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Occurrence of different species of *Plasmodium* in humans, non-human primates reservoirs and anopheline vectors in Atlantic Biome in the State of São Paulo, Brazil: a systematic review

Guilherme Galdino de Souza[1], Renata Tonhosolo[1*]

[1]Universidade Santo Amaro - Unisa, São Paulo/SP, Brasil.

ABSTRACT

OBJECTIVE

This study aimed to verify the participation of non-human primates (NHP), anophelines vectors and asymptomatic human carriers in the transmission of malaria in the state of São Paulo/Brazil, correlating the occurrence of different species of circulating *Plasmodium*.

METHODS

A systematic literature review was carried out using the PRISMA criteria. The search for scientific articles was carried out in the MEDLINE via PubMed, LILACS via BIREME and Scielo databases between 2010 and 2022; clinical trials, pictorial trials, literature reviews, case reports, among others that addressed the topic, available online in full text in English and/or Portuguese were analyzed.

RESULTS

Studies have shown that despite the predominance of *Plasmodium vivax* in human infections, the presence of *Plasmodium falciparum* has also been observed in some regions, highlighting the importance of comprehensive control strategies for both species. In view of this, the detection of asymptomatic cases is recommended in order to identify potential reservoirs of the disease, and this is even more necessary in non-endemic areas, such as the state of São Paulo, where non-immune patients may be vulnerable. In addition, the studies also suggest the relevance of ecology in transmission, with deforestation being associated with the maintenance of malaria.

CONCLUSION

The findings can help future research, as well as direct malaria control policies in São Paulo, providing a basis for strategies to tackle this disease in the region.

DESCRIPTORS

Plasmodium spp, Autochthonous malaria, Atlantic Forest, Asymptomatic carrier, Apes, Anopheles, Epidemiology.

Corresponding author:
Renata Tonhosolo.
Docente da Faculdade de Medicina, Universidade Santo Amaro_UNISA. R. Prof. Enéas de Siqueira Neto, 340 - Jardim das Imbuias, São Paulo – SP, Brasil.
E-mail: rtonhosolo@prof.unisa.br
ORCID ID: https://orcid.org/0000-0003-1138-2238

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INTRODUCTION

Malaria is an ancient disease that was attributed to the "bad air of the swamps". This idea was only modified in the year 1880 after the discovery of the presence of protozoa in the peripheral blood of feverish patients in Algeria, carried out by the French doctor Charles Louis Alphonse Laveran. These protozoa were later called *Plasmodium*, and there are currently 4 species recognized as etiological agents of malaria in humans (*Plasmodium malarie, Plasmodium ovale, Plasmodium vivax*, and *Plasmodium falciparum*), more recently, the simian parasite *Plasmodium knowlesi* has taken on a zoonotic character and infects humans in Southeast Asia. In addition, there are more than 26 species of *Plasmodium* ssp. known to be capable of infecting non-human primates[1,2].

The life cycle of *Plasmodium* ssp. consists of asexual and sexual phases in the vector and asexual phases in the vertebrate host. The exclusive vector of protozoan transmission in humans is female mosquitoes of the genus *Anopheles*. In Brazil, specifically in the Atlantic Forest Biome, the species most associated with the transmission of protozoa in humans and non-human primates (NHP) is the *Anopheles cruzii*. The mosquito vector generally develops in forests, mainly in bromeliads, with reproduction peaking in hot, rainy seasons[1-3].

The female mosquito vector infected by the protozoan inoculates sporozoites into the vertebrate host, which infects the hepatocytes and matures into schizonts. When liver cell lysis occurs, merozoites resulting from hepatic schizogony are released into the bloodstream, which infect red blood cells, thus initiating the erythrocyte cycle. In red blood cells, the merozoites transform into trophozoites and schizonts, and can also transform into gametocytes, the latter being ingested by the mosquito when it bites the vertebrate host. In the gastrointestinal tract of *Anopheles* ssp, the sporogonic cycle occurs, bring responsible for the formation of sporozoites, which reach the mosquito's salivary glands and are inoculated into a new host[1,4].

In humans, clinical manifestations are related to the erythrocyte phase, when the patient presents malarial attacks, which consist of the presence of a fever accompanied by tremors and a sensation of cold for approximately 15 to 60 minutes. When the fever reaches a high level (> 40°C) the patient begins to report a feeling of heat, finally experiencing intense sweating, with remission of the fever. Thus, it is stated that malaria has 3 phases: tremor, heat, and sweat. In addition, there may be other findings, such as anemia and hepatosplenomegaly and, in more severe cases, there is involvement of the central nervous system, lungs, liver, and kidneys[5].

