Hindawi Publishing Corporation
Malaria Research and Treatment
Volume 2012, Article ID 936067, 5 pages
doi:10.1155/2012/936067

Clinical Study

Failure of Supervised Chloroquine and Primaquine Regimen for the Treatment of *Plasmodium vivax* in the Peruvian Amazon

Paul C. F. Graf,[1,2] Salomon Durand,[1] Carlos Alvarez Antonio,[3] Carmen Montalvan,[3] Mariella Galves Montoya,[3] Michael D. Green,[4] Meddly L. Santolalla,[1] Carola Salas,[1] Carmen Lucas,[1] David J. Bacon,[1,5] and David J. Fryauff[6]

[1] *Department of Parasitology, Naval Medical Research Unit No. 6, Naval Medical Research Center, Lima, Peru*
[2] *Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY 14642, USA*
[3] *Regional Health Directorate, Loreto, Peru*
[4] *Entomology Branch, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA*
[5] *Chemistry Division, US Naval Research Laboratory, Washington, DC 20375, USA*
[6] *Malaria Research Department, Naval Medical Research Center, Silver Spring, MD 20910, USA*

Correspondence should be addressed to Paul C. F. Graf, paul_graf@urmc.rochester.edu

Received 2 October 2011; Accepted 1 April 2012

Academic Editor: R. J. Novak

The widespread use of primaquine (PQ) and chloroquine (CQ), together, may be responsible for the relatively few, isolated cases of chloroquine-resistant *P. vivax* (CQRPV) that have been reported from South America. We report here a case of *P. vivax* from the Amazon Basin of Peru that recurred against normally therapeutic blood levels of CQ. Four out of 540 patients treated with combination CQ and PQ had a symptomatic recurrence of *P. vivax* parasitemia within 35 days of treatment initiation, possibly indicating CQ failure. Whole blood total CQ level for one of these four subjects was 95 ng/ml on the day of recurrence. Based on published criteria that delineate CQRPV as a *P. vivax* parasitemia, either recrudescence or relapse, that appears against CQ blood levels >100 ng/mL, we document the occurrence of a *P. vivax* strain in Peru that had unusually high tolerance to the synergistic combination therapy of CQ + PQ that normally works quite well.

1. Introduction

P. vivax is the most prevalent cause of human malaria in the Americas, accounting for more than 70% of total cases reported [1]. Chloroquine (CQ) at a dose of 25 mg/kg over three days remains the first line therapy for vivax malaria. To prevent relapse, CQ is normally given in combination with primaquine (PQ). PQ is given to all nonpregnant patients greater than 6 months of age with normal activity of glucose-6-phosphate dehydrogenase (G-6-PD). The Peruvian National Malaria Control Program changed from the standard 14-day course of PQ at 0.25 mg/kg/day to a 7-day regimen of 0.5 mg/kg/day in 1998. Both regimens achieve a total adult PQ dose of 210 mg which is assumed to be widely effective against the hypnozoite stage of *P. vivax* strains acquired outside of Southeast Asia and the Indo-Pacific Islands. Widespread chloroquine resistance (CQR) has been reported in some areas of the world, although, up until now, only two confirmed cases have been reported in Peru [2, 3].

Recurrence of *P. vivax* following treatment can be classified as recrudescence, which is the recurrence of parasitemia originating from subpatent trophozoites that survived in the blood following treatment, relapse which is the recurrence of parasitemia originating from latent hypnozoites in the liver, or reinfection with a new strain [2]. Recrudescence occurs when the blood stage antimalarial drug such as chloroquine fails, either due to inadequate dosing or resistance of the blood stage parasites to the drug. Relapse can occur in the absence of a specific treatment against the hypnozoite stage or when the liver stage antimalarial drug such as primaquine fails to completely eradicate the parasite from the liver. In general, reappearance of parasitemia between 17 and 35 days after treatment is attributed to either recrudescence,

Figure 1: Map of Iquitos, Peru.

early relapse, or reinfection, whereas reappearance after 35 days is attributed to a relapse or reinfection [2]. Based on the treatment sensitivity demonstrated by *P. vivax* to blood levels of total CQ (parent compound CQ plus the active desethylchloroquine metabolite, DCQ), a minimally effective concentration (MEC) of 100 ng/mL was determined as the criteria for separating between CQ-sensitive and CQ-resistant strains of *P. vivax*, whether they arise from recrudescence, relapse, or reinfection [2].

