

# Assessment of Therapeutic Response of *Plasmodium vivax* and *Plasmodium falciparum* to Chloroquine in a Malaria Transmission Free Area in Colombia

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*In order to determine the frequency of therapeutic failures to chloroquine (CQ) in patients with malaria due to either Plasmodium falciparum or P. vivax, and to explore the usefulness of a malaria-free city as a sentinel site to monitor the emergence of drug resistance, 53 patients (44 infected with P. vivax and 9 with P. falciparum) were evaluated at the Laboratory of Parasitology, Universidad del Valle in Cali, Colombia. Patients received 25 mg/kg of CQ divided in three doses over 48 h; they were followed during 28 days according to WHO/PAHO protocols. While therapeutic failures to CQ in the P. vivax group were not detected, the proportion of therapeutic failures in the P. falciparum group was high (78%) and consistent with the reports from endemic areas in Colombia. The diverse origin of cases presenting therapeutic failure confirmed that P. falciparum resistant to CQ is widespread in Colombia, and further supports the change in the national antimalarial drug scheme. Monitoring of drug resistance in malaria free areas would be useful to identify sites requiring efficacy evaluation, and in some situations could be the most appropriate alternative to collect information from endemic areas where therapeutic efficacy studies are not feasible.*

Key words: malaria - *Plasmodium falciparum* - *Plasmodium vivax* - chloroquine - Colombia

The incidence of malaria has increased in the last 30 years in Colombia. In the 70's, an average of 281 cases per 100,000 inhabitants was diagnosed with malaria, while by the 90's incidence had increased to 607 cases per 100,000 inhabitants. Both *Plasmodium vivax* and *P. falciparum* are reported in a ratio of 3:2 respectively (Ministerio de Salud 1998).

Traditionally, chloroquine (CQ) was the first line drug for treatment of uncomplicated malaria in Colombia. It has been used at a dosage of 25 mg/kg over 48 h (maximum dosage: 1,500 mg) for both *P. vivax* and *P. falciparum*. For radical cure, in *P. vivax* infections primaquine (15 mg in adults) is added to CQ over 14 days, whereas in *P. falciparum*, CQ has been given simultaneously with a single dosage of sulfadoxine/pyrimethamine. This is followed by primaquine as a gametocytocide (45 mg as a single dose in adults). Due to reports of the presence of widespread and high level (> 25%) CQ-resistant *P. falciparum* in Colombia, in 1999 the Ministry of Health replaced CQ with amodiaquine in the multiple drug combination therapy (Ministerio de Salud 1999).

Therapeutic failures to CQ in patients with uncomplicated *P. falciparum* malaria in Colombia are well docu-

mented in some areas of the Pacific Coast, Amazon region, and in the department of Antioquia (Espinal et al. 1985, Blair 1986, Osorio et al. 1999). On the other hand, the susceptibility of *P. vivax* to CQ in Colombia has not been sufficiently studied. Nevertheless, recent reports of therapeutic failures to CQ in *P. vivax* cases from Brazil, Guyana and Guatemala (Padilla et al. 1998, Alecrim et al. 1999, Baird & Martin 1999) prompt the need to assess and monitor the susceptibility of *P. vivax* to CQ in other countries in the region.

Currently, the Colombian Ministry of Health is developing a surveillance system to monitor the efficacy of antimalarial drugs using a standardized methodology and as sentinel sites some endemic areas. Since the first reports of resistance to antimalarials have been in non-immune travelers, this study explored the potential use of a non-endemic area as a sentinel site to detect the emergence of resistance to antimalarial drugs. We evaluated the therapeutic response to CQ in patients with malaria due to both *P. falciparum* and *P. vivax*.

## PATIENTS AND METHODS

This study was conducted at the Parasitology Laboratory of the Universidad del Valle, in Cali, in the South Western region of Colombia (Figure). Between 600 and 1,400 malaria cases are reported annually in Cali, and more than 90% of them are diagnosed at this laboratory. The ratio of *P. vivax*/*P. falciparum* cases is similar to the rest of the country (3:2). In the city there is no local malaria transmission and most of the malaria cases reported originated from the Pacific Coast and the Amazon region of Colombia.