The thick smear test is considered the gold standard for diagnosis, it is carried out by collecting blood by digital or venipuncture without anticoagulant, followed by fixing the collected blood on a slide, and finally analyzing the whole in a microscope. The positivity for the disease is confirmed when the microscopist finds the *Plasmodium* in the visual field of the microscope. Therefore, this method is dependent on the training and experience of those who carry it out. There are also currently alternative diagnostic tests for malaria, including: Rapid Diagnostic Tests (TDRs), Polymerase Chain Reaction (PCR), and Loop-Mediated Isothermal Amplification (LAMP), the latter of which has greater sensitivity and specificity compared to the other methods mentioned[6,7].

Treatment against malaria aims to compromise the evolutionary cycle of *Plasmodium* ssp. by interrupting blood schizogony, destroying hypnozoites (latent form of the *P. vivax* and *P. ovale*), or stopping transmission by preventing the formation of gametocytes. Therapeutic schemes vary according to the species of the parasite and the comorbidities that patients present[8].

According to estimates, in 2018, there were 228 million cases of malaria in the world, and in the same period 405 thousand deaths were recorded. It is expected that in 2020, together with the COVID-19 pandemic, the number of cases increased to 241 million[9,10].

In Brazil, one of the main countries affected by parasitosis in the Americas, 99.7% of malaria cases are concentrated in the North region, in the states of the Legal Amazon. In 2019, more than 150,000 cases were reported, the majority of which resulted from *P. vivax* infection[9]. In areas outside the Amazon region, there is residual transmission of malaria in states with remaining areas of the Atlantic Forest (SP, MG, RJ, and ES). Even though there are few reports in extra-Amazonian regions, the parasitosis cannot be neglected, as lethality up to 128 times higher than that found in the Amazon region is observed[11]. It is important to highlight that in non-endemic or low endemic areas there is a greater risk of worsening and death of the patient due to the delay in diagnosis[9, 10].

The State of São Paulo is the extra-Amazonian region with the highest number of cases in Brazilian territory; in which four malaria transmission areas stand out: North Coast, South Coast, Vale do Ribeira, and metropolitan areas of São Paulo surrounded by Serra do Mar[11-13]. Autochthonous cases are also predominantly caused by *P. vivax*, transmitted in coastal municipalities and also in Juquitiba, Pedro de Toledo, Tapiraí, and, in the municipality of São Paulo itself, in the extreme South zone (Engenheiro Marcilac)[14]. Serious social and economic problems have arisen in these areas as a result of human occupation and deforestation, which in some areas may be a critical factor in the increase in the number of cases.

The peculiar epidemiological situation regarding malaria in the Atlantic Forest in the southern and southeastern regions of Brazil is characterized by atypical cases involving asymptomatic or oligosymptomatic individuals, the majority of whom are infected with *P. vivax*, which can act as a source of transmission. The pattern in these regions, is characterized by malaria with a poor clinical framework, which does not put the patient's life at risk and there is no need for urgent treatment; children and pregnant women may present more serious conditions. It is worth noting that there is still a lack of studies on the prevalence/occurrence of asymptomatic carriers for the other species of *Plasmodium* circulating in the region.

The etiological agents of malaria can infect other primates in addition to humans, mainly monkeys from the Cebidae and Atelidae families, which, when infected by the protozoan, become reservoirs and allow the cycle to continue, including among humans. *P. brasilianum* and *P. simium* are the agents responsible for the infection of these primates, which, respectively, present morphological, genetic, and immunological similarities with *P. malariae* and *P. vivax*[15].

In a study carried out in the Atlantic Forest, samples of NHP and anophelines were collected. In research on *Alouatta guariba clamitans*, the presence of *Plasmodium* was identified in 36% of the primates analyzed, with the species found being *P. vivax* and *P. malariae*. To carry out the research, 9,416 specimens of female anophelines were collected, of which 0.2% tested positive for the presence of Plasmodium, the species found being: *P. vivax*, *P. falciparum*, *P. chabaudi*, *P. Berghei*, and *P. malária*. In the study, *P. falciparum* was also found in the liver of rodents of the species *Oligoryzomys cf. flavescens*, a fact that calls into question the statement that there is no presence of the species *P. falciparum* in circulation in the Atlantic Forest[16].