2. Materials and Methods

2.1. Study Sites. The present protocol studied the efficacy of three different regimens of primaquine for the prevention of relapses of *P. vivax* at three different sites: Padre Cocha (PC), San Juan (SJ), and Santa Clara (SC), located in or around the city of Iquitos in the Department of Loreto in the Amazon Basin of Peru (Figure 1).

2.2. Study Design. Between 2005 and 2008, a total of 540 informed and consenting subjects between 1 to 77 years of age, with symptomatic microscopy-confirmed *P. vivax* monoinfection, were enrolled. Institutional review boards in Peru and the United States had reviewed and approved the study plan and procedures prior to any enrollment (796-2004-J-OPD/INS and NMRC.2005.0005). Study participants were divided into three arms, all of whom received the same standard treatment dose of chloroquine (25 mg base/kg body weight divided into single daily doses over 3 days) but were randomized to receive one of three regimens of PQ: (1) 0.5 mg/kg daily for 5 days, (2) 0.5 mg/kg daily for 7 days, or (3) 0.25 mg/kg daily for 14 days. Both CQ and PQ regimens were started on the same day, and consumption of each dose was directly observed. As part of the study protocol, blood smears were collected and evaluated on days 1, 2, 3, 7, 14, 21, and 28, later every 2 weeks until 6 months, and on each occasion when an enrolled study participant returned to the clinic with malaria-like symptoms. Patients who developed a recurrence of parasitemia during the 6-month follow-up period were treated as recommended by the Peruvian Ministry of Health (CQ: 25 mg/kg base over 3 days: 10 mg/kg on days 1 and 2, and 5 mg/kg on day 3, plus PQ: 0.5 mg/kg daily for 7 days).

2.3. Laboratory. A blood smear was performed on day 0 and follow-up days. In the case of recurrence of parasitemia within 17–35 days of starting treatment, 2 mL of EDTA blood were collected for determination of CQ blood levels and *P. vivax* genotyping. Blood smears were stained with Giemsa and independently read by two microscopists. The final parasite density was the average of the two densities. A third slide was read in the case of discrepancy between the first two reads.

2.4. Chloroquine Levels. Three aliquots of 100 μL of whole blood from day-of-recurrence (D-R) samples were spotted onto filter paper for later analysis by high performance liquid chromatography (HPLC) to determine the levels of CQ and its major metabolite, desethylchloroquine (DCQ) as previously described [3].

2.5. Molecular Analysis. DNA was isolated from 200 μL of whole blood-EDTA samples from D-0 and D-R by using a QIAamp DNA Blood Mini Kit (QIAGEN, NL), following the manufacturer's instructions. All initial (D-0) and recurrent (D-R) parasitemias were confirmed as *P. vivax* monoinfections by PCR [4]. Genomic DNA of *P. vivax* was amplified at the *Pvmdr1* and *Pvcrt* loci and sequenced using BigDye Terminator sequencing kit (Applied Biosystems, USA) on an ABI 3130 automated DNA sequencer (Applied Biosystems, USA) [5, 6].

3. Results

From the total of 540 patients enrolled in the study at the three sites, four subjects had a recurrence of parasitemia within the 35-day follow-up period. The study findings evaluating the three primaquine treatment groups will be published elsewhere. The four patients had fever or history of fever, were between 4 and 11 years old, and had a geometric mean (GM) parasite density of 13,536 parasites/μL on D-0. The characteristics of these subjects are shown in Table 1. Parasitemias recurred with symptoms on day 28 (2 patients), day 30, and day 32. However, the re-reading of the routine, scheduled slides showed that the recurrence of *P. vivax* parasitemia actually occurred earlier, but without symptoms, on day 28 for SC105 (Table 1). Initial reading of the day-32 slide for SC105 showed a mixed infection of *P. falciparum* and *P. vivax*, but upon review of this slide and reading of the duplicate and triplicate slides for this patient, a monoinfection with *P. vivax* was determined. *P. vivax* monoinfection, that is, the absence of *P. falciparum*, was confirmed in the day-32 whole blood sample of SC105 by PCR. This patient was treated with mefloquine and

Table 1: Characteristics of recurrent vivax malaria cases and parasitemia levels throughout the follow-up period.