All patients who attended the Parasitology Laboratory between October 1998 and August 1999 and who

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had a positive thick smear were divided into two groups according to the identified *Plasmodium* species: *P. vivax* and *P. falciparum*. Patients were included in two stages: *P. vivax* group was included from October 1998 to March 1999 and *P. falciparum* group from March 1999 to August 1999. Inclusion and exclusion criteria were identical for both groups. The inclusion criteria were: age between 10 and 64 years old, parasitaemia range from 1,000 to 50,000 parasites/ $\mu$ l, and availability to return to follow-up for up to 28 days. The exclusion criteria were: mixed infections, pregnancy, persistent vomiting of the study drug, any sign of severe malaria and previous diagnosis of a chronic illness such as diabetes or hypertension.

The institutional ethical review committees of CIDEIM and the Universidad del Valle approved this study. All patients participated voluntarily and under informed consent. In the present study, patients with *P. falciparum* malaria were included in 1999 when CQ was still recommended by the Ministry of Health as the first line treatment for uncomplicated *P. falciparum* malaria.

**Protocol** - The standard protocol recommended by PAHO to assess the therapeutic efficacy of antimalarial drugs in areas with low/moderate intensity of malaria transmission was used for *P. falciparum* (PAHO 1998). For *P. vivax*, a protocol that is being developed by PAHO to assess the therapeutic efficacy of CQ to treat *P. vivax* (PAHO 1998) was used. According to these protocols, patients were clinically and parasitologically evaluated on days 0, 1, 2, 3, 7, 14, 21, and 28 for *P. falciparum* and on days 0, 1, 2, 4, 7, 14, 21, and 28 for *P. vivax*.

Treatment responses in *P. falciparum* cases were classified in two ways: (1) parasitological response, graded as RIII, RII, early RI, and late RI/sensitive (WHO 1996), and (2) therapeutic response, according to the following classification: (a) early treatment failure (ETF), defined by any of the followings: (i) development of warning signs or severe malaria on days 1, 2 or 3, in the presence of parasitaemia; (ii) parasite density on day 2 higher than the day 0 count; (iii) parasite density on day 3 > 25% of count on day 0; (b) late treatment failure (LTF), defined by any of the followings: (i) development of warning signs or severe malaria after day 3 in the presence of parasitaemia; (ii) unscheduled return of the patient because of clinical deterioration in the presence of parasitaemia; (iii) presence of parasitaemia on any scheduled follow-up day on or after day 7; (c) adequate clinical response (ACR): did not meet criteria for early or late treatment failure and clearance of parasitaemia after day 3 through day 28.

For *P. vivax*, treatment failure was defined as the presence of any parasitaemia on day 4, or a negative slide on day 4 and a positive result on any subsequent day. Otherwise, treatment response was classified as sensitive.

As stated in the protocols, the sample size was calculated using the Double Lot Quality Assurance Sample Method (Lemeshow & Taber 1991). With an estimated proportion of treatment failures between 25% and 10%, an error of 5% and 80% of power, the minimum number of patients required in the study was 16 and the maximum 42. Adjusting the larger sample size to a 20% loss to follow-up, 50 patients were required in each study group.

Patients received 25 mg/kg CQ (a total of 1,500 mg in adults) over 48 h. Patients were under supervision of study personnel during 1 h after taking CQ. Primaquine was administered after the 28th day or in case of treatment failure. Primaquine treatment in *P. vivax* cases was not supervised. Patients classified as therapeutic failures in the *P. falciparum* group received amodiaquine plus sulphadoxine/pyrimethamine, and primaquine at the standard dosages.

In each follow-up day, two thick smears were taken and stained with 1% Giemsa. Parasite density was calculated by counting the number of parasites (asexual forms) found per 200 leukocytes, and assuming a total of 8,000 leukocytes/ $\mu$ l. A negative result was given after 200 fields were reviewed and no parasites were found. An experienced laboratory technician read the thick smears. A second reader reviewed the entire positive and 10% of the negative slides. And a third reader reviewed discordant results between the first and the second readers (positive/negative or parasite densities with a difference greater than 25%). The final result was either of the first two counts closest to that of the third reader.

Patients who completed the study, and did not take other antimalarial drugs during the follow-up, were included in the analysis. The Student's *t* test was used for comparison of continuous variables, the Chi-square test for the comparison of binary and categorical data, and the Pearson correlation to assess the relationship between the parasite density at enrollment and time to treatment failure.