Previous work began to describe the epidemiological context of the Atlantic Forest region with autochthonous transmission of malaria, in which anopheline vectors infected by species of *Plasmodium* spp. were found. A study on NHP pointed to the possibility of these being natural reservoirs for simian and also for human malaria. This evidence implies the epidemiological

context due to the simultaneous occurrence of different transmission situations, compromising the control of human malaria in these areas[17]. In addition to studies conducted on vectors and NHP reservoirs, recent data indicate that the region concentrates the largest number of reported cases of the human disease in the Atlantic Forest in the state of São Paulo; the identification/characterization of entomological and epidemiological aspects will provide understanding of malaria in this region[12,18-21] and the expansion of this study is necessary for the human population.

Preliminary studies indicated that between one and three quarters of the population in these foci present serological evidence of recent exposure to several variants of *P. vivax* or *P. malariae*, despite the small number of clinical cases of malaria, suggesting a high prevalence of asymptomatic infection, which is difficult to detect by traditional parasitological methods. Asymptomatic carriers, as previously mentioned, can serve as a source of infection for vectors, allowing the spread of the endemic disease[22,23]. In this way, recognition of the different species of *Plasmodium* circulating among humans, other primates, and in insect vectors, in the state of São Paulo, will contribute to determining control measures, as well as the most appropriate possible therapy, since treatment varies according to the species of protozoan that infects the individual[5,8]. Furthermore, determining the species of the parasite has epidemiological and environmental importance, as knowledge of reservoir animals allows prophylactic strategies to be adopted[1-3,4,9].

The present study aimed to carry out a systematic review of the literature on the participation of NHP, anopheline vectors, and asymptomatic human carriers in the residual transmission of malaria in the State of São Paulo, especially in regions where, in recent years, autochthonous cases of human malaria were reported, pointing to the occurrence of different species of *Plasmodium* circulating in remaining areas of the Atlantic Forest in the city of São Paulo. We expected to find a correlation of the infections by *Plasmodium* spp. among humans with those that also circulate among insect vectors and simians, demonstrating the presence of species other than *P. vivax* in the region, as it is believed that simians and insects, as well as asymptomatic humans, may contribute to the transmission dynamics of human malaria in the state of São Paulo.

METHODS

Protocol and registration, eligibility criteria, sources of information and searches

This systematic review followed the PRISMA criteria on the prevalence/occurrence of different species of *Plasmodium* circulating among asymptomatic human carriers, simians, and insect vectors in the State of São Paulo, Brazil. The search for scientific articles was carried out in MEDLINE via PubMed, LILACS via BIREME, and Scielo databases, with publication dates between 2010 and 2022. Clinical trials, pictorial essays, literature reviews, and case reports, among others that addressed the topic in accordance with the research objective and which were available *online* in full text for free in English and/or Portuguese were analyzed.

Study selection, collection process and data list

For all databases mentioned, the descriptors used to identify the articles were: "Malaria", "Brazil", "*Plasmodium*", "Primates", and "*Anopheles*" with the Boolean operator "AND" and "OR" in English and Portuguese, with the following provision: "(Malária OR Malaria) AND (Brasil OR Brazil) AND Plasmodium AND (Primata OR Primates OR Anopheles OR Portador Assintomático)", Only those published between 2010 and 2022 that were available in English and/or Portuguese with full and free text were filtered. On the PubMed platform, only those published by MEDLINE were filtered, and only those published via LILACS were filtered by BIREME.

The articles resulting from the three databases were tabulated in an Excel document, grouped into spreadsheets according to the research platform where they were found, and the article information was made available by the platform in CVS format. Subsequently, in another spreadsheet, the articles were organized in alphabetical order according to the title, so that duplicates could be removed, which was performed manually.

Among the remaining articles, those that refer in their title or summary to the remaining areas of the Brazilian Atlantic Forest and also those that make reference to the state of São Paulo were chosen. After reading the abstract and methods, articles were selected that presented asymptomatic human carriers, simians, or insect vectors located in the state of São Paulo as the research population, and the intervention was the identification of *Plasmodium* spp. in the researched population, any other articles that did not meet the criteria listed were excluded. The final search was carried out on January 18, 2023.