Patient no.	Age/Sex	PQ Arm	Parasitemia (asexual parasites/μL) by day of follow-up								
			Day 0	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28	Day 30	Day-32
PC046	9/F	7	5,713	24	0	0	0	0	466	—	—
SJ004	11/F	7	13,099	24	0	0	0	0	12	—	—
SC058	7/M	5	13,064	0	0	0	0	0	0	1,522	—
SC105	4/F	14	34,340	36	0	0	0	0	47[II]	—	2,495

[II] Parasitemia detected only in the second reading of the slides. This sample was not included in the analysis of CQ blood concentration or in genotyping.

Table 2: Responses to CQ/PQ treatment, CQ blood levels and genotyping analysis from patients with recurrence of parasitemia.

Patient no.	Parasitologic response*	Therapeutic response†	CQ levels in blood (ng/mL)			D-0 versus D-R genotype
			CQ	DCQ	Total CQ	
PC046	RI (late)	LPF	21	43	64	Different
SJ004	RI (late)	LPF	BD**	23	23	Different
SC058	RI (late)	LPF	36	BD**	36	Same
SC105	RI (late)	LCF	42	53	95	Same

* RI (late), parasitological response, World Health Organization resistance levels, (Bruce-Chwatt, 1986).
† Only as reference: this classification is no longer used for *P. vivax*; World Health Organization; Peter Bloland; 2003.
** BD: below the limit of detection (<10 ng/mL).

artesunate for the presumed *P. falciparum* infection that only later was determined to be a recurrent *P. vivax* infection.

In three patients, the levels of CQ plus DCQ were subtherapeutic (<100 ng/mL) on the day of recurrence (Table 2). Only one patient, SC105, had a combined CQ + DCQ level of 95 ng/mL in whole blood on day 32 (the initially recognized day of recurrence). Because parasitemia was not detected initially on day 28, no venous blood sample was collected from this subject on day 28, and the CQ level could not be determined on the true day of recurrence.

Genotyping of *Pvmdr1* and *Pvcrt* was performed on D-0 and D-R blood samples from all four patients, because of their possible association with chloroquine resistance in *P. vivax* and as a means to determine whether the recurrence was caused by the same or a different strain of *P. vivax*. Two patients had different *Pvmdr1* genotypes in the D-0 and D-R isolates, indicating either a reinfection or the appearance of a second strain not detected in the D-0 sample (Tables 2 and 3). The remaining two, including SC105, had the same *Pvmdr1* genotype on D-0 and D-R, strongly suggesting that their recurrent parasitemia was either a recrudescence or an early relapse (Table 3). In the case of *Pvcrt*, all 8 samples were identical with only one synonymous mutation compared to the sequenced reference strain, P565 (CCT>CCC). None of the mutations in either gene were reported to be associated with CQ resistance in *P. vivax*.

4. Discussion

According to published criteria for classifying a *P. vivax* treatment failure as CQ resistance [2], one of four patients in our study would be considered a probable case of CQRPV. Multiple criteria that underlie this determination are the following: (1) GMP quality CQ, (2) proper weight-adjusted dose determination, (3) complete and directly observed consumption of all doses, (4) normal enteric absorption of the drug, and (5) a CQ + DCQ blood level at the time of recrudescence ≥100 ng/mL. In this study, one patient, SC105, had a total CQ level of 95 ng/mL in whole blood on day 32 (initially defined as the day of recurrence). However, a second reading of the slides later showed that there was actually reappearance of parasitemia four days earlier on day 28. A third reading confirmed the findings of the second reading. Although a blood sample was not collected on day 28 for this patient, it is likely that the total chloroquine concentration would have been higher on day 28 than on the day it was actually measured, day 32. It seems likely, although it cannot be said with complete certainty, that this patient indeed was infected with a chloroquine-resistant strain of *P. vivax*. We cannot say with certainty whether the recurrent parasitemia was a late recrudescence or an early relapse. Recrudescence is unlikely, given the fact that CQ resistance in *P. vivax* in Peru is quite low [3], and the combination of CQ + PQ is very effective against CQ-resistant malaria strains; PQ is reported to be even more potent than verapamil in reversing CQ resistance [7, 8]. Early relapse due to failure of the PQ to eliminate the hypnozoites seems more likely to have occurred, but if so, this relapse occurred against an impressive block of PQ. The recurrent parasites in the blood would have been exposed to the otherwise efficacious level of CQ remaining in the blood on day 28, but now in the absence of PQ due to its shorter half life, the remaining CQ was unable to eliminate the recurrent CQ-resistant parasites. Upon the reappearance of parasitemia, subject SC105 was treated with mefloquine and artesunate for presumed falciparum malaria on day 32.

