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Research Article Advances in Public Health, Community and Tropical Medicine APCTM-111
ISSN 2691-8803

Epidemiology of Possible Relapsing Cases of Vivax Malaria in the Brazilian Amazon

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Received Date: April 22, 2020; **Accepted Date:** May 05, 2020; **Published Date:** May 14, 2020

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Abstract

Background: Differentiating between relapsing vivax malaria and a new episode of vivax malaria is very difficult in the Brazilian Amazon, where transmission has a seasonal, but continuous pattern.

Methods: In this study we made an attempt to identify relapsing *P. vivax* malaria in the city of Mâncio Lima, Acre, between 2009 and 2013. Relapsing malaria was defined as an episode of vivax malaria that occurred between 29 and 60 days after an initial vivax episode treated with antimalarial drugs. All malaria cases notified in Mâncio Lima between January 01st, 2009 and July 31st, 2013 were revised. Due to SIVEP-Malaria system requirements, the variables were extracted in two steps: in the first step, date of notification and patient's name was obtained and names were compared. In the second step, patient's name and address, age, mother's name, malaria species and parasitemia, treatment, and date of treatment were obtained.

Results: Between January 2009 and June 2013, about 1301 episodes of vivax malaria full filed the operational criteria for relapsing vivax malaria; these episodes occurred in 1003 patients. Most of relapse events occurred in males and in patients under 14 y. o. The annual frequency of relapsing events varied between 4.76% and 8.55%. From the 1114 initial episodes of malaria, 63.3% of the cases had parasitemia lower than 300 parasites/mm^3. There was no association between the number of relapsing events and sex ($p = 0.238$), place of residence ($p = 0.184$) or education ($p = 0.065$). However, relapses were more frequent in younger patients. ($R = - 0.081$, $p = 0.011$).

Conclusion: Relapsing vivax malaria seems to be a common event in the Brazilian Amazon.

Keywords: Acre; Amazon; Parasitemia; *Plasmodium vivax*; Relapses.

Introduction

Malaria is still a public health problem in several areas of the world. In the Americas, Brasil is the country with the highest number of cases of malaria, and most of them are concentrated in the Brazilian Amazon. *P. vivax* is the predominant species, while *P. falciparum* account for approximately 15% of the cases, and *P. malariae* is rarely seen **[1]**.

In 2011, there were 263.323 cases notified in the Amazon, most of them by *P. vivax*. The state of Acre notified 14.01% of these cases (36,905) and since 2005 this area of the Brazilian Amazon has the highest Annual Parasitological index (API) in the country (136.6 cases/1,000 habitants in 2006 and 68.6 cases/1,000 inhabitants in 2007).In Acre, most of the transmission is located in Jurua Valley, in the municipalities of Cruzeiro do Sul, Mâncio Lima, Rodrigues

Alves and Tarauacá. There were 36.927 cases in 2010 (31,760 vivax malaria), 22.671 in 2011 (19,334 vivax malaria) and 27.503 cases in 2012(21,507 vivax malaria) **[2, 3]**.

After the initial infection, sporozoites travel to the liver and undergo several changes, resulting in tissue schizonts that later transform into merozoite forms that invade red blood cells. In *P. vivax* infection, some sporozoites may remain dormant in the liver as hypnozoites forms. At some point, hypnozoites may activate and undergo the regular schizogony process, giving rise to merozoites that are ready to invade the red blood cells. This is then called relapsing malaria, which contributes to transmission perpetuation, and is one of the major causes of high incidence of vivax malaria in some parts of the world **[5, 6]**. It is possible that uninfected Anopheles mosquitos activate hipnozoites when biting humans. Another hypothesis for hypnozoite activation is that cytokines in response to other pathogenic processes (especially those involved in arousal of fever) may lead to relapsing malaria **[4, 7, 8]**.

