Chloroquine–Primaquine Therapeutic Efficacy, Safety, and Plasma Levels in Patients with Uncomplicated *Plasmodium vivax* Malaria in a Colombian Pacific Region

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Abstract. In Colombia, published studies for the treatment of uncomplicated *Plasmodium vivax* malaria with chloroquine–primaquine (CQ-PQ) are scarce. The aim of the study was to evaluate the therapeutic efficacy and safety profile of this combination. A clinical trial was performed in adults with uncomplicated *P. vivax* malaria using the 28-day World Health Organization validated protocol. Patients received supervised antimalarial treatment and the primary efficacy end point was the clinical and parasitological response. Safety was assessed through adverse events surveillance, and plasma levels of antimalarial drugs were measured. A total of 77 patients were included. Adequate clinical and parasitological response rate diagnosed by thick blood smear examination was achieved in 72 of 73 patients (98.6%) with a complete 28-day follow-up. There were two parasitological therapeutic failures (TFs) (2.9%) on day 28, established by polymerase chain reaction among 68 patients, one of them with a positive film. No adverse events were detected. After completing the antimalarial treatment, all patients reached adequate plasma concentrations of CQ and desethyl-chloroquine (DECQ), with medians of 302.9 and 104.0 ng/mL, respectively. Uncomplicated *P. vivax* malaria treatment with CQ–PQ standard treatment was effective and safe in the study population; TFs were not associated with low plasma levels of CQ and DECQ.

INTRODUCTION

Malaria is widespread in tropical areas worldwide and has a high morbidity burden. Some challenges impeding countries' ability to advance toward elimination include the lack of sustainable funding, conflict in malaria-endemic zones, mosquito resistance to insecticides, and the emergence of parasite resistance to antimalarial drugs.[1]

Approximately 216 million malaria cases were reported globally in 2016 by the World Health Organization (WHO) and the disease was considered endemic in 91 countries and territories. *Plasmodium vivax* was the predominant parasite in America, representing 64% of malaria cases; *Plasmodium vivax* malaria represented more than 30% of cases in Southeast Asia and 40% in the Eastern Mediterranean region.[1] In that year, *Plasmodium falciparum* and *P. vivax* were the most prevalent species in Colombia, totaling 57,515 cases confirmed with microscopy and 5,655 with rapid diagnostic tests, and 36 reported deaths.[1] In the recent years, malaria transmission has been focalized in the Chocó Department.

In 2017, a total of 52,957 infections were reported in Colombia and vivax malaria cases accounted for 42.3% of them, a consistent pattern seen from year 2014 and on.[2] National data about the infections before 2014 showed a higher incidence of *P. vivax* over *P. falciparum* infections from years 2000 to 2013.[3]

The chloroquine–primaquine (CQ–PQ) protocol, based on individual tablets of CQ and PQ, has been the standard first-line treatment for *P. vivax* infections nationally since 1946 because of the synergistic mechanisms of both drugs,[4,5] in accordance with WHO recommendations.[6] Chloroquine acts as a blood schizonticide for erythrocytic stages of plasmodium parasites and PQ is used as a tissue schizonticide for hypnozoites. It is expected that together they prevent recurrences due to recrudescence and true relapses.

Therapeutic efficacy studies are required to evaluate drug efficacy in vivo. Studies conducted at regular intervals in repeated locations, based on therapeutic outcomes (e.g., therapeutic failure [TF]), allow for the early detection of possible antimalarial drug resistance, with additional tests necessary to confirm its occurrence such as polymerase chain reaction (PCR) molecular analysis, in vitro assays, and drug concentration measurements. Presently, WHO defines treatment failure as "the inability to clear parasites from a patient's blood or to prevent their recrudescence after the administration of an antimalarial,"[7] an assessment performed by means of clinical evaluation and microscopy, from which resistance can only be suggested. On the other hand, resistance is defined as "the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject,"[7] with established minimum inhibitory concentrations (MICs) as the main reference points for in vivo studies determining parasite response to measured antimalarial drug blood levels. A clear distinction between the two is necessary because of the implications for public health.

