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PROGRAMA DE PÓS-GRADUAÇÃO EM
BIODIVERSIDADE E BIOTECNOLOGIA
DA REDE BIONORTE

MALÁRIA: PERFIL EPIDEMIOLÓGICO E DIVERSIDADE DE GENES ASSOCIADOS À RESISTÊNCIA AOS ANTIMALÁRICOS EM PARASITOS DO *PLASMODIUM FALCIPARUM* E DO *PLASMODIUM VIVAX*, NO ESTADO DE RORAIMA

JACQUELINE DE AGUIAR BARROS

BOA VISTA-RR
2024

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Tese de doutorado apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Biodiversidade e Biotecnologia da Rede BIONORTE, na Universidade Federal de Roraima, como requisito parcial para a obtenção do Título de Doutor em Biodiversidade e Biotecnologia.

Orientador(a): Profa. Dra. Fabiana Granja Co-Co-orientador(a): Profa. Dra. Maria de Fátima Ferreira da Cruz.

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BARROS, Jacqueline de Aguiar. Malária: perfil epidemiológico e diversidade de genes associados à resistência aos antimaláricos em parasitos do *Plasmodium falciparum* e do *Plasmodium vivax,* no estado de Roraima. 2024. 121 f. (Doutorado em Biodiversidade e Biotecnologia) – Universidade Federal de Roraima, Boa Vista, 2024.

RESUMO

A malária ainda é um problema de saúde significativo no Brasil, principalmente na Amazônia Legal, onde o estado de Roraima está localizado. Na perspectiva da eliminação da doença é importante identificar o perfil epidemiológico para estabelecer estratégias de controle de acordo com a realidade local. O surgimento de resistência dos plasmódios aos antimaláricos pode impossibilitar a estratégia de controle através do tratamento, assim, torna-se importante conhecer a diversidade de genes associados à resistência a estes fármacos. Assim, o objetivo desse estudo foi descrever o perfil da morbimortalidade da malária e a diversidade de genes associados ou potencialmente associados à resistência aos antimaláricos em parasitos de *P. falciparum* e *P. vivax* no estado de Roraima. Para atender os objetivos propostos realizou-se primeiro um estudo retrospectivo, pautado em dados secundários, no período de 2010 a 2020. As fontes para a coleta de tais dados foram o Sistema de Informação de Vigilância Epidemiológica da Malária, Sistema de Informações sobre Mortalidade e Sistema de Informação de Internação Hospitalar. Na segunda etapa da pesquisa realizou-se coleta de sangue e aplicação de questionários no Pronto Atendimento Cosme e Silva e Unidade de Saúde Sayonara, quando foram incluídos no estudo participantes com diagnóstico de malária por gota espessa, maiores de 18 anos e que assinaram o termo de consentimento livre e esclarecido. No laboratório procedeu-se com extração de DNA, PCR, purificação e sequenciamento de DNA. Os ensaios de monitoramento de quimiorresistência do *P. falciparum* foram realizados através da investigação de mutações no gene *pfk13.* Para o *P. vivax* foram pesquisados os polimorfismos nos genes *pvcrt-o* e *pvmdr1*. Os resultados mostraram que a malária importada da Venezuela e o garimpo na terra indígena Yanomami foram fatores que causaram o aumento da malária em Roraima. Homens jovens, da raça parda e que desenvolvem atividade de garimpagem foram os principais acometidos por malária na casuística estudada. O hábito de automedicação em área de garimpo é recorrente, o que pode selecionar parasitos resistentes aos antimaláricos. Não encontramos mutações no gene *pfk13* indicando que o *P. falciparum* circulante em Roraima é sensível ao tratamento com artemisinina. Porém, a presença de mutações nos genes *pvcrt-o* e *pvmdr1* em casos sem evidencias de recrudescências mostrou que esses genes não são bons marcadores moleculares para prever o aparecimento do fenótipo de resistência do *P. vivax* à cloroquina.

Palavras-chave: Diversidade gênica; Cloroquina; Artemisinina; *P. vivax*; *P, falciparum.*

BARROS, Jacqueline de Aguiar. Malaria: epidemiological profile and diversity of genes associated with resistance to antimalarials in *Plasmodium falciparum* and *Plasmodium vivax* parasites, in the state of Roraima. 2024. 121 p. (PhD in Biodiversity and Biotechnology) – Federal University of Roraima, Boa Vista, 2024.

ABSTRACT

Malaria is still a significant health problem in Brazil, especially in the Legal Amazon, where the state of Roraima is located. To eliminate the disease, it is important to identify the epidemiological characteristics to set control strategies according to the local reality. The emergence of resistance to antimalarials may impact the control strategy based on treatment, therefore, it is important to know the genes diversity associated with resistance to antimalarial. Thus, the objective of this study was to describe the morbidity and mortality profile of malaria and the diversity of genes associated or potentially associated with resistance to antimalarials in *P. falciparum* and *P. vivax* parasites in the state of Roraima. To meet the proposed objectives, a retrospective study was first carried out, based on secondary data, from 2010 to 2020. The sources for collecting such data were the Malaria Epidemiological Surveillance Information System, the Mortality Information System and the Hospital Admission Information. For the second research aim, blood was collected and epidemiological malaria questionnaires were applied at the Cosme e Silva Emergency Hospital and Sayonara Health Unit, when participants with malaria diagnosis by thick blood smears, over 18 years of age, and who signed the free and informed consent form, were included in the study. In the laboratory, DNA extraction, PCR, purification and DNA sequencing were carried out. *P falciparum* chemoresistance monitoring assays were performed through mutations in the *pfk13* gene. For *P. vivax*, polymorphisms in the *pvcrt-o* and *pvmdr1* genes were investigated. The results showed that malaria imported from Venezuela and mining in the Yanomami indigenous land are increasing the malaria cases in Roraima. Young, brown men who carry out mining activities are the most affected by malaria. The habit of self-medication in mining areas is recurrent, which can promote the appearance of parasite resistance to antimalarials. We did not find mutations in the *pfk13* sequences indicating that P. falciparum parasites circulating in Roraima are not resistant to artemisinin. However, the presence of *pvcrt-o* and *pvmdr1* mutations without recrudescence cases showed that these genes are not useful molecular markers to predict the emergence of P. vivax phenotypes resistant to chloroquine.

Keywords: Genetic diversity; Chloroquine; Artemisinine; *Plasmodium vivax*; *Plasmodium falciparum*.

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LISTA DE ABREVIATURAS E SIGLAS

ACT	Artemisinina
CBio	Centro de Estudos da Biodiversidade
CID-10	Classificação Internacional de Doenças
crt	*chloroquine resistance transporter*
DDT	dicloro-difenil-tricloroetano
dNTPs	Desoxinucleotídeos trifosfatados
DSEI-Leste	Distrito Sanitário Especial Indígena Leste
DSEI-Yanomami	Distrito Sanitário Especial Indígena Yanomami
IBGE	Instituto Brasileiro de Geografia e Estatística
IPA	Incidência Parasitária Anual
LaBMol	Laboratório de Biologia Molecular
LVC	Lâminas de Verificação de Cura
*mdr*1	*multi-drug resistance* 1
ODS	Objetivos de Desenvolvimento Sustentável
OMS	Organização Mundial da Saúde
ONU	Organização das Nações Unidas
PCR	*Polymerase chain reaction*
pfK13	*pfkelch*13
PNCM	Programa Nacional de Controle da Malária
SIH	Sistema de Informação de Internação Hospitalar
SIM	Sistema de Informações sobre Mortalidade
Sivep-Malária	Sistema de Informação de Vigilância Epidemiológica da Malária
SNM	Serviço Nacional de Malária
SNPs	Polimorfismos de nucleotídeo único
SNPs	S*ingle nucleotide polymorphism*
TCLE	Termo de Consentimento Livre e Esclarecido
UDAs	Unidades Distribuidoras de Antimaláricos
UFRR	Universidade Federal de Roraima

SUMÁRIO

1. INTRODUÇÃO

Apesar dos enormes esforços de controle ao longo de muitas décadas, a malária ainda é um problema de saúde significativo no Brasil, principalmente pela elevada incidência da doença e pelo potencial de gravidade clínica. Além disso, existem as consideráveis perdas sociais e econômicas em populações vulneráveis que vivem em condições precárias de habitação e saneamento (BRASIL, 2022; BRASIL, 2023).

A Amazônia Legal (composta por Acre, Amapá, Amazonas, Maranhão, Mato Grosso, Pará, Rondônia, Roraima e Tocantins) concentra 99,9% dos casos autóctones registrados no país, por apresentar condições ambientais que favorecem a proliferação do vetor (BRASIL, 2022).

A espécie parasitária mais frequente é o *Plasmodium vivax*, que apesar de possuir menor potencial de causar doença grave, pode causar infecções recorrentes, condição que dificulta seu tratamento. Já o *P. falciparum* embora menos frequente é a espécie responsável por causar casos mais graves da doença e óbitos (BRASIL, 2022).

Na perspectiva da eliminação, o Brasil apresentou a meta de eliminação da malária até 2035, considerando como marco intermediário a eliminação da transmissão de malária por *P. falciparum* até 2030. Os últimos cinco anos serão considerados apenas com transmissão de malária por *P. vivax* (BRASIL, 2023). Diante da diversidade de cenários de transmissão de malária no Brasil, torna-se importante obter informações que podem subsidiar a formulação de estratégias efetivas no controle da doença de acordo com a realidade local.

Roraima encontra-se em área endêmica para malária e vem apresentando um aumento no número de casos e na morbiletalidade nos últimos anos (BRASIL, 2020). Assim, se torna importante entender melhor o padrão epidemiológico e a dinâmica de transmissão dessa infecção no estado. Mesmo diante do cenário de aumento progressivo dos casos desta endemia, poucos estudos sobre a caracterização molecular de resistência aos antimaláricos e a diversidade desses genes em populações parasitárias de *P. vivax* e de *P. falciparum* circulantes no estado de Roraima foram realizados. Os genes *pvcrt-o* e *pvmdr1* de *P. vivax* são candidatos em potencial para marcadores da quimiorresistência do P. vivax à cloroquina (MELO et. al., 2014; JOY et. al., 2018). Enquanto o gene *pfk13* é reconhecido universalmente como marcador da quimiorresistência à artemisinina no *P. falciparum* (OMS, 2018).

Como o controle da malária é principalmente pautado no diagnóstico oportuno seguido de tratamento, é facilmente compreensível que o surgimento de parasitas resistentes aos

antimaláricos represente um sério obstáculo ao controle da malária. Dessa maneira, a vigilância molecular de polimorfismo desses genes pode contribuir para a identificação precoce de parasitos resistentes, compreensão da migração dos parasitos resistentes e das tendências evolutivas na propagação do padrão de resistência.

Assim, o estudo do perfil epidemiológico da malária em Roraima e a descrição da variabilidade dos genes associados a resistência à artemisinina e cloroquina, em parasitos de *P. falciparum* e *P. vivax*, respectivamente, podem subsidiar a formulação de estratégias efetivas no controle integrando saúde-doença-atenção, além de orientar políticas de saúde voltadas para a população de acordo com o seu contexto, no qual os problemas e as soluções realmente acontecem.

Para tal tivemos como

1.1 OBJETIVO GERAL:

Estudar o perfil epidemiológico da malária e a diversidade de genes associados ou potencialmente associados à resistência aos antimaláricos em parasitos de *P. falciparum* e *P. vivax* no estado de Roraima.

E como

1.2 OBJETIVOS ESPECÍFICOS:

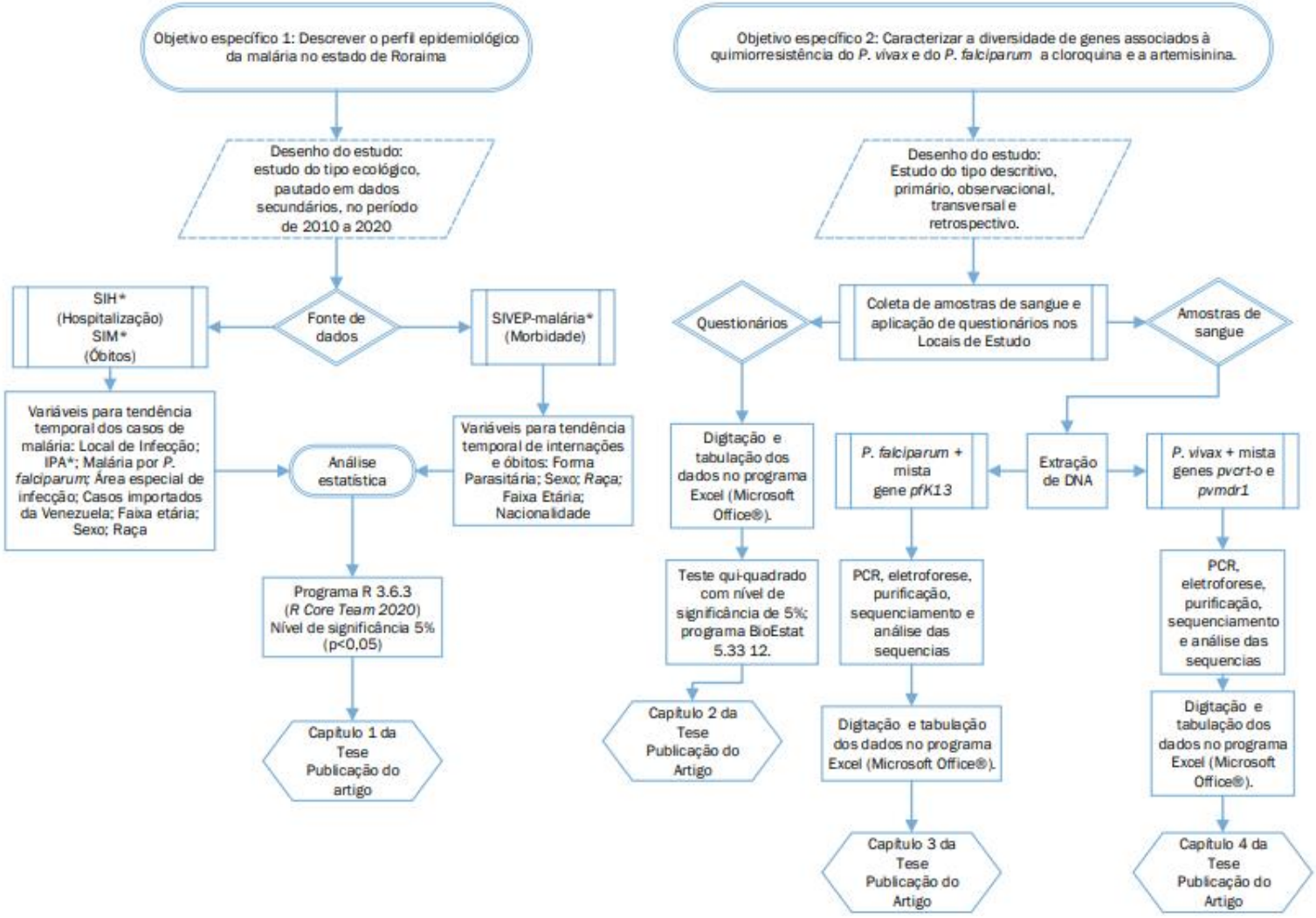
- Descrever o perfil epidemiológico da malária no estado de Roraima.
- Caracterizar a diversidade de genes *P. vivax* e do *P. falciparum* associados a quimiorresistência desses parasitos a cloroquina e a artemisinina, respectivamente.
- Fornecer dados para o Ministério da Saúde quanto ao perfil de quimiorresistência dos plasmódios.

Este documento foi organizado de forma a apresentar os resultados dos estudos realizados para atender aos objetivos propostos. O primeiro capítulo aborda o perfil epidemiológico da malária pautado em dados secundários. O segundo capítulo descreve o padrão epidemiológico da malária com base nos questionários aplicados no momento da coleta de amostras. O terceiro capítulo trata sobre a vigilância molecular de mutações no gene *pfk13* associadas com a resistência do *P. falciparum* à artemisinina, em amostras de parasitos do estado de Roraima. E o quarto capítulo apresenta a investigação de

polimorfismos nos genes *pvcrt-o* e *pvmdr-1* para se conhecer o potencial preditivo dessas mutações como marcadores moleculares da resistência à cloroquina no *P. vivax*.

Com os resultados do presente trabalho, espera-se contribuir para o fortalecimento da capacidade de resposta da saúde pública no controle da malária em Roraima e assim melhorar a qualidade de vida dessa população amazônica vulnerável à infecção causada pelo *P. vivax* e *P. falciparum.*

Figura 1. Desenho Experimental do Estudo



* SIH: Sistema de Informação de Hospitalização, SIM: Sistema de Informação de Mortalidade, Sivep-Malária: Sistema de Informações de Vigilância Epidemiológica da Malária, IPA: Incidência Parasitária Anual.

2. REVISÃO BIBLIOGRÁFICA

2.1 ASPECTOS HISTÓRICOS, EPIDEMIOLOGIA, ESTRATÉGIAS E DESAFIOS PARA ELIMINAÇÃO DA MALÁRIA

A malária acomete a humanidade há dezenas de milhares de anos, ao longo dos anos chegou a atingir praticamente todos os lugares da terra, sendo a exceção as regiões polares e subpolares. Epidemias como a da peste no século XIV, pode aparentar maior dramaticidade pela forma aguda de sua ocorrência, mas nenhuma outra doença se compara à malária pela tenacidade e perenidade com que flagela a humanidade. Além disso, seu impacto transcende a saúde, repercutindo em fatores de desenvolvimento econômico, social, migratórios e em conflitos militares (CAMARGO *et. al*., 1995).

As características clínicas da doença (calafrios precedendo a febre alta e intermitente, além do aumento do baço) permitem reconhecer a sua presença em escritos chineses de 3000 aC, nas tábuas cuneiformes mesopotâmicas (2000 aC) e em escrituras Vedas na Índia (1800 aC). Em todos esses relatos a ocorrência da malária está associada a regiões pantanosas, várzeas e alagadiços (CAMARGO *et. al*., 1995; ESTEVES, 2012).

O termo malária surgiu dessa relação de ocorrência de febre intermitente e a proximidade com áreas pantanosas, hipótese levantada pela primeira vez pelo médico grego Hipócrates, que também descreveu detalhadamente os sintomas da malária, diferenciando a febre malárica intermitente da febre contínua provocada por outras doenças, além de mencionar o aumento do baço (CAMARGO *et. al*., 1995; ESTEVES, 2012). Assim, no século XIV os italianos passaram a descrever a doença como *'mal'aria*' (ar ruim ou nocivo), 200 anos depois esse termo foi inserido na língua inglesa. Por sua vez, os franceses criaram o termo "paludismo", que vem do latim, *palus*, que significa pântano, para se referir à malária (TUTEJA, 2007; FRANÇA et. al., 2008).

A forma de tratamento da malária foi identificada primeiro que a identificação do agente etiológico e da forma de transmissão. Apesar da constatação de que os chineses, desde o século II, já usarem infusão da planta *Artemisia annua*, para cura da malária, a droga dela derivada – artemisinina - só entrou na farmacopeia ocidental no século XX. Por sua vez, o quinino, ou melhor, extrato da casca da Cinchona, ou quinaquina, uma rubiácea nativa dos Andes do Equador, Peru e Bolívia, foi o único e realmente importante tratamento da malária em nível mundial. O uso do quinino como antitérmico se origina no Peru por meados de 1600 e a partir daí chegou à Europa por mercadores e jesuítas (CAMARGO *et. al.,* 1995). A alternativa ao tratamento com quinina surgiu em 1934, com a descoberta da cloroquina por

Hans Andersag, porém a droga só foi reconhecida como eficaz e segura em 1946. Após a descoberta da cloroquina foram desenvolvidos o progranil e pirimetamina, largamente usados, porém a eficácia começou a diminuir nos anos 50. Em 1960, ocorreram os primeiros relatos de resistência à cloroquina em parasitos do *P. falciparum* na Tailândia e Colômbia e, posteriormente, em outros países endêmicos, como o Brasil (ESTEVES, 2012).

Em 1880, Charles Louis Alphonse Laveran, um cirurgião do exército francês na Argélia, examinando sistematicamente sangue de maleitosos, notou parasitos dentro das hemácias e que estas se rompiam liberando minúsculos organismos de forma filamentosa, simulando pequenos flagelos. Foi a descoberta do parasito da malária. Laveran acreditava que só havia uma espécie a *Oscillaria malarie*, mais tarde denominada *Laverania malariae*, que corresponde ao atual *Plasmodium falciparum* (ESTEVES, 2012; TUTEJA, 2007). Até então, acreditava-se que a malária era causada por uma bactéria *Bacillus malarie*, então a descoberta foi recebida com algum ceticismo pela comunidade científica. Porém, anos mais tarde a sua descoberta foi finalmente reconhecida e Laveran recebeu o Prêmio Nobel em 1907 (Figura 1).

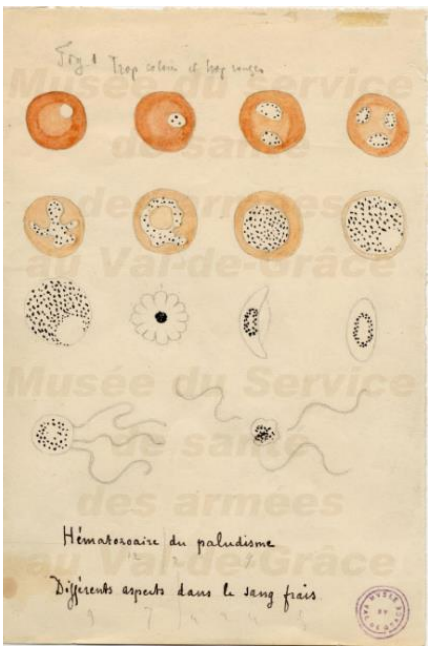


Figura 1. Ilustração elaborada por Laveran das diferentes fases de desenvolvimento do parasito observadas em sangue fresco (Fonte: www.cdc.gov)

Em 1886, Camillo Golgi identificou que havia duas formas diferentes nos ciclos de febre da malária que coincidiam com a ruptura das hemácias e liberação de novos parasitos na corrente sanguínea, definidas como terça e quartã. Em 1890, Giovanni Grassi e Raimundo Filetti identificaram duas espécies parasitárias e a definiram como *P. vivax* e *P. malarie*. O *P.*

falciparum foi, posteriormente, em 1897, caracterizado por William H. Welch como a espécie causadora da febre terçã maligna. Em 1922, John William Watson Stephens descreveu a quarta espécie do parasito, o *P. ovale*. Por sua vez, o *P. knowlesi* descoberto em Singapura em 1931 infectando macacos *Macaca fascicularis*, desde 2004, foi reconhecido também como causador de malária humana (SINGH *et. al.,* 2004; SINGH *et. al,* 2013). Mais recentemente, o *P. cynomolgi* - originalmente implicado apenas na malária simiana, também foi reconhecido como agente causal da malária humana (BYKERSMA, 2021). Quanto ao *P.simium,* até o momento as evidencias apontam para uma infecção humana de caráter zoonótico (MOURTIERT, *et. al.,* 2021; BRASIL *et. al,* 2017).

No que diz respeito à transmissão, somente em 1900, Ronald Ross, membro do Exército Britânico na Índia, demostrou (em pássaros) que o parasito era transmitido ao homem pela picada de mosquitos hematófagos, os pernilongos. Ao mesmo tempo na Itália, Giovanni Grassi, Amico Bignami e Giuseppe Bastianelli confirmaram a veracidade das observações de Ross, porém, descartaram a possibilidade dos mosquitos do gênero *Culex*, o pernilongo comum, transmitirem malária e confirmou que o vetor pertencia ao gênero *Anopheles*. Além disso, descreveram o ciclo completo de desenvolvimento do parasito no homem e no mosquito (CAMARGO, 1995; TUTEJA, 2007).

Com a identificação do agente etiológico, forma de transmissão e tratamento, o ciclo da malária estava fechado e de posse de todos esses conhecimentos os cientistas concentraram esforços para identificar estratégias para quebrar a cadeia de transmissão e controlar a malária. As estratégias foram várias e, entre elas, muitas regiões do mundo adotaram aquela voltada para a eliminação do mosquito, através de combate às larvas e criadouros, porém essa medida de eliminação fracassava a cada nova chuva, pela formação de novos criadouros. Robert Kock, já famoso pelos estudos com o bacilo da tuberculose, foi entusiasta da estratégia de administração em massa de quinino como única medida profilática, o que também não funcionou (CAMARGO, 1995).

Após os insucessos de combate ao mosquito adulto e suas larvas, inicia-se o conceito de que malária é essencialmente uma doença de caráter sócio-político-econômico que deve ser combatida com quinino e melhorias sanitárias. Assim, Estados Unidos e muitas regiões da Europa reduziam a doença, com obras de engenharia hidráulica para drenagem de águas alagadas, tratamento em massa e serviço de vigilância ativo contra malária. Porém, o desenvolvimento econômico-social como medida profilática para malária, não pode ser aplicado aos países subdesenvolvidos por estes não possuírem instrumentos/recursos para isso (CAMARGO, 1995).

O DDT (sigla de dicloro-difenil-tricloroetano) inseticida desenvolvido pela Geigy Suíça na Segunda Guerra Mundial, inicialmente para combate à piolhos, mas que depois mostrou ter ação residual de vários meses nas paredes das casas, iniciou uma nova era de combate à malária e renovou as esperanças de erradicação em nível mundial. Em regiões onde a cadeia de transmissão da malária já estava enfraquecida, como nos Estados Unidos e países da Europa, a erradicação da malária foi uma realidade alcançada. Por exemplo, a Itália em cinco anos acabou com a malária, depois de mais de 400 anos de luta. Porém, a meta de erradicação mundial da malária através do DDT, deparou-se com a resistência do mosquito ao inseticida e surgimento de nichos novos de espécies que inicialmente eram sem importância, além do impacto no equilíbrio ambiental pelo seu uso, por poder demorar até 30 anos para se degradar (CAMARGO, 1995).

No Brasil, em 1939, o *Anopheles gambie*, importado da África, causou um surto epidêmico de malária no Rio Grande do Norte e vale do Jaguaribe no Ceará, esforços governamentais e da Fundação Rockefeller envolveram grandes somas de dinheiro, treinamento de quatro mil pessoas, larvicida, borrifação das casas, diagnóstico e tratamento com quinino. Assim, em 1944, não havia mais nenhum An. gambie na região e a malária foi eliminada (CAMARGO, 1995). A campanha contra o *An. gambiae* no Nordeste brasileiro, pode ser considerado um momento fundamental na história da institucionalização do combate à malária no país, pois originou o primeiro serviço específico para essa finalidade: o Serviço de Malária do Nordeste (HOCHMAN *et. al.,* 2002).

