Which diagnostic test to use for Testing and Treatment strategies in *Plasmodium vivax* low-transmission settings: a secondary analysis of a longitudinal interventional study

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Summary

Background The lack of sensitive field tests to diagnose blood stages and hypnozoite carriers prevents Testing and Treatment (TAT) strategies to achieve *Plasmodium vivax* elimination in low-transmission settings, but recent advances in Polymerase Chain Reaction (PCR) and serology position them as promising tools. This study describes a PCR-based TAT strategy (PCRTAT) implemented in Saint Georges (SGO), French Guiana, and explores alternative strategies (seroTAT and seroPCRTAT) to diagnose and treat *P. vivax* carriers.

Methods The PALUSTOP cohort study implemented in SGO (September 2017 to December 2018) screened participants for *P. vivax* using PCR tests and treated positive cases. Serology was also performed. Passive detection of *P. vivax* infection occurred during follow-up. Participants were categorised into overlapping treatment groups based on 2017 PCR and serological results. Strategies were described in terms of participants targeted or missed, primaquine contraindications (pregnancy, G6PD severe or intermediate deficiency), and sociodemographic characteristics.

Findings In 2017, 1567 inhabitants were included, aged 0–92 years. A total of 90 (6%) were *P. vivax* carriers and 390 seropositive (25%). PCRTAT missed 282 seropositive individuals while seroTAT would have missed 21 PCR-positive cases. Primaquine contraindications ranged from 12% to 17% across strategies.

Interpretation Serology and PCR are promising tools for targeted treatment strategies in *P. vivax* low-transmission settings, when field compatible sensitive tests will be available. Both seem necessary to capture blood stages and potential hypnozoite carriers, while avoiding mass treatment. However, high primaquine contraindications rates need consideration for successful elimination.

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Disclaimer: This summary is available in French in the Supplementary Material.

Research in context

Evidence before this study

The lack of sensitive field-deployable tests and their inability to detect hypnozoites prevents the WHO from recommending Testing and Treatment (TAT) strategies for *Plasmodium vivax* elimination in low transmission settings. However, recent developments of serology tools capable of detecting recent *P. vivax* infections and promising advances in their field-applicability and the field-applicability of PCR tools, make the use of these tests conceivable for future implementation of these strategies to achieve elimination. Therefore, we searched PubMed for modelling data and existing real-life data on the implementation of TAT strategies using serology and/or PCR. We used the terms "Test and treat" OR "Screening and treatment", "vivax", "PCR" OR "serology" without date limits. We also read the WHO guidelines for malaria released in March 2023 and their cited references. While we found modelling data on the assessment of potential PCRTAT or seroTAT, no real-life data on implementation were available. Assumptions were made for those studies regarding primaquine eligibility rate and primaquine adherence. No study was found comparing the choice of using PCR versus serology versus both, to implement TAT strategies. Having conducted our own TAT strategy based on PCR with serology collection, we present data to contribute to the understanding of the on-the-ground implications of choosing among the different diagnostic tools available.

Added value of this study

This study is the first to provide data on what the implementation of a TAT strategy based on serology, PCR, or both would entail. It finds that these two tools complement each other in that each does not detect all cases detected by the other. Additionally, our study provides information on the relevance of using serology. Finally, our study provides real-life data on a high primaquine contraindication rate and emphasises the need to consider it when designing these interventions.

Implications of all the available evidence

The available data underscore the need to assess the real-world implementation and effectiveness of TAT interventions based on serology and/or PCR, and their comparison, in *P. vivax* low-transmission settings. Our data provide crucial information for forthcoming research aimed at selecting diagnostic tools for implementing this type of strategy. These results will be important for shaping future malaria elimination strategies in those areas. Additionally, our findings, along with existing literature, highlight the significant obstacle posed by contraindications to the standard primaquine regimen for the implementation of these strategies. Substantial efforts to improve the delivery, safe administration, and adherence to this treatment remain critical for achieving successful elimination.

Introduction

Plasmodium vivax accounts for approximately half of all malaria cases outside Africa.[1] These infections are responsible for a significant health and economic burden on affected populations.[2,3] While *P. vivax* is less lethal than *Plasmodium falciparum*, it causes substantial morbidity, particularly in children, where it can lead to severe malaria, severe anaemia, impaired cognitive development, and foetal growth disorders.[2,4] A notable aspect of *P. vivax* infections is its specific relapse mechanism as *P. vivax* forms dormant liver stages known as hypnozoites, which can persist for months and periodically trigger recurrent blood-stage malaria episodes if left untreated.[3,5] This relapse mechanism has been shown to account for 50–96% of infections in endemic areas.[5,6] While a specific treatment (primaquine) is required to clear hypnozoites, the medication has several limitations. It cannot be administered during pregnancy and breastfeeding, to infants under six months of age, and daily primaquine regimens cannot be used safely in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, due to the risk of haemolytic anaemia.[1] Additionally, the fight against *P. vivax* is complicated by factors such as transmission before symptom onset (early circulation of gametocytes in the blood during the infection), a high percentage of asymptomatic cases, the limited sensitivity of rapid diagnostic tests (RDTs), and the diverse behaviour of *Anopheles* vectors.[7–9] These unique characteristics make standard malaria control tools less effective against *P. vivax*. Additionally, there is currently no available vaccine against *P. vivax*.[10,11] Specific intervention strategies are therefore needed to reach elimination.