Therapeutic failure with CQ in *P. vivax* infections has been reported around the globe for many years, suggesting the emergence of resistance in various regions. The first reports of TF with CQ treatment were published in Myanmar in 1989, with subsequent reports of the same nature in other Asian countries.[8–12] In South America, there are only a few studies on the matter. In the early 2000s, publications reported Adequate Clinical and Parasitological Response (ACPR) to CQ in malaria vivax, with no recurrent parasitemia within the next 28 days after receiving treatment[13–15] alongside others with clinical treatment failures suggesting CQ resistance.[16–19] More recent studies show the presence of *P. vivax* isolates harboring CQ-resistant mutations in French Guiana and Brazil.[20,21]

Reported cases of TF with CQ alone in South America suggest that it is necessary to monitor the clinical and parasitological response to the drug because of a possible unrecognized emergence of resistant *P. vivax* strains in

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Colombia, although the extent of the threat to national malaria control efforts remains to be determined. In Colombia, the initial studies between 1998 and 2011 found a 100% ACPR to CQ alone in patients with malaria vivax monoinfection[22–26]; one study reported an 11% rate of TFs in 2001, defined by the authors as "clinical drug resistance"[27]; and one study addressed *P. vivax* in vitro susceptibility to various drug regimens between 2010 and 2012.[28] For the last 10 years, no national data were found on *P. vivax* response to standard treatment in relation to blood drug levels; there is a lack of information on the topic.

The studies conducted in other countries from 2008 to 2015 evaluated the standard treatment of CQ + PQ (28 or 42 follow-up days), reporting a 100% ACPR[29–32]; studies in 2003, 2009, and 2012 reported TF rates around 1%.[33–35] In Colombia, studies have shown no TF with this combination.[36–38]

The general objective of the study was to assess the therapeutic efficacy and safety of CQ combined with PQ for the treatment of uncomplicated patients with vivax malaria in a Colombian region and to measure the plasma level of CQ and its metabolite desethylchloroquine (DECQ). The aim was to contribute to the monitoring of the response to antimalarials and to formulate recommendations that enable the Ministry of Health to update current national antimalarial treatment guidelines.

PATIENTS AND METHODS

Study design and site. This was a therapeutic efficacy study with a single-arm prospective evaluation of the clinical and parasitological response to supervised treatment for uncomplicated *P. vivax* malaria. It was based on the Pan-American Health Organization (PAHO)/Red Amazónica de Vigilancia de Resistencia a Drogas Antimaláricas (RAVREDA) "Protocolo genérico para estudios in vivo de eficacia de los medicamentos antimaláricos en las Américas" protocol[39] and the "Guía práctica revisada para estudios de eficacia de los medicamentos antimaláricos en las Américas" guideline.[40]

The study was conducted in 2012–2013 in the municipalities of Quibdó (lat. 05°41′41″N; long. 76°39′40″W) and Tadó (lat. 05°15′48″N; long. 76°33′36″W), located at the Chocó Department. Quibdó is located on the Atrato River, with nearly 100,000 inhabitants, 35% living in rural locations. Tadó is located on the San Juan River, with approximately 20,000 inhabitants, 39% living in rural locations.[41] Malaria is endemic in the department, with a 2013 Annual Parasitic Index of 28.4 and 13,095 reported cases in 2013,[42] 4,232 of them in the municipality of Quibdó[43] and 1,814 in Tadó.[44]

Study population and inclusion criteria. Patients with uncomplicated vivax malaria seek care at the malaria diagnostic posts (outpatient service) in the Ismael Roldán Hospital in Quibdó and the San José Hospital in Tadó. Patients were eligible if they were diagnosed with *P. vivax* monoinfection, were aged 5–65 years, had parasitemia of at least 1,000 asexual parasites/μL (at least one parasite for every eight leukocytes), and had signs and symptoms of acute febrile disease with axillary temperature $\geq 37.5°C$, in the absence of another cause of fever. Exclusion criteria comprised danger signs, severe malnutrition, known underlying chronic or severe diseases (cardiac, renal, hepatic diseases, and human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), confirmed pregnancy, hypersensitivity to the medications tested, concomitant acute illness, and use of other antimalarial or antibiotic medication.

Sample size. The minimum sample size was established in 73 subjects, with an additional 20% to account for losses to follow-up, an expected 5% proportion of treatment failures, a level of confidence of 95%, and a 5% precision.

