>396</td>
<td>54</td>
<td>37 (147/396)</td>
<td>125</td>
<td>6</td>
</tr>
</tbody>
</table>

GR = gametocyte rate
GOR = gametocyte-only rate (percentage of subjects with gametocytes in absence of asexual stage parasites)
of infection and transmission potential, as suggested by the low symptomatic rate observed during this survey.

The apparent decline in malaria incidence during the 1990s might reflect reduced coverage in PCD despite increasing transmission, possibly due to stable or increasing naturally acquired immunity to malaria disease, resulting in a greater percentage of undetected, asymptomatic infections. A number of factors could be contributing to this apparent increased parasite burden in Vanuatu. The removal of single-dose primaquine from national malaria treatment guidelines in 1991 must be considered. Single-dose primaquine rapidly reduces gametocyte clearance time and has been shown to reduce the point prevalence of malaria when used in combination with potent blood schizonticides (10-12). Among 16 malarialmetric surveys conducted in Vanuatu between 1988 and 1992 just prior to and immediately following the change in treatment policy, the mean $P. falciparum$ gametocytaemic rate was 20%, and only 2% on Epi and 18% on Espiritu Santo. In 2002, these same locations had gametocyte rates between 48% and 60%. At the same time that primaquine was removed from the national malaria treatment formulary, SP was added in combination with chloroquine for treatment of falciparum malaria. Several reports implicate SP as having a gametocyte proliferative effect (13-15). Studies comparing gametocyte carriage rates to gametocyte-carrier mosquito infectivity have established a positive correlation between prevalence of gametocytaemia and malaria transmission burden within a community (16,17). It is therefore plausible that the systematic change in malaria treatment guidelines to combat chloroquine resistance has contributed to increased gametocyte carriage rates and increasing incidence of malaria over the ensuing decade.

In accordance with the general worldwide trend, around 1988, the Ministry of Health discontinued routine indoor residual house spraying with DDT and shifted towards permethrin-impregnated bednets as the primary means of vector control (1). Initial results were promising, but ITNs alone did not achieve the anticipated reduction in API to less than 10/1000 by year 2000, as targeted (7). The only anopheline species and vector of malaria present in Vanuatu is Anopheles farauti sensu stricto. In the nearby Solomon Islands, research indicates that this species has a 70-fold higher biting rate during the early evening hours (1830-2000) than in the later hours (2100-2400) (18). These data suggest that use of ITNs during normal sleep hours may have only minimal impact on reducing infective bites, a situation which is further compounded by the apparent increased gametocyte rates within the human
population.

With regard to this population’s apparent immunity against disease, the measured mesoendemic spleen rates were not consistent with the level of exposure anticipated in settings where symptomatic parasitaemia rates are low, as described here. Exposure-related protection against malaria-related morbidity and mortality has been demonstrated (19). In moderate to low transmission intensity areas of Tanzania, the odds of severe malaria decline with increasing age and consequent increasing infection exposure over time (20). In a cohort study of malaria-naive migrants to Papua, Indonesia, the likelihood of afebrile versus febrile parasitaemia did not increase significantly during the first 24 months of exposure until after the fourth sequential infection (21). Even assuming our extrapolated API of 425 (about 1 case per 2 person years), transmission intensity would probably be inadequate to establish and maintain immunity against disease. However, severe malaria rates and malaria-related mortality in children and adults in Vanuatu are extremely low (1,22) with only one death reported from 1999 to 2001 (GT, personal communication). Additional factors must therefore be contributing to the lower than expected morbidity rates observed in Vanuatu.

Based on the high gametocyte rates observed in this sample and, more specifically, the high gametocyte-only rates, we suspect heavy recent use of antimalarials, particularly SP. Although we did not specifically query individuals as to recent antimalarial use, both CQ and SP are widely available in the communities surveyed and are frequently used to treat febrile illness. The observed low symptomatic case rate could therefore represent partial immunity induced by intermittent drug suppression. Studies examining the impact of intermittent preventive treatment (IPT) with SP in Africa indicate that periodic administration of therapy in infants and children can reduce the incidence of clinical (symptomatic) malaria and severe anaemia by as much as 50-85% (23). A more recent study of the impact of intermittent SP therapy identified a sustained reduction in risk for clinical malaria that extended beyond the period attributable to the SP pharmacological effect, which authors have attributed to facilitated development of immunity against P. falciparum in the setting of intermittent treatment (24).

Previous studies on Vanuatu have identified a direct correlation between malaria incidence and G6PD deficiency rates (1, 5) consistent with the hypothesis that malaria drives selection for red blood cell polymorphisms, such as G6PD deficiency. Some studies suggest that G6PD deficiency protects not only against high parasite densities, but also against severe disease manifestations (25,26). In our study we did not observe severe malaria or deaths, although our method of sampling may have resulted in selection bias against more ill patients. The likelihood of patent parasitaemia was no greater in G6PD-deficient individuals than in those with normal G6PD activity (OR 0.77, 95% CI 0.3-1.94). We also observed no differences between symptomatic case rates and geometric mean parasite densities among G6PD-deficient (656/μl, SD 1237) and G6PD-normal (412/μl, SD 11696) individuals, although the number of cases was too low to make a fair comparison. It has been suggested that limited parasite diversity in remote island locations may explain the unusually mild nature of falciparum malaria in Vanuatu (27). Although non-immunity-related host and parasite factors have been established as contributors to the risk of severe malaria and malaria-related mortality, the low symptomatic case rates observed in this study and the apparent lack of exposure-related immunity warrant further investigation to assess the association of G6PD deficiency and other host-parasite factors with establishment of symptomatic versus asymptomatic infections.

We observed an unexpectedly high prevalence of plasmodial infections and high gametocyte rates in populations with apparent significant immunity to disease on Vanuatu. The removal of primaquine from and addition of sulfadoxine/pyrimethamine to the national malaria treatment guidelines are likely contributors to the sustained parasite burden. In addition to ITNs, supplemental vector control methods (eg, indoor residual spraying) may be necessary in some areas to further reduce infection burden. Decisions about new or revised antimalarial treatment policies should be based on empirical in vivo measurements of the efficacies of the currently recommended regimens. Although reinstitution of single-dose primaquine might
reduce malaria transmission, the risk of primaquine-induced haemolysis currently outweighs any potential impact on reducing further the already low malaria morbidity and mortality in Vanuatu.

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DISCLAIMER

The assertions herein are the views of the authors and do not reflect official policy of the US Department of the Navy, the US Department of Defense, or the US Government.

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