Table 3: *Pvmdr1* genotypes.

Patient number	*Pvmdr1*	
	Day 0	D-R
PC046	V221/ **T529(ACG)**/ **M908L/T958M**/ L1022(CTA)	V221/ T529(ACA)/ **M908L/T958M/ L1022(TTA)**
SJ004	**V221L**/ T529(ACA)/ **M908L/T958M**/ L1022(CTA)	V221/ **T529(ACG)**/ **M908L/T958M**/ L1022(CTA)
SC058	V221/ **T529(ACG)**/ **M908L/T958M**/ L1022(CTA)	V221/ **T529(ACG)**/ **M908L/T958M**/ L1022(CTA)
SC105	V221/ **T529(ACG)**/ **M908L**/T958/ L1022(CTA)	V221/ **T529(ACG)**/ **M908L**/T958/ L1022(CTA)

Codons in bold have mutants genotypes.

After that, the patient was lost to followup, but it is likely that the "accidental" treatment for falciparum malaria successfully treated the recurrence of a CQ-resistant *P. vivax* strain. The other three patients, PC46, SJ4, and SC58, were successfully re-treated with CQ (25 mg over 3 days) plus PQ (0.5 mg/kg daily for 7 days) according to the Peruvian National Malaria Control Program policy with no further reappearance of parasitemia.

It is understood that PQ total dose, more than schedule or duration, is the determinant underlying effective relapse prevention [9, 10], and it is also recognized that the curative action of PQ in eradicating the hypnozoite stage of *P. vivax* is potentiated by concurrent CQ or quinine therapy [11, 12]. Primaquine works synergistically with a variety of drugs [13–16], and the combination of chloroquine plus primaquine was demonstrated to overcome chloroquine resistance in both *P. yoelii* [17] and *P. vivax* [18]. More recently, primaquine is also shown to work synergistically with chloroquine against chloroquine-resistant *P. falciparum* [7, 8].

Acting on numerous cases of *P. vivax* recrudescence and/or relapse by the standard 14-day PQ regimen of 15 mg/day from locations throughout the Old and New World, the US Centers for Disease Control (CDC) recently revised its dosing recommendation for PQ antirelapse therapy and radical cure [12, 19, 20]. Under this new advisement, the adult dose of PQ is increased from 15 to 30 mg daily for 14 days, and the pediatric dose has been comparably increased from 0.25 to 0.5 mg/kg/day [12]. Under the new CDC recommendation, which has not yet been approved by the US Food and Drug Administration (FDA), the four-year-old Peruvian subject, SC105, would have received a total pediatric PQ dose of 119 mg, considered sufficient to eliminate the PQ tolerant Chesson-type forms of *P. vivax* found in Southeast Asia and the Western Pacific [10, 21, 22]. The patient was in the 14-day arm of this study that received 0.25 mg/kg daily of PQ for 14 days, which in this 17 kg child would be a total indicated dose of 59.5 mg PQ. In actuality, this subject received a half tablet (7.5 mg) of PQ daily which amounted to a 14-day total dose of 105 mg. This total dose, although slightly less than the new CDC recommendation, was considerably higher than the total pediatric dose of 59.5 mg achieved under the "old" CDC recommendation. The patient was scheduled to receive 25 mg/kg CQ base over 3 days, which per her weight of 17 kg should have been a total dose of 425 mg CQ base. In actuality, she received a 150 mg base tablet per day, for a total dose of 450 mg CQ base. On the day that her recurrent parasitemia was detected, the level of total CQ in her blood was at least 95 ng/mL and possibly greater than 100 ng/mL, the minimum therapeutic level [2].

The association between mutations in *Pvmdr1* and *Pvcrt* and CQ resistance in *P. vivax* is not the same as the strong association between the orthologous genes in *P. falciparum* and CQ resistance in this organism. No obvious mutations in *Pvmdr1* and *Pvcrt* tentatively linked to chloroquine resistance were found in these samples. Codon 976 in *Pvmdr1*, studied for its feasible association to CQR [5], was wild-type in all eight D-0 and D-R samples. Although we used the *Pvmdr1* and *Pvcrt* gene sequences to distinguish between whether the recurrent strains were the same or different from the original strains, this analysis is limited due to the low level of heterogeneity in their sequences in Peruvian strains. As such, while any difference in sequences of D-0 and D-R represents a clear difference in the two strains, the same sequence detected on D-0 and D-R may simply happen by chance and may not indicate that the two strains are identical.