Procedures. After obtaining informed written consent, complete medical history (symptoms, current medications, and previous use of antimalarial drugs) and biographic and contact details were noted. A complete physical examination was performed at the baseline and on days 1, 2, 3, 7, 14, 21, and 28 and a case record form was filled in for each patient. Body weight was recorded on day 0; temperature was measured using a thermometer with a precision of 0.1°C at baseline and on follow-up days, and additionally as clinically indicated. On each follow-up, clinical signs and symptoms were recorded, blood smears were performed to detect malaria parasites, and filter paper spot samples were taken for molecular diagnosis by PCR.

Microscopic blood examination and quality control. Thick and thin blood films for parasite count were obtained and examined at screening to confirm inclusion/exclusion criteria and labeled anonymously. Subsequent films were obtained on days 1, 2, 3, 7, 14, 21, and 28, and on other days if the patient spontaneously returned and parasitological reassessment was required. Blood smears of enrolled patients were examined by two different microscopists. If the difference in the quantification of parasitemia between the first two readings varied by more than 50% or species diagnosis was controversial, an additional reading was performed by a third microscopist. Each reader was blinded to the results of the others.

Molecular diagnosis by PCR. For molecular diagnosis, two to three drops of blood were collected on Whatman-3 filter paper at enrollment to confirm the diagnosis on day 0 and again on day 28 to detect parasitemia or to confirm complete clearance.

Samples were individually stored in a plastic bag and sent to the Malaria Group Laboratory at the University of Antioquia. The PCR diagnosis procedure is as follows: The filter paper was added with 0.5% saponin, washed three times with 1× phosphate buffered saline, and Chelex 100 was added; it was denatured at 56°C for 15 minutes and 100°C for 10 minutes; finally, the supernatant was recovered after a 15-minute centrifugation at 13,000 rpm. Genotyping was performed using a PCR protocol developed by Singh et al.[45] by a first amplification reaction with primers rPLU1 and rPLU5 for the fragment of the 18s-rRNA ribosomal subunit of the *Plasmodium* genus parasites. This PCR product was used for the second reaction (nested PCR) with primers rVIV 1 and rVIV 2 for the identification of *P. vivax* and rFAL1 and rFAL 2 for the detection of *P. falciparum*.

Antimalarial treatment. All enrolled patients received supervised treatment with CQ + PQ in accordance with WHO protocols. Patients were monitored for 28 days and given weight-adjusted doses of CQ once daily over 3 days: total dose of 25 mg/kg (10 mg the first day and 7.5 mg the next 2 days). Primaquine was administered during 14 days (0.25 mg/kg once a day), beginning on day 0. After supervised administration of the drug, patients were observed during 30 minutes for vomiting or adverse events. Patients who vomited within this period were then provided with another dose of the study drugs and were observed for 30 additional minutes. If a second vomiting episode occurred, the patient was excluded from the study and offered parenteral rescue therapy.

Concomitant treatment with acetaminophen was permitted to patients presenting axillary temperature ≥ 38°C.

For first-line therapy failures, patients received artemether–lumefantrine (AL) plus PQ. Artemether–lumefantrine was given twice daily over 3 days. Individual doses were calculated at 1.7/12/0.25 mg/kg of body weight for AL and PQ, respectively, following current national recommendations. In cases of persisting vomiting or complicated malaria, patients would have received parenteral sodium artesunate (2.4 mg/kg at 0, 12 and 24 hours, and then once a day for 3 days) followed by a complete AL treatment.

Antimalarial drugs concentration. Quantification of analytes was carried out in plasma from 5 mL of venous blood, obtained 1 hour after last CQ dose on day 2 and on day 28, to determine CQ and DECQ concentrations.[39]

Specimens were labeled anonymously (study number, day of follow-up, and date). The separation of CQ and DECQ was carried out by liquid chromatography using an Agilent C18 5μ chromatographic column, 250 × 4.6 mm, and a mobile phase composed of MeOH: tryethylamine 0.4% /Buffer: dibasic sodium phosphate 1.4 g/L and pH adjustment at three with phosphoric acid at a constant flow rate of 1 mL/minute and an injection volume of 20 μL, with programming by gradient.