In Brazil, treatment for vivax malaria (either relapses or new infections) includes Cloroquine for schizont forms and Primaquine for hypnozoites. The Ministry of Health provides free antimalarial medicines used throughout the national territory, through a national policy on malaria treatment. Diagnosis and treatment is performed by health agents specially trained for that, using a standard protocol. Malaria caused by *P. vivax* are treated with chloroquine at a total dose of1500 mg divided into three days, and primaquine in the 30 mg dose / day for 7 days or 15 mg dose/ day for 14 days, adjusting the dose when the patient is under 50 kg or over 70 kg .Although the Ministry of Health proposes both a short and a long course of treatment of Primaquine, most of the cases are treated with the short course of Primaquine, to enhance treatment adhesion. Pregnant women and children under 6 months old do not receive Primaquine, because of its hemolitic effects in the unborn and neonate. A special regimen to prevent relapsing malaria is advised, using weekly cloroquine at a lower dose for 12 weeks. Health agents are responsible to identify patients that are experiencing a relapsing malaria, or those eligible for vivax prophylaxis with cloroquine **[9]**.

Differentiating between relapsing vivax malaria and a new episode of vivax malaria is very difficult in endemic areas, because the clinical presentation tends to be the same. Molecular genotyping has been used to try to differentiate both processes based on genetic differences, but since Plasmodium is prone to antigenic variation as a way, even genotyping may fail to truly identify relapses from new infections **[10]**. At the same time, the cost and the complexity of molecular tests prevent them to be used in endemic areas as a regular tool for malaria treatment and control.

The Brazilian Ministry of Health demands that all patients treated for malaria have a second thick blood smear performed in the interval of 60 days after a treated episode of vivax malaria. Generally, a positive smear for *P. vivax* is considered to be a recrudescence (the permanence or resurgence of assexual forms of the parasite in the blood) when performed while drugs are still in the peripheral blood (up to 21 or 28 days) and it is considered to be a relapsing event when a positive smear is found between 21 and 180 days after treatment relapsing events due to hypnozoite activation can occur in different lag times according to the isolate; temperate isolates may relapse up to 420 days after initial infection,**[4]** but in endemic areas where transmission is frequent it is almost impossible to differentiate between a relapsing event and a new infection. Most researchers and policy makers consider that for transmission levels that prevail in the Brazilian Amazon, relapsing events occur more frequently in the first 60 days after treatment **[11]**. Thus, an operational criteria, defining relapsing malaria as the occurrence of vivax parasitemia between 29 and 60 days after initial *P. vivax* treatment has been adopted for some researches **[11-13]**. Although this is not the optimal criteria to differentiate between relapsing and new malaria, its simplicity and easiness of cost enables the health system to identify recurrent cases of vivax malaria which are possibly relapses, and have a criteria to use the prophylatic regimen, having an impact in malaria control **[5, 11, 14, 15]**.

In this study, we performed a review of vivax malaria notifications in a city with high transmission in the state of Acre, using an operational criteria to identify possible relapsing vivax malaria, and describes its epidemiological characteristics.

Materials and Methods

Study area and Population

This study was conducted in Mâncio Lima, Acre, in the western Brazilian Amazon region. Mâncio Lima is 5,453 km^2 in area and has 16,795 inhabitants living in urban (57.3%), rural or riparian (37.9%), and indigenous (4.8%) areas **[16]**. The city, located 38 km from Cruzeiro do Sul and 650 km northwest of Rio Branco, borders the municipality of Cruzeiro do Sul and Rodrigues Alves to the east, Amazonas state to the north, and Peru to the west. Mâncio Lima is an equatorial region surrounded by palm trees and rainforests **[17]**. Its monsoon season occurs from November to April, with an annual rainfall of 1,600-2,750 mm. The city's annual temperature ranges between 20 °C and 32 °C, and the annual relative humidity is 80-90%. In 2010, the human development index was 0.625. The economy's main sources of income are cattle-raising, fishing, and producing and selling banana and cassava products.

In 2010, there were 5,729 autochthonous malaria cases notified in Mâncio Lima, within a total population of 16,795, resulting in an incidence rate of 34.11%. About 40% of those cases occurred in the urban areas of the city. In 2013, Mâncio Lima registered 6,936 cases of malaria, of which 29.1% were *falciparum* malaria and 70.3% *vivax* malaria; only 0.6% of the cases were of mixed species **[3]**.

Case Definition and Selection Procedures

Malaria Diagnosis and Notification

In Brazil, all malaria cases are diagnosed by using a thick smear stained with Giemsa and examined under 700x magnification in at least 100 fields by experienced microscopists. Positive smears are notified in a National Surveillance system called SIVEP-Malaria, **[3]** including patients characteristics, parasitological results and treatment performed.