No início da década de 1940 a estimativa no Brasil era de 6 milhões de casos. O Serviço Nacional de Malária (SNM) foi criado em 1941, com a finalidade de organizar e executar o combate à malária, bem como realizar estudos e pesquisas sobre a doença. Nos primeiros anos de atividade as ações do SNM foram dirigidas ao combate às larvas do vetor, através de obras de drenagem, aterros e aplicações de substâncias larvicidas (verde-paris e o petróleo), além de inquéritos e investigações epidemiológicas e entomológicas. A partir de 1945 iniciou-se a aplicação de DDT combinado com o tratamento para controlar malária ao longo da faixa litorânea do Brasil. No final de 1947, o SNM deu início a uma grande campanha para controle da malária no Rio de Janeiro com uso em massa do DDT em domicílios urbanos e periurbanos, além da distribuição gratuita de cloroquina , administrada em dose única, através de uma ampla rede de Unidades Distribuidoras de Antimaláricos (UDAs), que além de incluir unidades de saúde, também contava com escolas públicas e particulares, fazendas, prefeituras municipais, agências de correios e igrejas (HOCHMAN *et. al.,* 2002).

Na década de 50, o programa de erradicação da malária, auxiliou na incorporação das técnicas de vigilância epidemiológica aos programas de controle de doenças transmissíveis em todo o mundo (GARRETT, 1995).

O SNM utilizou no período de 1952 até meados da década de 1960 o sal cloroquinado, estratégia idealizada por Mário Pinotti, para controlar a malária em localidades endêmicas do país, inclusive na Amazônia, onde não era possível utilizar inseticida de ação residual e nem contar com serviços de medicação. Porém, a resistência cada vez maior dos plasmódios à cloroquina, aliada a outros fatores, inviabilizou a continuidade da utilização dessa estratégia (HOCHMAN *et. al.,* 2002).

Após a campanha de erradicação da malária no Brasil, durante a década de 60, o número de casos da doença atingiu 51 mil casos no início dos anos 70, todos concentrados na região amazônica. A década de 70 também foi marcada pela intensificação da ocupação da Amazônia, com incentivos fiscais para empresários de outras áreas investirem na região, e então a situação epidemiológica se complicou. No início da década de 80 foram registrados 297 mil casos e em torno de 99,5% dos casos de malária ocorreram na região amazônica (TAUIL, *et. al.,* 1985).

No início da década de 90, a malária no Brasil concentrava-se em 79 municípios da região amazônica, onde os focos de transmissão estavam fundamentalmente ligados à atividade de garimpo e expansão da fronteira agrícola (BARATA, 1995). A partir dessa década também se observou um “efeito serrote” nos casos de malária, ou seja, redução em alguns anos e elevação em outros. Em 1993, o país notificou 483.367 casos de malária, o que representa uma redução de 14% na comparação com 1990, quando foram notificados mais de 560 mil casos. Em 1999 foram notificados 637.474 casos e em 2002 foram 349.896 casos, representando redução de 45% em relação a 1999. Porém, em 2005 foi verificado um aumento de 74%, em relação à 2002, com 607.751 casos (OLIVEIRA-FERREIRA *et. al.,* 2010). A partir de 2005, o número de casos de malária apresentou redução sustentada, chegando a 143.250 casos, em 2016, o menor número de casos em 37 anos (BRASIL, 2023).

Na região Amazônica a malária é doença de notificação compulsória regular e os casos suspeitos devem ser notificados às autoridades de saúde em até sete dias. A notificação deve ser realizada em ficha específica do Sistema de Informação de Vigilância Epidemiológica da Malária (Sivep- Malária). Também ficam registradas no sistema as Lâminas de Verificação de Cura (LVC), os respectivos diagnósticos e os tratamentos dos casos positivos (BRASIL, 2022).

Porém, na região extra-amazônica a malária é uma doença de notificação compulsória imediata, ou seja, todo caso suspeito deve ser notificado às autoridades de saúde

em até 24 horas. A notificação deve ser registrada no Sistema de Informação de Agravos de Notificação (SINAN), utilizando-se Ficha de Notificação e Investigação específica para Malária. Além disso, todos os exames de controle de cura devem ser registrados e a investigação deve ser finalizada no sistema no prazo máximo de 30 dias (BRASIL, 2022).

Estima-se que em 2022 ocorreram, globalmente, 249 milhões de casos de malária em 85 países endêmicos para malária (incluindo o território da Guiana Francesa). A região Africana contribuiu com 94% (233 milhões) destes casos, sendo que quatro países desta região concentraram quase metade dos casos: Nigéria (26,8%), República Democrática do Congo (12,3%), Uganda (5,1%) e Moçambique (4,2%). Por sua vez, os países da região do Mediterrâneo Oriental e do Sudeste Asiático foram responsáveis por 3% (8,3 milhões) e 2% (5,2 milhões) da carga de malária em 2022, respectivamente. Na região do mediterrâneo oriental 41% dos casos estavam concentrados do Sudão e no sudeste asiático 66% dos casos na Índia (WHO, 2023).

A região do Pacífico Ocidental foi responsável por 1,9 milhões de casos, sendo 90% destes na Papua Nova Guiné. Na Região das Américas ocorreram 0,55 milhões de casos e 73% destes estavam concentrados na República Bolivariana da Venezuela, Brasil e Colômbia. Vale ressaltar que nessa região a Argentina, Belize, El Salvador e Paraguai foram certificados como livres da malária em 2019, 2023, 2021 e 2018, respectivamente. Já a Região Europeia está livre da malária desde 2015 (WHO, 2023).

Em relação às mortes nas quais a malária foi a causa básica, estima-se que ocorreram 608.000 em 2022, distribuídas em 29 países. A região Africana concentrou 95% (580) dessas mortes e em quatro países dessa região ocorreram mais da metade do total de óbitos no mundo: Nigéria (31,1%), o República Democrática do Congo (11,6%), Nigeria (5,6%) e Estados Unidos República da Tanzânia (4,4%). Cerca de 78% do total de mortes por malária na região ocorreu em crianças menores de cinco anos (OMS, 2023).

Na região do Mediterrâneo Oriental foram 15.900 mortes por malária e na região do Sudeste Asiático 8.000 mortes pela doença, sendo que cerca de 94% delas ocorreram na Índia e na Indonésia. Na região do Pacífico Ocidental aconteceram 3.600 mortes, principalmente na Papua Nova Guiné. E a região das Américas registrou 343 óbitos (WHO, 2023).

No Brasil em 2022, foram notificados 128.951 casos de malária, 99,9% destes foram notificados em estados da Região Amazônica. Mais da metade dos casos concentraram-se nos estados do Amazonas com 43,19% (55.686 casos) e de Roraima com 20,41% (26.310 casos) (BRASIL, 2023). Segundo o Sistema de Informação de Mortalidade (SIM), neste ano 29 óbitos por malária foram relatados em Roraima.

A estratégia técnica global para a malária 2016–2030 foi aprovada pela Assembleia Mundial da Saúde em maio de 2015, fornecendo orientações aos países para acelerar o progresso rumo à eliminação da malária e estabelece a meta de reduzir a incidência e as taxas de mortalidade globais da malária em pelo menos 90% até 2030, usando como 2015 como ano base (OMS, 2015).

O primeiro pilar da Estratégia Técnica Global contra a malária 2016-2030, tem o objetivo de garantir acesso universal à prevenção, ao diagnóstico e o tratamento da malária; o segundo visa acelerar os esforços rumo à eliminação e à obtenção do status de país livre de malária e o terceiro pilar intenta transformar a vigilância da malária em uma intervenção básica nos países onde é endêmica (OPAS, 2018). Diante desse cenário, o Ministério da Saúde do Brasil, alinhado com os Objetivos de Desenvolvimento Sustentável (ODS) da Organização das Nações Unidas (ONU), lançou em 2015 o Plano de Eliminação da Malária por *P. falciparum* até 2030 (BRASIL, 2023).

O Brasil ampliou seus objetivos lançando, em 2022, o Plano Nacional de Eliminação da Malária, com as metas de chegar a menos de 68 mil casos em 2025 e menos de 14 mil casos em 2030. Possui ainda como marcos intermediários, até 2030, a redução de óbitos para zero e eliminar a transmissão de malária por *P. falciparum*. O ano de 2035 é considerado o limite para a meta de eliminação (BRASIL, 2023).

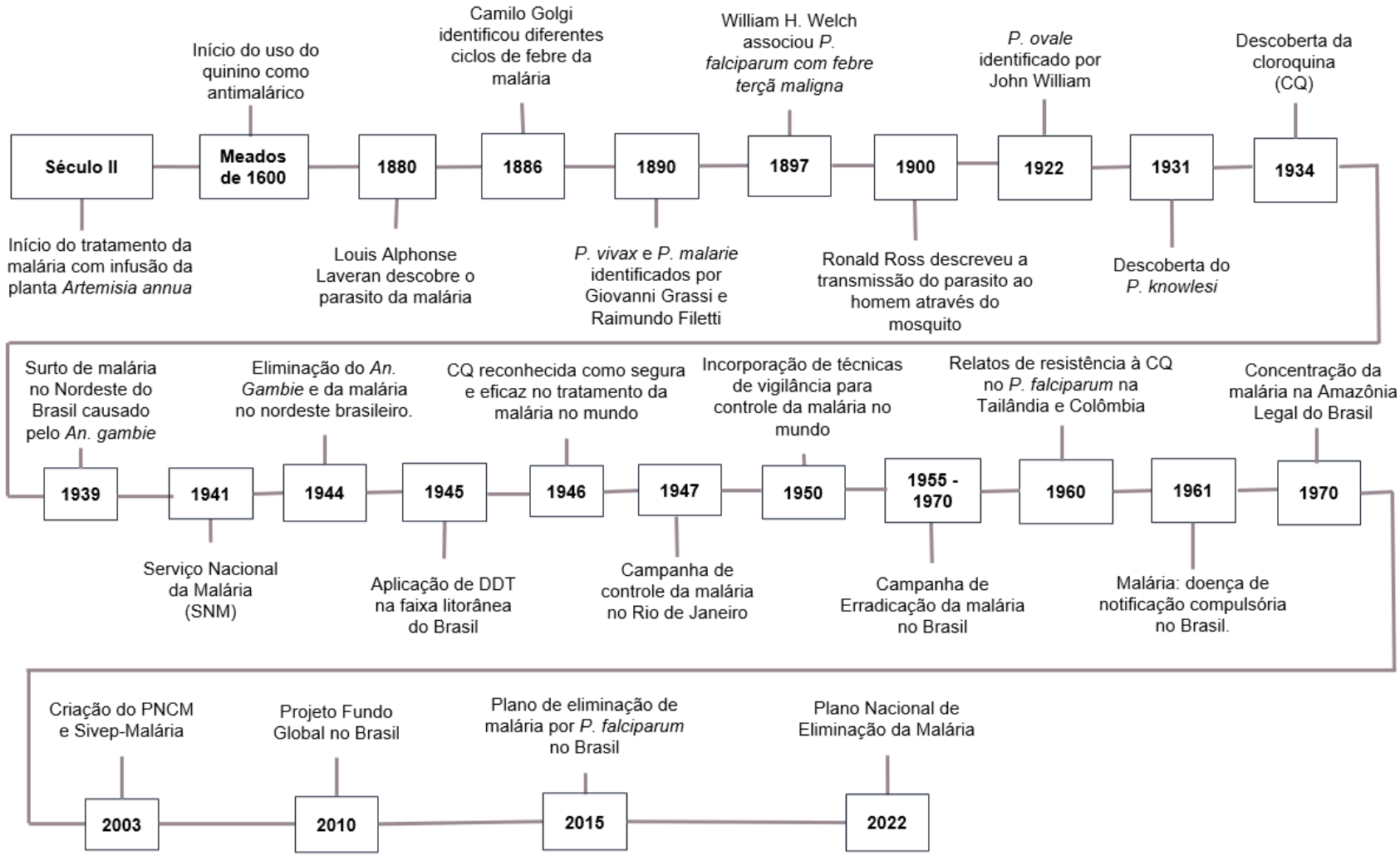
A proposta de estratificação de risco da malária leva em consideração que o risco da malária em cada município e as situações de ocupação humana e ambiental na Amazônia é de grande heterogeneidade. As localidades podem ser urbanas ou rurais, nesta última categoria se incluem as seguintes áreas especiais: garimpo, áreas indígenas, áreas de assentamento e demais categorias rurais (sítios, fazendas, seringais, entre outros) (BRASIL, 2023).

As áreas de garimpo e áreas indígenas representam um desafio adicional para a meta de eliminação, pois ambas estão frequentemente localizadas em áreas remotas e de difícil acesso, o que demanda adaptações locais para manter a continuidade das ações de prevenção e controle da malária. No que diz respeito especialmente ao garimpo, são urgentes estratégias para aumentar o acesso ao diagnóstico e tratamento oportunos (BRASIL, 2023).

Os conhecimentos adquiridos nos países que alcançaram a meta de eliminação da malária possuem o potencial de fornecer informações para fortalecer estratégias em outros países. Avanços tecnológicos, inovações em medicamentos, vacinas e controle de vetores também são consideradas ferramentas que podem fortalecer as estratégias de eliminação (WHO, 2015).

Porém, o caminho para a eliminação da malária também apresenta desafios que precisam ser enfrentados pelos programas nacionais contra malária, tais como: a) falta de financiamento e dificuldade de manter o compromisso político local; b) desempenho inadequado dos sistemas de saúde e sistemas fracos de vigilância, monitoramento e avaliação; c) risco desproporcional de malária em áreas de difícil acesso aos serviços de saúde; d) infecção assintomática ou não diagnosticada, que se tornam invisíveis para os sistemas de saúde e podem reforçar a cadeia de transmissão da doença; e) surgimento de resistência do vetor aos inseticidas e; f) surgimento de resistência do parasito aos antimaláricos (WHO, 2015).

Figura 2. Linha do tempo dos marcos históricos da malária e estratégias de eliminação da malária no Brasil



2.2 BIOLOGIA DOS PARASITOS DA MALÁRIA

A malária é causada por protozoários do gênero *Plasmodium* transmitidos ao homem pelas fêmeas infectadas de mosquitos do gênero *Anopheles*. Somente sete de aproximadamente 100 espécies desses protozoários são responsáveis por infectar seres humanos: *P. falciparum*; *P. vivax*; *P. ovale curtisi*, *P. ovale wallikeri*, *P. malariae*, *P. knowlesi*, *P. cynomolgi* e *P. simium* (FRANÇA, *et. al*., 2008; BYKERSMA, 2021; BRASIL *et. al.,* 2017; MAHITTIKORN *et. al.,* 2021).

Pela taxonomia clássica os parasitos exclusivamente humanos da malária foram divididos em dois subgêneros: a) o subgênero *Plasmodium*, incluindo *P. vivax, P. malariae* e *P. ovale* e; b) subgênero *Laverania*, apenas com o *P. falciparum*. Por sua vez, a filogenia genômica mostrou que esses dois subgêneros refletem grupos monofiléticos que se originaram na África e que o subgênero *Plasmodium* envolve espécies do Sudeste Asiático que provavelmente resultaram de radiações adaptativas de múltiplas espécies de uma única linhagem ancestral, evento que deu origem ao *P. vivax* (ESCALANTE, *et. al.,* 2022).

Há muitas diferenças entre a biologia das espécies de *Plasmodium* que causam malária em humanos, um exemplo é a idade dos glóbulos vermelhos que serão invadidos. O *P. falciparum* invade hemácias de qualquer idade. Por sua vez, o *P. vivax* e o *P. ovale* invadem somente os glóbulos vermelhos jovens ou reticulócitos. Ao contrário, o *P. malariae* invade glóbulos vermelhos velhos (NEVEU, *et. al.,* 2021; HANG *et. al.,* 2021).

Outra diferença é o tempo de produção de gametócitos (forma infectante do parasito para o vetor) e da sua vida útil, que são componentes fundamentais na cadeia de transmissão da doença. O *P. falciparum* passa por cinco estágios de desenvolvimento em 9 a 12 dias e tem o tempo de maturação mais longo, permanecendo infeccioso por vários dias em comparação com outros parasitos da malária humana. No *P. vivax* a produção de gametócitos pode começar com a primeira geração de merozoítos e podem ser detectados dentro de 3 dias após a observação dos primeiros parasitas assexuados (BOUSEMA*, et. al*, 2011). Assim, diagnóstico e tratamento oportunos (em até 48 horas) é uma importante estratégia de controle e tem grande impacto para quebrar a cadeia de transmissão da malária (BRAGA, *et. al.,* 2007).

O *P. vivax* e o *P. ovale* desenvolvem estágios dormentes ou hipnozoítos (o grego *hypnos*, sono) que causam recaídas tardias da doença após a infecção primária, que ocorrem após períodos variáveis de incubação, em geral nos seis primeiros meses após o tratamento, mesmo sem nova picada do mosquito ou ida do indivíduo à área endêmica. Tais estágios não são encontrados em *P. falciparum* ou *P. malariae* (BRAGA *et. al.,* 2007). Os eritrócitos infectados por *P. falciparum* podem aderir ao endotélio de capilares e vênulas, processo

conhecido como sequestro, o qual está ligado a apresentações clínicas graves da doença, como a malária cerebral (FRANÇA *et. al.,* 2008; MAXON *et. al,* 2020).

O ciclo biológico dos plasmódios em mamíferos compreende dois hospedeiros, o mosquito do gênero *Anopheles*, no qual ocorre a fase sexuada do ciclo, e o vertebrado (homem e/ou macaco), no qual ocorre a fase assexuada, inicialmente no fígado e, posteriormente, nas hemácias. O ciclo no humano inicia-se quando a fêmea infectada do anofelino, durante o repasto sanguíneo, inocula juntamente com a sua saliva, os esporozoítos (forma infectante do parasito para o homem) que estavam acumulados nas suas glândulas salivares. Aproximadamente 15 a 200 esporozoítos são inoculados sob a pele do hospedeiro, através da corrente sanguínea os esporozoítos chegam aos hepatócitos iniciando a primeira fase do ciclo, denominada fase pré-eritrocítica ou tissular, na qual se diferenciam em trofozoítos pré-eritrocíticos, que se multiplicam por reprodução assexuada do tipo esquizogonia, originando os esquizontes teciduais. Posteriormente, milhares de merozoítos irão romper os hepatócitos, cair na corrente sanguínea e invadir os eritrócitos, dando início à segunda fase do ciclo, chamada de esquizogonia sanguínea. É na fase sanguínea que aparecem os sintomas da malária (Figura 3) (BRAGA *et. al.,* 2007).

A fase pré-eritrocítica dura aproximadamente uma semana para o *P. falciparum* e *P. vivax* e cerca de duas semanas para o *P. malariae*. Nas infecções por *P. vivax* algumas populações de esporozoítos se desenvolvem rapidamente no fígado e caem na corrente sanguínea, enquanto outras viram hipnozoítos e ficam em estado de latência nos hepatócitos (BRAGA *et. al.,* 2007).

No ciclo eritrocítico as diferentes espécies de plasmódio possuem preferencias por estágios específicos de maturação dos eritrócitos o que implica diretamente nas respectivas parasitemias. O desenvolvimento intra-eritrocítico do plasmódio ocorre por esquizogonia, resultando em merozoítos que irão romper as hemácias e invadir novos eritrócitos, dando início a ciclos repetitivos de multiplicação eritrocitária. Esses ciclos repetem-se, geralmente, a cada 48 horas nas infecções por *P. vivax, P. ovale* e *P. falciparum* e a cada 72 horas nas infecções por *P. malariae*. Depois de algumas gerações de merozoítos nas hemácias alguns se diferenciam em formas sexuadas, os gametócitos (BRASIL, 2022; BRAGA *et. al.,* 2007).

Durante o repasto sanguíneo, a fêmea do anofelino ingere as formas sanguíneas do parasito presentes no sangue do hospedeiro, mas somente os gametócitos (forma infectante para o mosquito) serão capazes de evoluir no inseto, dando origem ao ciclo sexuado ou esporogônico. No intestino médio do mosquito o gametócito feminino transforma-se em macrogameta e o masculino, por um processo denominado exflagelação, dá origem a oito microgametas. Após 20 a 30 minutos um microgameta fecundará um macrogameta, formando

o ovo ou zigoto. Dentro de 24 horas após a fecundação, o zigoto passa ao estágio de oocineto, que se movimenta por contrações do corpo e atravessa a membrana que envolve o alimento e atinge a parede do intestino médio, na qual se encista na camada epitelial do órgão e passando a ser chamado oocisto. Inicia-se então o processo de divisão esporogônica e, após um período de nove a 14 dias, ocorre a ruptura da parede do oocisto, sendo liberados os esporozoítos que serão disseminados por todo o corpo do inseto através da hemolinfa até atingir as células das glândulas salivares. Estes esporozoítos atingirão o canal central da glândula e ingressarão no ducto salivar para serem injetados no hospedeiro vertebrado, juntamente com a saliva, durante o repasto sanguíneo infectante (BRAGA *et. al.,* 2007).

2.3 ASPECTOS CLÍNICOS DA MALÁRIA

O período de incubação da malária pode variar de acordo com o parasito causador da doença: *P. falciparum* (8 a 12 dias); *P. vivax* (13 a 17 dias); e *P. malarie* (18 a 30 dias) (BRASIL, 2020).

O espectro clínico da malária pode variar de manifestações assintomáticas, com poucos sintomas até quadros grave e letal. Os sintomas clássicos da malária são febre, calafrios, cefaleia, vômito, anorexia, fadiga, diarreia e anemia. Mas nem sempre o quadro clínico é característico da doença. Assim se o indivíduo esteve em área de risco para transmissão de malária, deve procurar uma unidade de saúde (FRANÇA *et. al,* 2008; BRASIL, 2020).

Se não tratada oportunamente a doença pode evoluir para edema pulmonar, complicações renais, icterícia e obstrução de vasos sanguíneos no cérebro (nos casos graves da doença), situação que poderá levar à morte do indivíduo (FRANÇA *et. al*., 2008).

As manifestações clínicas de malária grave são: dor abdominal intensa (ruptura de baço, mais frequente em *P. vivax*); mucosas amareladas (icterícia); mucosas muito hipocoradas (avaliada fora do ataque paroxístico febril); redução do volume de urina a menos de 400 mL em 24 horas; vômitos persistentes que impeçam a tomada da medicação por via oral; qualquer tipo de sangramento; falta de ar (avaliado fora do ataque paroxístico febril); extremidades azuladas (cianose); aumento da frequência cardíaca; convulsão ou desorientação e prostração (BRASIL, 2020).

A mortalidade por malária grave é de 10% a 20%, mesmo com o tratamento ideal, e atinge 100% se não for tratada. Além disso, a malária grave é mais provável em mulheres

grávidas no segundo e terceiro trimestres em comparação com outros adultos. A mortalidade em gestantes é de aproximadamente 50% (PLEWES, 2019).

2.4 TRATAMENTO DA MALÁRIA

A ação dos fármacos antimaláricos visa atingir o parasito em pontos específicos de seu ciclo evolutivo (Figura 3), que podem ser didaticamente resumidos em:

a) Interrupção da esquizogonia eritrocítica (sangue), responsável pela patogenia e manifestações clínicas da infecção. As drogas com ação esquizonticidas sanguíneas (formas assexuadas) são: cloroquina, mefloquina, quinino, derivados de artemisinina, lumefantrina e clindamicina.

b) Destruição de formas latentes do parasito no ciclo tecidual (hipnozoítos) das espécies *P. vivax* e *P. ovale*, evitando assim as recaídas. Atualmente, a primaquina é o único medicamento disponível no Brasil que consegue atingir a forma hepática. A tafenoquina deverá ser implementada em breve no nosso país por ser dose única.

c) Interrupção da transmissão do parasito, pelo uso de drogas que impedem o desenvolvimento de formas sexuadas dos parasitos (gametócitos). As drogas gametocidas são: primaquina e derivados da artemisinina (BRASIL, 2020; SIQUEIRA *et. al.,* 2020).

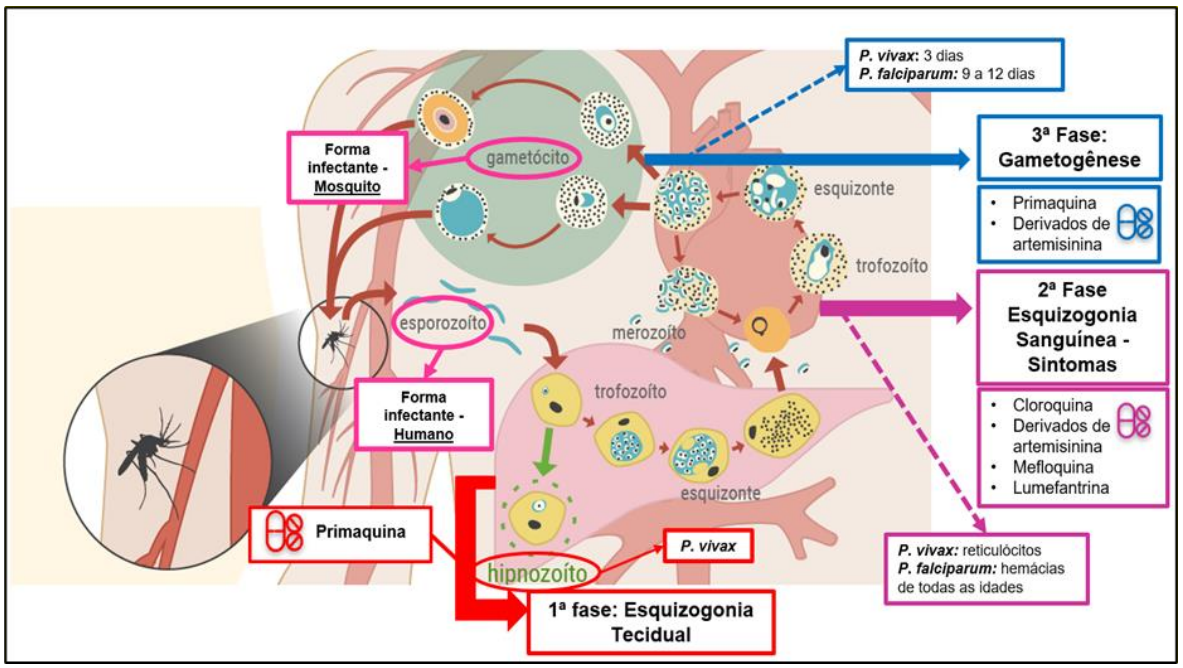


Figura 3. Ciclo biológico do *Plasmodium* no hospedeiro humano e local de ação dos fármacos antimaláricos (Fonte: Adaptado de Siqueira, A *et. al.,* 2020)

No Brasil, o Guia de Tratamento da Malária, recomenda para o tratamento da doença não complicada pelo *P. vivax* e *P. ovale*, a combinação da cloroquina por três dias (10mg/kg no dia 1 e 7,5 mg/kg nos dias 2 e 3) e primaquina (0,5/kg/dia, por sete dias), com o objetivo de curar tanto a forma sanguínea, quanto a forma hepática (cura radical), evitando assim a recrudescência e recaída, respectivamente. A primaquina é contraindicada para gestantes e crianças com menos de 6 meses de idade. Para as gestantes é indicado o tratamento com cloroquina por três dias e cloroquina profilática (5mg/kg/dose, até no máximo dois comprimidos) semanalmente até um mês de aleitamento, para evitar recaídas. A malária por *P. malarie* é semelhando ao tratamento da malária por *P. vivax* (cloroquina por três dias), todavia, não há necessidade de uso de primaquina (BRASIL, 2020).