The detection of the analytes was performed with a diode array detector, monitoring the wavelength of 331 nm for CQ and DECQ, whereby the CQ was detected at a time of retention of 13 minutes and DECQ of 9.3 minutes. Liquid–liquid extraction was used to obtain the analytes from plasma. The normality of the data was calculated statistically from the calibration curves, which showed not to have a normal distribution by means of the Shapiro–Wilk normality test with $P = 0.585$ for CQ and $P = 0.597$ for DECQ. This methodology was verified by determining the parameters of precision, accuracy, recovery, and linearity because it was a new adaptation of the method previously used by Zuluaga and others.[46,47]

Follow-up and loss. Follow-up visits and procedures were scheduled as per protocol on days 1, 2, 3, 7, 14, 21, and 28. Patients were instructed to return to the health center at any time if they had fever or any general danger sign as described under the exclusion criteria. When clinically indicated, patients were evaluated out of schedule and treated appropriately. The study team made home visits as follow-ups for study participants who were late for their scheduled visits. Patients who failed to return on day 1 or 2, or missed one dose of the treatment, were withdrawn from the study definitively. After day 3, patients who failed to return on day 7 but were present on day 6 or 8 (likewise on days 13/15, 20/22, and 27/29) could still be included in the study group.

Outcomes. Treatment outcomes were assessed on the basis of parasitological and clinical results and were classified according to the WHO protocol as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), or ACPR[48]:

1. Early treatment failure: Development of danger signs or severe malaria on day 1, day 2, or day 3 in the presence of parasitemia
   - Parasitemia on day 2 higher than day 0 count irrespective of axillary temperature
   - Parasitemia on day 3 with axillary temperature ≥ 37.5°C
   - Parasitemia on day 3 ≥ 25% of count on day 0
2. Late clinical failure

   - Development of danger signs or severe malaria on any day from day 4 to day 28 in the presence of parasitemia, without previously meeting any of the criteria of ETF
   - Presence of parasitemia and axillary temperature ≥ 37.5°C (or history of fever) on any day from day 4 to day 28, without previously meeting any of the criteria of ETF
3. Late parasitological failure: Presence of parasitemia on any day from day 7 to day 28 and axillary temperature < 37.5°C, without previously meeting any of the criteria of ETF or LCF
4. Adequate clinical and parasitological response: Absence of parasitemia on day 28 irrespective of axillary temperature, without previously meeting any of the criteria of ETF or LCF or LPF

Statistical analysis. IBM's statistical software SPSS (24th version, International Business Machines Corp., New York, NY) was used for data management and analysis. Data were analyzed using two methods: the Kaplan–Meier analysis and the per protocol analysis.

Ethical considerations. The trial was conducted according to good clinical practice guidelines and the international ethical standards for biomedical research with human subjects established by the WHO, and the norms of the Ministry of Social Protection of Colombia (Resolution 8430 of 1993) were followed; the Ethical Committee of the Faculty of Medicine of the University of Antioquia approved the study protocol (Act 006, 2012). Written consent was obtained from all adult patients and from the parents or guardians of the children who participated in the study. Children older than 12 years of age signed an informed assent form. The principal investigators had no affiliation with any of the malaria diagnostic centers where the study was conducted.

RESULTS

Baseline characteristics of participants. During the study period, 80 patients were screened, 77 continued on day 21, and 73 completed the study on day 28; three were removed from the study because of protocol violation and four were lost to follow-up. Demographic and clinical baseline data of the participants are summarized in Table 1. Other symptoms not shown in Table 1 included vomiting in 16 patients (20.8%), coughing in 10 (10.4%), diarrhea in six (7.8%), exanthema in three (3.9%), sore throat in two (2.6%), and dyspnea in one (1.3%).

The frequency of symptoms did not differ significantly between the patients who completed the study and those lost to follow-up (chi^2 test: $P > 0.05$), except for vomiting, diarrhea, sore throat, and dyspnea that were absent in the latter. Parasitemia did not present a statistically significant difference between both groups (Mann–Whitney U-test, $P > 0.05$).

Efficacy results. One patient was withdrawn from the study because of incorrect diagnosis at admission which was established by PCR after day 28. The treatment outcomes in 73 patients who completed the follow-up are summarized in Table 2. On day 1, microscopic parasitemia was identified in 70 patients; the average parasite load was 941 parasites/μL (SD = 1,519; median = 556). On day 2, the average in 18 patients was 154 parasites/μL (SD = 150; median = 119). On day 3, four patients remained positive, all with parasite counts of 40/μL. Adequate clinical and parasitological response, defined by a negative thick blood smear on day 28, was found in 72 cases.