Data Extraction and Identification of Relapsing Cases

All malaria cases notified in Mâncio Lima between January 01st, 2009 and July 31st, 2013 were revised. Due to SIVEP-Malaria system requirements, the variables were extracted in two steps: in the first step, date of notification and patient's name was obtained and names were compared. Notifications with the same patient's name or very similar names (e.g. Jose da Silva and Jose da Silva Santos) were selected for further analysis. In the second step, patient's name and address, age, mother's name, malaria species and parasitemia, treatment, and date of treatment were obtained. Cases were matched again using patient's name, address, age and mother's name. Only cases that matched patient's name, mother's name and at least one more criteria (age or address) were considered to have occurred in the same patient. In situations where mother's name was missing, a match of patient's name, age and address was required in order to consider the cases to belong to the same patient. A relapse of *vivax* malaria was defined as a *vivax* malaria case occurring in the same patient between 29 and 60 days after an initial diagnosis of vivax malaria was made, and treatment was initiated.

Statistical Analysis

A database was created with SPSS 20.0 software (SPSS Inc., Chicago, IL). The distribution of the independent variables was identified using a Student's t-test to compare the means, and a chi-square test was used for comparing the frequencies or proportions with the $\alpha = 0.05$ critical level.

Results

There were 24,797autchtonous malaria cases notified between January 2009 and June 2013 in Mâncio Lima. About 87.43% (n = 21,679) were *vivax* malaria, 12.21% were *falciparum* malaria (n = 5,428) and 0.36% were mixed infection *(P. vivax* and *P. falciparum*) **(Table 1)**.

There were 1003 patients that presented at least one episode of vivax malaria with at least one subsequent episode of relapsing vivax malaria, according to the operational criteria. These 1003 patients contributed to 1114 episodes of vivax malaria (index events) and 1301 episodes of relapsing vivax malaria.

There were 548 male patients (54.6%) and 455 female patients (45.4%). Mean age range was 18.42, with a minimum of 3 months old and a maximum of91 yeas old. Age quartiles were 7 years old, 14 years old, and 26 years old. These patients had no formal education in 3.9% of the cases, between 1 and 4 years of education in 30.6% of the cases, between 5 and 8 years of education in 37.7% of the cases. About 1.1% had more than 8 years of education, and 1.9% had an unknown status. About 24.8% of the identified patients were children in pre-school age (under 6 years old) **(Table 1)**.

Gender	**N**	**%**
Male	548	54.6
Female	455	45.4
Education		
Pre-school age	249	24.8
No schooling	39	3.9
≤ 4 years	307	30.6
5 to 8 years	378	37.7
≥ 9 years	11	1.1
Ignored	19	1.9
Place of residence		
Urban	361	36
Rural	315	31.40%
Riverine	327	32.60%
Age		
<7 y.o	286	28.5
8 to 14 y.o	216	21.5
15 to 25 y.o	238	23.7
≥26 y.o	263	26.2

Table 1: Epidemiological characteristics of subjects with a *P. vivax* malaria episode that classified as a possible relapsing event between 2009 and 2013, Mâncio Lima, Acre, Brazil.

Place of residence of patients with relapsing vivax malaria was urban in36.0% of the cases, rural in 31.4% of the cases, or riverine in 32.6% of the cases **(Table 1)**. Among urban localities identified in the notification form from relapsing patients, the most frequent ones were Guarani (8.3%), Sao Francisco (5.8%), Sao Vidal (5.7%), and Iracema (5.6%) **(Table 2)**. Parana do Pentecoste (7.8%) was the rural locality with the highest number of patients (7.8%), while Sao Domingo (8.8%), Republica Nukini (5, 8%) and Bom Sossego (3, 9%) were the riverine locations with the highest number of relapsing patients.

URBAN DISTRICTS	n	%
Guarani	83	8.3
São francisco	58	5.8
São vidal	57	5.7
Iracema	56	5.6
Others	107	10.7
RURALAREAS		
Riverine areas	89	8.9
Indigenous areas	77	7.7
Small farms	348	34.7
Settlements	85	8.5
Larger farms	43	4.3

Table 2: Prevalence of relapses according to place of residence, 2009-2012, Mâncio Lima, Acre, Brazil.