Em caso de recorrência por malária por *P. vivax* entre os dias 5 e 60, após o início do tratamento, há suspeita de falha da cloroquina ou da primaquina, ou de ambos. Nesses casos, o tratamento recomendado é o uso de artemeter/lumefantrina ou artesunato/mefloquina durante três dias, e primaquina (0,5 mg/kg/dia) por 14 dias, o que torna o esquema mais eficaz contra os hipnozoítos (BRASIL, 2020).

Segundo a OMS, a malária por *P. falciparum* deve ser tratada com derivado de artemisinina (ACT). No Brasil há a disponibilidade de artesunato 25 mg + mefloquina 50 mg e artemeter 20 mg + lumefantrina (120 mg), sendo a dose definida de acordo com o peso do paciente. Porém, a primeira combinação possui a vantagem de ser somente uma dose diária por três dias, além da maior meia vida da mefloquina permitir profilaxia pós-tratamento, sem risco aparente de selecionar parasitos resistentes. A segunda combinação é recomendada em duas doses diárias por três dias (por exemplo, após café da manhã e após o jantar). A primaquina deve ser administrada em dose única no primeiro dia de tratamento, na dose de 0,5 mg/kg, o que garante a eliminação de gametócitos maduros no sangue, quebrando a cadeia de transmissão. Gestantes e crianças menores de 6 meses recomenda-se o uso de ACT, sem a primaquina (BRASIL, 2020).

Na infecção mista por *P. falciparum* e *P. vivax* (ou *P. ovale*), o tratamento deve incluir, além do ACT, à primaquina por sete dias (BRASIL, 2020).

Malária grave em adultos e crianças (incluindo menores de 6 meses, gestantes em todos os trimestres gestacionais e período de amamentação) devem ser tratados com artesunato intravenoso ou intramuscular, por no mínimo 24 horas, e no máximo sete dias, até que o paciente possa tomar medicação oral preconizada de acordo com a espécie parasitária, respeitando as restrições de primaquina (BRASIL, 2020).

2.5 RESISTÊNCIA AOS ANTIMALÁRICOS

O aumento da resistência aos antimaláricos é um enorme obstáculo para a meta de eliminação da malária. As mutações que conferem resistência podem surgir nos estágio de reprodução sexuado do parasito no vetor, no qual ocorrer diploidia e meise. Mas também pode ocorrer nas fases de reprodução assexuada que ocorrem nos hepatócitos e nos eritrócitos do hospedeiro. Porém, os parasitos resistentes nem maior probabilidade de surgir em altos níveis de parasitemias das formas assexuadas do parasito e subdoses de medicamentos (PONGTAVORNPINYO *et. al.,* 2009).

A informação sobre a eficácia do tratamento para malária e o monitoramento do surgimento de parasitos resistentes aos antimaláricos são essenciais para manter o progresso em direção à eliminação. Além disso, servem para orientar a política nacional de tratamento antimalárico em países onde a malária é endémica. Esses estudos devem ser projetados para antimaláricos recomendados contra *P. falciparum* e *P. vivax* (OPAS, 2018).

Como estratégia para combater a ameaça de resistência aos antimaláricos, a OMS conta com uma rede sentinela de países endêmicos para malária e de outros parceiros globais para reforçar a vigilância da eficácia dos medicamentos antimaláricos e resistência, e garantir que os tratamentos mais eficazes sejam selecionados para a política nacional de tratamento (WHO, 2023)

A eficácia dos antimaláricos pode ser monitorada através de estudos de eficácia terapêutica, que são considerados o padrão ouro para determinar políticas nacionais de tratamento. Além desta, a resistência dos parasitos da malária aos medicamentos pode ser avaliada através de outras ferramentas. Para alguns medicamentos, mutações associadas à redução da sensibilidade já foram identificadas (WHO, 2023).

O *P. falciparum* multirresistente é uma ameaça para a eliminação da malária, segundo a OMS este parasito é caracterizado pela resistência a mais de dois compostos antimaláricos de diferentes classes químicas (WHO, 2018). Nesse contexto, a resistência à cloroquina foi relatada em 1960 (PAYNE, 1987) e a sulfadoxina-pirimetamina em 1970 (Cortese JF, et. al., 2002). Para evitar a emergência de *P. falciparum* resistente à artemisinina a OMS recomendou em 2001 as terapias combinadas à base de artemisinina (ACTs) como tratamento de primeira linha para a malária não complicada por *P. falciparum*, assim como para malária por *P. vivax* resistente à cloroquina (OMS). Porém mutações relacionadas com resistência à artemisinina foram identificadas no Sudeste Asiático (Straimer J., *et. al.,* 2015) e na América do Sul (Chenet SM, *et. al,* 2016) (Figura 4).

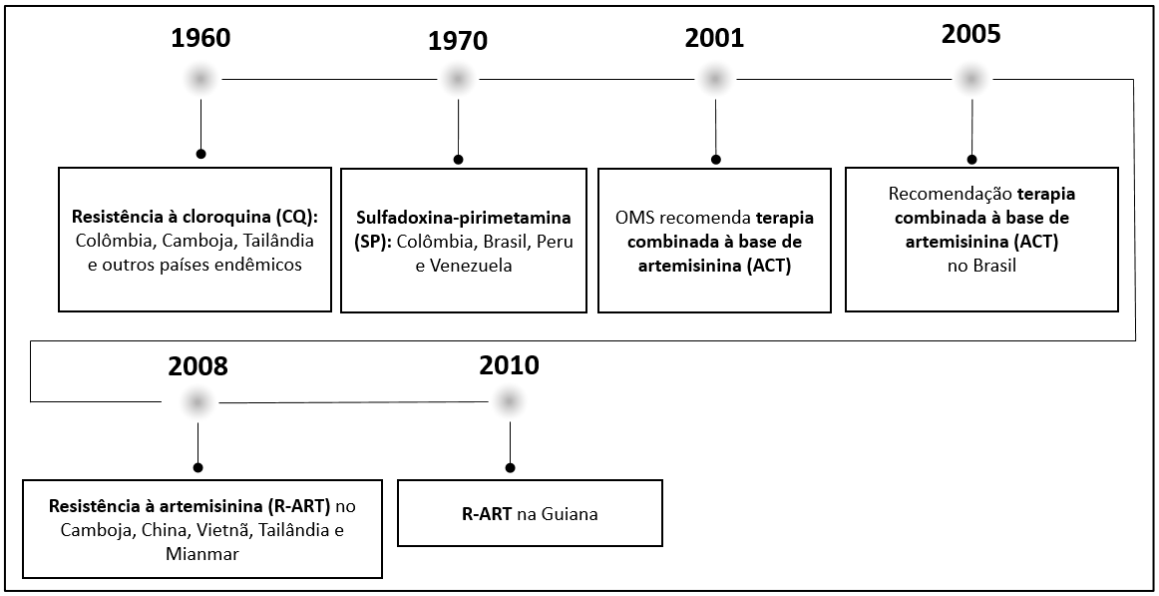


Figura 4. Linha do tempo da detecção de resistência à antimaláricos no *P. falciparum*

A resistência à artemisinina é definida como a eliminação retardada do parasito após tratamento com monoterapia com artesunato ou AC representando, portanto, a resistência parcial (WHO, 2018). Desde 2014, a vigilância global de resistência à artemisinina no *P. falciparum* é realizada pelo monitoramento de mutações no domínio de hélice do *Kelch* 13 (*pfk13*) (WHO, 2018; Pacheco MA, *et. al.,* 2020).

A validação de uma mutação *pfK13* como marcador de resistência à artemisinina requer que a mutação seja relacionada com a depuração lenta do parasito e com a sensibilidade reduzida ao medicamento em estudos *in vitro*. Por outro lado, a mutação é rotulada como marcador candidato/associado quando houver apenas documentação de eliminação tardia, sem validação com estudos *in vitro* (Quadro 1).

Tabela 1. Mutações candidatas/associadas e validadas da hélice *K13*

Mutações no gene pfk13 validadas para ART-R em estudo *in vitro* e *in vivo*	**Mutações no gene *pfk13* associadas com ART-R**
C580**Y**, R561**H**, R539**T**, I543**T**, P553**L**, M476**I**, N458**Y**, Y493**H**, F446**I**, P574**L,** P441**L**, T449**A**, C469**F/Y**, A481**V**, R515**K**, P527**H**, N537**I/D**, G538**V**, V568**G**, R622**I** e A678**V**	K479**I**, G533**A**, R575**K**, M579**I**, D584**V**, P667**T**, F673**I** e H719**N**

Fonte: WHO, 2018.

A Figura 5 mostra a panorâmica atual de resistência à artemisinina no *P. falciparum*, fornecido pela Rede Mundial de Resistência aos Antimaláricos / WorldWide Antimalarial Resistance Network (WWARN) que consolida dados provenientes de estudos clínicos e laboratoriais (WWARN, 2024).

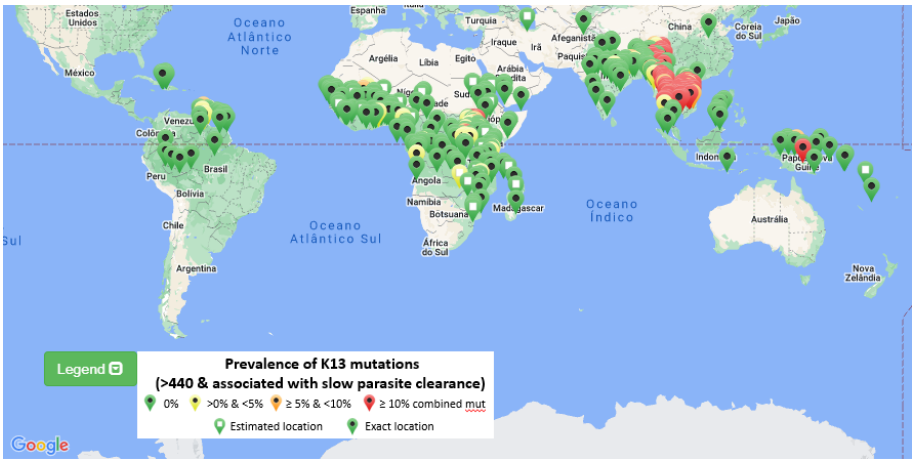


Figura 5. Prevalência de mutações na região em hélice do gene *Kelch 13* associadas a resistência à artemisinina

Na malária causada pelo *P. vivax*, a resistência à cloroquina é um problema crescente. Porém, diferente do *P. falciparum,* ainda não há marcador molecular validado para monitorar resistência à cloroquina nesse parasito, provavelmente, pela impossibilidade de experimentos *in vitro* pela ausência de cultivo contínuo para o *P. vivax* (MENARD *et. al.,* 2017)

Acredita-se que os mecanismos de ação e de resistência aos antimaláricos sejam semelhantes no *P. vivax* e *P. falciparum*. Assim, no *P. vivax* os estudos se baseiam nos genes ortólogos (genes que possuem a mesma função e uma origem comum) *pfcrt*, *pfmdr1, pfdhs e pfdhpr* do *P. falciparum* (SÁNCHES, 2010; SILVA, *et. al.,* 2018). A Figura 6 mostra visão global de ensaios clínicos voltados para a identificação dos tipos de resistência ao *P. vivax* (WWARN, 2024).

Figura 6. Ensaios clínicos voltados para a detecção de resistência à cloroquina em parasitos do *P. vivax*

O gene *pvcrt-o* foi caracterizado há quase duas décadas (Nomura *et. al.,* 2001). O polimorfismo de sequência é praticamente limitado ao *locus pvcrt-o* com a maioria dos polimorfismos com frequências < 5% (Ferreira, 2020). A inserção de Lisina (AAG) no primeiro éxon (aminoácido 10) chamada de inserção K10 estaria associada a uma redução na metade da concentração inibitória máxima de CQ (IC50) e foi assim identificada como possível marcador molecular de RCQ no *P. vivax* (Melo, 2014; Joy *et. al.,* 2018) (Figura 7).

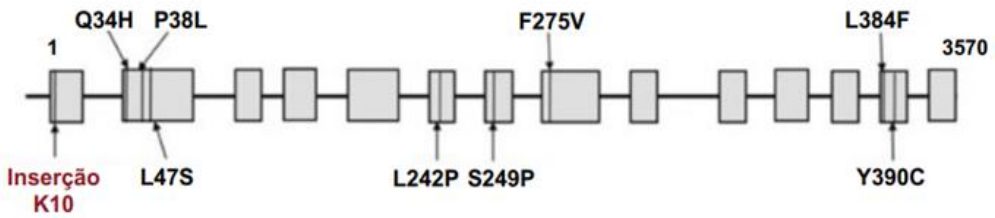


Figura 7. Substituições de aminoácidos e Inserção de Lisina (AAG) no primeiro éxon (aminoácido 10) do gene *pvcrt-o* (Adaptado de Cheong *et al.,* 2021)

O *pvmdr1* foi caracterizado depois em 2005 (Brega *et al.,* 2005). As mutações de aminoácidos Tirosina (Y) substituída por Fenilalanina (F) na posição 976 (Y976**F**), Fenilalanina (F) substituída por Leucina (L) na posição 1076 (F1076**L**) e Treonina (T) substituída por Metionina (M) na posição 958 (T958**M**) foram relacionadas com a RCQ (SUWANAEUCK, 2008; JOY *et al,* 2018; CHEONG, 2020) (Figura 8).

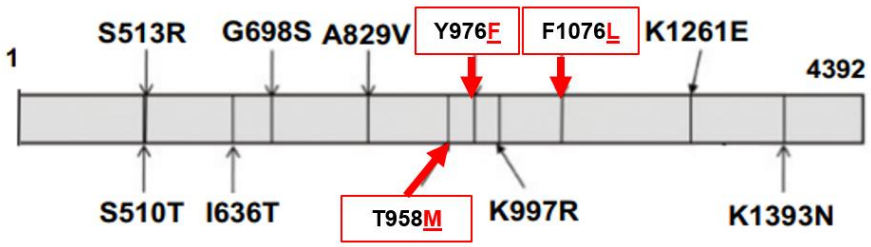


Figura 8. Substituições de aminoácidos no gene *pvmdr1* candidatas a marcadores moleculares de resistência do *P.vivax* à cloroquina (Adaptado de Cheong *et al.,* 2021)

3. CAPÍTULO 1

O presente capítulo refere-se ao primeiro objetivo específico proposto nesta tese de doutorado: “Descrever o perfil epidemiológico da malária no estado de Roraima”. Trata-se de análise da malária, no período de 2010 a 2020, no banco de dados do Sistema de Informação de Vigilância Epidemiológica da Malária (Sivep-Malária), Sistema de Informação Hospitalar (SIH) e Sistema de Informação de Mortalidade (SIM), para identificar “quem”, “quando” e “onde” as pessoas adoecem por malária em Roraima.

Este artigo foi publicado na Revista *Malaria Journal*, classificação Qualis A2 em Biotecnologia, no dia 11 de novembro de 2022, com o título: *“Gold miners augment malaria transmission in indigenous territories of Roraima state, Brazil”,* DOI 10.1186/s12936-022-04381-6. Disponível em: https://pubmed.ncbi.nlm.nih.gov/36447220/

CASE STUDY Open Access

Gold miners augment malaria transmission in indigenous territories of Roraima state, Brazil

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Abstract

Background: Endemic malaria is present in all 15 municipalities of Roraima state, Brazilian Amazonia. Knowledge of epidemiological data of specific populations can guide health policies to formulate effective strategies for integrated control of health-disease care. This study aims to ascertain when, where and who fell ill with malaria in Roraima state from 2010 to 2020.

Methods: This descriptive study was based on statistical secondary surveillance data through the analysis of relationships underlying numbers of cases, hospitalizations and deaths using the Malaria Epidemiological Surveillance Information System, Mortality Information System and Hospitalization Information System.

Results: From 2010 to 2020, there were 138,504 autochthonous cases, 26,158 Venezuelan imported cases, 3765 hospitalizations, and 77 deaths from malaria reported in Roraima. Annual parasitic incidence and the number of hospitalizations showed impressive changes over the period, but without significantly correlating with number of deaths. The proportion of *Plasmodium falciparum* infections had significant shifts throughout this study. Malaria prevalence in indigenous and mining areas has been increasing since 2014.

Conclusion: The presence of miners in indigenous areas is a reality that has been contributing to the increase of malaria cases in Roraima. The need to implement health policies that also meet this contingent is reinforced.

Keywords: Brazilian Amazon, Mining, Malaria, *Plasmodium*, Roraima, Indigenous

Background

Malaria is an acute febrile illness caused by the protozoan parasites of the *Plasmodium* genus. Two species are of special relevance to public health: *Plasmodium vivax*, the most spread species worldwide, is predominant in Brazil, while *Plasmodium falciparum*, which predominates in the global setting, is associated with severe malaria, complications of pregnancy and accounts for almost the totality of deaths. In turn, *P. vivax* generally induces uncomplicated malaria, although relapse occurrences may show significant associated morbidity [1, 2]. Transmission occurs through a bite from an infective female *Anopheles* mosquito. In 2020, 241 million cases were estimated globally, and 627,000 deaths were caused by malaria. This represents about 14 million more cases and 69,000 more deaths in 2020 than in 2019 [3]. In Brazil 145,188 cases of malaria were reported in 2020, representing a reduction of 7.8% when compared to 157,452 cases notified in 2019 [4].

Malaria still stands out as a major public health problem in Brazil and around the world, exerting a substantial impact on hospitalizations, complications of childbirth, premature mortality, and work and school absence, despite effective treatment currently available. Besides, malaria is closely related to poverty, exerting a negative impact on the quality of life and socio-economic condi- tion

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of people leaving in endemic areas [5, 6].

Brazil has a successful background in malaria eradication campaigns from the 1950s and 1960s. However, despite several decades of effort to control infections, the prevalence of malaria in Brazil is still high, with 99% of cases located in the so-called Legal Amazon Region, which comprises the states of Acre, Amazonas, Amapá, Pará, Rondônia, Roraima, Tocantins, Mato Grosso, and Maranhão [5].

During the 1950s, the malaria eradication programme helped to incorporate techniques of epidemiological surveillance to control transmissible diseases throughout the world [7]. The third pillar of the world strategy against malaria 2016–2030 intends to transform malaria surveillance into a basic intervention in the countries where it is endemic [8]. Based on this scenario, Brazil's Ministry of Health aligned with the Sustained Development Objectives (SDO) of the United Nations launched in 2015, the Plan for Malaria Elimination until 2030 [4].

A crucial step for descriptive epidemiology in identifying the general pattern of an infection and risk groups is the description of the disease in three basic categories: temporal distribution, spatial distribution and distribution according to personal attributes [9].

Roraima is the northernmost state in Brazil and has unique geographic, social and environmental characteristics that make the goal of eliminating malaria even more challenging in its territory. In particular, the movement of people on the international land borders with Venezuela and Guyana increases the risk of imported malaria cases, which are intensified by humanitarian crises, such as the one that occurred recently in Venezuela [10, 11]. In addition, there is currently a recurring threat of illegal gold and diamond mining in the Yanomami and Raposa Serra do Sol Brazilian indigenous areas, respectively. The first is located in Roraima and Amazonas states, along the Venezuela border; it is estimated that there are 20,000 illegal miners in this Yanomami indigenous territory. The second is located on the edge of the crystalline shield of the Guianas, situated along the international political border that is also constantly crossed by miners in both directions [12, 13]. In these indigenous areas, the way environment is altered by open-pit mining, digging hollows and benches throughout the landscape, produces a multiplicity of mosquito breeding places. Besides, shirtless gold miners working during periods of high vectorial activity together with the presence of asymptomatic disease carriers has been contributing to the high incidence of malaria in mining areas. In addition, the great mobility of gold miners also constitutes another risk factor, as it facilitates the renewal of susceptible populations, due to the iterative flow of infected/non-infected people [9].

A set of features, such as landscapes, human presence and vector distribution, intervenes in the epidemiology of malaria. In Northern Brazilian Amazonia, Roraima's landscapes include 83% of forests and 17% of savannahs. The greater risk for malaria is associated with the prairies, where dense ombrophile forest is the dominant vegetation and in the outskirts of the alluvial forest, followed by a minor risk in the savannah patches. Endemic malaria is present in all the 15 municipalities of the Roraima state, emphasizing the importance of surveillance strategies in all of them [14].

In view of the above, an investigation was carried out for guiding at state level (where problems and solutions really happen) the formulation of effective strategies in the integrated health-disease-care control of this population. To this end, secondary data sources were used because such methodology offers lower costs and faster results when compared to primary data collection, and also because these data are available in government sources, thus offering large arrays of data on a national scale.

The main proposal is to analyse malaria cases in Roraima from 2010 to 2020, to find out when, where and who gets sick from malaria. This period precedes the decade that planned to eliminate vivax malaria by 2035 and falciparum malaria by 2030 in Brazil.

Methods

An ecological time-series study was based on secondary data concerning the number of cases, hospitalizations and deaths from malaria that occurred in Roraima from 2010 to 2020.

With a land area of 223,644.527 sq km, the state of Roraima is at the northern tip of Brazil, and borders Venezuela, Guyana and the Brazilian states of Pará and Amazonas. According to the Brazilian Institute of Geog- raphy and Statistics (IBGE), the state's population was estimated at 631,181 inhabitants in 2020. Roraima has 15 municipalities and 104,509,087 sq km of its territory con- sisting of two main indigenous reservations: the Special Indigenous Health District Yanomami (DSEI-Yanomami) covering five municipalities: Alto Alegre, Amajari, Caracaraí, Iracema, and Mucajaí, and the Special Indigenous Health District Leste (DSEI-Leste) comprising 11 municipalities: Boa Vista, Alto Alegre, Amajari, Bonfim, Cantá, Normandia, Pacaraima, Uiramutã, São João da Baliza, São Luiz do Anauá, and Caroebe. Alto Alegre and Amajari are shared by two indigenous districts. The municipality of Rorainópolis is the only one that has no indigenous area within its territory (Fig. 1).

Malaria is a notifiable disease in Brazil, and its diagnosis and treatment are exclusively available through the Brazilian federal healthcare net of *Sistema Único de*

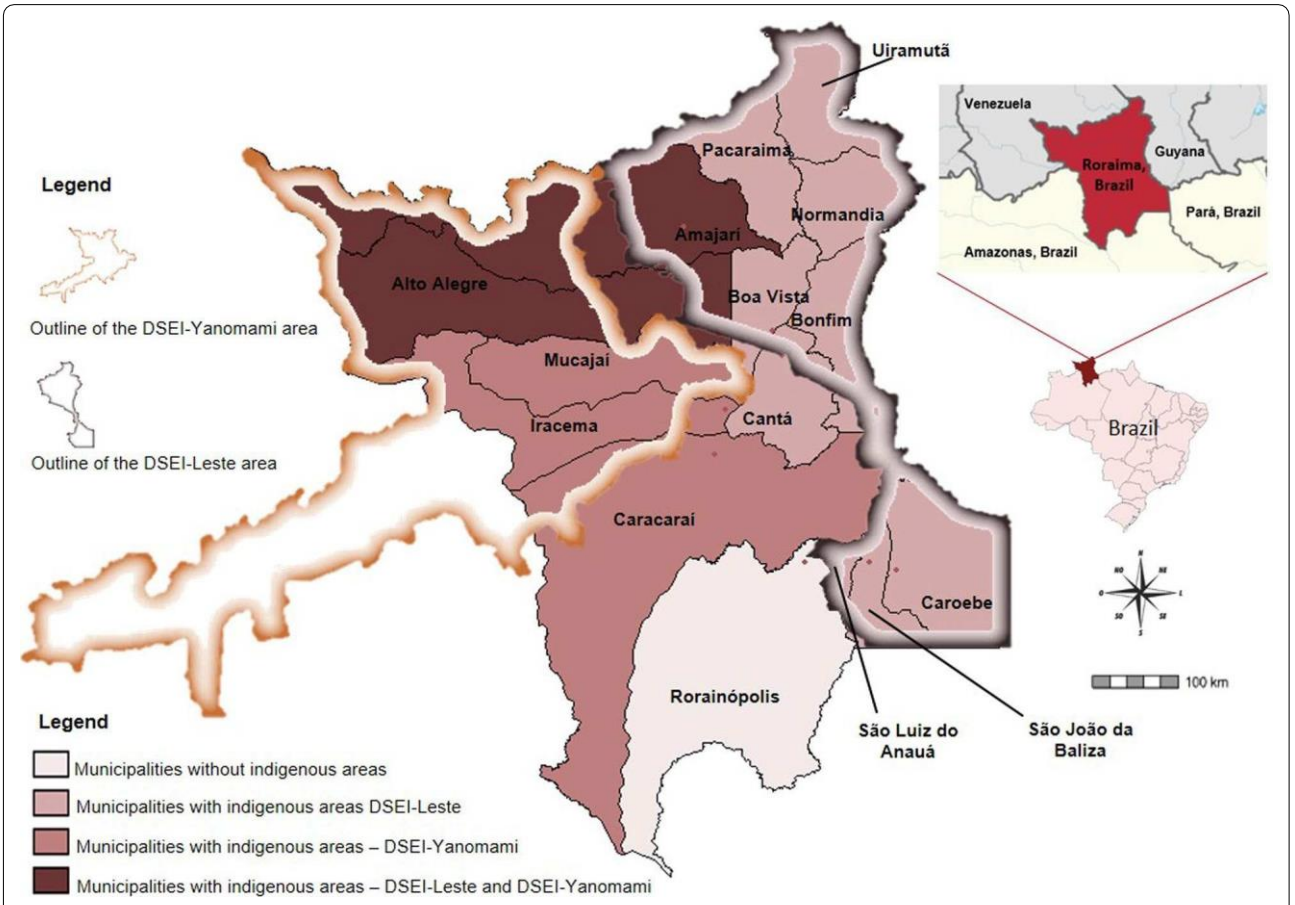

Fig. 1 The 15 municipalities of Roraima state showing Leste (DSEI-Leste) and Yanomami (DSEI-Yanomani) Special Indigenous Health Districts (DSEIs)

Saúde (SUS)/Health Unic System, including the Health Units of the 15 municipalities of Roraima and indigenous areas. In these municipalities, health facilities are distributed in 253 laboratories: Alto Alegre (36), Amajarí (24), Boa Vista (34), Bonfim (15), Cantá (21), Caracaraí (13), Caroebe (06), Iracema (8), Mucajaí (14), Normandia (17), Pacaraima (21), Rorainópolis (10), São João da Baliza (4), São Luiz (4), and Uiramutã (26). The gold standard for malaria diagnosis is the microscopic examination of a thick blood smear (TBS). The great majority of the cases were diagnosed by microscopy. Rapid diagnostic tests (RDTs) are only realized when the malaria diagnosis by TBS could not be realized in a period of fewer than 24 h and/or in remote places where there is no possibility of installing a microscopy service.