## RESULTS

A total of 63 patients were included in the study, 50 with *P. vivax* and 13 with *P. falciparum*. Although the calculated sample size was equal for both groups, inclusion in the *P. falciparum* group was stopped due to the high proportion of treatment failures detected. Fifty-three patients completed the study: 44 in the *P. vivax* and 9 in the *P. falciparum* group. Nine patients who were lost to follow up and one who took another antimalarial drug were not included in the analysis. In general, most patients were women more than 15 years old who came from the Pacific Coast region (Figure and Table). The most frequent symptoms were: fever (100%), headache (98%), chills (96%), and bone pain (80%). Thirty-seven patients (70%) had malaria during the 12 previous months. At the time of diagnosis 18 (36%) patients in the *P. vivax* group had not visited an endemic area since the previous malaria episode, suggesting that the present episode was a relapse.

Among the 9 *P. falciparum* patients who completed the study, 7 (78%) (95% CI: 40-97) were classified as therapeutic failures: 5 LTF and 2 ETF. Two patients (22%) were classified as adequate therapeutic response. The two patients classified as ETF did not meet the parasitological criteria for resistance. According to the parasitological response, 5 (55%) (95% CI: 21-86) were CQ resistant: 1 RII, 1 early RI, and 3 late RI. There was an inverse correlation between parasitaemia at enrollment and the day of treatment failure. Patients with higher parasitaemia failed earlier than patients with lower parasitaemia ( $r = -0.78$ ;  $P = 0.035$ ).

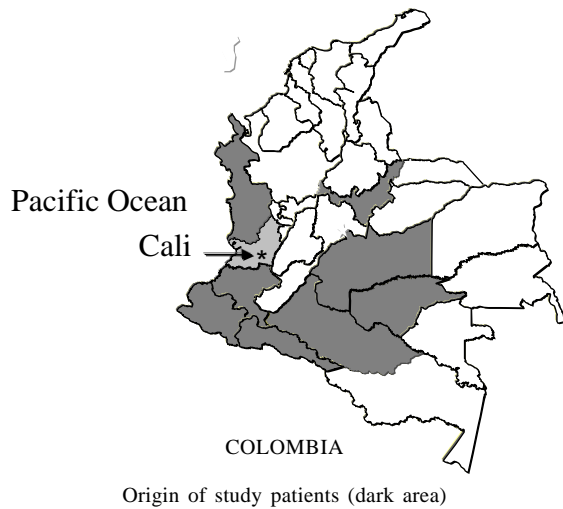


TABLE  
Demographic data and parasitaemia according to *Plasmodium* species

Characteristic	<i>P. falciparum</i> nr = 10 <sup>a</sup>	<i>P. vivax</i> nr = 44
Sex	1	37
Female	9	7
Male	30	28
Age mean(years)	30	28
10-14	0	36
15-44	9	4
45-64	1	4
Origin (regions)		
Pacific Coast	9	39
Amazon Region	1	1
Orinoquia Region		3
Andean Region		1
Geometric mean of parasitemia/ul	5,766	3,802
Range	2,240-23,200	1,000-25,509

a: one patient lost on day 14

All patients infected with *P. vivax* cleared parasites by day 4 and were negative in all subsequent controls. However, five patients returned spontaneously to the clinic with a new *P. vivax* malaria episode within 2 to 6 months after treatment. These cases had not visited an endemic area since the last malaria episode and were classified as relapses.

### DISCUSSION

The World Health Organization is promoting the creation of national and international networks for monitoring antimalarial drug resistance (WHO 2000). The strategy consists of establishing sentinel sites in endemic areas within a country where periodic testing of the efficacy of antimalarial treatment is carried out. In countries with low intensity malaria transmission, the number of sentinel sites to be included in the national network is constrained by the economic and human resources avail-

able but mainly by the availability of sites with a sufficient number of eligible patients. Inclusion of non endemic areas as sentinel sites for monitoring antimalarial drug resistance would help to optimize the resources by signaling sites requiring efficacy evaluation and, in some situations, could be the most adequate alternative to gather information from endemic areas where local studies are not feasible.