The latest recommendations from the World Health Organization (WHO) have highlighted various tools for *P. vivax* control, including Mass Drug Administration (MDA) for both blood-stage and hypnozoite cure, Targeted Drug Administration (TDA), and Testing and Treatment (TAT). MDA for reducing *P. vivax* transmission is recommended with very low certainty, based on a systematic review that found a potential short-term impact of blood-stage targeted drugs on *P. vivax* transmission (without hypnozoiticidal drug).[1,12] Regarding Mass Relapse Prevention (MRP), which involves mass treatment with hypnozoiticidal drug alone, WHO has issued a conditional recommendation against it, with very low certainty evidence due to the risk of severe harm in case of G6PD deficiency.[1] The only exception is for small focal outbreaks in areas with very low G6PD

deficiency rates and when combined with blood-stage treatment.[1] However, this option is also costly and not well accepted by populations.[10] While WHO does not recommend TAT, it acknowledges that it might be appropriate in transmission foci with very low transmission levels when MDA is not ethically acceptable or feasible. The only study that investigated the effect of TAT in a low-transmission setting with *P. vivax* did not find any impact, but it relied on microscopic diagnostics. When compared with Polymerase Chain Reaction (PCR) results, microscopy-based diagnosis missed 72% of blood-stage infections.[9]

Current recommendations are based on available data and expert discussions, but additional data are still required to further assess novel malaria control strategies that target both blood-stage parasites and hypnozoites to prevent relapses and facilitate elimination. To implement these strategies, the primary challenge lies in the absence of diagnostics tools to identify hypnozoites accurately. Administering primaquine treatment without detecting hypnozoite raises concerns due to the risk of severe side effects in individuals with G6PD deficiency. The second challenge is the lack of a rapid diagnostic test with enough sensitivity to allow for the detection of asymptomatic *P. vivax* carriers and interrupt transmission. Recent alternative strategies include the use of PCR or serology to conduct TAT with hypnozoiticidal drugs in low transmission settings.[8,13,14] PCR appears promising to increase the sensitivity of blood-stage infection detection but misses hypnozoites (PCRTAT).[13] Serological tools have been developed to diagnose blood-stage *P. vivax* infection in the previous 9 months, which could detect individuals who may have developed hypnozoites during their last infection and could experience relapses.[11] Therefore, using serology to target people to treat (seroTAT) could detect recent infections but might miss some early blood-stage infections. While mathematical modelling has indicated that seroTAT strategies would be effective, their feasibility and effectiveness in real life have not been studied yet.[8,14] An unexplored option is the use of a dual PCR and serology strategy (seroPCRTAT) to combine benefits from both methods. The main limitations of those new strategies are the high cost of those diagnostic tools, the need for a laboratory with technological expertise, and the lack of point-of-care tests for easy field implementation. However, advances in biological tools may make their use feasible in the near future, underscoring the need for additional data on their relevance.

This study aims to describe a real-life PCR TAT implemented in an Amazonian *P. vivax* low transmission setting and look at the feasibility and implications of theoretical alternative strategies that could have been implemented (TAT using RDT, serology, or dual PCR-serology) in a specific malaria transmission context.

Methods

Study area

French Guiana is a low malaria transmission region, with an incidence rate of 55 per 100,000 inhabitants in 2020.[15] The majority of cases are due to *P. vivax* (97%), and are predominantly located in specific areas in the forest and on the border with Brazil along the Oyapock river.[15,16] Nearly half of the *P. vivax* cases were previously reported to be caused by hypnozoites in patients who had not benefited from radical cure using primaquine.[5] The main vector is *Anopheles darlingi*, with a highly adaptable behaviour for egg-laying and biting, contributing to the persistence of malaria in the area.[16] In Saint Georges de l'Oyapock (SGO), along the Oyapock River, after a substantial decrease in the number of cases from 2007 to 2016, epidemics occurred in 2017 and 2018, with mainly *P. vivax* autochthonous cases (263 cases of *P. vivax* and 7 cases of *P. falciparum* reported in 2017, and 135 cases of *P. vivax* reported in 2018).[15,17]

SGO is a small town (4245 inhabitants) divided into 12 central neighbourhoods and three remote hamlets located in the forest along the river accessible only by boat. The neighbourhoods are very diverse in population and housing conditions. SGO is heavily tied to Oiapoque, its counterpart on the Brazilian bank of the Oyapock River, which is linked to gold mining activities, serving as a path or support for gold miners.[18] Slash and burn farming, fishing, and hunting are the other main activities and resources in the area.

Study design

Our study is a secondary analysis of a longitudinal interventional study, called PALUSTOP. PALUSTOP was implemented among a cohort of 1567 individuals from September to December 2017 (first survey) with a one-year follow-up from September to December 2018 (second survey) to assess the effectiveness of a mass testing and treatment (TAT) strategy based on PCR in SGO.[17] A total of 12 out of the 15 neighbourhoods of SGO were selected based on the high malaria incidence recorded by the regional surveillance system in 2015 and 2016.

During the first survey, a target of 1500 individuals (children and adults) from these 12 neighbourhoods were invited to participate. Those who accepted to be enrolled provided consent. All enrolled participants were tested by RDT, PCR, serology and G6PD activity, and responded to a questionnaire. Blood samples and temperature were collected by nurses and the questionnaire was administered by trained community health workers (CHW) to decrease the risk of misunderstanding (cultural and language barriers). The CHWs went door-to-door to invite participants to visit the temporary neighbourhood sites that were set up specifically for the study.

All individuals with a positive result from RDT or PCR received treatment following the national and international guidelines, regardless if they were symptomatic or

not.[17,19] *P. falciparum* infections were treated with artemether-lumefantrine and *P. vivax* infections with chloroquine for 3 days (total of 25 mg/kg) followed by 14 days of primaquine (15 mg/day for adults or 0.25mg/kg/day for children) in the absence of medical contraindications.[19] Serology testing was conducted for research purposes only, thus, seropositive participants did not receive any treatment.

Regarding the participants' follow-up, all individuals included in the first survey were invited to participate in the second survey. The same data were collected with additional data on their malaria history since the first survey. They were also tested by RDT, PCR, and serology and treated if positive in RDT or PCR tests.

Passive surveillance was also implemented in the only healthcare center of the area to document clinical malaria cases diagnosed by RDT and thick blood smears occurring among study participants before and in between the two surveys.

Additional data on the PALUSTOP study implementation and the PALUSTOP study population has been previously published.[17,20] The PALUSTOP protocol has been registered at the French National Agency for the Safety of Medicines and Health Products (Agence nationale de sécurité du médicament et des produits de santé ID RCB N° 2015-A00928-41) (Supplementary Material).

For our observational ancillary study, we focused on the cohort's diagnostic tests results, exposures and sociodemographic characteristics at inclusion, as well as the malaria episodes occurring during the follow-up, without considering the result of the intervention itself.