The malaria cases were obtained through Malaria Epidemiological Surveillance Information System (SIVEP-Malaria). The annual parasitic incidence (API) was used to estimate the risk of malaria infection within a given population in a given period of time. The degree of risk of API is expressed as very low risk (< 1 case), low risk (< 10 cases), medium risk (10–50 cases), or high risk (> 50 cases).

The data on mortality and hospitalizations due to malaria were collected in Mortality Information System (SIM) and Hospitalization Information System (SIH), respectively. Both were identified on those systems through codes B50 to B54, according to International Disease Classification (CID-10). The variables concern- ing parasite species were described as: a) *P vivax*: which could also include results of *Plasmodium malariae*; b) *P. falciparum:* which could also include mixed plasmodial infections; and, c) unspecified malaria when the parasitic species was not identified in the registry.

The following variables were correlated for temporal analysis: site of infection (non-indigenous area, DSEI- Leste and DSEI-Yanomami), API, plasmodial species (*P. falciparum* and *P. vivax*), special areas of infection (indigenous, settlement, urban, mining), Venezuelan imported cases, age group, gender, and race. Each variable, numerical or qualitative, was investigated in the 15 municipali- ties of Roraima in each year of the investigation period (11 years), resulting in 165 statistical units.

Regarding the temporal trend of hospitalizations throughout the study period, the following variables in each year of the study were considered: parasitic species, gender, race, and age group. In relation to deaths, the comparable variables were parasitic species, gender, race, age group, and nationality.

The statistical analysis was performed through the program R 3.6.3 (R Core Team 2020). A linear model estimated by Generalized Least Squares (GLS) was applied to control the temporal autocorrelation among observations repeated over time (assuming an autoregressive structure of first-order at the municipality level). All the depend- ent variables were analysed in their original scales, except in falciparum malaria cases which were surveyed in a log scale in order to incorporate the high heteroscedasticity observed in this relation. Graphics were shown only when the associations were statistically significant ($p < 0.05$).

Results

During the period 2010 to 2020, 167,968 cases of malaria were notified and 138,504 of them were identified as autochthonous (database consulted on 29 March, 2022). Among the autochthonous cases, 35% (58,597) occurred in indigenous areas: 19,102 in DSEI-Leste and 39,495 in DSEI-Yanomami lands. In the same period, 3,765 hospitalizations and 77 deaths from malaria were reported in Roraima (Table 1).

The API presented a significant shift during the decade under study ($p < 0.001$). In this period, the highest API was recorded in 2010 (42.56/1000 inhabitants), fol- lowed by a reduction from 2011 (26.12/1000 inhabitants) to 2013 (10.10/1000 inhabitants), and a new rising trend from 2014 on (11.72/1,000 inhabitants), which lasted until 2020 (46.55/1000 inhabitants) (Table 1).

The non-indigenous and the DSEI-Leste indigenous areas of Roraima have shown an increasing number of malaria cases from 2017 on, with a rate of + 123.81 and + 143.63 in relation to 2016, respectively. DSEI-Yanomami, in turn, presented an increase in the num- ber of malaria cases from 2014 on, at a rate of + 60.26 in comparison to the preceding year (Table 1).

The number of malaria hospitalizations changed significantly over the period of the study ($p = 0.002$). The year 2010 also presented a higher frequency of hospitalizations due to malaria (490), and a decrease followed after- wards, with rates of 36.33 and 16.34% in 2011 and 2012, respectively. In 2013 there was a net increase of 11.9% in relation to the previous year, followed by a reduction of 24.65% in 2014. As of 2015, the number of hospitalizations began to get higher again, achieving 7.27%, a trend that was kept until 2018, which showed an increase of 34.7% in relation to the previous year. Nevertheless, 2019 was marked by a reduction of 3.82% in comparison to 2018.

The number of malaria deaths did not change significantly over the period 2010–2020 ($p = 0.25$). Between 2010 and 2017, fewer than six deaths per year were reported. However, in the years 2018 and 2019, 18 and 12 deaths due to malaria were registered, respectively (Table 1).

The number of Venezuelan imported cases increased significantly from 2016 ($p = 0.007$), reaching a peak in 2018 with 4478 cases. However, in 2019 and 2020 a reduction of 48.95 and 63.68% of Venezuela's imported cases was observed. The incidence of cases in urban and

Table 1 Population, malaria autochthonous cases, Roraima annual parasitic incidence (API), malaria cases in indigenous (DSL and DSY), and non-indigenous areas, hospitalizations and deaths in Roraima from 2010 to 2020

Year	Population	Autochthonous cases	API p < 0.001)	Non indigenous	DSEI-Leste	DSEI-Yanomami	Hospitalizations p = 0.002	Deaths p = 0.25
2010	451,227	19,055	42.23	13,288	2,407	3,360	490	4
2011	460,165	11,860	25.77	7,963	1,603	2,294	312	2
2012	469,524	5,923	12.61	4,375	1,076	472	261	2
2013	488,072	4,828	9.89	3,599	625	604	292	5
2014	496,936	5,713	11.5	4,145	602	966	220	2
2015	505,665	6,176	12.21	3,636	381	2,159	236	4
2016	514,229	5,716	11.12	3,024	368	2,324	254	1
2017	522,636	11,183	21.4	8,647	895	1,641	369	4
2018	576,568	18,346	31.82	11,426	3,199	3,721	497	18
2019	605,761	20,322	33.55	9,025	3,383	7,914	478	16
2020	631,181	29,382	46.55	10,779	4,563	14,040	356	19
Total		138,504	-	79,907	19,102	39,495	3,765	77

Sources: IBGE; SIVEP-MALÁRIA; SIM; SIH

rural areas as well as in settlements declined between 2010 and 2014, but it increased again from then on ($p < 0.001$). The same was true for the indigenous territories and mining areas, which showed a remarkable rise in cases from 2016 ($p = 0.007$). When comparing the number of cases that occurred in 2016 and 2020, an increase of 1,090% in indigenous and 75,576% in mining areas was observed (Table 2).

In 2010, 10 municipalities were classified as high risk (Cantá, Amajari, Alto Alegre, Caracaraí, Bonfim, Iracema, Pacaraima, São João da Baliza, Rorainópolis, Mucajaí). In the following years, the number of high-risk municipalities declined, remaining only the municipalities of Amajari and São João da Baliza. In 2017, how- ever, a new rise of cases occurred, totalling five high-risk municipalities (Cantá, Rorainópolis, Caracaraí, Iracema, Alto Alegre). In 2020, Roraima had again 11 of its municipalities included in the category of high transmission risk (Alto Alegre, Amajari, Pacaraima, Iracema, Uiramutã, Mucajaí, Cantá, Caroebe, São João da Baliza, Caracaraí. Rorainópolis), two classified as medium risk (São Luiz and Bonfim), and two as low risk (Normandia and Boa Vista) (Fig. 2).

Malaria caused by *P. vivax* was predominant during the investigation period. The proportion of *P. falciparum* cases was higher in 2011, reaching 10.67% of the occurrences; the proportion shift of cases caused by *P. falciparum* over the study period was significant ($p = 0.01$).

In terms of gender, during the decade of the research, the infection rate was greater in men than in women, but statistical analysis has pointed out that the gender ratio showed no significant shift over the period of study ($p = 0.35$). As for the age group, the cases of malaria seem to present over time a slight increasing trend in the age estimate of most people who contracted malaria, although such evidence is not clear ($p > 0.064$) (Table 3).

Plasmodium vivax had been doubtlessly the more prevalent parasite species, but even so a tendency of increase in the rate of hospitalizations due to *P. falciparum* seemed to exist over time, although without a statis- tical meaning. ($p = 0.42$).

Over the period of research, the average age group of hospitalizations ranged from 20 to 39 years ($p = 0.006$). In terms of gender, there was no discrepancy among the hospitalization cases over the years ($p = 0.71$) (Table 4). The correlation between deaths by *P. falciparum* ($p = 0.77$) and gender ($p = 0.18$) was not statistically relevant, and the average age groups of malaria deaths were between 20 to 39 years and 40 to 59 years ($p = 0.018$).

In 2019 and 2020, 75 and 73.7% of malaria deaths were caused by *P. vivax*, respectively. In 2018 and 2019, deaths in Venezuelan people represented 61.11 and 37.5%, respectively. In 2020 all deaths occurred in Brazilian peo- ple (Table 5).

Discussion

An overview of malaria epidemiology in the Amazon region from 2003 to 2012 showed the greatest reduction of cases in 2012 when 241,806 cases of malaria were registered, representing a reduction of 60.1% compared to 2005 and 9.1% in relation to 2011 [15]. The malaria cases reduction number observed in Roraima from 2011 to 2013 was also recorded in other Brazilian states of the Amazon Region, mainly in the years 2012

Table 2 Imported cases of malaria diagnosed and reported in Roraima, and autochthonous cases in Roraima, according to the special infection area (AEI)

Variables	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Imported											
Venezuela ($p = 0.007$)	1406	877	1149	2112	1220	1260	2470	2323	4478	2285	830
Guyana	1347	1351	1296	1619	714	554	772	575	610	433	266
French Guiana	27	16	6	4	6	4	0	5	7	21	6
Suriname	18	20	3	5	5	2	4	5	2	4	5
Other Countries	8	1	8	7	7	3	8	4	7	2	1
Total	2806	2265	2462	3747	1952	1823	3254	2912	5104	2745	1108
Autochthonous (AEI)											
Indigenous áreas ($p = 0.007$)	2559	7105	5929	3959	1554	1238	1578	2606	2726	11,441	18,765
Rural áreas ($p < 0.001$)	5360	6595	6206	4058	1889	1783	2076	2005	1662	4437	4196
Settlements ($p < 0,001$)	2447	2909	4513	2065	1092	785	986	564	728	2275	1869
Urban ($p < 0.00$)	743	1443	2181	1711	1333	959	1008	970	569	1042	1170
Mining ($p = 0.007$)	11	128	10	7	5	6	4	6	10	851	3027
Total	11,120	18,180	18,839	11,800	5873	4771	5652	6151	5695	20,046	29,027

Source: SIVEP-MALARIA

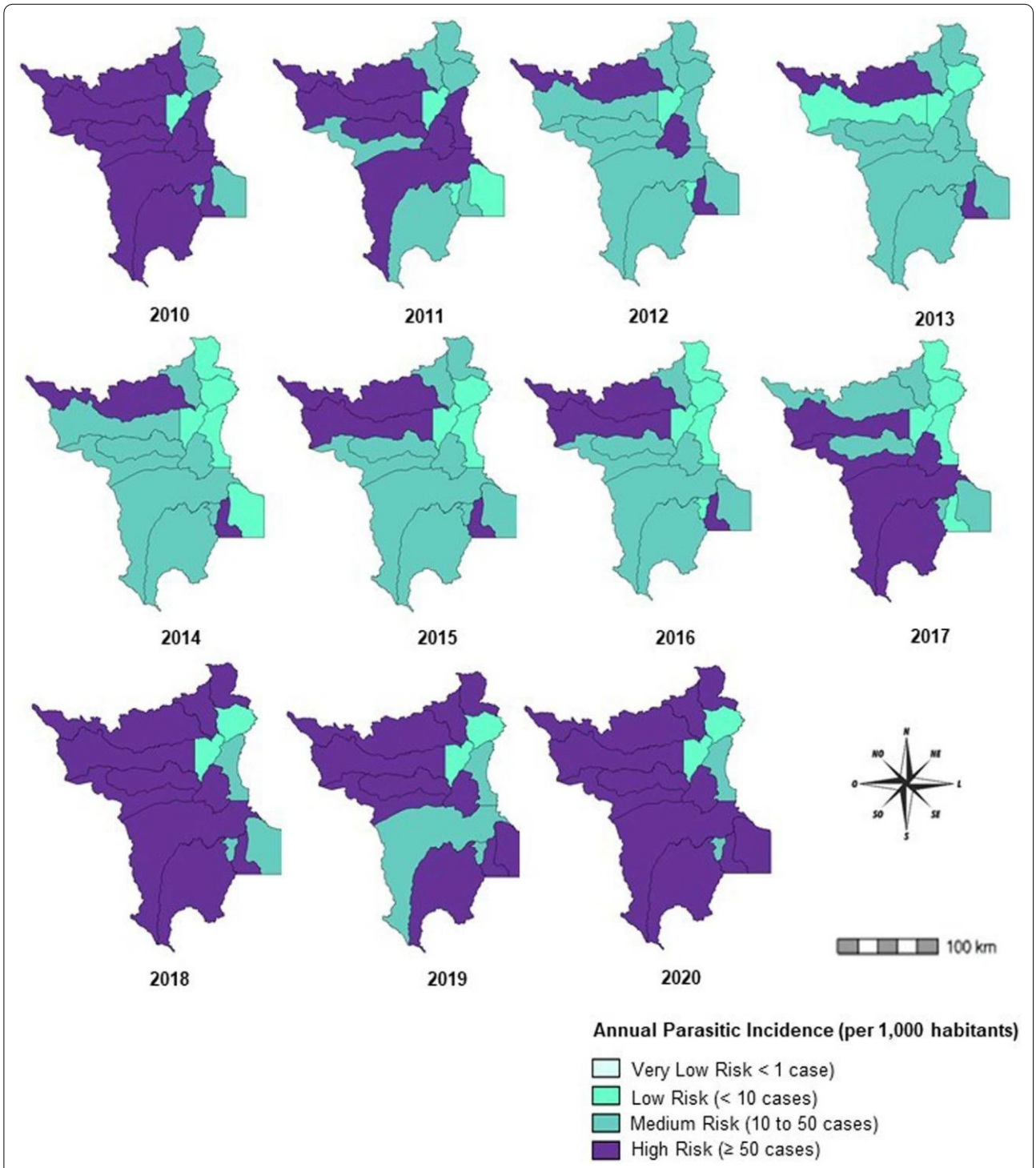

Fig. 2 Annual Parasitic Incidence (API) of Roraima state according to the municipality of infection, 2010 to 2020. Sources: SIVEP-MALARIA; IBGE

and 2013. This reduction, however, was not homogeneous: the states of Pará, Rondônia and Amazonas presented 69, 40 and 8% of reduction, respectively, in 2013 compared to 2012; meanwhile, in Roraima this reduction rate was 18.64% in 2013 when compared to 2012 [4]. In 2013, the API of 9.89/1000 inhabitants was the lowest identified in the study period. It was the only

Table 3 Roraima state: Autochthonous malaria cases by parasite species, gender and age group, from 2010 to 2020

Variables	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Species											
P.vivax	17,192	10,594	5396	4449	5335	5997	5212	10,961	17,583	19,407	23,276
P.falciparum	1863	1266	527	379	378	179	504	222	763	915	6106
% P.falciparum (p = 0.01)	9,78	10,67	8,90	7,85	6,62	2,90	8,82	1,99	4,16	4,50	20,78
Gender (p = 0.34)											
Male	11,591	7142	3753	3131	3665	3701	3391	7065	11,562	12,716	17,273
Female	7463	4717	2170	1697	2048	2475	2325	4118	6784	7606	12,109
Age Group (p > 0.064)											
0 to 9	5107	3367	1343	995	1234	1785	1612	2075	3880	4755	7644
10 to 19	4785	2853	1451	1069	1317	1525	1427	2593	4164	4536	6700
20 to 39	5821	3578	1923	1649	1836	1672	1596	3599	5979	6918	9895
40 to 59	2713	1720	969	905	1026	937	844	2254	3418	3372	4176
≥ 60	627	339	237	210	300	257	237	661	904	739	967

Source: SIVEP-MALARIA

Table 4 Roraima state: Malaria hospitalizations according to parasite species, gender and age group from 2010 to 2020

Variables	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Species											
P. falciparum (p = 0,42)	85	61	40	64	50	39	61	71	119	91	96
P. vivax	376	247	208	221	166	187	185	289	358	379	264
Unspecified	29	4	13	7	4	10	8	9	20	8	16
Gender (p = 071)											
Male	175	114	120	93	88	89	100	153	195	176	159
Female	315	198	141	199	132	147	154	216	302	302	217
Age group											
0 to 9	105	73	47	48	37	62	46	75	116	95	90
10 to 19	93	64	38	44	25	31	40	77	87	87	54
20 to 39 (p = 0.006)	236	133	113	165	113	99	114	145	221	233	183
40 to 59	38	29	40	29	32	30	42	50	55	52	39
≥ 60	18	13	23	6	13	14	12	22	18	11	10

Source: SIH

year in which the Roraima state was classified as low risk for malaria transmission.

Conceivably, this reduction was a result of the actions adopted by the National Programme of Malaria Control (PNCM) of 2005, including: new schedules of *P. falciparum* treatment involving the use of artemisinin-based combination therapy (ACT) and primaquine; use of long-lasting insecticidal bed nets; supervision in diagnosis stations; quality control and monitoring of diagnosis performance; use of RDTs; detection systems and epidemic alert; the project of expanded access to prevention and control measures against malaria for Vulnerable Populations of Brazilian Amazon in 2009 (sponsored by the Global Fund to Fight AIDS, Tuberculosis and Malaria), and the Project of Municipal Supporters for Malaria Control in 2012; and, strengthening local team skills in epidemiological investigation, aiming to promote a progressive reduction of malaria cases [15, 16].

It is noteworthy that even considering that the PNCM actions in place in Roraima, API increased 211.67% infection risk in the time span 2014 to 2020, diverging from the reduction of malaria cases observed in the rest of Brazil in the same period. In addition, data from the Ministry of Health pointed to a decline of 19.1% from 2017 to 2020 [4]. In fact, the distribution over time of malaria cases in Roraima from 2010 to 2020 presented a significant variation over the decade, although during the same period API has shown a medium degree of infection risk (API 10–49.9/1000 inhabitants). However, after the reduction in the number of malaria cases in 2012 and

Table 5 Roraima state: Malaria deaths according to parasite species, sex, nationality and age group from 2010 to 2020

Variables	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Specie											
P. falciparum (p = 0.77)	1	1	0	2	1	2	0	1	9	2	3
P. vivax	2	1	2	1	1	0	0	1	7	12	14
Unspecified	1	0	0	2	0	2	1	2	2	2	2
Gender (p = 0.18)											
Male	3	1	1	3	1	2	1	3	10	8	10
Female	1	1	1	2	1	2	0	1	8	8	9
Nationality											
Venezuelan	0	1	0	0	0	1	0	1	11	6	0
Brazilian	4	1	2	5	2	3	1	3	7	10	19
Age Group (p = 0.018)											
0 to 9	1	1	0	0	0	0	0	0	1	6	8
10 to 19	0	1	0	1	0	1	0	1	3	3	2
20 to 39	2	0	1	1	0	1	0	2	8	4	2
40 to 59	0	0	0	1	2	1	0	1	5	2	3
≥ 60	1	0	1	2	0	1	1	0	1	1	4

Sources: SIM

2013 in Roraima, the cases increased by 18.13% in 2014, specifically in the DSY areas, due to the return of illegal mining activities, mainly on the banks of the Uraricoera, Mucajaí and Couto de Magalhães rivers. In reality, mining activities on the banks of the Uraricoera river already existed from 1987 to 1989 in such a way that up to 2,003 mining rafts were counted near the indigenous community of Waikás. These mining operations were shut down in 1991 after the conclusion of the indigenous territories' boundary demarcation. But, in 2010 new gold mining rafts returned to the Uraricoera. Despite the efforts of local leaders, gold miners refused to leave the region, alleging that mining activity was the only source of income for their families. By the end of 2016, 133 open- pit gold-mines were identified in the Yanomami area, and were opened with greater momentum from 2018 [13, 17]. The scenario of increasing malaria cases triggered by illegal gold mining in the indigenous Yanomami areas and the resulting impact on the local health system has established several meetings of teams involved with malaria control in the Roraima DSEIs municipalities and Ministry of Health/SESAI representatives, in an effort to find effective solutions.

In 2018 a proposal of registering gold miners inside Yanomami lands in the SIVEP-Malaria platform was put forward, in order to allow the stratification of transmission data by origin in indigenous areas with or without mining activities, as control actions adopted in each case are distinct. This record from 2019 in Roraima, showed the increase in the number of autochthonous cases of malaria in mining areas. However, the registration of malaria cases from mining locations is far from reality, reinforcing the need for professional training for malaria notifiers, as well as the investigation of the likely source of infection of the reported cases. It is worth noting that the illegality of gold mining activities in Roraima hinders state efforts to control the disease in the municipalities and DSEI-Yanomami, in terms of safety and logistics issues.

The access routes to the mining areas are mainly through the rivers and forest areas of the municipalities of Alto Alegre, Amajari, Mucajaí, Caracaraí, and Iracema, or by plane through clandestine hidden air- strips in rural areas. The API spatial analysis of these municipalities during the study period showed that Amajari and Alto Alegre presented the largest periods under high infection risk. Amajari presented a high infection risk over the whole decade, except for 2017 when it presented a medium risk. Alto Alegre, in turn, was under high risk of infection for seven years, except for 2012 and 2014, when it showed medium risk, and in 2013 low risk.

The pressure of illegal mining is greater in the Yanomami indigenous areas, however, more recently, this activity has also been identified in the Raposa Serra do Sol Indigenous Land, in the DSEI-Leste, located in the municipalities of Normandia, Pacaraima and Uiramutã, between the Tacutu, Maú, Surumu, and Miang rivers and on the Venezuelan border [18]. A survey carried out in 2020 showed the existence of illegal diamond mining in the municipalities of Uiramutã and Pacaraima [13]. During the study period, these

municipalities showed a high risk of malaria transmission from 2018, which remained until 2020.

According to the World Health Organization, malaria cases in the Americas fell from 894,000 in 2019 to 653,000 cases in 2020. Part of this reduction was attributed to movement restrictions during the COVID-19 pandemic and to the lack of fuel affecting mining activities. Such restrictions could also have affected access to care and case detection [3]. The malaria scenario in Roraima during the COVID-19 pandemic was different, with a 44% increase of autochthonous malaria cases in 2020 when compared to 2019. Interestingly, in 2020, there was also a 30% increase in mining activities in Yanomami indigenous land, mainly in the Waikas and Kanayanau regions. The new illegal mining centres were located mainly in the channels of the Uraricoera river, which concentrates 52% of the total area of illegal mining in the Yanomami indigenous land. Illegal mining was also identified on the banks of the Parima, Mucajaí, Couto de Magalhães, and Catrimani rivers [17].

Malaria control in the gold mines of Roraima is a major challenge. In addition to the difficulty in controlling the vector, mining is illegal and is carried out in areas of difficult access, making timely diagnosis and treatment considerably difficult [19]. Another problem in mining areas is that people infected with malaria often self-medicate with erratic regimens, often using just a dose called 'incubator' to quickly eliminate symptoms and return to mining. These non-curative underdoses favour parasites resistant to antimalarials [20, 21]. In addition, miners use drugs of dubious quality, such as Artecom® (artemisinin based medication), which is not registered by a drug regulatory authority or by the WHO prequalified programme and is therefore illegal in French Guiana and neighbouring countries [22].

Malaria control in illegal miners goes beyond the domain of public health, and it is necessary to include other government bodies in the debate on gold mining in indigenous lands, which as in any other economic activity must take principles of sustainability, preservation of biodiversity and guarantee the cultural and social rights of indigenous peoples to ensure social wellbeing and health of indigenous and non-indigenous populations.

In 1961, the Bolivarian Republic of Venezuela (herein- after called Venezuela) was the first country certified by WHO to eradicate malaria; nevertheless, as of 2012 the country's situation turned alarmingly. Economic collapse in Venezuela led to a lack of anti-malarial medication and the failure of other control measures, resulting in a rise in case numbers, both in endemic and non-endemic regions, affecting neighbouring countries with imported malaria cases, including infections due to *P. falciparum* [23]. The economic crisis has driven many people to illegal gold mining, where they contract malaria and spread the disease when returning home. Cases of imported malaria proceeded mainly from Bolivar and Amazonas, Venezuelan states bordering Brazil.

On the Brazilian side, the Pacaraima municipality has a population of 11,667 inhabitants, including the indigenous population. The seat of the municipality, the only non-indigenous area in the municipality, is located at an average altitude of 900 m and has vegetation cover of steppe savannah, thus presenting a negligible risk for malaria transmission. However, the indigenous areas of São Marcos, located alongside highway BR-174, offers favourable environmental conditions for the mosquito vector. Venezuelan immigration has contributed to the recrudescence of autochthonous cases in these native communities because they serve as shelters for refugees when they move to the capital, Boa Vista, along the BR-174, and in 2018 to 2020, the municipality of Pacaraima presented a high risk of malaria infection. The biggest reduction in 2020 can be explained by the Ven- ezuelan border closing during the period of the Covid-19 pandemic. On the other hand, an increase in autochthonous cases, mainly in indigenous localities of DSEI-Leste, located in the municipality of Pacaraima, was recorded.

Indigenous reserves occupy 70% of areas in the municipalities of Normandia, Uiramutã, Alto Alegre, Pacaraima, and Iracema. In these municipalities, malaria infections are concentrated in the indigenous area, with little or no transmission in non-indigenous areas.