Table 2 shows the frequency of relapsing cases among those notified. The frequency of relapsing events varied between 4.76% and 8.55%, with a mean frequency of 6.01% during the study period.

Year	Period	N. Vivax malaria	N. Relapses	% Relapses
2009	Jan-Dec	4867	271	5.57
2010	Jan-Dec	5358	255	4.76
2011	Jan-Dec	4200	289	6.88
2012	Jan-Dec	4413	243	5.51
2013	Jan-Jun	2841	243	8.55
TOTAL		**21679**	**1302**	**6.01**

Table 3: Prevalence of *P. vivax* malaria and relapses between 2009 and 2013, Mâncio Lima, Acre.

Among the 1003 patients identified with a possible relapsing episode, 898 subjects (89.5%) had only one index case of vivax malaria, 98 (9.8%) had two index cases of vivax malaria, and 7 patients had three index cases of vivax malaria,. These 1003 patients contributed to 1114 episodes of vivax malaria (index events) and 1301 episodes of relapsing vivax malaria **(Table 4)**.

	N	%	AVERAGE	Minimum	Maximum
R1-M1	1003	100	44.72	29	60
R2-R1	134	13.4	43.39	29	60
R3-R2	26	2.6	45.46	29	59
R4-R3	6	0.6	48.16	35	60
R5-R4	1	0.1	51	51	51
R6-R5	1	0.1	50	50	50
RN-M1	1171		47.12	29	60
R1-M2	104	100	44.74	29	60
R2-R1	11	10.6	42.45	31	60
R3-R2	3	2.9	45.66	35	57
R4-R3	1	1	46	46	46
R_N-M2	119		44.71	29	60
R1-M3	7	100	38.85	31	50
R2-R1	3	42.9	41	33	56
R3-R2	1	14.3	59	59	59
R_N-M3	11		46.28	31	59

R_N-M_N			46.03	29	60
M1-M3 - index malaria; R1-R6 - relapsing events after a single index Malaria.					

Table 4: Interval between an index malaria case and subsequent relapse events, Mâncio Lima, Acre, 2009-2013.

The majority of patients (n = 789) had only one relapsing malaria per index malaria. However, some patients had up to six relapsing events for a single malaria index. About 151 patients had two relapsing events, 46 had 3 relapsing events and 17 patients had between 4 and 6 relapsing events during the duration of the study.

The interval between the index malaria event and a relapsing event, or between relapsing events was on average46 days. From the 1114 initial episodes of malaria, 25.8% had parasitemia lower than 200 parasites/mm^3, 37.5% had parastemia between 200 and 300 parasites/mm^3, 13.8% had parasitemia between 301and500 parasites/mm^3, and 21.3% between 501 to 10.000 parasites/mm3. In 18 malarias only (1.6%) parasitemia was over 10,000 parasite/mm^3.

Cloroquine (3-day regimen) and primaquine (7-day regimen) was used as treatment in 1101 episodes. Five patients were treated with cloroquine only (four children under 6 months old and one child that was 4 years old), and in ten patients another treatment (not specified in the notification form) was used.

There was no association between the number of possible relapsing events and sex (p = 0.238), place of residence (p = 0.184) or education (p = 0.065). However, there was a significant negative correlation between age and the number of possible relapsing events (R = - 0.081, p = 0.011), being relapses more frequent in younger patients. Also, there was a small, but significant positive correlation between parasitemia in the first episode and parasitemia in the first relapse (R = 0.229, P < 0.001).

Discussion

Relapsing vivax malaria has been described in Brazil **[11, 12, 15]** and in other countries **[18-21]**. However, studies are scarce and hard to compare because of different study designs, clinical heterogeneity and lack of study quality in some of them, resulting in different conclusions about relapse rates and associated factors around the world.

Vieira et al. (2014) **[12]** reported a frequency of 31.5% of relapses (defined as a positive thick smear between 30 and 60 days after a initial treated *P. vivax* episode) in 2008 and 20.1% in 2012 in Rondonia, in the Brazilian Amazon. Simoes et al. (2012) **[11]** fond a relapse rate of 23% in Porto Velho for the year 2009, identifying as risk factors gender (male patients), higher parasitemia at diagnosis and shorter duration o symptoms. In other countries, relapse rates up to 65%have been described **[22, 23]**.