Variables and data sources

Malaria status definition

P. vivax carriers refer to all participants diagnosed by PCR (*P. vivax* only) during the first survey (2017) whatever their RDT or serological result. PCR detection and identification of four *Plasmodium* species which can infect humans (*P. falciparum, P. vivax, P. ovale,* and *P. malariae*) was performed using a real-time approach previously published.[21] The sensitivity was set at 0·25 parasites/µL for *P. vivax*.

Regarding the serology, participants were split into three groups depending on their serological status during the first survey: seropositive, seronegative, or no data. Serology was implemented using a multiplex Luminex Magpix platform to measure antibodies against eight *P. vivax* proteins that allows for the identification of individuals who were infected by *P. vivax* in the 9 months prior to blood sampling. Measured IgG antibody responses were analysed using a Random Forests classification algorithm for the assignment of seropositivity status. Further details are provided in a previously published study.[22] For the current study, we retained the serological classification output obtained with the algorithm parameters set with a specificity of 90% and sensitivity of 63%. These diagnostic performance parameters were deliberately selected to prioritize high specificity over sensitivity, to reduce the number of individuals who would receive primaquine treatment in the context of a SeroTAT intervention when they are not hypnozoite carriers (overtreatment).

Finally, the RDT test used was the SD BIOLINE® Malaria Ag Pf/Pan test (pfHRP2/pLDH-based, Standard Diagnostics), commonly used in French Guiana.

G6PD deficiency characterisation

G6PD deficiency was assessed at the time of enrolment using a colorimetric method (Cerba®). G6PD deficiency status was defined as normal, intermediate, or severe G6PD deficiency following the WHO definition (severe below 30% of the adjusted male median G6PD, intermediate between 30% and 80% for female, normal above 30% for male and 80% for female).[23]

Primaquine contraindication

In the PALUSTOP study, primaquine was given to *P. vivax* carriers who were not pregnant, not breastfeeding and did not have severe or intermediate G6PD deficiency. This decision was made considering the benefit-risk balance for this population in our local context (asymptomatic cases in remote area).

For our analysis, primaquine contraindication group will refer to all individuals with either pregnancy or G6PD severe or intermediate deficiency, as no data was collected regarding breastfeeding except for the PCR-positive and treated individuals.

Sociodemographic and exposure variables

Sociodemographic data (age, sex, occupation, years spent at SGO and nationality) and exposure variables (slash and burn farming, fishing, hunting, visits to high-risk area, and visiting gold mining sites) were self-reported by the participants when answering the survey questionnaire administered by CHWs and used to describe the participants. Variables were chosen based on the suspected exposures linked to *Plasmodium* spp. carriage in the area.[17,18] The high-risk area mentioned in this data were the indigenous Oiapoque territories located on the Brazilian side of the border, where a significant number of SGO inhabitants regularly visit during holidays and where an epidemic occurred during the same period, potentially linked to the SGO epidemic.[17,18]

Potential treatment groups upon alternative campaign strategy

To compare the PCRTAT strategy implemented in PALUSTOP with other theoretical TAT strategies, we defined three potential strategies based on RDT, PCR and/or serological results:

1. RDTTAT, targeting all the participants with a positive RDT, whatever their serological or PCR result.

2. seroTAT, targeting all the participants with positive serology for *P. vivax*, whatever their RDT or PCR result.
3. seroPCRTAT, targeting all the participants with a PCR and/or a serology positive *for P. vivax*, whatever their RDT result.

For each strategy, participants were split into potential primaquine targeted group or not, based on their RDT, PCR and serological results and the presence or absence of primaquine contraindication (Fig. 1, Supplementary Table S1).

For the seroPCRTAT strategy, the potential primaquine target group was split into the PCRTAT treated group (with positive PCR, whatever their serological result, named *PQ-PCRTAT*) and potential additional seroPCRTAT treated group (PCR negative seropositive, named *Additional PQ-seroPCRTAT*). All the seronegative participants with PCR negative were grouped and named *No PQ-seroPCRTAT*.

P. vivax infection and carriage in the year following the 2017 assessment

P. vivax infection in the year following the 2017 baseline assessment was defined as a malaria infection between the first and the second survey reported by the participants during the second survey (*P. vivax* or undetermined, without information on the diagnostic tool used) and/or malaria infection detected at SGO healthcare centre between the first and the second survey (*P. vivax* or undetermined detected by RDT and thick blood smears, at least 7 days after the PALUSTOP first survey).

P. vivax carriage at one year was defined as *P. vivax* diagnosed by PCR during the second survey (from September 2018 to December 2018).

P. vivax infection in the year before enrolment

P. vivax infection in the year before the 2017 test was defined as a malaria infection in 2017 reported by the participants during the first survey and/or malaria infection detected at SGO healthcare centre in the 9 months preceding their inclusion in the first survey (*P. vivax* or undetermined detected by RDT and thick blood smears).

Statistical analysis

We compared socio-demographic and exposure variables between potential treatment groups for alternative campaign strategies using frequencies and percentages for categorical variables, and medians and interquartile range for quantitative variables.

The socio-demographic and exposures of the PCRTAT-treated group and potential additional seroPCRTAT treated or untreated groups were also compared using Chi-2 test (or Fisher's exact test) for qualitative characteristics and Student's t-test (or Mann–Whitney test) for quantitative measures.

All the missing data (whether due to loss to follow-up or otherwise) were retained in the description to provide a comprehensive overview of the feasibility and implications of conducting these strategies in real life.