The measures of malaria prevention and control in indigenous areas are also a challenge to public health, due among other reasons to environmental changes and to nomadic behaviour and cultural characteristics such as hunting, fishing, farming, and bathing in rivers and streams [5]. Some studies point out that in the Amazon, the risk of indigenous people getting sick from malaria is twice that of non-indigenous people. These studies indicate that in the period 2003 to 2012 the epidemic municipalities were characterized by having indigenous populations, settlements, gold mining, and international borders [6, 15, 24].

Timely diagnosis and treatment have a greater impact on the control of malaria caused by *P. falciparum*, since *P. vivax* infections present gametocytes (the infectious mosquito stage) from the first days of infection; in turn, *P. falciparum* gametocytes are found in the bloodstream only after seven days of infection [5]. Thus, a significant percentage rise of infections due to *P. falciparum*, reported over the observation period, highlights the lack of access to opportune treatment, mainly in gold miners' malaria cases. If not timely treated, malaria can evolve to its severe forms, causing hospitalizations and deaths. The percentage of hospitalizations due to malaria, not only in

Brazil but also in endemic areas of the world, is directly proportional to the provision of timely diagnosis and adequate treatment.

Plasmodium vivax infections have been increasingly associated with an important, multisystemic impact on individual health, mainly in the presence of co-morbidities [25]. Vivax malaria cases may also develop into more serious forms of the disease, either because of inexperienced outpatient care, late diagnosis, inadequate/incomplete treatment, or even drug resistance [26, 27].

The number of deaths caused by malaria in Roraima can be considered high when compared to national data. While from 2000 to 2017 there was a progressive reduction of deaths nationwide (from 245 to 34), in Roraima 18 deaths caused by malaria were reported in 2018 alone, although from 2010 to 2017 the mortality rate aver- aged three deaths per year, with up to 24 deaths within a period of eight years. Besides this increase, in 2019 deaths due to malaria in Roraima made up 46% of the total reported for Legal Amazon [4]. These data are prob- ably due to a larger number of imported cases from Venezuela (11 deaths) and the increase of malaria infections in gold mining areas among Brazilians (7 deaths) in 2018. In 2019, 16 deaths, six in Venezuelans and 10 in Brazilians were reported in Roraima. These figures exemplify how difficult is timely diagnosis and treatment in Venezuela and in mining areas.

This study, like any study based on secondary data, may have some limitations related to possible underreporting, incompleteness and inadequate registration.

Conclusions

Malaria remains a serious health problem in Roraima, and two factors are responsible for the increase of malaria cases in the decade of the study.

The first, related to cases imported from Venezuela, exemplifies the challenge of controlling malaria in border areas, making it essential to maintain and strengthen surveillance for early identification of any change in malaria epidemiological patterns of importation or reintroduction in border localities.

The second is related to illegal mining in indigenous areas that returned in 2010 and with greater impetus from 2018 onwards. In 2020, during the Covid-19 pan- demic, the pressure of this activity was more intense in the Yanomami indigenous area but also occurred in the indigenous region of Raposa Serra do Sol. Malaria control challenges in indigenous areas include the cultural diversity of indigenous peoples, housing (that sometimes does not allow for vector control) and the presence of illegal mining. It is mandatory that new health policies include this contingent, providing resources and strate- gies in order to reduce infections.

Actions to control malaria in illegal mining transcend the health area for formulation of public policies and coordination with other institutions at municipal, state and federal levels is required, especially in relation to strategies to increase access to diagnosis and timely treatment. Health education for this population should also be considered, on the importance of carrying out complete treatment to avoid an increase in hospitalizations and deaths on taking medication of dubious origin to treat malaria and without adequate diagnosis, to the importance of monitoring molecular markers of resistance to anti-malarials in Roraima, in order to control the spread of *P. falciparum* and *P. vivax* parasites resistant to anti-malarials currently available.

Abbreviations

SDO: Sustained Development Objectives; IBGE: Brazilian Institute of Geog- raphy and Statistics; DSEI-Yanomami: Special Indigenous Health District Yanomami; DSEI-Leste: Special Indigenous Health District Leste; SIVEP-Malaria: Malaria Epidemiological Surveillance Information System; SIM: Mortality Infor- mation System; SIH: Hospitalization Information System; CID-10: International Disease Classification; API: Annual Parasitic Incidence; GLS: Generalized Least Squares; PNCM: National Programme of Malaria Control; SUS: Health Unic Sys- tem; ACT: Artemisinin combination therapy; WHO: World Health Organization.

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Author contributions

Conceptualization: JAB; formal analysis: JAB, FG, PP, PM, MFFC; investigation: JAB, FG and MFFC; software: JAB and PP; writing original draft: JAB, FG and MFFC; supervision: MFFC, FG; review and editing: JAB, MFFC, FG, PM. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of *Universidade Federal de Roraima* (CEP/UFRR): CAAE 24122619.6.0000.5302, 17 March, 2020.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

4. CAPÍTULO 2

Este capítulo também se relaciona com o objetivo específico de “Descrever o perfil epidemiológico da malária no estado de Roraima”, porém sob a ótica da análise da descritiva dos questionários aplicados aos participantes do estudo no momento da coleta. Com o objetivo de investigar o provável local de infecção, principal atividade desenvolvida e aspectos epidemiológicos dos indivíduos infectados, além dos aspectos clínicos e laboratoriais.

O artigo foi publicado na Revista Cadernos de Saúde Pública, classificação Qualis A1 em Biotecnologia, no dia 29 de março de 2024, com o título: *“A snapshot of a representative Brazilian state of illegal mining in indigenous areas during the era of malaria elimination”*, DOI: 10.1590/0102-311XEN224023. Disponível em: https://cadernos.ensp.fiocruz.br/ojs/index.php/csp/article/view/8629.

TITLE: A snapshot of a representative Brazilian state of illegal mining in indigenous areas during the era of malaria elimination

SHORT TITLE: Illegal mining in Brazilian indigenous malaria endemic areas

KEY WORDS: mining, indigenous, Amazon, malaria, Yanomami

ABSTRACT:

Malaria is a public health problem and the cases diagnosed in the capital of Roraima have the potential to characterize the burden of the disease in the state. This study aimed to describe the epidemiological, clinical and laboratory aspects of malaria cases diagnosed in Boa Vista. For this purpose, a descriptive, cross-sectional study was carried out in two health units in the city, with individuals diagnosed and who agreed to respond the questionnaire. Of the total of 206 participants, characterized as men, mixed race and young, 96% (198) reported illegal mining activity. Among the group of miners, 66 % (131) came from other states of Brazil or other countries. The mines were mainly located in the Yanomami Territory in Roraima. *P. vivax* infection occurred in 74% (153) of participants. In the miner's group, there were reports of hospitalizations for severe malaria, previous malaria attacks and delays in treatment after the onset of symptoms. Although 73 % (145) of miners reported knowing how malaria was transmitted, only 54% (107) used mosquito nets or repellents. The use of Artecom® and chloroquine by miners is not for the complete treatment but only to relieve symptoms for returning to gold mines highlighting the importance of molecular surveillance to antimalarial resistance. Indigenous are considered a vulnerable malaria population and miners promote the increase of malaria in Roraima indigenous land. Therefore, access to diagnosis and treatment in indigenous areas invaded by miners is imperative to confront this disease that ravages indigenous communities and threatens public health on a large scale to achieve the goal of eliminating malaria in the state.

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INTRODUCTION

Malaria is an acute febrile infectious disease, caused by the protozoan of the genus *Plasmodium*, transmitted by the female mosquito of the genus *Anopheles* [1,2]. The disease is endemic in 85 countries (including the territory of French Guiana) located in tropical and subtropical regions of the world. There are an estimated 249 million cases and 608,000 deaths from malaria in the world in 2022. In the Americas region, the Bolivarian Republic of Venezuela, Brazil and Colombia accounted for more than 73% of the 0.6 million cases that occurred in this region [2].

In Brazil, malaria is endemic in the Legal Amazon (Acre, Amapá, Amazonas, Maranhão, Mato Grosso, Pará, Rondônia, Roraima and Tocantins states), which accounts for 99% of cases of the disease in the country. In 2021, there were 139,107 cases and 61 deaths from malaria recorded in the country [3]. That same year, Roraima presented 26,005 autochthonous cases and 25 deaths from the disease [4].

Despite being a preventable and treatable disease with medicines available free of charge in the Unified Health System (SUS) health network, morbidity and mortality data show that malaria is still an important public health problem. The disease has a negative impact on both the health and livelihoods of people around the world, especially on the poorest and most vulnerable populations. Furthermore, it poses challenges for its elimination and demands successful strategies to achieve the goal of eliminating malaria transmission in Brazil by 2035 [5].

In the period from 2010 to 2020, the period that precedes the decade of work for Brazil to achieve the elimination target, cases of malaria imported from Venezuela and mining in the Yanomami indigenous area were identified as the factors responsible for the increase in cases of the disease in Roraima [6]. The diagnosis of malaria occurred mainly in Boa Vista, the state capital. And in this scenario, in addition to the clear impact on the municipal healthcare network, there is also the risk of outbreaks and epidemics in Boa Vista. In fact, the municipality has a climate favorable to the presence of the main vectors of malaria, in addition to there being large rivers, streams, and lakes in the urban area of the city, characteristics that favor the existence of breeding sites [7].

In 2022, according to data from the Malaria Epidemiological Surveillance Information System (Sivep-malaria), among the 26,204 autochthonous cases registered in Roraima, 12,010 were associated with mining activities, and 86% (10,329) of them were diagnosed/notified in Boa Vista, a Roraima municipality classified as low risk for malaria transmission (IPA < 10 cases/1,000 inhabitants). In Boa Vista 1% (206) were considered autochthonous and 99% were imported cases from other Roraima municipalities: Alto Alegre (75%), Mucajaí (17%) and Amajari (2%) or neighboring countries (mainly Venezuela and Guyana) [8].

The health units with the highest number of case notifications were the Cosme and Silva Emergency Service and the Sayonara Basic Health Unit, representing 31% (4,490) and 14% (2,075) of cases, respectively, representing almost 50% of the malaria cases notified in Boa Vista [8].

In the group of individuals seeking a malaria diagnosis in Boa Vista, it is possible to identify multiple factors that can characterize the epidemiological pattern of the malaria burden in the state of Roraima, which has the potential to support the formulation of effective public policies to control malaria disease following local reality [9].

Mining activity is provided by law in Brazil. The problem is that mining in the Amazon almost operates without a license, with mercury, within Indigenous Lands and Conservation Units and without recovering the environmental damage caused and, therefore, represents an illegal activity.

Given the representativeness of malaria cases diagnosed in Boa Vista, the objective of this work was to investigate where the infection occurs and the main activity of infected individuals through an epidemiological survey, as well as the clinical and laboratory aspects of malaria cases reported in the two units that concentrate the largest number of malaria notifications.

METHODOLOGY

The state of Roraima, located in the extreme north of Brazil, shares an international border with Venezuela and the Republic of Guyana, as well as a national border with the states of Amazonas and Pará. According to the Brazilian Institute of Geography and Statistics (IBGE), in 2021 the state had a population of 652,713 inhabitants. The state capital, Boa Vista, concentrates 67% of the population, with 436,591 inhabitants, and is bordered by the municipalities of Pacaraima, Normandia, Bonfim, Cantá, Mucajaí, Alto Alegre and Amajari (Figure 1). It comprises 15 municipalities and 104,509.087 Km² of its territory consists of indigenous reservations. The Yanomami represent the largest indigenous reserve in Brazil. The Special Indigenous Health District Yanomami (DSEI-Yanomami) in Roraima covers 5 municipalities: Alto Alegre, Amajari, Caracaraí, Iracema and Mucajaí. All mining sites in Roraima are illegal and are located within indigenous lands. In Roraima, the largest mining areas in indigenous lands are in Yanomami territories.

Boa Vista has 15 malaria diagnostic centers located in the urban area of the city, 06 in Hospital/Urgency and Emergency Units; 01 in Maternal and Child Hospital and 08 in Primary Health Care (PHC) units. The study was carried out in two health units located in the west of Boa Vista: Emergency Service Cosme e Silva and Basic Health Unit Sayonara Maria Dantas (Figure 1). These notification Units were chosen because they have the highest Boa Vista number of malaria notifications according to Sivep-malaria.

To achieve the proposed objective, a descriptive, cross-sectional study was carried out from December 2021 to June 2022, during the driest season of the year in Roraima, when malaria transmission is favored, consequently leading to an increase in demand. by diagnosis. Individuals over 18 years of age and who were diagnosed with malaria through thick bood film were included in the study. Minors under 18 years of age, indigenous people living in villages; individuals who were unable to read the Free and Informed Consent Form (ICF) and individuals who refused to sign the ICF did not participate.

This work was approved by the Research Ethics Committee of the Federal University of Roraima (CEP/UFRR): Opinion No. 3,920,373, issued on March 17, 2020. Regarding the inclusion of indigenous people in the study, the Ethics Committee allowed only the inclusion of non-village indigenous people who speak Portuguese and reside in Boa Vista, after the research project had been presented to the Association of Indigenous People for obtaining a letter of consent which was attached in the submission process to the CEP/UFRR.

After signing the ICF, a specific questionnaire was applied containing questions related to the individual (gender, age, race, origin, main activity in the 15 days before the onset of symptoms, knowledge about the forms of malaria transmission and prophylaxis) and the disease (symptoms, parasitic form causing the infection and history of the disease). The questionnaires were typed and tabulated in the Excel program (Microsoft Office®).

The maps were created using the QGIS program version 3.28.10, mining areas in Roraima were obtained from Mapbiomas [10]. Geopolitical limits of Brazil and Indigenous Lands were accessed on the IBGE website [11].

Statistical analysis was performed using the chi-square test with a significance level of 5%, using the BioEstat 5.33 program [12].

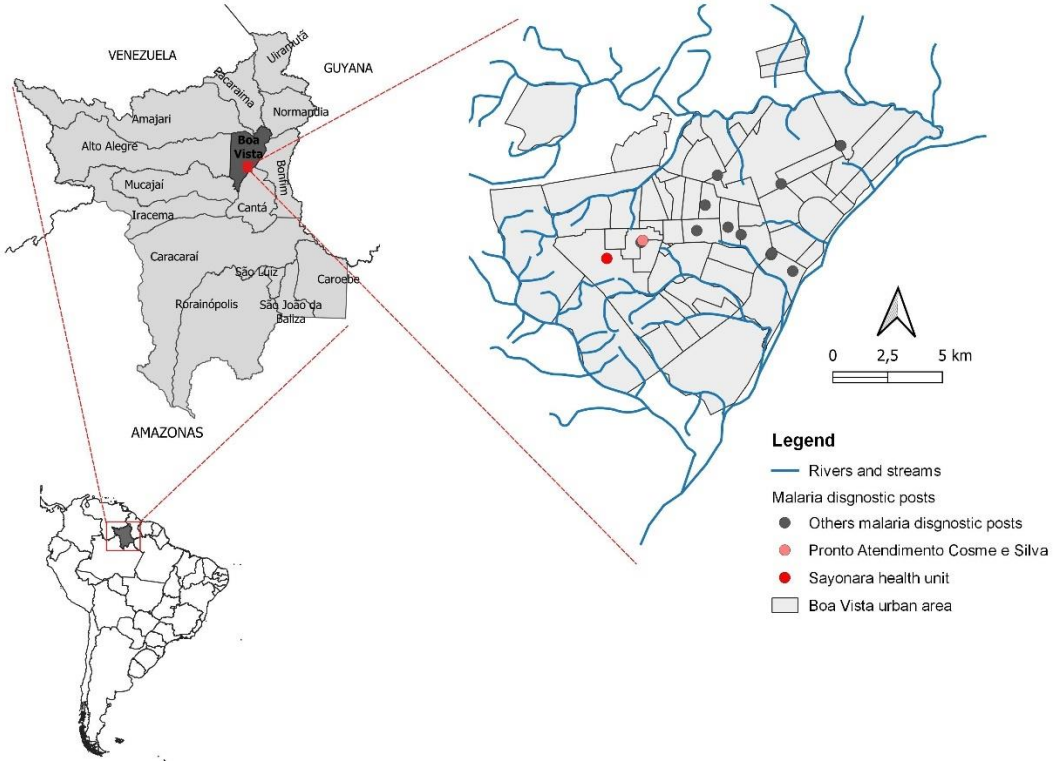

Figure 1. Location of the municipality of Boa Vista showing the urban area, rivers networks and streams, and malaria diagnostic units, highlighting the 2 where the samples were collected, Roraima, Brazil. Preparation of the map: the authors.

RESULTS

According to Sivep-malaria, during the study period - December 2021 to June 2022 - 7,379 malaria cases were notified in Boa Vista and 43% of them were diagnosed in the health units selected as strategic points for this research.

When analyzing participants' responses about the main activity carried out in the 15 days before the onset of symptoms, 04 activities were identified. The mining activity was the predominant one ($p < 0.0001$) with 198 (96%) participants, followed by agriculture in 06 (3%), hunting/fishing in one (0.5%) and tourism in one (0.5%).

The age of the participants ranged from 18 to 67 years old, and the age groups 18 to 29 and 30 to 39 years old were prevalent in participants who reported mining activity ($p < 0.0001$). Of the total number of participants, the male gender was more frequent than the female gender ($p < 0.0001$) with 169 (82%) men, and the brown race with 119 (58%) individuals was the most frequent ($p < 0.0001$). The predominant symptoms were fever (90.3%), headache (87.4%), chills (67.5%), abdominal pain (54.9%) and myalgia (53.4%) compared to the others less frequently cited as sweating, diarrhea, dyspnea, low back pain and no symptoms ($p < 0.0001$). At least one symptom was reported by 202 participants (98%). The same symptomology was described by the miners and this group was the only one with participants who revealed not having symptoms 04 (2%) (Table 1).

Participants were most affected by *P. vivax* (p < 0.0001), which occurred in 153 (74.3%) individuals, followed by 42 (20.4%) by *P. falciparum* and 11 (5.3%) by mixed malaria (*P. falciparum* + *P. vivax*), making up 24.7% of *P. falciparum* single or mixed infections.

The majority of participants 166 (81%) reported previous episodes of malaria (p < 0.0001). There were no reports of hospitalizations due to malaria in the majority of participants (p < 0.0001). Hospitalizations were reported only in the miners group by 40 participants (20%).

The non-use of prophylactic measures and the use of repellent were the most cited when asked about prophylactic measures (p < 0.0001). Furthermore, the group of participants in mining activity was the only one who reported the use of antimalarials as a form of prophylaxis with 14 (6%) reports (Table 1).

Table 1. Characterization of study subjects, according to the main activity carried out 15 days for age group, gender, race/color, symptoms, parasitic species and epidemiological variables related to malaria, Boa Vista, Roraima.

Variables	Illegal mining (n=198)		Agriculture (n=6)		Hunting/Fishing (n=1)		Turisme (n=1)		Total (n=206)	
	nº	%	nº	%	nº	%	nº	%	nº	%
Age group (years)										
18 - 29	92	46.5	3	50.0	0	0	0	0	95	46.1
30 a 39	60	30.3	1	16.7	1	100	0	0	62	30.1
40 a 49	26	13.1	1	16.7	0	0	1	100	28	13.6
50 a 59	17	8.6	1	16.7	0	0	0	0	18	8.7
≥ 60	3	1.5	0	0.0	0	0	0	0	3	1.5
Gender										
Male	164	82.8	3	50.0	1	100	1	100	169	82.0
Female	34	17.2	3	50.0	0	0	0	0	37	18.0
Race/color										
White	32	16.2	2	33.3	0	0	0	0	34	16.5
Black	39	19.7	2	33.3	0	0	0	0	41	19.9
Asian	7	3.5	0	0.0	0	0	0	0	7	3.4
Multiracial	115	58.1	2	33.3	1	100	1	100	119	57.8
Indigenous	5	2.5	0	0.0	0	0	0	0	5	2.4
Symptoms										
Fever	179	90.4	5	83.3	1	100	1	100	186	90.3
Headache	173	87.4	6	100	1	100	0	0	180	87.4
Chill	136	68.7	2	33.3	1	100	0	0	139	67.5
Abdominal pain	109	55.1	2	33.3	1	100	1	100	113	54.9
Myalgia	105	53.0	4	66.7	1	100	0	0	110	53.4
Nausea/Vomit	87	43.9	2	33.3	0	0	0	0	89	43.2
Sweating	78	39.4	2	33.3	1	100	0	0	81	39.3
Diarrhea	36	18.2	1	16.7	0	0	0	0	37	18.0
Dyspnea	27	13.6	0	0.0	0	0	0	0	27	13.1
Low back pain	8	4.0	0	0.0	0	0	0	0	8	3.9
No symptoms	4	2.0	0	0.0	0	0	0	0	4	1.9
Species										
P. vivax	147	74.2	4	66.7	1	100	1	100	153	74.3

P. falciparum + mixed malaria	51	25.8	2	33.3	0	0	0	0	53	25.7
Have you had malaria before?										
Yes	161	81.3	4	66.7	0	0	1	100	166	80.6
No	37	18.7	2	33.3	1	100	0	0	40	19.4
Have you ever been hospitalized?										
Yes	40	20.2	0	0	0	0	0	0	40	19.4
No	158	79.8	6	100	1	100	1	100	166	80.6
Do you know how malaria is transmitted?										
Yes	145	73.2	5	83.3	1	100	0	0	151	73.3
No	53	26.8	1	16.7	0	0	1	100	55	26.7
Medidas profiláticas										
Mosquito nets	47	23.7	1	16.7	0	0	0	0	48	23.3
Repellent	60	30.3	1	16.7	0	0	0	0	61	29.6
Antimalarials	12	6.1	0	0	0	0	0	0	12	5.8
No prevention	79	39.9	4	66.7	1	100	1	100	85	41.3

Data source: primary data

Concerning the timeliness of treatment, starting treatment within 96 hours and over 96 hours are the most frequent in relation to other periods, that is, up to 24 or 48 hours (p = 0.0024) and 57 (29.4%) gold miners started treatment within a period of more than 96 hours after the onset of symptoms (Table 2).

Table 2. Characterization of the case series by the main activity 15 days before the onset of symptoms, according to the period of initiation of treatment after the appearance of symptoms, Boa Vista, Roraima.

Onset of symptoms / treatment	Illegal mining (n=194)		Agriculture (n=6)		Hunting/Fishing (n=1)		Turisme (n=1)		Total (n=202)	
	nº	%	nº	%	nº	%	nº	%	nº	%
Up to 24 hours	32	16.5	0	00.0	0	0	0	0	32	15.8
Up to 48 hours	43	22.2	1	16.7	0	0	0	0	44	21.8
Up to 96 hours	62	32.0	4	66.7	1	100	0	0	67	33.2
Over 96 hours	57	29.4	1	16.7	0	0	1	100	59	29.2

Data source: primary data

Regarding gold miners, 95.5% (189/198) were infected in indigenous territories of three Roraima municipalities (Alto Alegre, Mucajaí, and Amajari): 81% (153/189) of them in illegal mines located along the Uraricoera River in Alto Alegre; 17.5% (33/189) in illegal mines located along the Mucajaí River and its tributary Couto de Magalhães in the municipality of Mucajaí and; 1.5% (3/189) in mines also located along the Uraricoera River but in the municipality of Amajari (Figure 2). In addition, imported cases were identified in patients who came from mining sites in Venezuela (4/198), Guyana (4/198) and Pará (1/198). Likewise, in participants who reported agricultural activity, the probable location of infection was also Alto Alegre (1/6) and Mucajaí (1/6), in addition to the municipalities of Cantá (1/6), Caracaraí (1/6) and Caroebe

(2/6). The participant who reported hunting/fishing occupation as well as that one with tourism activity became infected in the municipality of Mucajaí (2/2).

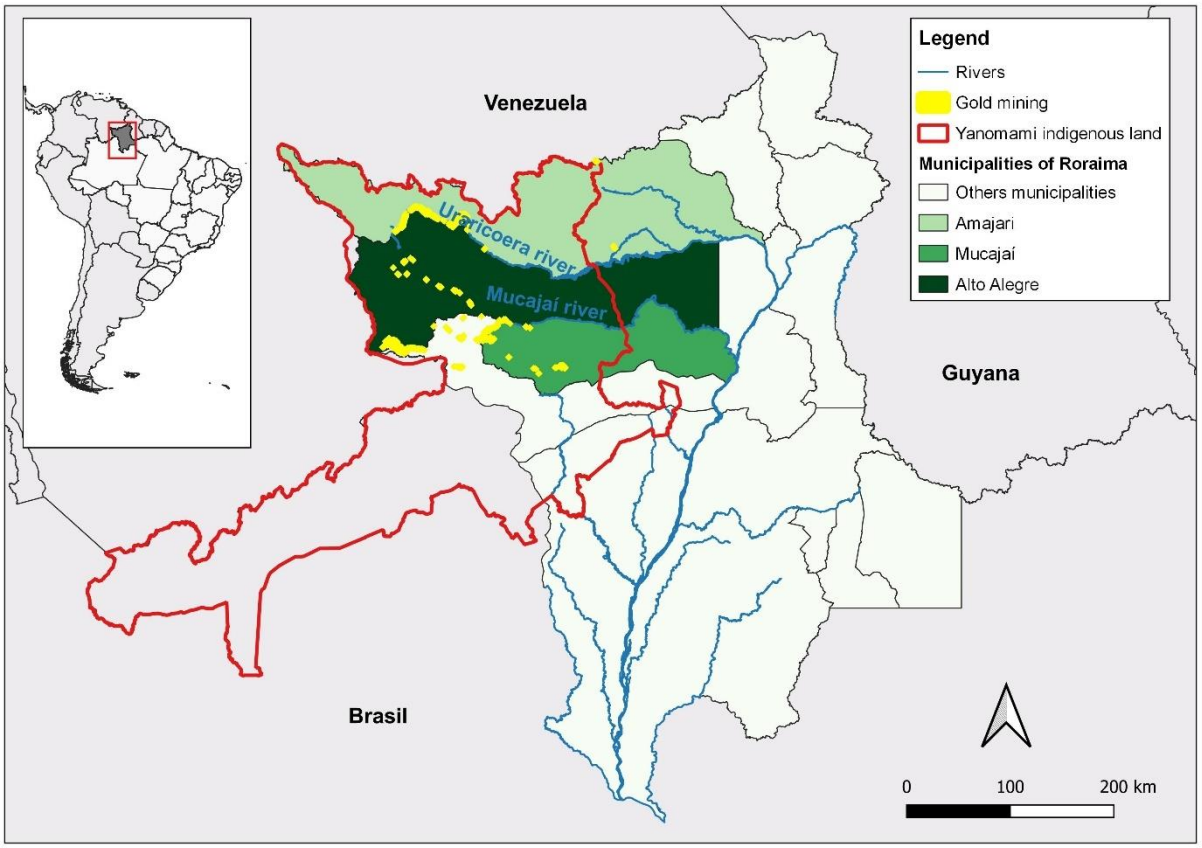

Figure 2. Municipalities of infection of participants who carried out mining activities 15 days before the onset of symptoms, Boa Vista, Roraima. Data source: primary data. Preparation of the map: the authors.