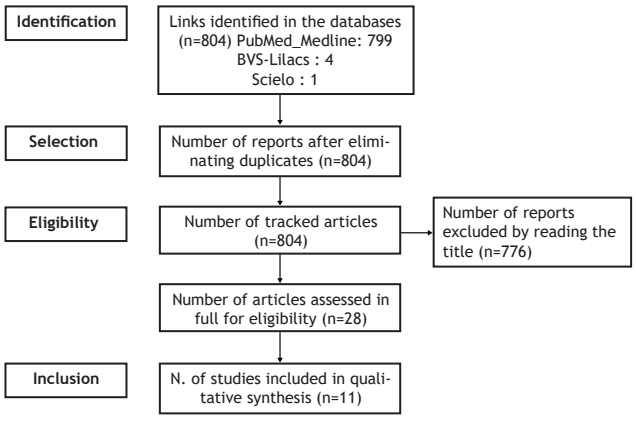
Risk of bias in each study, Summary measures, and Risk of bias between studies

The summarization of the results was nominal qualitative, in which the research was grouped according to the population researched: asymptomatic human carriers, simians, or insect vectors. As the same article can have more than one population, it may be included in more than one group.

RESULTS

After searching the databases, on January 18, 2023, the article selection process was carried out according to the PRISMA flowchart, as shown in Figure 1.

Figure 1. Article selection flowchart, PRISMA format.



After selection of the articles, some important studies were identified for analysis, as presented in Table 1.

Table 1. Articles selected in the current study.

No.	Authors	Title	Type of study	Studied Population
1	Farinas MLRN, Aschar M, Costa-Nascimento MJ, Di Santi SM.	An algorithm based on molecular protocols to improve the detection of Plasmodium in autochthonous malarial areas in the Atlantic Forest biome.	Retrospective cross-sectional study.	955 blood samples collected in active case detection.
2	Medeiros-Sousa AR, de Oliveira Christe R, de Castro Duarte AMR, Mucci LF, Ceretti-Junior W, Marrelli MT.	Effects of anthropogenic landscape changes on the abundance and acrodendrophily of Anopheles (Kerteszia) cruzii, the main vector of malaria parasites in the Atlantic Forest in Brazil.	Epidemiological study.	15,764 mosquitoes belonging to 80 species/taxa in 15 genera.
3	de Rezende Dias G, Fujii TTS, Fogel BF, Lourenço-de-Oliveira R, Silva-do-Nascimento TF, Pitaluga AN, Carvalho-Pinto CJ, Carvalho AB, Peixoto AA, Rona LDP.	Cryptic diversity in an Atlantic Forest malaria vector from the mountains of South-East Brazil.	Epidemiological study.	52 sequences (two alleles from each individual) were analyzed for the Clock gene.
4	Laporta GZ, Burattini MN, Levy D, Fukuya LA, de Oliveira TM, Maselli LM, Conn JE, Massad E, Bydlowski SP, Sallum MA.	Plasmodium falciparum in the southeastern Atlantic forest: a challenge to the bromeliad-malaria paradigm?	Epidemiological study.	921 specimens of Anopheles.
5	Kirchgatter K, Tubaki RM, Malafronte Rdos S, Alves IC, Lima GF, Guimarães Lde O, Zampaulo Rde A, Wunderlich G.	Anopheles (Kerteszia) cruzii (Diptera: Culicidae) in peridomiciliary area during asymptomatic malaria transmission in the Atlantic Forest: molecular identification of blood-meal sources indicates humans as primary intermediate hosts.	Epidemiological study.	13,462 female Anopheles.
6	Sallum MA, Daniel-Ribeiro CT, Laporta GZ, Ferreira-da-Cruz Mde F, Maselli LM, Levy D, Bydlowski SP.	Finding connections in the unexpected detection of Plasmodium vivax and Plasmodium falciparum DNA in asymptomatic blood donors: a fact in the Atlantic Forest.	Narrative review.	Individuals exposed to Plasmodium transmission.
7	Dos-Santos JC, Angerami RN, Castiñeiras CM, Lopes SC, Albrecht L, Garcia MT, Levy CE, Moretti ML, Lacerda MV, Costa FT.	Imported malaria in a non-endemic area: the experience of the university of Campinas hospital in the Brazilian Southeast.	Retrospective case series.	224 patients with confirmed parasitological diagnosis of malaria.
8	Bacci MR, Santos JA, Zing NC, Bragatto FB.	Fever of unknown origin and the role of Plasmodium vivax in Sao Paulo.	Case report.	A 58-year-old patient, of Asian origin, with persistent fever, was admitted to the emergency room of the Complexo Hospitalar São Bernardo do Campo of Faculdade de Medicina do ABC, Brazil, in January 2013.
9	Duarte AM, Pereira DM, de Paula MB, Fernandes A, Urbinatti PR, Ribeiro AF, Mello MH, Matos MO Jr, Mucci LF, Fernandes LN, Natal D, Malafronte RS.	Natural infection in anopheline species and its implications for autochthonous malaria in the Atlantic Forest in Brazil.	Epidemiological study.	6,703 anopheline females.
10	Laporta GZ, Ramos DG, Ribeiro MC, Sallum MA.	Habitat suitability of Anopheles vector species and association with human malaria in the Atlantic Forest in south-eastern Brazil.	Epidemiological study.	8,288 females of An. bellator, An. Cruzii, and An. Marajoara.
11	Couto RD, Latorre Mdo R, Di Santi SM, Natal D.	[Autochthonous malaria notified in the State of São Paulo: clinical and epidemiological characteristics from 1980 to 2007].	Epidemiological study.	18 variables from the malaria notification form in the State of São Paulo.