TABLE 1
Demographic and clinical characteristics of 77 follow-up patients

		n	%
Age, years: mean (SD) median	25.8 (14.4) 22.0		
Gender: male		40	51.9
Ethnicity:			
African descent		48	62.3
Mestizo		22	28.6
Native American		7	9.1
Malaria: number of episodes in the previous year			
0		22	28.6
1		22	28.6
2–4		28	36.4
> 4		5	6.4
Current malaria episode:			
Evolution time, days: mean (SD) median	4.7 (2.9) 4.0		
Parasitemia day 0, asexual forms/µL: mean (SD) median	8,726 (8,818) 5,504		
Symptoms during current malaria episode			
Fever		77	100
Cephalea		77	100
Shivers		77	100
Diaphoresis		77	100
Musculoskeletal pain		53	68.8
Nausea		36	46.8
Asthenia		33	42.9
Abdominal pain		30	39
Anorexia		29	37.7

One patient presented LPF diagnosed by thick blood smear and by PCR: a 12-year-old male patient, resident in Bosque Latina in the municipality of Quibdó, with no history of malaria during the previous year. With an initial parasitemia of 8,078 parasites/µL and a negative thick blood smear on day 2, on day 28, he presented 880 parasites/µL of *P. vivax*. Plasma drug concentrations showed values as follows: day 2 CQ 324.4 ng/mL, DECQ 102.8 ng/mL; day 28 CQ 83.1 ng/mL, DECQ 28: 63.7 ng/mL.

Another patient (16-year-old male, resident in Caraño Piñal) had a positive PCR for *P. vivax* on day 28. With an initial parasitemia of 18,376 parasites/µL and a negative thick blood smear on days 2 and 28, plasma drug concentrations showed values as follows: day 2: CQ: 258 ng/mL, DECQ 85.1 ng/mL; day 28: CQ 52.2 with undetectable DECQ.

PCR analysis on day 28 was performed on 68 samples, with two positive results for *P. vivax*, accounting for 2.9% of the analyzed samples. The PCR-corrected per-protocol analysis showed an ACPR of 97.1%. Parasitic genetic comparisons between day 0 and 28 were not performed. The median of the parasitemia on the initial day did not differ statistically ($P > 0.05$; Mann–Whitney U-test) between patients with ACPR (median = 6,117) and those considered with parasitological

TABLE 2
Treatment outcomes on day 28 in 73 patients

	Thick blood film		PCR	
Treatment outcomes	n	%	n	%
Adequate clinical and parasitological response	72	98.6	66	97.0
Early treatment failure	0	–	0	–
Late clinical failure	0	–	0	–
Late parasitological failure	1	1.4	2	3.0

failure using PCR, with parasite counts of 8,078 and 18,376/µL (median = 13,227).

Safety of antimalarials. No serious adverse events were identified during the follow-up in relation to treatment and no cases of complicated malaria occurred.

Antimalarial drugs blood concentration. Plasma concentrations are presented in Table 3. The amount of blood was not sufficient to perform the measurements in all patients; however, data were obtained for the two analytes in 58 participants (58/77; 75.3%) on day 2. The median values of CQ on day 2 among patients with a negative PCR on day 28 and those with a positive test were 302.9 ng/mL ($C_{min} = 131$; $C_{max} = 730$) and 291.2 ng/mL ($C_{min} = 258$; $C_{max} = 324$), respectively. The median values of DECQ on day 2 among patients with a negative PCR on day 28 and those with a positive test were 105.4 ng/mL ($C_{min} = 39$; $C_{max} = 366$) and 94.0 ng/mL ($C_{min} = 85$; $C_{max} = 103$), respectively. No significant differences were found between these values (Mann–Whitney U-test; $P > 0.05$).

DISCUSSION

Current treatment for malaria vivax with CQ is believed to have remained effective in Colombia for the last 72 years. In general, there is little evidence about emergent resistance of *P. vivax* to antimalarials because of the difficulty of carrying out in vitro tests with this plasmodium. Efficacy studies, carried in vivo, are scarce, although there are reports of TFs in other regions of the world such as in Southeast Asia and Africa. In our country, data are limited, despite *P. vivax* being historically most prevalent in national registered malaria cases up to year 2013.