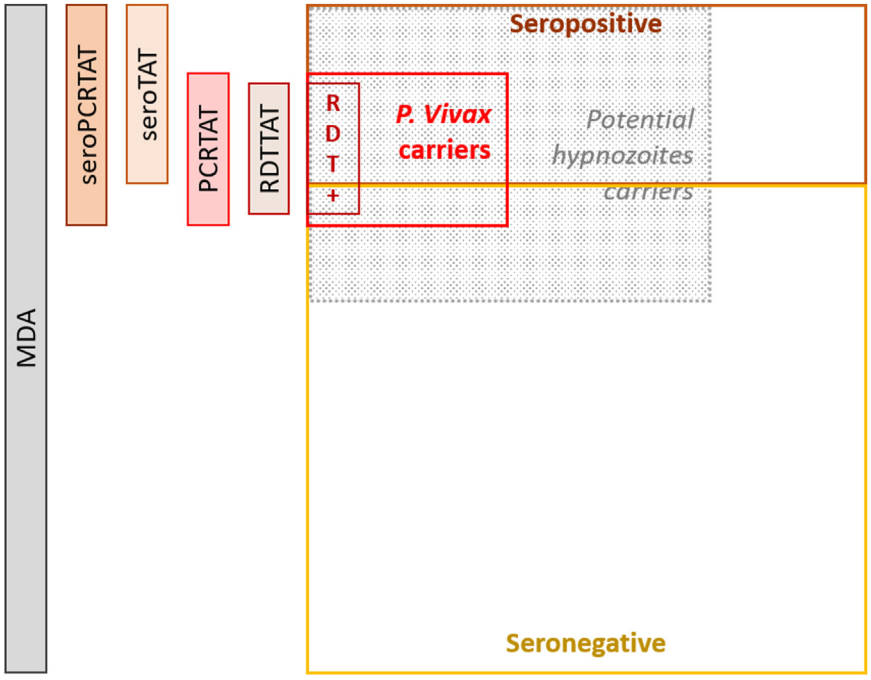

Fig. 1: Potential treatment groups upon alternative campaign strategy depending malaria status.

The statistical analyses were performed using R software version 4.1.2. (Copyright© 2022, R Foundation for Statistical Computing). Figures were formatted and treated using Microsoft PowerPoint.

Ethics approval and consent to participate

The study was approved by the Comité de Protection des Personnes du Sud-Ouest et Outre-Mer 4 N° AM-36/1/CPP15-024. Prior to the study, written informed consent was obtained from all participants. Formal written consent was obtained from the parent/guardian for child participants. The database of the PALUSTOP prospective study was anonymised and declared to the French regulatory commission (Commission Nationale Informatique et Libertés, CNIL, n°917186). The passive follow-up database was anonymised and declared to the CNIL (authorization N°2135463). Samples collected by the National Reference Centre were registered by the French Ministry for Research (declaration number DC-2010-1223; collection Nu2).

Role of the funding source

The PALUSTOP study was funded by European Funds for Regional Development, Synergie funding contract N° GY0012082, the French Guiana Regional Health Agency, the Pan American Health Organization, WHO, and the French Ministry for Research. The funders had no role in study design, data collection and analysis, interpretation, decision to publish, or in the writing of the manuscript.

Results

Population description

A total of 1567 participants were included in the study enrollment in 2017. The median age of the 1567 participants was 18 years (Interquartile range, IQR [7; 35]) in 2017, with participants ranging from 0 to 92 years old; 53% of participants were female (Table 1). The main occupations of adult participants were unemployed (38%), informal work (21%), and food-producing activities (22%). Among participants, 57% were French and 43% Brazilian, with large variation according to age groups.

	Variable	N = 1567[a]
Sociodemographic characteristics	Male	731 (47%)
	Age in 2017	18 (7, 35)
	Years spent in Saint Georges (*NA = 1*)	9 (3, 17)
	Occupation (*NA = 1*)	
	Preschool	149 (10%)
	Student	572 (37%)
	Unemployed	333 (21%)
	Fixed job	93 (6%)
	Food producing activity	182 (12%)
	Retired	36 (2%)
	Informal job	201 (13%)
	Nationality (*NA = 1*)	
	French	888 (57%)
	Brazilian	668 (43%)
	Other	10 (1%)
Exposures	Slash and burn farming (*NA = 1*)	729 (47%)
	Hunting (*NA = 1*)	253 (16%)
	Fishing (*NA = 1*)	439 (28%)
	Visiting gold mining sites (*NA = 1*)	53 (3%)
	Visits to high-risk area (*NA = 1*)	228 (15%)

NA: not available (missing data). [a]N (%), Median (IQR).

Table 1: **Sociodemographic characteristics of the study population.**

PCRTAT strategy implementation

Among the 1567 participants, 6% were *Plasmodium* spp. carriers (n = 100, with 90 PCR positive for *P. vivax* and 10 for *P. falciparum*) while 4% had no PCR result (n = 61, mainly due to refusal of blood test). Among the PCR positive, 71% were asymptomatic carriers, meaning no fever or history of fever in the past 48 h.

All *Plasmodium* spp. carriers received anti blood-stage treatment. A total of 79 participants (88%) were treated with primaquine.

A total of 289 participants (18%) had missing data regarding potential clinical infection in the following year while 335 participants (21%) had missing data regarding potential *P. vivax* carriage at one year (Fig. 2). Among those participants with missing data, 290 were lost to follow-up between the two surveys (around 57% of them moved out while 7% declined to participate in the second survey). The remaining missing data was due to missing PCR results or missing questionnaires (Fig. 2). The 335 participants lost to follow-up or without PCR results in 2018 (second survey) had similar sociodemographic profiles than the others but were less exposed to malaria contamination risk factors (farming, vector density around the house) (Supplementary Table S2).

Among the 1567 participants, 4.0% had *P. vivax* clinical infection in the following year (n = 62), while 1·9% were carrying *P. vivax* in the second survey in 2018 (n = 30). Among the 62 participants with a *P. vivax* clinical infection in the following year, 74% (n = 46) were diagnosed at SGO healthcare center, and 26% (n = 16) were reported by the participants.