Also, in the present study, about 50% of the events classified as relapsing malaria occurred in patients up to 14 years old. Possible explanations for that is the low dose of Primaquine that was prescribed and poor treatment compliance in children and adolescents **[24, 25]**.

In a cohort study in Brazil in which patients were treated with a total dose of 210 mg of Primaquine over 14 days, the inci4dence of relapses was 2.4 relapses per 100 person-months. Factors associated with relapse was female sex, higher parasitemia at baseline, shorter number of days with symptoms prior to baseline, and lower mg/kg dose of primaquine. Only patients over 14 years old were included in the study, making it impossible to evaluate response according to age. Female sex was associated with relapses, but the study included mostly male patients (76%).

Doses and regimens of primaquine varies around the world. The total dose of primaquine seems to be more important than the daily dose, so regimens can vary in length because the daily dose is different. A long time ago, a primaquine regimen of 15 mg/day for 14 days in adults was adopted as standard anti-relapse therapy, however further studies showed that at least 22.5 mg/day was required to prevent relapsing malaria with certain strains **[26]**. Most countries now adopt a 30mg/day regimen for 7 days or 60 mg/day for 7 days, both resulting in a total dose of 420 mg **[26]**. This total dose seems to be efficient against relapsing malaria.

In Brazil, a shorter course of primaquine was adopted (7 days instead of 14 days) to enhance adherence, but the daily recommended dose was fixed in a maximum of 0.5 mg/kg/day per 7 days, which results in 35 mg/day for an adult with 70 kg, and a total dose inferior to 22.5 mg/kg when the patient is under 45 kg. Also, for many years the Brazilian guidelines did not recommend adjusting the dose of Primaquine for those patients over 70 kg. Only recently guidelines were changed and an adjusted dose for obese patients has been adopted **[9]**. This policy may explain why relapsing vivax malaria is more frequent among children and adolescents under 14 years old, and also in obese patients, since the dose of Primaquine was not adjusted to obese patients before and serum levels might be below recommended therapeutic dose. Santos et al. **(2010)[24]** reported two cases of relapsing vivax malaria that occurred in obese patients living in non-endemic areas in Brazil(Brasilia) that received supervised treatment with standard sub doses of Primaquine, and who were cured only after adjusting the dose for their weight. Also, after initial treatment with chloroquine most symptoms (such as fever and myalgia) disappear, which may promote discontinuation of treatment with primaquine by the patient, even in the 7-day regimen **[15, 27]**.

The inadequate use of Primaquine has been associated with relapsing events. Goller et al. (2007) **[26]** analyzed data from Brazil, India and Thailand, and concluded that in

comparison with no primaquine treatment, the chance of relapse decreased by approximately 80% for a total adult primaquine regimen of 210 mg and by 95% for regimens of 315 mg and 420 mg. The risk of relapse in Thailand was 10 times greater than in India and two times greater than in Brazil. Also, a three-fold increase in the likelihood of successful treatment of each additional milligram of primaquine per kilogram of body weight was reported by the authors. Higher doses of primaquine were required to prevent *P. vivax* relapse in Thailand than in Brazil and India. According to the authors, **[26]** these differences in relapsing rates in each country depend on local response to primaquine and the local epidemiology of relapsing malaria, which may in turn vary according to local individual and parasite genetic characteristics, resulting that some strains of *P. vivax* may require higher doses of Primaquine than others.

Whether or not primaquine resistance is occurring in Brazil is still a matter of debate. Since there are a few studies about relapsing malaria and the effect of Primaquine performed in our country and there is no standardized in vitro test to assess Primaquine resistance, a definitive conclusion has not been reached yet. Duarte et al. (2001) **[28]** conclude that since low doses of primaquine were associated with relapse, it is not possible to advocate Primaquine resistance in Brazil until doses are properly adjusted by weight. Villalobos-Salcedo et al. (2000) **[15]** performed a clinical trial in the Amazon with 73 patients, where the standard Primaquine treatment (14 days) was compared to a shorter regimen with lower doses of Primaquine. Possible relapses events were identified in 6.5% of patients who received the standard 14-day regimen and 26.7% of those receiving the shorter regimen with lower Primaquine doses.