The interest of this study was to evaluate recurrences during the first month after the beginning of treatment, which were expected to correspond to recrudescences. Although PQ was given to patients, evaluating the frequency of relapses (expected to occur after the first month) was not the purpose of this study. It has been found that the combination of PQ–CQ compared with CQ alone reduced early recurrences before day 42 by 90%, probably explained by the prevention of early relapses; PQ possibly contributes to reducing recrudescence through its blood schizonticidal activity.[49]

Because of difficulties in carrying out in vitro studies with *P. vivax* to test resistance, in vivo efficacy studies offer an acceptable alternative to approach this matter, requiring continuous surveillance through therapeutic outcomes because various reports suggest *P. vivax* emerging CQ resistance around the globe.

This study identified a low frequency of TF to CQ–PQ combination (1.4%) which correlates to a study conducted with children aged 4–10 years in the region of Urabá, which found a 2.6% TF rate to CQ. The other efficacy studies of CQ monotherapy in the country have shown a 100%

TABLE 3
Plasma concentration of antimalarials on days 2 and 28

Analyte (ng/mL)	n	Mean (95% CI)	Median	Lower–upper
CQ day 2	58	325.2 (290.2–360.1)	302.9	131.2–730.3
CQ day 28	46	78.2 (64.7–91.7)	70.3	34.6–262.7
DECQ day 2	58	111 (96.2–125.7)	104	39.2–366.4
DECQ day 28	38	48.3 (42.1–54.6)	44.9	29.3–125.7

CQ = chloroquine; DECQ = desethylchloroquine; CI = confidence interval.

efficacy.[23–26,36–38] A study published in 2001[27] showed TF in three of 27 (11%) patients in the regions of Urabá and Llanos Orientales; this high proportion of TF could be due to a lack of application of the WHO standardized protocol with little clarity on treatment supervision. It also described a case with uncleared parasitemia during follow-up that was not confirmed by a molecular method (a rapid test was used), which could correspond to a *P. falciparum* infection.

When therapeutic efficacy was analyzed with PCR-diagnosed *P. vivax* infections, we found a 2.9% rate of TF corresponding to two patients with positive results on day 28. Parasite genotyping was not performed in this study and it was not possible to exclude reinfections or relapses as the cause of these cases.

It is clear that CQ given at the doses used in this study remains within therapeutic concentrations for up to 1 month,[50] theoretically guaranteeing complete clearance of sensible plasmodia in erythrocytic stage. This study established adequate CQ and DECQ plasma concentrations on day 2 in 58 patients. On day 28, CQ and DECQ levels were measured for 46 and 38 patients, respectively. Two patients presented TF diagnosed by PCR, both within CQ therapeutic range (CQ + DECQ $\geq$ 100 ng/mL),[50] similar to results in other locations.[18,51,52] Therapeutic failures in the presence of CQ levels above its MICs, are very suggestive of a case of CQ-resistant *P. vivax*.

Limitations in this study include plasma-level measurement on day 7 and molecular parasite comparison between days 0 and 28. The lack of previous data regarding plasma drug levels and CQ treatment efficacy in the country and specifically in the analyzed communities hinders the ability to compare evolution of decreased drug efficacy, with the implied incapacity to timely shift drug regimens and avoid an increase in disease burden. In addition, measuring the CQ and DCQ on day 7 of the study and the day of the TF is recommended to allow for comparison with other studies. There is also need for parasite genotyping as an integral part of efficacy studies to precisely distinguish TF cases from newly acquired infections and to allow a more accurate understanding of the transmission dynamics and prevalence within communities, especially in those with high endemicity where the disease poses greater challenges. Serial studies should be carried out within world and national malaria elimination initiatives to monitor changes in epidemiology and to identify possible CQ-resistant strains of the parasite, particularly in sites with already-identified cases, a real risk since reports of near-complete CQ TF for *P. vivax* have already been found in other countries.

CONCLUSION

This report of a low CQ–PQ TF rate for uncomplicated *P. vivax* infection in the presence of adequate plasma drug concentrations suggests CQ resistance of this parasite in Colombia. The results show that the combination of CQ–PQ retains high efficacy for uncomplicated vivax malaria in the region. There is a patent need for active surveillance and further studies determining antimalarial drugs resistance in the country.

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