Potential treatment groups upon alternative campaign strategy

Among the 1567 participants of the first survey, 25% of the participants were seropositive (n = 390), 67% were seronegative (n = 1055) and 8% had no serological result (n = 122). Regarding the RDT results, only 1% were

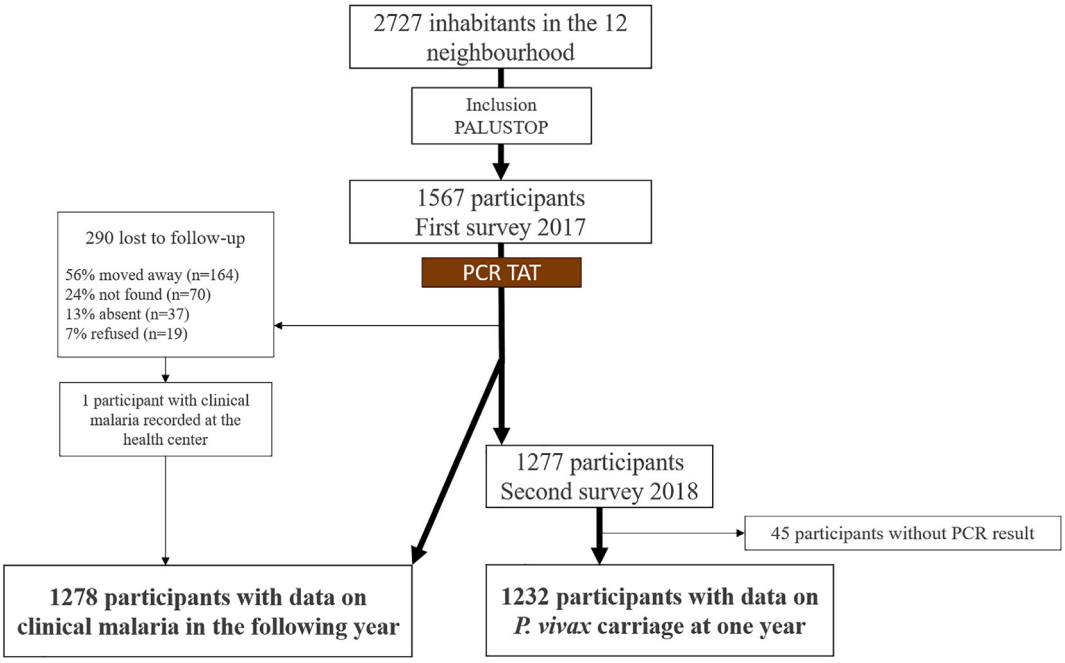

Fig. 2: Flowchart of the study.

found to be *Plasmodium* spp. carriers (n = 13, with 12 *Plasmodium* spp. and one *P. falciparum*), 97% had negative RDT (n = 1527), and 2% had no RDT result (not interpretable results or test not done) (n = 27).

Looking at the consistency of the biological results, 74% of the 90 participants with PCR positive for *P. vivax* had a positive serology (n = 67) (Fig. 3). Among the *Plasmodium* spp. positive RDT (n = 12), eight were seropositive and PCR positive, two were seropositive but PCR negative, one was PCR positive but seronegative, and one was seronegative and PCR negative (Supplementary Table S3).

Regarding the different potential treatment groups upon alternative campaign strategies, 90 participants

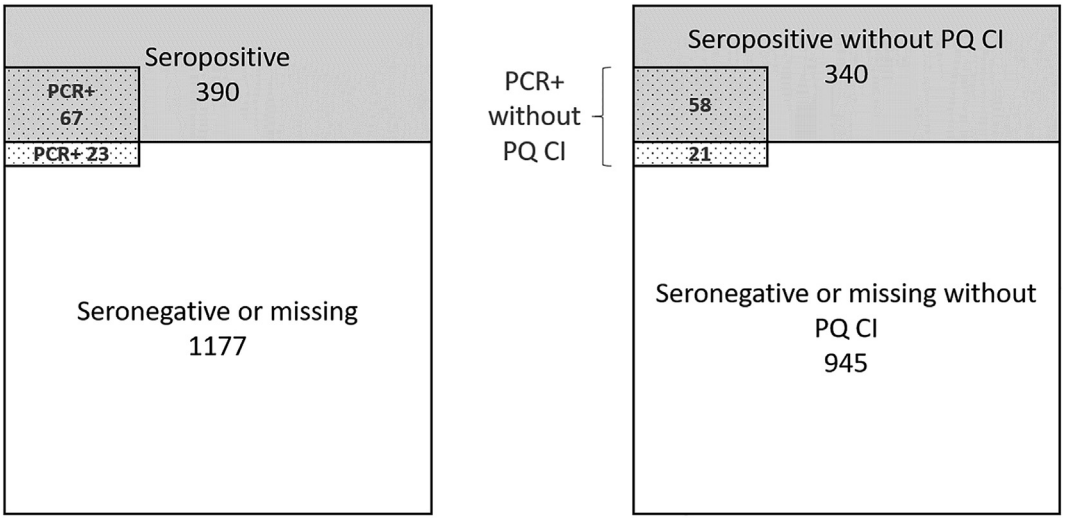

Fig. 3: Consistency of biological results: repartition of participants RDT, PCR, and serological results and number of participants with primaquine contraindication (PQ CI) shown for each group. Interpretation: 90 participants were PCR positive: 67 seropositive and 23 seronegative or with missing serological results. Of the 90 PCR positive, 11 had contraindications to primaquine (12%). 390 participants were seropositive whatever their PCR result. Among all seropositive, whatever their PCR result, 50 had contraindications to primaquine (13%).

have been targeted for blood-stage treatment in PALUSTOP. This would have been 12 participants in the case of RDTTAT, 390 in the case of seroTAT, and 413 in the case of seroPCRTAT. Excluding the primaquine contraindication group, the number of participants targeted for primaquine would have been 10, 340, and 361 participants for RDTTAT, seroTAT, and seroPCRTAT, respectively (Table 2). PCRTAT have missed 282 seropositive individuals who could have been targeted for primaquine while seroTAT would have missed 21 PCR-positive individuals. RDTTAT would have missed 72 PCR-positive individuals and 332 seropositive individuals (Fig. 3).

Among the 340 participants that could have been targeted in the case of seroTAT, 27% (n = 93) might have already been treated by primaquine. Indeed, 70 participants were diagnosed with malaria in SGO healthcare center in the past 9 months (*P. vivax* or undetermined), and 23 had no information in SGO healthcare center but self-reported a malaria infection (*P. vivax* or undetermined) in 2017.