The majority of participants with mining activity came from Roraima 67 (34%) and Maranhão 60 (33%), followed by Pará 33 (17%), Venezuela 22 (11%) and Amazonas 7 (3.5%) ($p < 0.000$). The states of Piauí (2), Amapá (2) and Ceará (2) were the origins of 1% of the participants, while Goiás, Minas Gerais and Rondônia contributed each one with one participant (0.5%) in (Figure 3).

Concerning participants who carried out agricultural activities, around 33% (02) came from Roraima and the states of Pará, Amazonas, Bahia and Venezuela. The participant who carried out hunting/fishing occupation was from Pará and the participant who reported tourism was from Piaui.

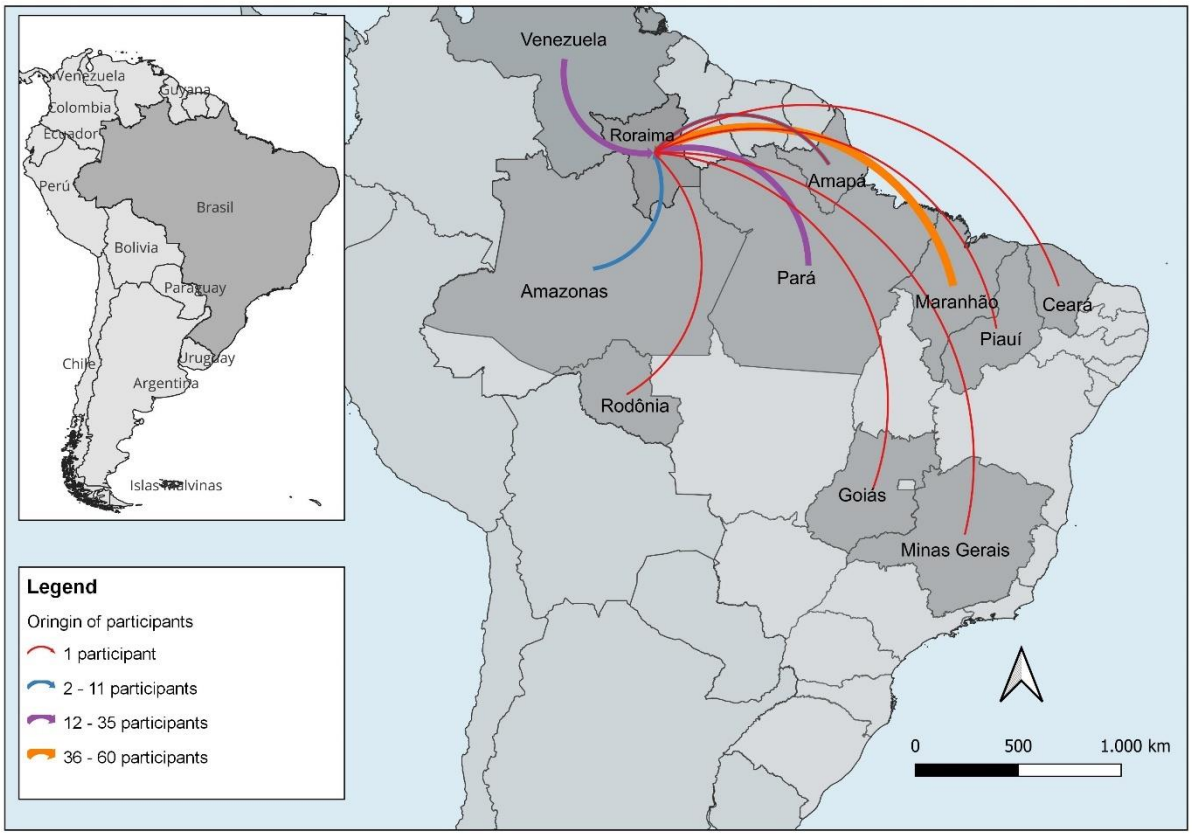

Figure 3. Origin of study participants who carried out illegal mining activity 15 days before diagnosis, Boa Vista, Roraima. Data source: primary data. Preparation of the map: the authors.

DISCUSSION

The present work is a descriptive study. It is important to highlight that the selected units are representative of the malaria-diagnosed cases in Roraima. Our casuistic comprises cases of passive surveillance/search. Therefore, we may be underestimating the number of cases because individuals who carry out self-treatment in mining, without seeking health facilities, could only be identified by active search in illegal mining areas in Yanomami lands. However, this active search is made difficult by the location of the mines in remote forest areas whose access is often via clandestine take-off and landing strips, besides the risks to the researchers' lives in these conflict areas.

Identifying the place of infection is essential to guide the planning of malaria prevention and control actions. In Brazil, cases of malaria in urban, and rural areas and settlements have decreased since 2017, contrasting with cases in miners and indigenous that increased around 257% and 62%, respectively [6,14].

In the present study, 96% of the participants reported mining activity at least 15 days before the onset of symptoms, strongly suggesting that they were infected in indigenous lands where all Roraima illegal mines are located.

The presence of miners in the Yanomami indigenous territories contributed to the increased number of malaria cases in Roraima in recent years. Even in 2020,

during the Covid-19 pandemic, there was a 44% increase in autochthonous malaria cases in Roraima, and in the Yanomami Indigenous land, which corresponds to a 77% increase when compared to 2019, beyond the increased number of hospitalizations and deaths. In this same period, there was also a 30% increase in illegal mining in the Yanomami indigenous area [6]. The mines are very close to the indigenous communities. The Uraricoera River concentrated 52% of the entire area degraded by illegal mining; gold exploration takes place in Waikás, Araçá, and Korekorema indigenous communities. The mines along the Mucajaí River (and its tributary Couto de Magalhães) represent almost 25% of the total degraded illegal mining; gold exploration occurs in indigenous communities borders of Kayanau and Papiu[15].

Indigenous reserves are considered the most preserved areas and are responsible for keeping the forest standing [13]. The illegal mining activity in Yanomami lands has multiple impacts on traditional communities and the ecosystem [13]. Disintrusion is necessary, but it is not a simple or low-cost process. It is needed to develop a robust political, economic, and public security project. Evidence of the presence of organized crime (called narco mining) makes the process even more difficult [13, 16], as well as health actions to control malaria in these areas.

Mining is a major challenge for the elimination of malaria in the next decade in Brazil. In addition to requiring health and environmental interventions, there is a need to formulate control policies that include the specificity of this contingent of individuals, despite the illegality of mining activity [6, 14, 17], because controlling malaria in the mining population will reduce the impact of malaria on indigenous people.

The miners can increase malaria transmission by three main factors: a) modification of the environment, with the use of hoses and combustion engines to extract the sediment, forming pools of water that serve as breeding grounds, enhancing the reproduction of the mosquito vector, especially *Anopheles darlingi*; b) more exposition to mosquitoes by work outdoors for long periods a day and sleep in tents with incomplete walls; and c) the intense mobility between the mining areas and their areas of origin or other areas, promoting transmission and the occurrence of outbreaks [18, 19, 20, 21].

The number of women in mining, although smaller, should also be considered for strategies to eliminate malaria, especially by the possibility of pregnancy due to the potential for severe malaria disease in this condition, when not treated promptly, such as, maternal and fetal anemia; premature birth; low birth weight and fetal problems; maternal and neonatal death [22, 23, 24].

Fever, followed by headache, was the most frequent symptom among study participants. Fever remains the main symptom for health education strategies based on the identification of malaria symptoms. Therefore, the advice to seek the health

unit if you feel feverish and have been in an area at risk for malaria transmission continues to be an important guideline for timely diagnosis and treatment, highlighting that cultural differences must be considered so that this strategy is effective, especially in territories with multi-ethnic borders [25, 26].

In the group of miners, 81% (161) had previous malaria attacks and 2% (04) had no symptoms at the time of diagnosis. It was not possible to define whether previous episodes of malaria were related to relapse, recrudescence, or reinfection. Furthermore, miners reported that soon after the diagnosis and treatment for malaria, they returned to the mine, often on the same day, therefore, being constantly exposed to areas where malaria is transmitted.

The silent condition of asymptomatic carriers represents a challenge to the elimination of malaria [24, 27], therefore, surveillance must also track the movement of human carriers. The asymptomatic participants engaged in this study were relatives of the patients who had the malaria diagnosis at Boa Vista. Since they came also from mining areas, the malaria microscopic test was realized.

Regarding the parasitic form, 53 cases of infection by *P. falciparum* and *P. falciparum* plus *P. vivax* (mixed malaria) were identified, of which 51 were in participants who were mining. In the Americas region, *P. vivax* malaria represents 75% of cases [21]. However, in mining areas, *P. falciparum* appears to be more frequent than in other ones [17, 27]. It is noteworthy that among the 11 cases of mixed *P.falciparum/P.vivax* infections, 10 were in participants with mining activities. In this context, updating microscopists or adopting more precise diagnostic procedures is crucial for adequate treatment. If mixed infections are misdiagnosed as a *P. vivax* monoinfection and treatment is prescribed only for this parasite, the increase in *P. falciparum* parasitemia may evolve into severe malaria [28].

For miners, absenteeism due to malaria causes a major financial impact. Thus, self-medication and the use of sub-doses of medication as a form of prophylaxis or quick relief of symptoms are common in this group [20, 29]. In the national malaria treatment protocol, chloroquine is the first-line treatment for uncomplicated malaria caused by *P. vivax*. A combination of chloroquine is prescribed for three days (10 mg/kg on day 1 and 7.5 mg/kg on days 2 and 3) and primaquine (0.5/kg/day, for seven days), aiming to cure both the blood form and the hepatic form (radical cure) respectively, thus avoiding relapse due to hypnozoites [30]. However, the medication used by the miners who participated in the present study was Artecon®, which could be purchased at the mine itself. This drug is not recommended by local pharmaceutical authorities and the World Health Organization and it is illegal in Brazil, French Guiana, and neighboring countries [27, 31].

In this way, the increase in the number of infections, in addition to the

emergence of resistance to antimalarials could occur, reinforcing the need for molecular surveillance through antimalarial resistance markers in mining areas, especially in the case of artemisinin [17, 32, 33, 34].

The National Malaria Program considers treatment with antimalarials to be opportune when it is carried out, from the onset of symptoms, within 48 hours for autochthonous cases and within 96 hours for imported cases [5], but most miners reported starting treatment after 96 hours or more. Here, in the group of miners, 29% (57) were diagnosed after 96 hours,

In this scenario, it is important to introduce diagnosis and treatment in mining to interrupt the transmission and, consequently, reduce the burden of malaria in indigenous areas. However, in Roraima, given the illegality of mining activity together with the “narco mining" [16], it is not safe for the health team to do diagnosis and treatment in mining areas.

The Malakit Project was a successful and innovative strategy with an impact on the reduction of malaria cases in miner populations in French Guiana and Suriname. This intervention came with the proposal to provide miners with training on malaria, kits for self-diagnosis through rapid testing and self-treatment [31, 35]. In Roraima, this strategy could be adopted at health units diagnosing malaria in miners, to overcome lack of security in conflict areas.

It is noteworthy that 66% of the miners in this study came from other Brazilian areas, including those of the extra-Amazonian region, or Venezuela. The mobility of miners is a challenge to eliminating malaria since infected people cross borders and increase the possibility of reintroducing malaria into areas where malaria has been reduced or eliminated as well as the spread of malaria parasites [33, 34, 36].

CONCLUSION

The snapshot of malaria diagnosed in Boa Vista shows that the burden of malaria transmission in the state occurs in the mines located in the Yanomami Indigenous Land. The presence of miners increases the number of malaria cases in Roraima, including in Yanomami Indigenous.

The disintrusion of miners from Yanomami territories is urgently needed and requires a robust and complex long-term government project. Meanwhile, Public Health in Brazil can carry out strategies to control malaria among miners to protect the indigenous people and decrease the transmission in Roraima. These strategies will have an impact on reducing malaria cases in the Yanomami indigenous population and, consequently, achieving the goal of eliminating malaria by 2035. To this end, surveillance must be also implemented with cooperative policies between municipalities and federal units in Brazil and neighboring countries to detect the mobility routes of miners’ populations.

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5. CAPÍTULO 3

O presente capítulo apresenta a investigação de mutações associadas com a resistência do *P. falciparum* à artemisinina no gene *pfk13*, em amostras de parasitos do estado de Roraima, para atender ao segundo objetivo específico proposto na presente tese de doutorado: Caracterizar a diversidade de genes *P. vivax* e do *P. falciparum* associados a quimiorresistência desses parasitos a cloroquina e a artemisinina, respectivamente. Este capítulo segue as normas da revista *Journal of Environmental Research and Public Health* (ISSN 1660-4601), Qualis A1 em Biotecnologia. O artigo foi publicado na edição *especial: "Epidemiology, Surveillance, and Control of Frontier Malaria", a qual pertence à seção "Infectious Diseases, Chronic Diseases, and Disease Prevention*", DOI: https://doi.org/10.3390/ijerph21060679.

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Molecular Surveillance of Artemisinin-Resistant *PlasModiuM falciparuM* Parasites in Mining Areas of the Roraima IndigenousTerritory in Brazil

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Abstract: Multidrug- and artemisinin-resistant (ART-R) *Plasmodium falciparum (Pf)* parasites representa challenge for malaria elimination worldwide. Molecular monitoring in the Kelch domain region *(pfk13)* gene allows tracking mutations in parasite resistance to artemisinin. The increase in illegal miners in the Roraima Yanomami indigenous land (YIL) could favor ART-R parasites. Thus, this study aimed to investigate ART-R in patients from illegal gold mining areas in the YIL of Roraima, Brazil. A questionnaire was conducted, and blood was collected from 48 patients diagnosed with *P. falciparum* or mixed malaria (*Pf* + *P. vivax*). The DNA was extracted and the *pfk13* gene was amplified by PCR. The amplicons were subjected to DNA-Sanger-sequencing and the entire amplified fragment was analyzed. Among the patients, 96% (46) were from illegal mining areas of the YIL. All parasite samples carried the wild-type genotypes/ART-sensitive phenotypes. These data reinforce the continued use of artemisinin-based combination therapies (ACTs) in Roraima, as well as the maintenance of systematic monitoring for early detection of parasite populations resistant to ART, mainly in regions with an intense flow of individuals from mining areas, such as the YIL. This is especially true when the achievement of falciparum malaria elimination in Brazil is planned and expected by 2030.

Keywords: Amazon; malaria; chemoresistance; *pfk13*; Guiana Shield

1. Introduction

Plasmodium falciparum is the world's leading and most lethal cause of malaria, making it one of the main challenges to global public health. In 2022, it was responsible for 97%(249 million) of cases and 95% (608,000) of deaths from malaria worldwide [1].
In the Americas region, *P. falciparum* was responsible for 28% of the total 552,000 casesin 2022. Venezuela, Brazil, and Colombia accounted for around 73% of malaria cases in this region [1]. In Brazil, this parasite was responsible for 15.92% of the 142,522 cases reported

in the country in 2023, according to data from the Malaria Epidemiological Surveillance Information System (Sivep-Malaria) [2].

Roraima is one of the states that make up the Brazilian Amazon, where 99% of malaria cases occur [3]. According to Sivep-Malaria, the proportion of *P. falciparum* malaria in the state was 29% of the total of 34.555 malaria cases reported in 2023 [2].

In the global strategy to combat malaria, the World Health Organization (WHO) has presented targets for 2016 to 2030, including reducing malaria cases by at least 90% by 2030and eliminating malaria in at least 35 countries [4]. In this context, Brazil launched the *P. falciparum* malaria elimination plan in 2015 and proposed eliminating malaria by 2035 in 2022. One of the intermediate targets of the Brazilian National Plan is the elimination of *P. falciparum* malaria by 2030 [5].

A major obstacle to eliminating malaria is the emergence of *P. falciparum* multidrug resistance [6]. In fact, in 1960, only a decade after starting large-scale use of chloroquine,

P. falciparum resistance to this drug was detected in Colombia, Cambodia, and Thailand, and it spread rapidly to other endemic countries, including Brazil [7]. In the 1970s, the combination of sulphadoxine and pyrimethamine (SP) was introduced to treat *P. falciparum* infections in South America, but chemoresistance was also soon reported in Colombia, Brazil, Peru, Venezuela, and Bolivia [8–11]. In the 1980s, mefloquine (MQ) was proposed in Brazil as a therapeutic alternative for non-severe malaria, but soon MQ-resistant parasites were reported [12].

Following the reports of treatment failure and the spread of multidrug-resistant *P. falci- parum* parasites, the WHO recommended ART-based combination therapies (ACTs) to treat uncomplicated *P. falciparum* malaria [13]. In addition, the use of ART as monotherapy for malaria treatment was suspended to prevent the emergence of drug resistance [14]. ACTs combine two active substances with different mechanisms of action: an ART derivativeand another antimalarial drug. The first has a short plasma half-life (1 to 2 h) and aims to rapidly reduce the parasite biomass. The second antimalarial drug has a longer plasma half-life (days to weeks) and is designed to eliminate the remaining parasites [6,15].

The Brazilian National Malaria Control Program (PNCM) introduced treatment withACTs for uncomplicated *P. falciparum* malaria in 2005, which led to an increasing decline in malaria in the Brazilian Amazon region [16]. The same epidemiological dynamic can be observed in other endemic countries worldwide [17].

The detection of ART resistance in Southeast Asia, first in Cambodia in 2008 and then inChina, Vietnam, Thailand, and Myanmar, has highlighted an obstacle in the global malariaelimination effort [18]. The emergence of this resistance can be attributed to monotherapy with unregulated ART or artesunate (AS), which had been available in this region since the mid-1970s, as well as the availability of these drugs in the private health sector [19].

In 2010, a mutation associated with ART resistance was identified in Guyana, a South American country. Genomic analyses indicate that the mutation in Guyana did not spreadfrom Southeast Asia but occurred independently [20].

The state of Roraima, together with the western part of Amapá, the northern Amazon, and Pará in Brazil, and the territories of Guyana, Suriname, French Guiana, Venezuela, and Colombia, is part of the Guiana Shield. This region is considered a potential source for the emergence of malaria resistance in South America, as the subsoil rich in gold and other minerals attracts prospectors to the indigenous forest areas and, therefore, is increasing the size of the human population [21–23].

In the last decade, the proportion of *P. falciparum* infections in Roraima has increased significantly, probably due to delayed diagnosis and treatment of Venezuelan migrants and gold miners living in illegal mining areas in the YIL [24]. In addition, Venezuela's economic collapse led to a shortage of antimalarial drugs and the search for treatment in neighboring countries, particularly Brazil, increasing imported malaria cases, including *P. falciparum*infections [25].

In Roraima, there was a 44% rise in malaria cases in 2020 [24]. In the same year, illegalmining in the YIL increased by 30%. More than half (52%) of the mines in this region are located on the Uraricoera River, but mines have also been found on the banks of the Mucajaí, Couto de Magalhães, Parima, and Catrimani rivers [26].

The mines are located in isolated forest areas without access to diagnosis and treatmentby the Brazilian Unified Health System (SUS). For this reason, miners with a fever tend totake antimalarials of dubious or even illegal origin to avoid having to stop mining. This scenario may favor the selection of ART-resistant parasites and underlines the need for molecular surveillance of antimalarial resistance in this region [24,27,28].

Surveillance of molecular markers of antimalarial resistance is an important strategy for detecting treatment failure and should be implemented to detect resistant parasites earlyand prevent their spread [29–31]. In 2014, mutations in the propeller domain of the Kelch 13 gene on chromosome 13 (*pfk13*) were associated with delayed elimination of parasites in vitro and in vivo, and this molecular marker has been used for global surveillance of artemisinin resistance (ART-R) [6,32]. The following mutations have been validated by in vitro and in vivo studies: C580**Y**, R561**H**, R539**T**, I543**T**, P553**L**, M476**I**, N458**Y**, Y493**H**, F446**I**, and P574**L**. The mutations P441**L**, T449**A**, C469**F/Y**, A481**V**, R515**K**, P527**H**, N537**I/D**, G538**V**, V568**G**, R622**I**, and A678**V** are considered ART-R molecular markers. In addition, less common *pfk13* variants such as K479**I**, G533**A**, R575**K**, M579**I**, D584**V**, P667**T**, F673**I**, and H719**N** have also been associated with delayed parasite elimination [6].

Considering that the future efficacy of ACTs is endangered by the emergence of resistance artemisinin, this study aims to investigate single nucleotide polymorphisms (SNPs) associated with *P. falciparum* ART-R in the *pfk13* gene in the state of Roraima.

2. Materials and Methods

The study site was the municipality of Boa Vista (2°49′10″ N and 60°40′23″ W, the capital of Roraima, which is located in the far north of Brazil and is the only capital abovethe equator [33]. Almost the entire Roraima state is part of the Guiana Shield, along withthe western part of Amapá and the northern parts of the Amazonas and Pará in Brazil. In addition to Brazil, Guyana, Suriname, French Guiana, southern Venezuela, and eastern Colombia comprise the Guiana Shield. There is an intense migratory flow of miners in this Shield and C580**Y** and R539**T** mutations in the *pfk13* gene have already been identified in Guyana [20] (Figure 1).

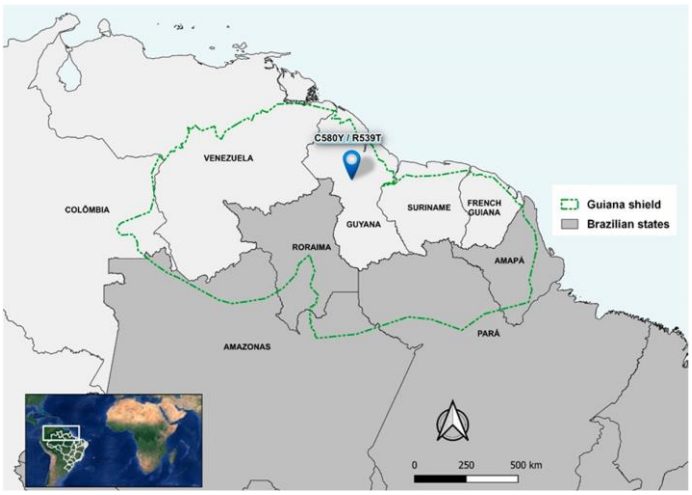


Figure 1. Location of Roraima in the Guiana Shield and mutations in the *pfk13* gene (C580**Y** and R539**T**) in Guyana. Maps were produced by the authors.

According to estimates by the Brazilian Institute of Geography and Statistics (IBGE),the city currently has 436,591 inhabitants and is home to 67% of the state's population. It borders the municipalities of Normandia, Pacaraima, and Amajari to the north; Mucajaí and Alto Alegre to the south; Bonfim, Cantá, and Normandia to the east; and the municipality of Alto Alegre to the west.

Boa Vista is home to the state's most sought-after public health institutions. The twohealth facilities with the highest number of malaria reports, according to Sivep-Malaria, were selected as sample collection sites: Emergency Service Cosme e Silva and Basic Health Unit Sayonara Maria Dantas, located in the West Zone of the city (Figure 2). This urban area has the highest employment density, 70% of the city's 56 neighborhoods, and mostof the population has a low monthly income. The West Zone emerged in the 1990s due to interregional migration flows, rural exodus and gold prospectors who moved to Boa Vista after mining was banned in the early 1990s [34]. The samples were collected from December 2021 to June 2022, during the transition period from the rainy to the dry season, when the mosquito population increases seasonally [34].

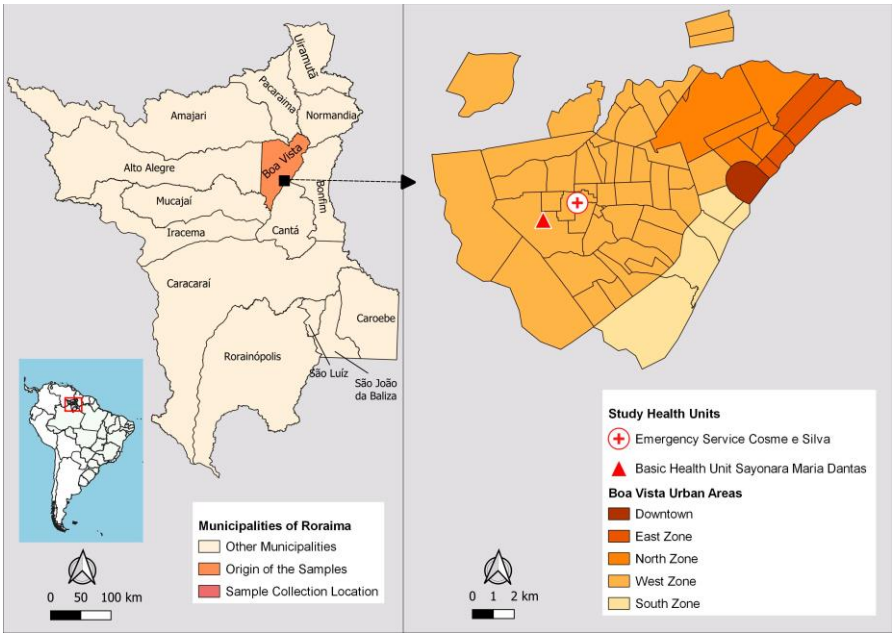


Figure 2. Maps showing the location of Boa Vista, the urban areas, and the study areas. The maps were created by the authors.

This study was approved by the Research Ethics Committee of the Federal Universityof Roraima (CEP/UFRR): CAAE 24122619.6.0000.5302, on 17 March 2020. Participants signed an informed consent form (ICF) to participate in this study. Individuals over the age of 18 who had been diagnosed with *P. falciparum* or mixed malaria (*P. vivax* + *P. falciparum*) by a thick blood smear were included. Those excluded from this study were children under 18, indigenous villagers, individuals who could not read, and those who refused to sign the informed consent form. Participants in this study were asked questions related to the individual and the disease. Blood was collected by venipuncture of 5 mL of peripheral blood. Part of the blood (around 50 microliters) was transferred directly from the syringe to filter paper (Whatman 903 Protein Saver Cards, Merck (Sigma), Darmstadt, Germany) and the rest was placed in a vacutainer tube containing EDTA (Becton, Dickinson & Company, Franklin Lakes, NJ, USA).