Pedro et al. (2012) **[29]** reported longer relapsing intervals in patients that acquired *P. vivax* malaria during travels to endemic areas and who returned to areas without malaria transmission, such as Rio de Janeiro, in Brazil. The median time to relapse was 108 days, and the maximum interval was 369 days. De Araujo et (2012) **[30]** also reported a longer relapsing interval in patients living in non-endemic areas in Brazil who acquired malaria elsewhere (up to 185 days) These findings from non-endemic areas in which the acquisition of new vivax infections was nearly impossible suggest that the rate of relapsing vivax malaria in the Brazilian Amazon might be even higher than that calculated using the operational criteria of 29 to 60 days, and that relapsing might be mostly associated with Primaquine sub dosing.

In fact**,** Pedro et al (2012) **[29]** also reported as the main factor associated with these relapses was non-weight-adjusted primaquine dosing, and a total dose of at least 3.6 mg/kg was found as the threshold for preventing relapsing episodes, which is superior to what is recommended in the National protocol. Based on these findings, the authors recommended a revision of the Brazilian National guidelines for treatment of *P. vivax* malaria. However, this would require a new policy for screening of patients with G-6-P-d- deficiency, because of the risk of hemolysis caused by higher levels of Primaquine **[31, 32]**.

Recent data from Taylor et al. (2019) **[33]** confirm this hypothesis. Analysis of data on 1441 recurrent P. vivax infections in 1299 patients on the Thailand-Myanmar border observed that, without primaquine radical curative treatment, 3 in 4 patients relapse. In contrast, after supervised high-dose primaquine only 1 in 40 relapse, in a much lower rate (~3%) than estimated previously.

The classification of recurrent *P. vivax* episodes into recrudescence's, relapses and new infections is not easy. Molecular genotyping has been used to help differentiating these events. However, relapses may originate from reactivation of the same parasite clone that gave origin to the initial malaria episode, or from a heterologous hypnozoite **[10, 20, 21]**. Orjuela-Sanchez et al. (2009) **[9]** described high rates of *P. vivax* recurrence (26-40% 180 days after treatment) in two rural cohorts in the Brazilian Amazon who were exposed to low levels of malaria transmission after a vivax malaria episode treated with chloroquine-primaquine. About 25% of these occurrences were detected up to 50 days after baseline treatment, therefore can be classified as 'relapse events' by the operational criteria. However, genetic analysis indicate that these frequent events were genetically diverse, indicating fast haplotype replacement. De Araujo et al. (2012) **[30]** also found evidence of multiple-clone activation of hypnozoites in relapsing *P. vivax* malaria from Brazil, and studies in other countries have also pointed to the same findings **[18-20, 34]**.

Acknowledgments

Authors´ Contributions: FMA and MdSN conceived the study and designed the study protocol; FMA, ADEC, JVP, CBB, SASM, BMD, ESG, ACM, AAR and ARS revised the notifications, analyzed data and identified relapsing events; FMA performed the statistical analysis under the supervision of MdSN. FMA, ESG and MdSN drafted the manuscript. All authors critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. MdSN is the guarantor of the paper.

Acknowledgements: The authors wish to thanks local and state health authorities for research assistance.

Funding: This work was supported by the Federal University of Acre (UFAC, Brazil), FUNTAC (Edital FDCT grant number 04/2012), CNPq (Edital Universal 2011) and Fundação de Amparo à Pesquisa do Acre (FAPAC, Brazil) through the Research Program for SUS Announcement MS / CNPq / FDCT-FUNTAC / SESACRE 2013 (grant number 14/2013). Research fellowships were awarded by ConselhoNacional de Desenvolvimento e Pesquisa (CNPq) and Federal University of Acre.

Competing Interests: The authors declare they have no conflict of interest.

Ethical approval: The study was approved by the Ethics Committee for Research with Human Beings at the Federal University of Acre (protocol number 23107.016975/2011-28). The procedures followed were in accordance with the ethical standards of the Helsinki Declaration. We obtained informed consent from the legal guardian of each participant prior to the study.

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Citation: *Araújo FM, Pacheco JVC, Cayotopa ADE, Schlosser AR, Martins AC, et al. (2020) Epidemiology of Possible Relapsing Cases of Vivax Malaria in the Brazilian Amazon. Adv Pub Health Com Trop Med: APCTM-111.*