Looking at the different potential treatment groups upon alternative strategies, the participants targeted in PCRTAT seemed younger than those targeted in seroTAT but older than those targeted in RDTTAT. The participants targeted by each of the strategies seemed to have more risk factors compared to the whole population, with a higher proportion of individuals presenting each risk factor for individuals targeted by PCRTAT than those targeted for seroTAT (refer to descriptive data in Supplementary Table S4).

Relevance of treating seropositive in addition to PCR-positive

A total of 79 *P. vivax* carriers were treated by primaquine in the PALUSTOP PCRTAT approach (*PQ-PCRTAT*), while 282 additional participants could have been additionally treated by primaquine if a seroPCRTAT was implemented (*Additional PQ-seroPCRTAT*) (Table 3, Supplementary Table S5). Those 282 participants were older (median age 29 years old, IQR [16; 46]) than both the *PQ-PCRTAT* (median age 23 years old, IQR [12; 36]) and *No PQ-seroPCRTAT* (median age 13 years old, IQR [6; 31]) ($p < 0.001$). They were also more often Brazilian (50% versus 38–40% in the two other groups, $p = 0.027$). Regarding exposures at risk of malaria, the *Additional PQ-seroPCRTAT* group had increased exposure compared to the *No PQ-seroPCRTAT* for all the risk factors previously identified: outdoor activities (farming, fishing, hunting) and visits to high-risk area (Table 3). Among those 282 participants, 23% (n = 64) were aware that they had recently experienced an infection and might have already been treated by primaquine.

Looking at malaria history after the first survey, 18% of the participants in *PQ-PCRTAT* had clinical malaria in the following year (n = 14), while it was 9% of the *Additional PQ-seroPCRTAT* (n = 25) and 2% of the *No PQ-seroPCRTAT* (n = 19) (Table 4). *P. vivax* carriage in the second survey represented 10% of the participants in *PQ-PCRTAT* (n = 8), 4% of the *Additional PQ-seroPCRTAT* (n = 10), and 1% of the *No PQ-seroPCRTAT* (n = 8).

Among the 57 participants who received primaquine in PALUSTOP PCRTAT and for whom no clinical malaria was recorded in the following year, nor was *P. vivax* carriage detected at one year, only 42 would have been targeted in seroTAT. This means that the remaining 16 would not have received primaquine and might have developed relapses. On the other hand, in the PCRTAT, 32 participants who were not targeted in PCRTAT but who would have been targeted in seroTAT developed malaria in the following year (Table 4).

Discussion

In the real-life implementation of PCRTAT, 79 participants received primaquine out of the 90 *P. vivax* carriers. By not utilising serology, we missed 282 additional seropositive participants who could have been treated (23% were aware that they had recently experienced an infection). Conversely, in the case of seroTAT, 21 participants with positive PCR would have been missed. The number of *P. vivax* carriers missed by serology can be partially explained by the 63% sensitivity of the serological assay, which was chosen to favour the specificity to detect recent infections in the past 9 months

Strategy	PCRTAT[a]	seroTAT[a]	RDTTAT[a]	seroPCRTAT[a]
Participants targeted (n)	90	390	12	413
Primaquine contraindication (n (%))	11 (12%)	50 (13%)	2 (17%)	52 (13%)
Pregnancy	5	9	1	10
G6PD severe deficiency	1	4	0	5
G6PD intermediate deficiency	6	38	1	38
Missing G6PD	0	2	0	2
Participants targeted with primaquine among eligible (n (%))	79 (88%)	340 (87%)	10 (83%)	361 (87%)
Participants targeted with primaquine among total (n (%))	79 (5%)	340 (22%)	10 (1%)	361 (23%)

[a]Testing and Treatment strategy based on PCR (PCRTAT), serology (seroTAT), rapid diagnostic test (RDTTAT), or serology and PCR (seroPCRTAT).

Table 2: **Number of participants targeted for blood-stage treatment and primaquine in potential treatment group of each TAT strategy.**

	PQ treated in PALUSTOP[a] (n = 79)	Additional PQ treated if seroPCRTAT[a] (n = 282)	Not targeted if seroPCRTAT[a] (n = 1154)	p-value[b]
Male	44 (56%)	165 (59%)	516 (45%)	<0.001
Age in 2017	23 (12, 36)	29 (16, 46)	13 (6, 31)	<0.001
Nationality				
French	49 (62%)	139 (49%)	682 (59%)	0.027
Brazilian	30 (38%)	142 (50%)	462 (40%)	
Other	0	1 (0·4%)	9 (0·8%)	
			NA = 1	
Slash and burn farming	61 (77%)	166 (59%)	475 (41%)	<0.001
			NA = 1	
Hunting	26 (33%)	96 (34%)	127 (11%)	<0.001
			NA = 1	
Fishing	42 (53%)	128 (45%)	257 (22%)	<0.001
			NA = 1	
Visiting gold mining sites	7 (9%)	19 (7%)	26 (2%)	<0.001
			NA = 1	
Visits to high-risk area	29 (37%)	59 (21%)	131 (11%)	<0.001
			NA = 1	

PQ: Primaquine; seroPCRTAT: Testing and Treatment strategy based on serology and PCR screening; NA: not available (missing data). [a]N (%), Median (IQR). [b]Pearson's Chi-squared test, Kruskal–Wallis rank sum test, Fisher's exact test.

Table 3: **Description of the different treatment groups.**

(90%). Moreover, serology might miss some early blood-stage infections diagnosed by PCR. However, opting for an algorithmic with greater sensitivity would increase the proportion of PCR-positive individuals detected by serology, but with a trade-off of a greater degree of overtreatment. This highlights the challenge of striking the right balance in implementing real-life TAT strategies to effectively progress towards *P. vivax* elimination.