The samples were transported to the Molecular Biology Laboratory of the Biodiversity Research Center of the Roraima Federal University. The tubes containing blood were centrifuged at 3000 *g* for 10 min to remove the plasma. The "red blood cell concentrate" (containing leukocytes and platelets) was added to the cryopreservation solution glycerolyte 57 (Baxter Inc., Illinois, USA) volume by volume (*v*/*v*), followed by the aliquoting of eachsample. The aliquots with the cryopreservation solution were stored at 20 ˚C until DNAextraction.

Deoxyribonucleic acid (DNA) was extracted using the column technique (centrifuga-tion method), using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, from 500 µL of the "red blood cell concentrate".

The *pfk13* gene fragment was amplified by nested polymerase chain reaction (PCR) [35]. The following primers were used in the first reaction: K13 F: 5´CGGAGTGACCAAATCTGG GA3´ and K13 R: 5´GGGAATCTGGTGGTAACAGC3´. A mixture of PCR reagents was prepared with 13.75 µL of ultrapure water, 5 µL Taq hotfirepol®, (Solis, Tartu, Estonia), 0.625 µL of each of the primers (10 pmol), and 5 µL of the *P. falciparum* DNA sample was added to each mixture. The PCR conditions in the thermal cycler were as follows: initialization at 95 ˚C for 15 min; 30 cycles of denaturation at 95 ˚C for 30 s, annealing of the primers at 58 ˚C for 2 min, and extension at 72 ˚C for2 min; and final elongation at 72 ˚C for 10 min.

The following primers were used for the second PCR: K13 N_F: 5´GCCTTGTTGAAAG AAGCAGA3´ and K13 N_R: 5´GCCAAGCTGCCATTCATTTG3´. A mixture was prepared with 37.5 µL ultrapure water, 10 µL Taq 5 hotfirepol®, 1.25 µL of each of the primers (10 pmol), and 5 µL of the first PCR amplified product was added to each mixture. The PCR conditions were as follows: initialization at 95 ˚C for 15 min; 40 cycles of denaturation at 95 ˚C for 30 s; annealing at 60 ˚C for 1 min and extension at 72 ˚C for 1 min; and final elongation at 72 ˚C for 10 min.

The final 849 base pairs (bp) amplified product (amplicon) was analyzed after elec-trophoresis in a 2% agarose gel, stained with Bluegreen (LGC), using a DNrBio-Imagining System/Model: MiniBIS Pro (UV) photo-documentation system (DNR, Jerusalem, Israel).A 100 bp molecular weight marker was used to check the size of the fragments (Figure 3).

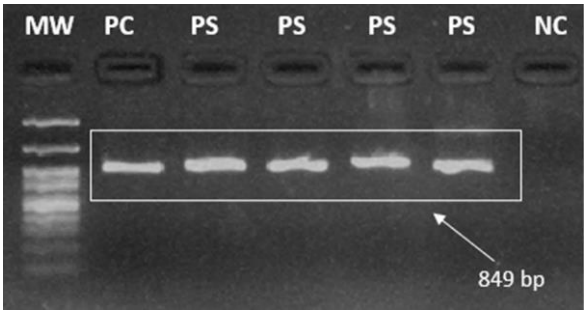


Figure 3. Representative illustration of a 2% agarose gel highlighting the amplicons from *pfk13* nested PCR. Legend: MW = Molecular Weight (100 bp), PC = Positive Control, PS = Positive Sample,NC = Negative Control. Image taken by the authors.

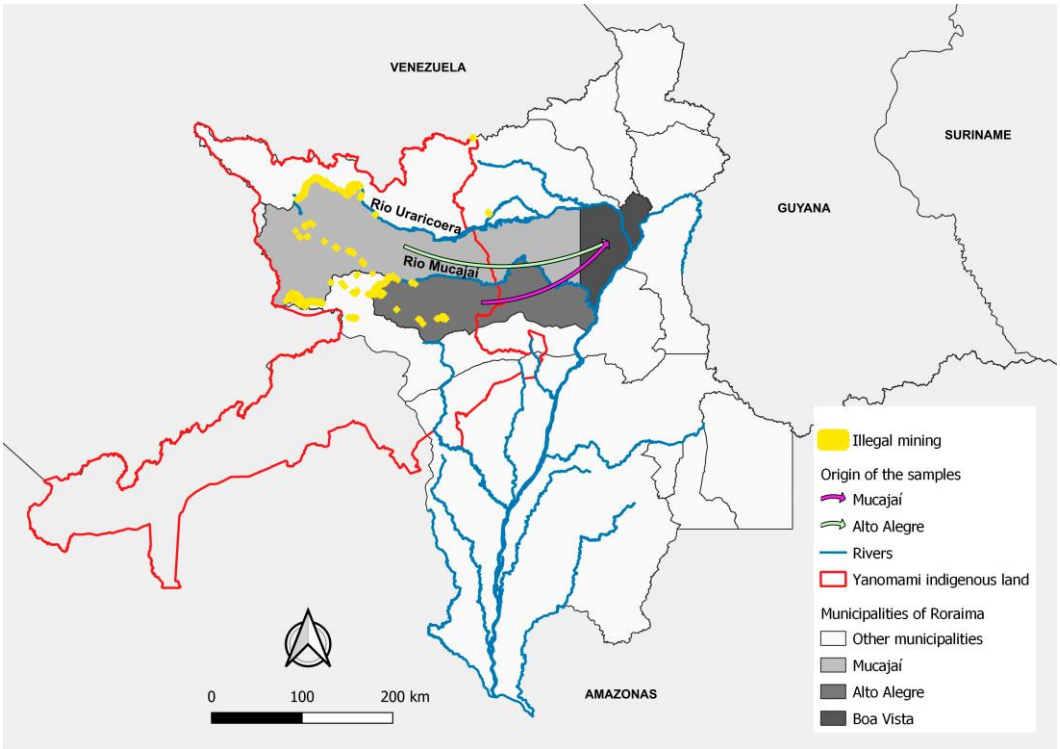
The amplicons were purified using the Wizard® Kit, according to the manufacturer's instructions. The sequencing reaction was carried out using the Big Dye kit® TerminatorCycle Sequencing Ready Reaction version 3.1 (Applied Biosystems, Carlsbad, CA, USA),according to the manufacturer's instructions. The amplicons were subjected to Sanger-type sequencing using capillary electrophoresis on the ABI PRISM DNA Analyzer 3730 (AppliedBiosystems, Carlsbad, CA, USA) of the PDTIS/Fiocruz genomic platform and ABI PRISM DNA Analyzer 3500 (Applied Biosystems, Carlsbad, CA, USA) of the LabMol/CBio/UFRR.

The entire amplified fragment DNA sequence (codons 427 to 709) was analyzed using the ClustalW multiple sequence aligner in the BioEdit® version 7.7.1 software (North Carolina State University, Raleigh, NC, USA), using the prototype 3D7 as the reference sequence (GenBank PF3D7_1343700: Reference 000002765 *Plasmodium falciparum* Genome Sequencing Consortium, release date: PlasmoDB # 29, 2016-october-12, available in https: //www.ncbi.nlm.nih.gov/nuccore/). The maps were created using the QGIS program version 3.28.10. Mining areas in Roraima were obtained from Mapbiomas [36]. The geopolitical limits of Brazil and the indigenous lands were accessed on the IBGE website [37].

3. Results

A total of 53 samples were collected, 42 from *P. falciparum* and 11 from mixed *P. falciparum* + *P. vivax* malaria infections. Of the total samples collected, 83% (44) were from men, ages 18 to 55, with a median of 36 years. Regarding the main activity performed 15 days before the symptoms, 96% (51) of participants reported mining and only 4% (02) agriculture. Detailed epidemiological information on these patients has recently been published [38].

Approximately 97% (48) of the samples were amplified by nested PCR for the *pfk13*gene, and all amplicons were purified and sequenced. Of the total sequenced samples, 46 (96%) were from patients reporting mining activity, and only 2 (4%) were from thosewho reported agricultural activities. The mines were mainly located in the YIL in the municipalities of Alto Alegre (40) and Mucajaí (5) (Figure 4). In addition, one participantcame from gold mines in Itaituba/PA (1). The patients who reported working in agriculture were likely infected in the municipalities of Caracaraí (1) and Alto Alegre



(1).

Figure 4. Origins of the samples, locations of the mines, rivers, Yanomami indigenous land, munici-palities of infection, the Guiana Shield, and the study site. Map made by the authors.

After aligning the nucleotide sample sequences with the 3D7 *P. falciparum* reference genome strain (PF3D7_1343700), no mutations were found in the *pfk13* gene, neither in the codons associated with ART-R nor in the other codons analyzed. Thus, in the amplified fragment, all *P. falciparum* sequence samples were identical to the wild-type 3D7 reference sequence (Figure 5).

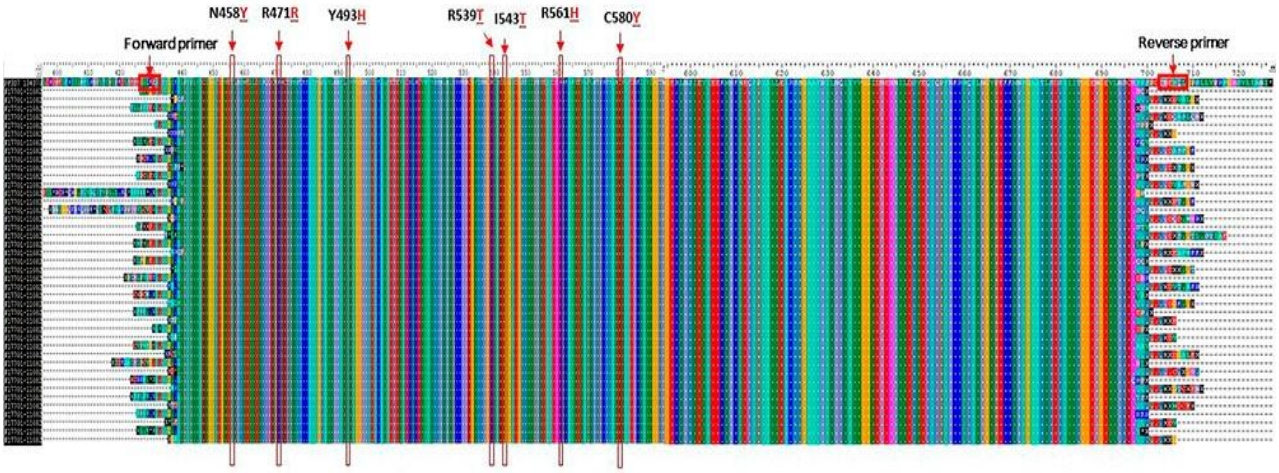


Figure 5. Alignment of *pfk13* gene sample sequences. SNPs associated with ART resistance are highlighted in red. The red rectangle at the beginning of the sequence corresponds to the primer sequences. Each color represents one amino acid.

4. Discussion

It is known that gold miners are considered at risk for malaria and that *P. falciparum* is widely distributed in several gold mining regions, especially in the Guiana Shield [23,39], where conditions are favorable for the selection of antimalarial-resistant parasites.

Slow elimination of the parasite after treatment with ACT characterizes partial resis- tance to ART. In the Brazilian endemic areas, parasites in the blood on day 3 (D3) after the start of therapy (D0) are rare in patients treated with ACT. Therefore, assessing *P. falciparum* clearance on D3 after initiation of treatment is an important parameter for monitoring ART-R [6]. However, in the present study, participants' parasitemia was not assessed on day D3 after starting treatment with ACT because it was difficult to follow the vast majority of participants, who were miners that traveled to Boa Vista only for diagnosis and returned to the mines in YIL just after receiving the medication.

In recent years, illegal mining has increased in Roraima in the YIL, mainly in Mucajaí,Uraricoera, Catrimani, and Parima river areas. More than half (52%) of the total area damaged by mining is concentrated along the Uraricoera River [26]. Access routes to themining areas are mainly via the rivers and forest areas of the municipalities of Alto Alegre,Amajari, Mucajaí, Caracaraí, and Iracema, or by plane via clandestine airstrips hidden in rural areas. The gold miners in this region do not have access to malaria diagnosis and treatment through the SUS, which is exclusively for the indigenous peoples living there [24]. Therefore, to avoid losing working days through travel to distant cities, gold miners buy the drug Artecom® (dihydroartemisinin-piperaquine; Chongqing Tonghe Pharmaceutical Co. Ltd., Chongqing, China), which is not registered in Brazil and enters illegally through the Suriname, Guyana and French Guiana borders. They claimed that a single dose of this drug relieves the symptoms for a few days even though it cannot clear all the parasites. Then, this indiscriminate drug use makes miners a risk group for selecting *P. falciparum* parasites resistant to ART [39].

Currently, the only treatment for *P. falciparum* malaria is the ACTs. Consequently, the emergence of resistance to ART would have a devastating impact on the global goal of malaria elimination. It could also lead to the risk of selecting resistant parasites against partner drugs [6,40].

Molecular surveillance of *pfk13* helix domain polymorphism in endemic countries can play an important role in early warning of ART-R parasites [6,23,41,42]. The C580**Y**mutation in the *pfk13* gene is relevant for molecular surveillance of ART resistance in Southeast Asia [18,43]. This mutation is present in the vast majority of resistant parasites in Cambodia and reaches a prevalence of up to 70% at the border between Thailand and Myanmar [6]. In 2010, the C580**Y** mutation was also identified in 5% (5/98) of parasite samples from Guyana [20]. The appearance of this mutation in South America raised thequestion of whether there would be a risk of mutant alleles spreading to different hotspots in the Amazon basin through intensive migration across the Venezuela, Guyana, and Brazil borders as a result of illegal gold mining. However, between 2016 and 2017, the prevalence of this allele decreased to 1.6% (14/864) [42]. The in vitro competitive co-cultivation of *pfk13* mutant (C580**Y** and R539**T**) and non-mutant parasites from Guyana showed that the mutants

have a growth deficit compared to the non-mutant wild parasites [42]. This disadvantage in the fitness of the ART-R mutant parasite could be one of the reasons whythese *pfk13* gene mutants have not been fixed and, thus, have not spread from Guyana toneighboring countries in recent years [42]. However, we must bear in mind that the risk of the spread of such mutants in the Amazon basin, especially those from the Guiana Shield,is proportional to the intensity of mining activities whose main migration patterns include Venezuela, Guyana, and the state of Roraima in Brazil [23].

The World Health Organization drew attention to the presence of *P. falciparum* parasites carrying the C580**Y** mutation in infected Chinese travelers in Equatorial Guinea and Ghana upon their return to their country and hypothesized that these mutations were likely to have originated in Africa rather than Southwest Asia, but there was no evidence that this mutation had spread to parasite populations in African areas [6,44]. In fact, non- synonymous *pfk13* mutations are present at low frequencies in Africa [40,45]. Of the 35 non-synonymous mutations detected on that continent, the R561**H** was the most frequent, followed by A578S, which appears to be the most prevalent worldwide [40,45–47]. The R539**T** and P553**L** variants were identified in a sample from Angola, and the M476**I** in a sample from Equatorial Guinea [46]. R561**H**, M579**I**, and C580**Y** mutations can confer in vitro artemisinin resistance in African parasites [45].

Although 97% of the samples in our study came from individuals infected in miningareas, all sequences analyzed showed non-mutated/wild-type genotypes in the helix–loop domain of *pfk13*. This finding corroborates a study carried out in Roraima, which also described the absence of polymorphisms in the *pfk13* gene in parasite samples collectedbetween 2016 and 2017 in the municipalities of Boa Vista, Pacaraima, and Rorainópolis [48].Samples of *P. falciparum* from the Brazilian Amazon basin collected between 1984 and 2011, i.e., in the period before and after the introduction of ART treatment in Brazil, alsoshowed no mutations in the *pfk13* gene [41], as reported in more recent studies in this region [49,50].

In 2013, the mutant *pfk13* A481**V** parasite, which is associated with delayed elimination of *P. falciparum* in patients in Southeast Asia [51], was detected in one of the 575 samples of *P. falciparum* malaria from Manaus/Brazil, but without clinical signs of ART-R [23]. The A504**D** mutation observed in 2018 in Colombia also did not lead to ART-R phenotypes [51]. Thus, further work is needed before these mutations can be considered markers for chemoresistance to ART in samples from South America.

As the P413**A** mutation in the BTB/POZ domain of the *pfk13* gene was recently identified in parasites from an African isolate of *P. falciparum* subjected to ART in vitro pressure [52], we intend to investigate mutations in the BTB/POZ domain, in addition tothose here investigated in the *pfk13* gene helix domain [53].

Finally, considering that the *pfCoronin* gene is the main driver of reduced susceptibility to ART in Senegalese parasites developed in vitro [54], we intend to extend our investiga- tions to this gene, due to the possibility of synergism with *pfk13* in cases of partial resistanceto ART [54,55].

5. Conclusions

Given the results of this study, treatment with ART derivatives can continue to be used as the first-line treatment for *P. falciparum* malaria in Brazil. We recommend continuingmolecular surveillance to track ART resistance in Roraima, as mutant parasites could be introduced and/or selected due to the influx of miners into the Guiana Shield, which consists of Brazil, French Guiana, Suriname, Guyana, Venezuela, and Colombia.

Author Contributions: Conceptualization: J.d.A.-B., M.d.F.F.-d.-C., F.G.; Data curation: J.d.A.- B., D.d.S.e.S., A.C.C.; Formal analysis: J.d.A.-B., M.d.F.F.-d.-C., F.G., R.d.A.-F., L.T.d.Q., N.K.A.- d.-O.M.; Funding acquisition: M.d.F.F.-d.-C., F.G.; Investigation: J.d.A.-B., D.d.S.e.S., M.d.F.F.-d.-C.; Methodology: J.d.A.-B., D.d.S.e.S., R.d.A.-F., L.T.d.Q., M.d.F.F.-d.-C., F.G.; Project administration: J.d.A.-B., M.d.F.F.-d.-C., F.G.; Resources: M.d.F.F.-d.-C., C.T.D.-R., F.G.; Software: J.d.A.-B., A.C.C.; Supervision: M.d.F.F.-d.-C., F.G.; Writing—original draft: J.d.A.-B., M.d.F.F.-d.-C., F.G.;Writing—review and editing: M.d.F.F.-d.-C., C.T.D.-R., F.G., N.K.A.-d.-O.M., R.d.A.-F., L.T.d.Q. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The Research Ethics Committee of the Federal University of Roraima (CEP/UFRR) approved this study on 17 March 2020, CAAE 24122619.6.0000.5302.

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The original data (sequences) presented in the study are openly available (deposited) in Genbank™ with accession numbers PP584057-PP584104.

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Conflicts of Interest: The authors declare no conflict of interest.

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6. CAPÍTULO 4

O presente capítulo apresenta a investigação de polimorfismos nos genes *pvcrt- o* e *pvmdr-1* para se conhecer o potencial preditivo dessas mutações como marcadores moleculares do fenótipo de resistência à cloroquina no *P. vivax.* Atendendo, assim, o segundo objetivo específico proposto: Caracterizar a diversidade de genes *P. vivax* e do *P. falciparum* potencialmente associados a quimiorresistência desses parasitos a cloroquina e a artemisinina, respectivamente.

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Investigation of Mutations in the *crt-o* and *Mdr1* Genes of *PlasModiuM vivax* for the Molecular Surveillance of Chloroquine Resistance in Parasites from Gold Mining Areas in Roraima, Brazil

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Abstract: *Plasmodium vivax* causes the largest malaria burden in Brazil, and chloroquine resistance poses a challenge to eliminating malaria by 2035. Illegal mining in the Roraima Yanomami Indigenous territory can lead to the introduction of resistant parasites. This study aimed to investigate mutations in the *pvcrt-o* and *pvmdr-1* genes to determine their potential as predictors of *P. vivax* chloroquine- resistant phenotypes. Samples were collected in two health centers of Boa Vista. A questionnaire was completed, and blood was drawn from each patient. Then, DNA extraction, PCR, amplicon purification, and DNA sequencing were performed. After alignment with the Sal-1, the amplified fragment was analyzed. Patients infected with the mutant parasites were queried in the Surveillance Information System. Among the patients, 98% (157/164) of participants were from illegal mining areas. The *pvcrt-o* was sequenced in 151 samples, and the K10 insertion was identified in 13% of them. The *pvmdr1* was sequenced in 80 samples, and the **M**YF haplotype (958**M**) was detected in 92% of them and the TYF was detected in 8%, while the **MYL** was absent. No cases of recrudescence, hospitalization, or death were found. Mutations in the *pvcrt-o* and *pvmdr-1* genes have no potential to predict chloroquine resistance in *P. vivax*.

Keywords: Amazon; malaria; chloroquine; *pvcrt-o*; *pvmdr1*; mining

1. Introduction

Plasmodium vivax is the second most important malaria pathogen in the world, being responsible for 2.8% of the 249 million malaria cases in 2022. Outside of Africa, it is the main *Plasmodium* species, and it has the widest geographical distribution in the world. It causes significant morbidity in Southeast Asia, North and South America, and the Middle East [1].
In the Americas, 72% of malaria cases are caused by *P. vivax*, and four countries accounted for 80% of estimated cases in 2022: the Bolivarian Republic of Venezuela (28%), Brazil (27%), Colombia (18%), and Peru (6%) [1].

In 2023, 142,522 malaria cases were reported in Brazil, of which 99.97% were autochthonous in the states of the legal Amazon region (Acre, Amapá, Amazonas, Maranhão, Mato Grosso, Pará, Rondônia, Roraima, and Tocantins), and 82.6% were caused by *P. vivax*. In the same year,34,555 cases were reported in Roraima, 71% of which were caused by *P. vivax* [2].

To achieve the goal of malaria elimination by 2035, the National Malaria Program (PNCM) envisages only *P. vivax* transmission for the last five years after the eliminationof malaria transmission by *P. falciparum* in 2030 [3]. However, the unique biology of *P. vivax* poses additional challenges to the elimination target, such as relapses caused by hypnozoites, the ability to infect mosquitoes before symptoms appear, and asymptomatic infections in endemic areas [4].

Adding to this context, the emergence of resistance to chloroquine (CQR), the drug of firstchoice for the elimination of the blood stages of *P. vivax*, may represent a further obstacle tocontrol strategies based on the use of this antimalarial drug [5,6]. Indeed, three decades after the emergence of *P. falciparum* CQR, such resistance in *P. vivax* was first reported in Papua NewGuinea in 1980, and subsequent studies have shown an increase in this resistance, accompaniedby reports of severe and fatal *P. vivax* malaria in these regions [7,8].

In addition to Southeast Asia, particularly in Thailand [9] and Cambodia [10], *P. vivax* CQR has been documented in East Africa, Ethiopia [11,12], South America, parts of the Guiana Shield, and the Cooperative Republic of Guyana [13].

In Brazil, CQR was first detected in 1999 in a patient in Manaus in Amazonas state [14]. Later studies in the same city reported a 10.1% failure rate for treatment with CQ in 2007 [15] and 5.2% in 2014 [16]. More recently, 1.1% treatment failure following supervised treatmentwith CQ and primaquine (PQ) was reported in the city of Oiapoque in the state of Amapá,on the border with French Guiana [17].

The resistance mechanisms in *P. vivax* are still not fully understood, probably also becausethis parasite species lacks continuous in vitro cultivation methods [18,19]. In this context, monitoring with molecular markers has emerged as a practical and cost-effective field tool forantimalarial drug resistance monitoring compared to in vivo and in vitro tests [18].

Molecular markers that can be considered for predicting a malaria resistance phe- notype in *P. vivax* have been identified based on the orthologs' resistant-related genes in *P. falciparum* [20,21]. Thus, the multidrug resistance gene 1 of *P. vivax* (*pvmdr1*) and theCQ resistance transporter gene (*pvcrt-o*) are orthologous to the *pfmdr1* and *pfcrt* genes in *P. falciparum* [18,22,23]. Alterations in the *pvmdr1* sequences are thought to confer CQR byreducing the transport of CQ into the digestive vacuole (DV), where the parasite digests host cell proteins and converts hemoglobin heme to nontoxic hemozoin [24].

The mutant *pvcrt*, in turn, would act as an efflux pump in the active transport of CQ out of the DV and away from its target (converting heme to hemozoin) [18]. The lysine insertion (AAG) in the first exon (amino acid 10), referred to as the K10 insertion in the *pvcrt-o* gene, would be associated with a reduction of half of the maximum inhibitory concentration of CQ (IC50) and has been identified as a possible molecular marker for CQR in *P. vivax* [25,26]. Concerning *pvmdr1*, the amino acid mutations Y976F, F1076L, and T958M have been linked to CQR [21,26,27].

In Roraima, the malaria burden in the state has increased, especially since 2018, dueto the increased migration flow from Venezuela and Guyana, together with the boost of illegal mining in the Yanomami Indigenous land. In the same period, there has been an increase in hospitalizations and deaths by *P. vivax* malaria [28], and it is known that the clinical severity of malaria could be related to the appearance of CQR [29].

Since little is known about the *P. vivax* genotypes of *pvmdr1* and *pvcrt-o* genes circulat- ing in the parasites of Brazilian endemic areas, such as those of Roraima, this study aimedto investigate the polymorphism of these genes in regions with a great influx of people.

2. Materials and Methods

This study was approved by the Research Ethics Committee of the Federal University of Roraima CAAE 24122619.6.0000.5302 (CEP/UFRR, acronym in Portuguese; opinion n. 3,920,373, issued on 17 March 2020). The CEP/UFRR allowed only the inclusion of non-village Indigenous people who speak Brazilian Portuguese and reside in Boa Vista. Additionally, the research project was demanded to be presented to the Kannu Kadan Indigenous Association for obtaining a letter of consent, which was attached to the sub- mission process to the CEP/UFRR. The samples were collected at the Emergency ServiceCosme e Silva and Sayonara Health Unit from December 2021 to June 2022, due to the seasonal increase in the number of malaria cases in Roraima during this period. Both health centers are located in the west zone of the city and have the highest number of malaria reports according to the Malaria Epidemiological Surveillance Information System (Sivep-Malaria) (Figure 1).