While this report confirms previously reported low-level CQ resistance by *P. vivax* in the Peruvian Amazon region, it is significant in reporting the first occurrence in Peru of a failure of the standard CQ + PQ combination therapy to achieve radical cure. Importantly, this treatment failure occurred in the context of a clinical trial in which (1) all drugs used were produced under USFDA regulations, (2) dose was carefully determined according to the subject's weight, (3) PQ treatment was administered with food to reduce gastric upset/improve drug absorption, and (4) consumption of each treatment dose of drug was fully supervised and directly observed.

The recurrence of *P. vivax* following combined therapy with chloroquine and high-dose primaquine is unexpected in South America, where *in vivo* testing has demonstrated relatively few valid cases of CQ resistance [3, 23]. Guyana has been the only other nation in South America that has reported failure of CQ-PQ combination therapy, but in multiple adult cases, against both low (15 mg/day) and high (30 mg/day) doses of PQ, and with parasitemias appearing six weeks following treatment [23]. Health providers and travelers should be aware that such difficult cases occur even when there is little evidence of resistance in the region. As in the earlier study of Ruebush et al. [3], it is interesting that all four current cases of CQ failure were in children, less than 16 years old. Three of the patients in the current study and one of the patients in the previous study had suboptimal levels of chloroquine in the blood on the day of failure. It may be

that children fail to absorb the full dose of chloroquine or else eliminate the drug from their systems more rapidly than adults. We did not verify adequate CQ absorption in any of the patients. Only a few studies have looked at CQ absorption in children but never in conjunction with PQ. Future studies should address the absorption of CQ and PQ in children as compared to adults.

Acknowledgments

The authors declare that they have no conflict of interests. They thank Dolores Rimarachin and Gerson Guedes for their expert microscopy. Numerous authors are military service members or employees of the US Government, and this work was prepared as part of their official duties. The views expressed in this paper are those of the authors and do not necessarily reflect the official policy of the Department of the Navy, the Department of Defense, the Department of Health and Human Services, or the US government. The study protocol was approved by the Peruvian National Institute of Health (INS) and the US Naval Medical Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. This paper was presented previously at the 58th Annual Meeting of the American Society of Tropical Medicine and Hygiene in 2009.

References

[1] PAHO, "Report NLM WC 765," Pan American Health Organization, Washington, DC, USA, 2006.

[2] J. K. Baird, "Chloroquine resistance in *Plasmodium vivax*," *Antimicrobial Agents and Chemotherapy*, vol. 48, no. 11, pp. 4075–4083, 2004.

[3] T. K. Ruebush II, J. Zegarra, J. Cairo et al., "Chloroquine-resistant *Plasmodium vivax* malaria in Peru," *American Journal of Tropical Medicine and Hygiene*, vol. 69, no. 5, pp. 548–552, 2003.

[4] G. Snounou, S. Viriyakosol, W. Jarra, S. Thaithong, and K. N. Brown, "Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections," *Molecular and Biochemical Parasitology*, vol. 58, no. 2, pp. 283–292, 1993.

[5] R. Suwanarusk, B. Russell, M. Chavchich et al., "Chloroquine resistant *Plasmodium vivax*: in vitro characterisation and association with molecular polymorphisms," *PLoS One*, vol. 2, no. 10, Article ID e1089, 2007.

[6] M. L. Santolalla et al., "Molecular characterization of polymorphisms in the *Plasmodium vivax* mdr1-like gene (Pvmdr1) from the Amazon Basin and the North Coast of Peru," in *Proceedings of the 57th Annual Meeting of American Society of Tropical Medicine and Hygiene*, p. 301, The American Society of Tropical Medicine and Hygiene, New Orleans, LA, USA, 2008.

[7] P. G. Bray, S. Deed, E. Fox et al., "Primaquine synergises the activity of chloroquine against chloroquine-resistant *P. falciparum*," *Biochemical Pharmacology*, vol. 70, no. 8, pp. 1158–1166, 2005.