In the present study, the standard WHO/PAHO in vivo tests (WHO 1996, PAHO 1998) were used to assess the therapeutic efficacy of CQ to treat both uncomplicated *P. falciparum* and *P. vivax* malaria, and to explore the usefulness of non-endemic areas as sentinel sites. The study population were composed of both "immune" (residents of endemic areas) and non-immune patients (travelers from non endemic areas to endemic areas), coming from different endemic regions (mainly the Pacific Coast) of Colombia. The proportion of therapeutic failures to CQ among *P. falciparum* patients was very high (78%) and consistent with reports from endemic areas (Espinal 1985, Blair 1986, Osorio 1999). Due to the wide distribution of the origin of the cases, rates of drug resistance for any given area can not be drawn from these results. However, they showed that CQ resistance is widespread and further support the change in the national antimalarial drug scheme to treat uncomplicated *P. falciparum* malaria. If in vivo studies in endemic areas had not been conducted, it would have been mandatory to conduct such studies in the areas where the cases originated. Studies in endemic areas are needed to assess whether a change in the local antimalarial drug policy is indicated. However, there would be some areas, such as those burdened with conflict, remote access or where follow-up of patients is not possible and hence standard in vivo tests can not be conducted. In such situations, a study conducted in a non-endemic area that is restricted to travelers to/from the area of interest would be suitable to estimate the level of resistance in the endemic area.

Studies among travelers could overestimate the level of drug resistance in an endemic area. In the present study clinical failures were more common than parasitological failures. Therefore a 100% correlation between therapeutic and parasitological classifications of response to treatment observed in studies in endemic areas was not detected (Osorio 1999). This could be explained by the fact that patients from non-endemic areas may be more susceptible to clinical deterioration with lower parasitaemias than patients from endemic areas who have been exposed to malaria or have had previous subclinical infections. Although further studies are needed to quantify the difference in therapeutic response among travelers to, and residents of endemic areas, we believe that the threshold of 25% failure, that is used to change local antimalarial drug policies, is a reasonable guide for the evaluation of treatment response of patients diagnosed in referral centers in non-endemic locations.

If travelers are more susceptible to treatment failures than residents of endemic areas because they lack acquired immunity, studies in non-endemic areas will be more sensitive. In the present study, treatment failure to CQ among patients infected with *P. vivax* was not detected.

We used the threshold of 25% to study resistance to CQ *P. vivax* since this is the level of resistance that has been established by WHO as a limit for modifying the antimalarial drug policy for *P. falciparum* (WHO 1996). Based on the lot quality assurance sampling method recommended by the PAHO protocol (PAHO 1998) and according to the results the true prevalence expected of treatment failure in patients attending the parasitological laboratory in Cali is less than 10%. Therefore, the presence of a low level of CQ resistance in *P. vivax* in the region can not be ruled out. Future studies would require calculation of the sample size based on low levels of expected resistance (less than 25%) to be able to detect treatment failures if it exists.

Periodic in vivo testing is not the only method that can be used to monitor antimalarial drug resistance in non-endemic areas. Passive detection of treatment failures (by advising to return if symptoms persist or reappear) or routinely examining of slides after treatment (day 4 or 7) could also be applicable according to the circumstances. Since these methods do not ensure full compliance with the medication and many patients do not return, they can not be used to quantify the efficacy of the antimalarial regimen. Nevertheless, when treatment failure is suspected they would help to determine that a standard in vivo study is needed and/or to signal endemic areas where further studies will be required. The finding that five patients participating in the study spontaneously returned with a new episode of *P. vivax* infection classified as a relapse demonstrates the feasibility of passive detection, although treatment with primaquine was not supervised and follow up was not done in all patients. The high proportion of relapse (36%) among patients at enrollment into the study prompt for an evaluation of the radical therapy with primaquine considering compliance with treatment as well as a possible failure of primaquine therapy.

Passive case detection and periodic in vivo tests are not mutually exclusive and both methods can be used in non-endemic areas to monitor the emergence and spread of drug resistance. Additionally, the feasibility to follow patients with low or no acquired immunity to malaria up to 28 days or more without the risk of re-infection is an advantage for early detection of emerging drug resistance. Consequently, we consider that non-endemic areas could be included as sentinel sites in the national network for monitoring antimalarial drug resistance in Colombia.

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