Additionally, we observed an elevated risk of clinical malaria infection in the following year among both the *PQ-PCRTAT* and the *Additional PQ-seroPCRTAT* groups compared to the *No PQ-seroPCRTAT* group. While our results do not allow for an estimate of the effectiveness of a strategy solely based on serology or a combined approach using both serology and PCR, they do suggest that the *Additional PQ-seroPCRTAT* group, similarly to PCR-positive individuals, is more susceptible to infection. Our findings do not allow us to conclusively determine whether this increased risk is primarily due to relapse or re-infection, because of the epidemic context in SGO at that time. The finding of higher rates of clinical *P. vivax* malaria in the primaquine-treated group suggests that there is substantial heterogeneity—individuals are PCR-positive because they are at high risk of exposure to infectious mosquitoes and when they are treated with primaquine they are likely to be reinfected by mosquitoes, even if they have been effectively treated. Nevertheless, this result should be interpreted with

PCR status	Serological status	Treatment group	Clinical malaria in the following year and/or *P. vivax* carriage at one year	Clinical malaria in the following year	*P. vivax* carriage at one year
PCR positive without PQ CI	Seronegative or no data (n = 20)	*PQ treated in PALUSTOP PCRTAT (PQ-PCRTAT)*	5 (25%)	3 (15%)	2 (11%) *NA = 1*
	Seropositive without PQ CI (n = 59)		17 (29%)	11 (19%)	6 (11%) *NA = 5*
PCR negative or no data	Seronegative or no data (n = 1154)	*Not targeted if seroPCRTAT (No PQ-seroPCRTAT)*	25 (2%)	19 (2%)	8 (1%) *NA = 261*
	Seropositive without PQ CI (n = 282)	*Additional PQ treated if seroPCRTAT (Additional PQ-seroPCRTAT)*	32 (11%)	25 (9%)	10 (4%) *NA = 58*
p-value (Fisher's exact test)			<0.001	<0.001	<0.001

PQ CI: Primaquine Contra-indication; Treatment group: Testing and Treatment based on PCR (PCRTAT), or Serology and PCR (seroPCRTAT) screening.

Table 4: **Clinical malaria and/or *P. vivax* carriage in the following year (health centre data and PCR status at one year) depending PCR and serological status in the first survey.**

caution, as the primaquine delivery system in French Guiana can lead to significant delays in primaquine administration, potentially allowing for relapse to occur before treatment is received. Moreover, it is well known that patient adherence to the 14-day primaquine regimen can be challenging.[24] In our study, all patients received the first dose of primaquine at home by the study team, but no follow-up was conducted to ensure that patients diligently followed the 14-day regimen.

To our knowledge, there is no existing literature comparing PCRTAT and seroTAT strategies, and TAT strategies have not been extensively studied in *P. vivax* low-transmission settings.[1] Regarding the assessment of PCRTAT, we found only one study that used modelling to predict its limited effectiveness for *P. vivax* because it does not target the blood-stage PCR-negative population carrying hypnozoites.[13] In the case of seroTAT, while no real-life seroTAT study has been published, serological testing has been utilised in combination with other criteria, such as cases in neighbouring areas, to target focal MDA programmes for the elimination of *P. vivax* in China.[25] The other published seroTAT studies used modelling to compare TAT strategies with microscopy or RDT diagnostic tools versus serological tools.[8,14] These studies found that seroTAT was more effective than microscopy or RDTTAT strategies, and slightly less effective than MDA. Notably, the model was based on the development of serological markers capable of detecting recent infections with a sensitivity and specificity of 80%.[26] Finally, we did not find any published study evaluating a dual seroPCRTAT strategy. While PCRTAT allows for identifying most of the individuals carrying blood-stage *P. vivax*, seroPCRTAT (considering both PCR and serological results) could allow for the identification of individuals carrying blood-stage *P. vivax* as well as potential hypnozoite carriers and prevent relapse for individuals with high-risk behaviour, expanding the identification of a group that would benefit from treatment. Regarding RDTTAT strategies, the very low number of positive RDT results among the PCR-positive participants in our study reinforces the well-known low sensitivity of RDT for *P. vivax*, particularly for active case detection which includes a large part of asymptomatic cases. This limitation makes available RDTs unsuitable as a stand-alone diagnostic tool for TAT interventions in *P. vivax* low transmission areas, despite their ease of use.[27]

In addition to TAT strategies, serology has also been proposed in reactive drug administration and targeted interventions for specific groups.[8] These additional interventions are recommended by the WHO in low-transmission settings to achieve malaria elimination.[1] Those interventions could also benefit from PCR and serology to test and target individuals to treat, reducing the use of mass treatment strategies. Moreover, the relationship we found between serology and at-risk behaviours for malaria infection suggests its usefulness as a surrogate marker for exposure. Defining eligibility for targeted preventive treatment intervention based on a biological criterion could facilitate population screening, thereby avoiding potential stigma and misclassification that may influence participants' self-reporting of potentially sensitive information, especially when exposure likely results from illegal activities.

Feasibility of implementing TAT strategies in real-life

Safe and effective implementation

In *P. vivax* low transmission settings, the primary concern raised in the literature is the risk of haemolysis when administering primaquine to individuals with G6PD deficiency. In French Guiana, this risk is mitigated by the mandatory requirement for G6PD testing before primaquine administration. The development of rapid diagnostic tests for G6PD will enable quicker and cost-effective identification of individuals with contraindications.[10,28] By conducting G6PD deficiency screenings for all study participants, PALUSTOP underscores another viable solution applicable to specific geographic areas.

Another concern highlighted in the literature regarding preventive drug administration pertains to the extent of intervention coverage and population adherence.[1,8] In the PALUSTOP study, a 100% treatment coverage for *P. vivax* carriers without primaquine contraindications was guaranteed by the presence of a dedicated team of CHWs, nurses, and doctors.

While new drugs targeting hypnozoites, such as Tafenoquine, will probably increase individual compliance, there is no suitable treatment regimen for patients with G6PD deficiency and it is not available in France. For both safety and population adherence, TAT strategies offer a compelling compromise compared to MDA, maintaining efficacy while considering the risk/benefit balance of preventive drug administration interventions.

Field tests availability

One of the remaining challenges in effectively implementing TAT interventions is the feasibility of conducting serology and PCR in the field and remote areas. For some areas, these limitations can be overcome with laboratory tests, as demonstrated in our study. However, the human and logistical resources required make this challenging without dedicated funding and long-term logistical support.