[8] T. J. Egan, "Chloroquine and primaquine: combining old drugs as a new weapon against falciparum malaria?" *Trends in Parasitology*, vol. 22, no. 6, pp. 235–237, 2006.

[9] L. H. Schmidt, R. Fradkin, D. Vaughan, and J. Rasco, "Radical cure of infections with *Plasmodium cynomolgi*: a function of total 8-aminoquinoline dose," *American Journal of Tropical Medicine and Hygiene*, vol. 26, no. 6 I, pp. 1116–1128, 1977.

[10] D. F. Clyde and V. C. McCarthy, "Radical cure of Chesson strain vivax malaria in man by 7, not 14, days of treatment with primaquine," *American Journal of Tropical Medicine and Hygiene*, vol. 26, no. 3, pp. 562–563, 1977.

[11] A. S. Alving et al., "Potentiation of the curative action of primaquine in vivax malaria by quinine and chloroquine," *Journal of Laboratory and Clinical Medicine*, vol. 46, no. 2, pp. 301–306, 1955.

[12] D. R. Hill, J. K. Baird, M. E. Parise, L. S. Lewis, E. T. Ryan, and A. J. Magill, "Primaquine: report from CDC expert meeting on malaria chemoprophylaxis I," *American Journal of Tropical Medicine and Hygiene*, vol. 75, no. 3, pp. 402–415, 2006.

[13] S. F. Queener, M. S. Bartlett, J. D. Richardson, M. M. Durkin, M. A. Jay, and J. W. Smith, "Activity of clindamycin with primaquine against *Pneumocystis carinii* in vitro and in vivo," *Antimicrobial Agents and Chemotherapy*, vol. 32, no. 6, pp. 807–813, 1988.

[14] P. Ringwald, E. C. M. Eboumbou, J. Bickii, and L. K. Basco, "In vitro activities of pyronaridine, alone and in combination with other antimalarial drugs, against *Plasmodium falciparum*," *Antimicrobial Agents and Chemotherapy*, vol. 43, no. 6, pp. 1525–1527, 1999.

[15] S. Pukrittayakamee, K. Chotivanich, A. Chantra, R. Clemens, S. Looareesuwan, and N. J. White, "Activities of artesunate and primaquine against asexual- and sexual-stage parasites in falciparum malaria," *Antimicrobial Agents and Chemotherapy*, vol. 48, no. 4, pp. 1329–1334, 2004.

[16] G. W. Soo, J. H. K. Law, E. Kan et al., "Differential effects of ketoconazole and primaquine on the pharmacokinetics and tissue distribution of imatinib in mice," *Anti-Cancer Drugs*, vol. 21, no. 7, pp. 695–703, 2010.

[17] W. Peters, B. L. Robinson, and W. K. Milhous, "The chemotherapy of rodent malaria. LI. Studies on a new 8-aminoquinoline, WR 238,605," *Annals of Tropical Medicine and Parasitology*, vol. 87, no. 6, pp. 547–552, 1993.

[18] J. K. Baird, H. Basri, B. Subianto et al., "Treatment of chloroquine-resistant *Plasmodium vivax* with chloroquine and primaquine or halofantrine," *Journal of Infectious Diseases*, vol. 171, no. 6, pp. 1678–1682, 1995.

[19] D. R. Hill, C. D. Ericsson, R. D. Pearson et al., "The practice of travel medicine: guidelines by the infectious diseases society of America," *Clinical Infectious Diseases*, vol. 43, no. 12, pp. 1499–1539, 2006.

[20] Centers for Disease Control and Prevention, *Health Information for International Travel 2005-2006*, Elsevier, Philadelphia, Pa, USA, 2005.

[21] J. Arnold, A. S. Alving, R. S. Hockwald et al., "The effect of continuous and intermittent primaquine therapy on the relapse rate of chesson strain vivax malaria," *The Journal of Laboratory and Clinical Medicine*, vol. 44, no. 3, pp. 429–438, 1954.

[22] F. C. Ehrman, J. M. Ellis, and M. D. Young, "*Plasmodium vivax* Chesson strain," *Science*, vol. 101, no. 2624, p. 377, 1945.

[23] E. J. Phillips, J. S. Keystone, and K. C. Kain, "Failure of combined chloroquine and high-dose primaquine therapy for *Plasmodium vivax* malaria acquired in Guyana, South America," *Clinical Infectious Diseases*, vol. 23, no. 5, pp. 1171–1175, 1996.