DISCUSSION

The studies analyzed in this systematic review[24-32] offer a comprehensive view of the epidemiology of malaria in the State of São Paulo. The predominance of *Plasmodium vivax* in human infections was evidenced in most studies, with rates varying in different regions of the state. For example, Bacco et al.[26] reported that 85% of cases in the Ribeirão Preto region were due to *P. vivax*. This trend was consistent in several regions of the state [24,29,30,32]. However, the study by Laporta et al.[31] in the Vale do Ribeira region showed that around 30% of infections were caused by *Plasmodium falciparum*, which causes more severe cases. This reinforces the importance of control strategies that consider both *Plasmodium* species. Furthermore, Santos et al.[30] and Couto et al.[34] highlighted the presence of asymptomatic cases, highlighting the need for active surveillance to identify and treat these individuals, who can act as reservoirs in the transmission of malaria.

Some of the research converges on the importance of surveillance and preventive measures for malaria in non-endemic areas in Brazil [24,29,30]. The study of Costa et al.[18] highlights that non-immune patients in non-endemic areas are more vulnerable, highlighting the need to establish reference services for effective diagnosis and treatment. This finding is corroborated by the study of Santos et al.[30], which highlights the high fatality rate outside the Amazon region compared to the endemic region.

Despite this, the data from the studies have important limitations due to the retrospective nature of many of them, which may have led to underreporting of cases, and the lack of data on comorbidities or co-infections that could influence the results[24,26,29,30]. Furthermore, most of the studies were conducted in specific geographic areas, limiting the generalization of results to the entire state of São Paulo[31,32].

In relation to primates, the study of Duarte et al.[32] detected *Plasmodium* in different *Anopheles* species, suggesting a possible zoonotic transmission. The detection of *Plasmodium malariae* in mosquitoes promotes the hypothesis of transmission cycles involving non-human primates, thus requiring further investigation. Therefore, it is necessary to understand the interactions between non-human primates, mosquito vectors, and humans for a complete understanding of the dynamics of malaria transmission in these regions.

The studies of Laporta et al.[33] and Duarte et al.[32] emphasize the importance of ecology in the transmission of malaria. The first study emphasizes how different species of *Anopheles* are associated with geographic areas and environmental conditions, with different geographic regions having different species of mosquito vectors, each with its own transmission characteristics, highlights the complexity of the cycle dynamics. On the other hand, the second study reports that deforestation in the Atlantic Forest may be contributing to the increase in malaria cases in the area, as environmental alterations can affect the habitat of mosquito vectors and hosts (humans and other primates), creating favorable conditions for the transmission of malaria.

CONCLUSION

In conclusion, the analysis of the studies shows that despite the predominance of *Plasmodium vivax* in human infections, the presence of *Plasmodium falciparum* was also observed in some regions, highlighting the importance of comprehensive control strategies for both species. Therefore, detection of asymptomatic cases is recommended to identify poten-

tial reservoirs of the disease, which is even more necessary in non-endemic areas, such as the state of São Paulo, where non-immune patients may be more vulnerable. Furthermore, studies also suggest the relevance of ecology in transmission, with deforestation being associated with the maintenance of malaria. These findings can help future research, as well as direct malaria control policies in São Paulo, providing a basis for strategies to combat this disease in the region.

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