Field-compatible, sensitive tests will be essential to expand the potential use of these interventions to remote areas where they are most needed.[8] A rapid diagnostic test based on serology is currently under development using different antibodies that have proven effective as indicators of *P. vivax* infection in the preceding 9–12 months.[29] Regarding the efficacy of current *P. vivax* serology, the available serological markers should only be used in low-transmission settings, as they have lower specificity for detecting recent

infections in areas with higher transmission.[26] Field-compatible PCR tests are also under development,[30] which could facilitate the implementation of PCRTAT strategy and enable seroPCRTAT strategies. Also, the cost of these tools must be kept under control to allow for everyday practice implementation. The sensitivity of those tests will also be crucial for their use in TAT strategies because the individuals missed by testing might contribute to the continuous spread of malaria and halt the elimination strategy. Those factors will need to be considered and assessed in cost-effectiveness analyses when data on the effectiveness of those strategies become available.

Campaign target

PALUSTOP PCRTAT intervention was implemented during the day, posing challenges in recruiting individuals occupied with work, farming, or fishing. In addition, the mobility of the local population across the border for several days makes it impossible to include specific individuals, despite their potentially higher risk of malaria.

Not all of the *P. vivax* carriers in PALUSTOP PCRTAT study received primaquine as 12% had contraindications. If we had targeted both *P. vivax* PCR- and seropositive individuals, 13% would have had hypnozoiticidal drug contraindications (n = 52, 43 G6PD severe or intermediate deficiencies and 10 pregnancies). These percentages were expected in our context, considering the demographics of the SGO population, which includes a younger demographic individual (increased pregnancy and breastfeeding rate), as well as a mix of populations of various ethnicities (increased risk of G6PD deficiency). Additionally, the number of participants with primaquine contraindications increased, as we chose to exclude individuals with intermediate G6PD deficiency. This decision was made due to the perceived elevated risk associated with G6PD deficiency, considering that serology serves just as a proxy for potential hypnozoite carriage. Specific primaquine protocols for individuals with G6PD severe or intermediate deficiency exist but they are challenging to implement due to the longer treatment times and the need for intense monitoring.

It is crucial to consider the intervention target and local primaquine contraindications rate when implementing TAT strategies, as individuals who will not get the treatment could potentially contribute to disease transmission and reduce the strategy's overall effectiveness. Modelling studies should incorporate this rate into the calculation of the theoretical effectiveness of TAT strategies, as well as in research on the implementation plan for TAT (period, frequency, etc.).

Limitations

This study presents an exploratory analysis of possible TAT strategies that were not pre-planned when the PCRTAT strategy was implemented. This design has several limitations, including the risk of false positive associations due to the increased number of unplanned statistical tests and the lack of outcome data (e.g., malaria episodes) or consideration of confounding factors (e.g., exposure). Consequently, we chose to present mainly descriptive data, which could be useful for the upcoming discussion about TAT strategies, rather than conducting further explanatory analyses that could be biased by the design.

Regarding the lack of outcome and confounding factors data, a major limitation of our study is the absence of tangible information regarding *P. vivax* infection in the following year when such cases were diagnosed outside the SGO healthcare centre. Given the specific context of SGO and its access to healthcare, along with the mobility of SGO inhabitants, we can presume that these cases might have been diagnosed mainly across the border in Brazil. To mitigate this potential bias, we relied on self-reported malaria cases during the second survey as a means of capturing these cases, but this method has also its own limitations, including recall bias. Another limitation is the lack of data on PCR status during the second survey, with around 21% lost to follow-up, mainly among those who were PCR-negative in the first survey. While these individuals shared similar sociodemographic characteristics, they had lower exposure to factors that increase the risk of contamination, such as farming activities and visits to high-risk areas compared to the remaining participants. Data was also missing regarding breastfeeding which probably underestimate primaquine contraindication.

We chose not to conduct further stratified analyses by age and sex, as age-stratified behaviours and exposures are available in a previous study,[20] and information on G6PD sex distribution will be published in a separate paper dedicated to this topic. Data on race/ethnicity were not collected, as this is not permitted in French territories.

Finally, the higher median age of participants in the group targeted for seroTAT could indicate the contribution of age in addition to potential recent infection in the past 9 months to the observed seropositivity.

Conclusions

Serology and PCR are promising tools for TAT strategies but have their own limitations as they miss potential *P. vivax* or hypnozoites carriers. SeroPCRTAT could be a promising option to implement targeted treatment strategies in the context of low transmission of *P. vivax* as it might allow the detection of *P. vivax* and potential hypnozoite carriage (and avoid relapse if treated), and also enable the identification of populations with increased exposure. The development of point-of-care serological and PCR tests also argues in favour of their use for TAT strategies and reactive drug

administration, while avoiding the unnecessary treatment of the whole exposed population in the area.

Contributors

EM, FD, and LM designed the PALUSTOP study, while EM, LM, AS, JL, JG, MW and HT designed the secondary analysis. EM collected data on the field. YL and LM performed the PCR screening. YL performed the serological analysis. YL, LM, MW, SP, and IM analysed the biological data. EM, JL, JG and HT analysed the epidemiological data. HT wrote the first draft of the manuscript. All authors (AS, JL, JG, EM, FD, LM, YL, MW, SP and IM) contributed to reviewing and editing of the manuscript and read and approved the final version prior to submission.

Data sharing statement

Individual participant data that underlie the results reported in this article (text, tables, figures, and appendices) are not publicly available as specific authorisation from the Commission Nationale de l'informatique et des Libertés (CNIL) is required for their transfer. If a specific authorisation is obtained from the CNIL, de-identified participant data may be obtained from the corresponding author upon reasonable request only for conducting individual participant data meta-analysis approved by an independent review committee. The study protocol is available upon request. All the data will be available immediately following publication and ending 36 months following article publication. Proposals should be directed to helene.trehard@univ-amu.fr; to gain access, data requestors will be required to sign a data access agreement.

Declaration of interests

M.W. and I.M. are inventors on patent PCT/US17/67926 on a system, method, apparatus and diagnostic test for *P. vivax*. The other authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lana.2024.100883.

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