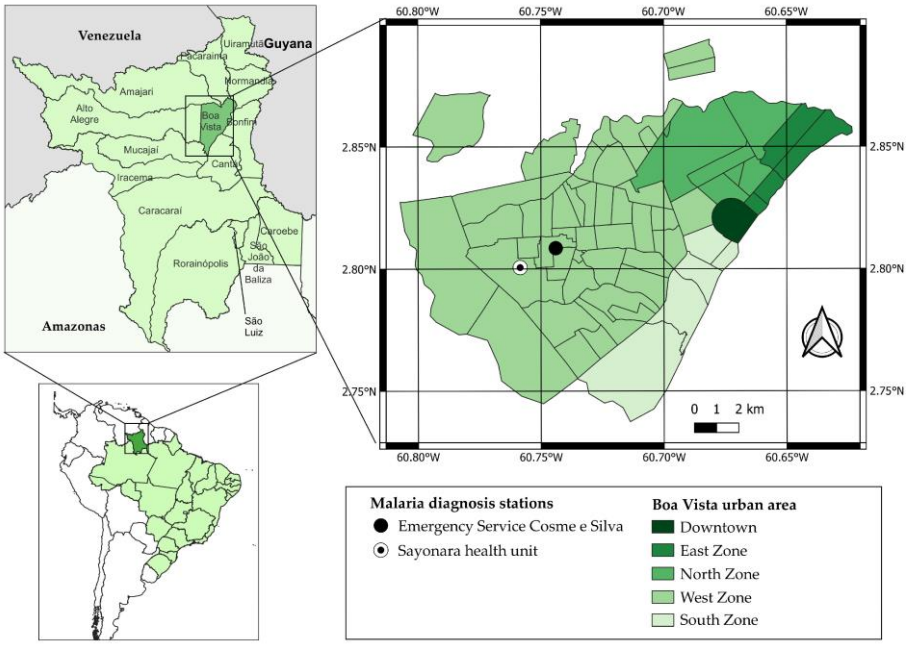

Figure 1. Location map of Roraima, Boa Vista, the urban area of Boa Vista, and sample collectionsites.

Individuals over the age of 18 who had been diagnosed with *P. vivax* malaria or mixed malaria (*P. vivax* + *P. falciparum*), and had been diagnosed through thick blood smears, were included. The non-inclusion of individuals under 18 years old does not representa limitation of this study, because the considerable increase in malaria cases occurred inmining areas where children under 18 years of age do not have access. Individuals who could not read or refused to sign the free and informed consent form (TCLE) were also excluded from this study. After signing the consent form, an epidemiologic questionnairewith questions about the person and malaria was completed.

Blood was collected by venipuncture of 5 mL of peripheral blood. A portion of the blood(approximately 50 microliters) was transferred directly from the syringe to filter paper (Whatman903 Protein Saver Cards, Merck, Burlington, MA, USA) and the remainder to a Vacutainer tube(Becton, Dickinson & Company, Franklin Lakes, NJ, USA) containing EDTA.

All participants were treated according to the National Malaria Control Program (PNCM) protocol for non-severe malaria, which includes the administration of a combina- tion of CQ for 3 days (10 mg/kg on day 1 and 7.5 mg/kg on days 2 and 3) and PQ for 7 days (0.5 mg/kg/day) [30]. According to the PNCM, cure control should be assessed through the cure verification slide (CVS) on days 3, 7, 14, 21, 28, 42, and 63 after the start of treatment, according to theoperational capacity of the local health network. Collections on D3 and D28 should be prioritized for P. vivax infections. The day the diagnosis is made and treatment begins isconsidered day zero (D0) [31,32].

To identify the CVS of the participants infected by parasites with target mutations, a search was carried out in Sivep-Malaria for one year before and one year after the date of sample collection. To investigate malaria hospitalization cases after diagnosis, a search was carried out on Sivep-Malaria at the Notification Unit of the Roraima General Hospital, astate reference for severe malaria. The deaths were investigated by a search carried out on the Mortality Information System (SIM). Relapse or recurrence was considered the reappearance of asexual parasitemia with or without symptoms after treatment due to the following: (i) recrudescence (incomplete clearance of asexual parasites after antimalarial treatment within 28 days); (ii) relapse(arising from hypnozoites between 28 and 60 days); or (iii) reinfection (after 60 days) [31,32].

The blood samples collected in vacutainer tubes and on filter paper were transported to the Molecular Biology Laboratory (LaBMol) of the Center for Biodiversity Studies (CBio)at the Federal University of Roraima (UFRR). The samples collected in the tubes were centrifuged at 3000 g for 10 min to remove the plasma and the cryopreservation solution glycerolyte 57 (Baxter, Minato City, Japan) was added to the "red blood cell concentrates" (containing leukocytes and platelets) volume by volume (v/v), followed by aliquoting each sample. The aliquots with the cryopreservation solution were stored at 20 °C in racks and packed in individually labeled plastic bags until the deoxyribonucleic acid (DNA)was extracted.

DNA extraction was carried out using the column technique (centrifugation method), using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, from a volume of 500 µL of the sample.

The methodology used for the PCR (polymerase chain reaction) of the *pvmdr1* gene was performed according to the protocol previously described, with the following primers: F: 5′-ATAGTCATGCCCCAGGATTG-3′ and R: 5′-ACCGTTGGTCTGGACAAGTAT-3′ [33].

A mixture of PCR reagents was prepared for a final volume of 50 µL, with 26.75 µL of ultrapure water, 6 µL of MgCl2 (25 mM), 5 µL of PCR Buffer II (10), 5 µL of deoxynu-cleotide triphosphates (dNTPs) (8 mM), 1 µL of each primer (10 pmol), and 0.25 µL of AmpliTaq Gold DNA Polymerase (250 U). Finally, 5 µL of DNA was added to each mixture.The PCR conditions in the thermal cycler included the following: initiation at 95 °C for 10 min; 40 cycles with denaturation at 94 °C for 15 s; primer annealing at 60 °C for 30 s; extension at 72 °C for 1 min; and final elongation at 72 °C for 7 min. The final product was a 762 pb fragment amplified to analyze T958 **M**, Y976**F**, and F1076**L** in the *pvmdr1* gene.

The PCR reaction to amplify the *pvcrt-o* gene was based on the protocol previously described, with the following primers: F: 5′-AAGAGCCCGTCTAGCCAT CC-3′ and R: 5′-AGTTTCCCTCTACAC CCG-3′ [21]. To the reagent mixture with a final volume of42 µL, 23.75 µL of ultrapure water, 4 µL of $MgCl_2$ (25 mM), 4 µL of PCR Buffer II (10x), 2 µL of deoxynucleotide triphosphates (dNTPs) (8 nM), 2 µL of each of the primers (10 pmol),and 0.25 µL of AmpliTaq Gold DNA Polymerase (250 UI) were added. A total of 4 µL of DNA was added to each PCR reagent mixture. The PCR conditions in the thermal cycler included initial heating at 95 ˚C for 10 min, followed by 35 cycles with denaturation at 94 ˚C for 30 s, primer annealing at 61 ˚C for 1 min, extension at 72 ˚C for 1 min, and final elongation at 72 ˚C for 7 min. The final product of the PCR reaction (amplicon) generated a fragment of 1186 bp.

Three controls were used to ensure the accuracy and reliability of the results duringthe amplification of the target genes. The positive control consisted of a *Plasmodium vivax* sample diagnosed by molecular and microscopic examinations, whose target sequence amplification was confirmed by DNA sequencing. The negative control comprised a sample from a clinically healthy individual with no malaria history. Additionally, a blank controlcontaining all test reagents but not the DNA, replaced by ultrapure water, was used.

To obtain the target sequences of the isolates, the PCR products were purified using theWizard® Kit, Promega, Madison, WI, USA, according to the manufacturer's instructions. The sequencing reaction was performed using the Big DyeTM Terminator Cycle Sequencing Ready Reaction version 3.1 kit (Applied Biosystems, Waltham, MA, USA), and 3.2 pmol of forward and reverse primers were used separately in the reactions.

To investigate SNPs in the *pvmdr1* and *pvcrt-o* genes, the sense and antisense sequences of the samples and the reference sequence were aligned and analyzed with a ClustalW multiple sequence aligner in BioEdit software version 7.7.1 (North Carolina State University,Raleigh, NC, USA). The Salvador 1 (Sal-1) strain was used as the reference sequence (GenBank Accession No. AF314649.1 for *pvcrt-o* and No. AY571984.1 for *pvmdr1*).

The information from the questionnaires and the laboratory analysis results were entered and tabulated in the Excel program (Microsoft Office 365 v16.78.3®). The maps were drawn up using the QGIS program version 3.28.10. Mining areas in Roraima were obtained from Mapbiomas [34]. The geopolitical limits of Brazil and Indigenous lands were accessed on the IBGE website [35].

3. Results

Samples were collected from 164 participants, of whom 153 had *P. vivax* and 11 (7%) had mixed malaria (*P. vivax* + *P. falciparum*).

The participants ranged in age from 18 to 67, and 82% (135/164) were men. The most commonly reported symptoms were fever at 91.5% (150/164), headache at 86% (141/164), chills at 67% (110/164), abdominal pain at 55% (90/164), and nausea/vomitingat 43% (70/164). Detailed epidemiological information on these patients was recently published [36].

Regarding the main activity carried out in the 15 days before the onset of symptoms,96% (157/164) of the participants reported gold mining, mainly in the municipalities ofAlto Alegre with 76% (120/157) of the participants, and Mucajaí with 18.5% (29/157) of them. Agriculture accounted for 3% (5/164) of the participants, with 40% (2/5) in the mu- nicipality of Mucajaí and 20% (1/5) in the Alto Alegre, Cantá, and Caroebe municipalities. Hunting/fishing and tourism, with a percentage of 0.6% (1/164), occurred in participants from the municipalities of Alto Alegre and Mucajaí, respectively (Table 1).

Table 1. Distribution of study participants according to the main activities performed in the 15 days before symptoms and the probable site of infection (n = 164).

Probable Site of Infection	Illegal Mining		Agriculture		Hunting/Fishing		Tourism		Total	
	N	%	N	%	N	%	N	%	N	%
Alto Alegre	120	76.4	1	20	1	100	0	0	122	74.4
Amajari	1	0.6	0	0	0	0	0	0	1	0.6
Cantá	0	0.0	1	20	0	0	0	0	1	0.6
Caroebe	0	0.0	1	20	0	0	0	0	1	0.6
Mucajaí	29	18.5	2	40	0	0	1	100	32	19.5
Guyana	3	2	0	0	0	0	0	0	3	1.8
Venezuela	4	2.5	0	0	0	0	0	0	4	2.5
Total	157	100	5	100	1	100	1	100	164	100

The *pvcrt-o* gene was amplified in 94% (154/164) of the samples, and 99% (151/154) of the amplified products were sequenced. Of all the samples sequenced for the *pvcrt-o* gene, 87% (131/151) were identical to the Sal 1 strain, used as a wild-type reference forCQ-sensitive parasites. The lysine insertion (codon AAG) at position 10, called the K10 insertion, was identified in 13% (20/151) of the sequenced samples. The K10 insertion was detected in 25% (1/4) of the samples from participants engaged in agriculture, 100% (1/1) of those engaged in hunting/fishing, and 12% (18/145) of those engaged in illegal mining(Table 2). Despite the small number of samples, the K10 insertion was more frequent in prospectors

(18) than in farmers (1) or hunters/fishermen (1).

Table 2. Distribution of K10 insertions in the *pvcrt-o* gene (n = 151), according to the main activitycarried out by patients in the 15 days before symptoms occurred.

K10	Agriculture		Illegal Mining		Hunting/ Fishing		Tourism		Total	
	N	%	N	%	N	%	N	%	N	%
With	1	25	18	12.4	1	100	0	0	20	13.2
Without	3	75	127	87.6	0	0	1	100	131	86.8
Total	4	100	145	100	1	100	1	100	151	100

The two participants who carried parasites with a K10 insertion in the *pvcrt-o* geneperformed farming activities (1) and hunting/fishing activities (1) in the municipalities of Mucajaí and Alto Alegre, respectively, while the three farmer participants who carried parasites without a K10 insertion were probably infected in the municipalities of Cantá, Caroebe, and Mucajaí. The only participant who reported tourism carried parasites without a K10 insertion in the *pvcrt-o* gene, and the probable place of infection was the municipality of Mucajaí (Tables 1 and 2).

Of all the participants who reported mining activities in the Yanomami Indigenous area of Roraima, the municipality of Alto Alegre ranked first, with 14% (15/109) of the parasites having a K10 insertion. The second-ranked municipality was Mucajaí, where 7% (2/28) of *P. vivax* samples showed K10 insertion. The only sample from a mining site in the municipality of Amajarí had no K10 insertion (Tables 1 and 2).

Among the 110 individuals infected in Alto Alegre, 15% (16/110) had parasites carry- ing a K10 insertion. Of all the samples with probable infection in Mucajaí, 10% (3/31) had a K10 insertion. No K10 insertion was detected in the four samples from the municipalities of Amajarí, Cantá, and Caroebe. K10 was also not detected in samples of the four participants infected in Venezuela, but it was noted in 1/3 of the samples from participants infected in Guyana (Figure 2). In short, the K10 mutant was present in Alto Alegre, Mucajaí, and Guyana, and seems to be more fixed in Alto Alegre.

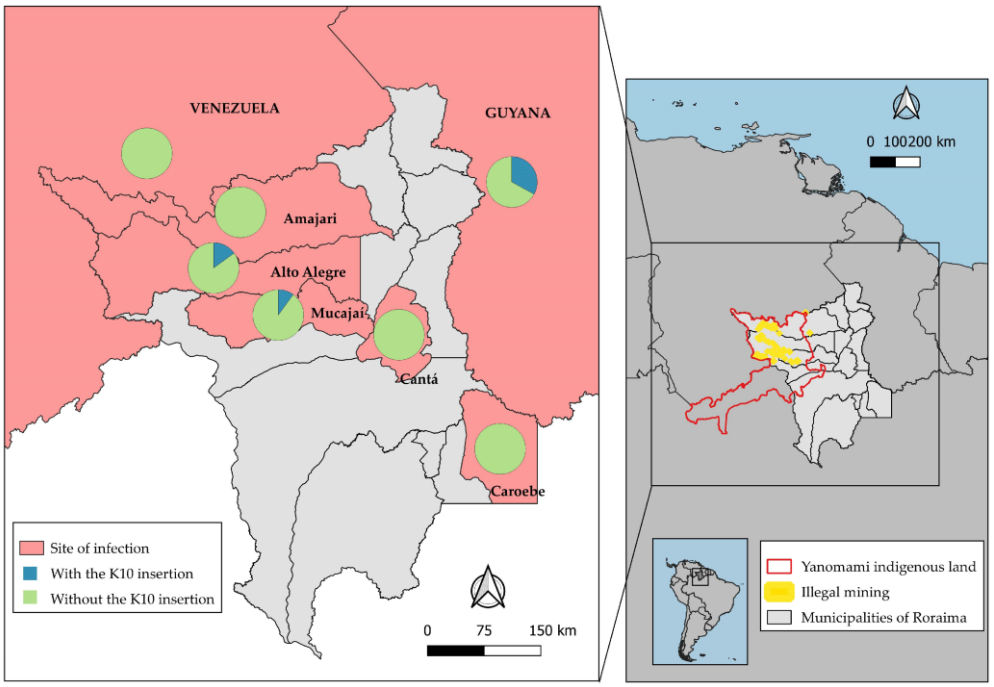

Figure 2. Distribution of K10 insertions (codon AAG) in the *pvcrt-o* gene in *P. vivax* samples, accordingto probable site of infection (n = 151).

The *pvmdr1* gene was sequenced in 80 samples. Mutations in the entire fragment were analyzed, including the three codons (T958**M**, F1076**L**, and Y976**F**) potentially associatedwith the phenotype of RCQ in *P. vivax*. The T958**M** mutation (**M**YF haplotype)—in other words, threonine replaced by methionine at codon 958—was found in 92.5% (74/80) of thesequenced samples, while the wild-type TYF haplotype was identified in 7.5% (6/80) of the samples. The F1076**L** and Y976**F** mutations were absent in all the samples (Table 3).

Table 3. Haplotype distribution in the *pvmdr1* gene (n = 80), according to the main activity carriedout by the study participants in the 15 days before symptoms occurred.

Haplotypes	**Agriculture**		**Illegal Mining**		**Hunting/ Fishing**		**Total**	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
MYF (**958M**)	1	100	73	94	0	0	74	92.5
TYF	0	0	5	6	1	100	6	7.5
Total	1	100	78	100	1	100	80	100

When relating the sequenced samples of the *pvmdr1* gene and the main activity carried out by the patient in the 15 days before the onset of symptoms, the only participant whoreported agriculture carried a parasite with the single mutation **M**YF haplotype (958M) from the municipality of Caroebe. The only participant who reported hunting/fishing carried aparasite with the wild TYF haplotype from the municipality of Alto Alegre. Concerning goldmining, 94% (73/78) of the participants carried parasites with the **M**YF haplotype from themunicipalities of Alto Alegre (55), Mucajaí, and the neighboring country Venezuela (1), and 6%(5/78) with the wild TYF haplotype from Alto Alegre (3) and Mucajaí (2) (Table 3 and Figure 3).

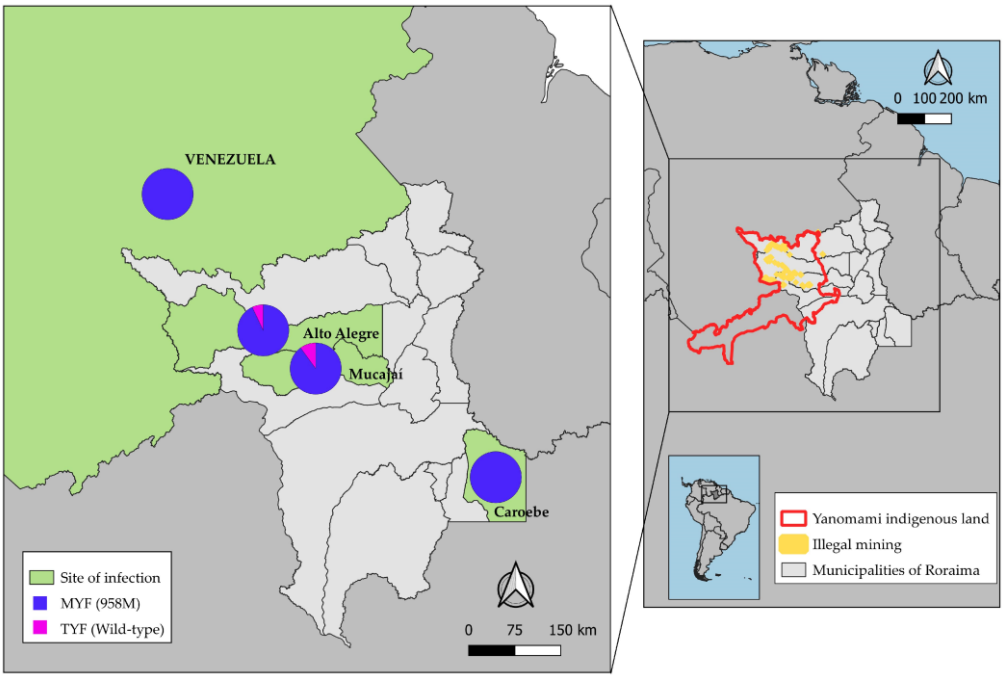

Figure 3. Distribution of *pvmdr1* gene haplotypes in *P. vivax* samples, according to probable site ofinfection (n = 80).

The distribution of the *pvmdr1* gene haplotypes by probable site of infection reveals that the **M**YF haplotype is predominant in the municipalities of Alto Alegre (93%; 55/59) and Mucajaí (90%). The only sample from the municipality of Caroebe and neighboring

Venezuela also had the **M**YF haplotype. The wild TYF haplotype was only present in a small number of samples from Mucajaí (11%) and Alto Alegre (5%) (Figure 3).

Regarding mining activities, the parasite haplotype was the **M**YF in the only samplefrom Venezuela. In parasites transmitted in mining Yanomami Indigenous areas of Alto Alegre, 95% (55/58) of the samples carried the **M**YF haplotype. Similarly, in infections from the Mucajaí, the MYF haplotype predominated (89%; 17/19) (Figure 3).

A total of 79 samples were sequenced for both the pvcrt-o and pvmdr1 genes. In addition to parasites with a single mutation in pvcrt-o or pvmdr1, five patients wereinfected with parasites carrying double mutants: insertion of K10 in the pvcrt-o gene andthe **M**YF (958**M**) haplotype in the pvmdr1 gene. These patients reported mining activitiesin the 15 days preceding symptoms in the municipalities of Mucajaí (1) and Alto Alegre (4).These data show that around 86% (68/79) of the parasite samples carried the **M**YF haplotype and lacked the K10 insertion. In contrast to the K10 insertion, the MYF mutant parasites are present in all studied municipalities (Figure 4).

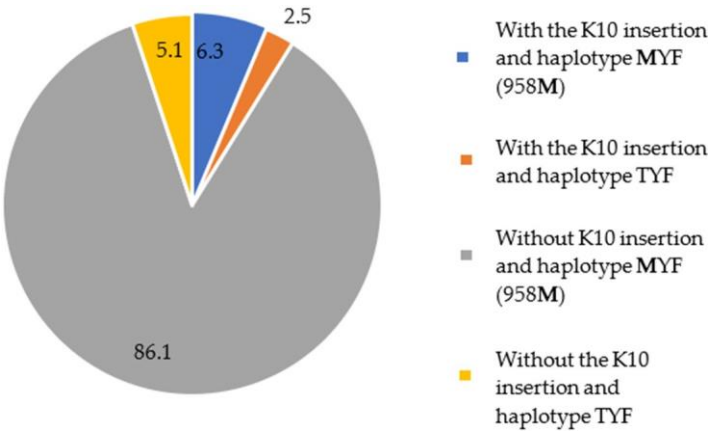

Figure 4. Percentage of *pvcrt-o* and *pvmdr1* gene alleles in the 79 samples sequenced for both genes.

No case notifications in the 28 days preceding or following the date of sample collectionof the 18 patients carrying parasites with the pvcrt-o K10 insertion were registered in Sivep-Malaria, therefore showing no recrudescence episodes. However, probable cases of relapse in 11% (2/18) and reinfection in 39% (7/18) of those who reported mining activities were recorded (Table 4).

Table 4. Distribution of parasites with a K10 insertion in the *pvcrt-o* gene, according to activities in thelast 15 days, and notifications in Sivep-Malaria about hospitalization, death, and use of antimalarials for prophylaxis (n = 20).

Variables	Agriculture (n = 1)		Illegal Mining (n = 18)		Hunting/Fishing (n = 1)		Total (n = 20)	
	N	%	N	%	N	%	N	%
Sample Collection	1	100	9	50	1	100	11	55
Recrudescence (<28 days)	0	0	0	0	0	0	0	0
Probable Relapse (29 to 60 days)	0	0	2	11	0	0	2	10
Probable Reinfection (>60 days)	0	0	7	39	0	0	7	35
Hospitalization	0	0	0	0	0	0	0	0
Death	0	0	0	0	0	0	0	0
Use Antimalarials Prophylaxis	0	0	1	5.6	0	0	1	5

None of these participants were hospitalized or died during this study. The useof antimalarials as prophylaxis was reported by only one participant in mining in the municipality of Mucajaí (Table 4).

We also searched for registers in Sivep-Malaria of participants carrying parasites with the **M**YF haplotype of the pvmdr1 gene within 28 days before or after the date of sample collection, and no cases in the recrudescence period were observed in these patients.However, among them, the miner patients had 12% (9/73) cases of probable relapse and 29% (21/73) of reinfection. There were no records of hospitalization or death among these participants. Only 12% (9/73) of the participants with mining activities in the municipalitiesof Alto Alegre (7) and Mucajaí (2) reported use of antimalarials as prophylaxis.

4. Discussion

The emergence of CQR in *P. vivax* presents a significant challenge for eliminating malaria in Brazil by 2035. This species is responsible for the largest malaria burden in thelegal Amazon. Moreover, the complex biology of *P. vivax* and the limited availability of laboratory research tools make it difficult to identify cases of antimalarial resistance in this parasite. Monitoring molecular markers to identify mutations related to antimalarial resistance over time can provide essential information to identify effective treatment policiesand help determine the change of first-line drugs according to the local reality [22].

Detecting *P. vivax* CQR is complex due to the difficulty in distinguishing whether relapses or recurrences of the disease are due to relapse (related to hypnozoites), recrudes- cence (related to antimalarial resistance), or reinfection [7]. This difficulty is greater when monitoring occurrences in individuals conducting mining activities, as they often return to the place of infection. The illegal mining sites in Roraima are located in isolated areas of forest in the Yanomami Indigenous land, which comprises the municipalities of Amajarí,Alto Alegre, Mucajaí, Iracema, and Caracaraí. In these locations, the miners have no access to the Brazilian Health Unic System (SUS) healthcare diagnosis network [28]. When theytravel to Boa Vista for malaria diagnosis and treatment, the gold miners return to the mines immediately after receiving diagnosis and antimalarial drugs, making it impossible to monitor the clinical efficacy of CQ through the negativity of parasitemia (CVS).

Thus, to identify the cure and whether there was any progression to the severe form of the disease, resulting in hospitalization or death, we consulted the SUS Health Infor- mation Systems (SIS), the Mortality Information System (SIM), and SIVEP-Malaria. This search proved to be an important strategy for investigation integrating research with local surveillance and minimizing SUS costs for monitoring the patients.

Few single-nucleotide polymorphisms (SNPs) have been reported in the *pvcrt* gene, unlike *pfcrt*, its ortholog in *P. falciparum*. The most common polymorphism in the *pvcrt* gene is a lysine insertion (codon AAG) at position 10 (K10), which was proposed to be associated to CQR [22,27,37].

In this study, we found a K10 insertion in the *pvcrt-o* gene in 13% (20/151) of the sequenced parasite samples, and no recrudescence, hospitalization, or death episodes in the patients infected with parasites carrying this mutation was noticed. Interestingly, theK10 insertion was only identified in parasites from locations with intense mining activity,such as Alto Alegre and Mucajaí, and in Roraima and the neighboring country of Guyana, suggesting that this mutant is becoming established in these areas because of an intenseflow of individuals from different areas and possibly reflecting the greater diversity of *P.vivax* in areas with greater transmission.

In previous studies with patients from Acre, Amazonas, Amapá, Pará, Rondônia, and Roraima, the K10 insertion was not also associated with CQR [38]. The same was true in Manaus, where the K10 insertion was not related to in vitro *P. vivax* resistance toCQ [39], and in French Guiana, where no polymorphism in the *pvcrt-o* and *pvmdr1* geneswas identified in CQR parasites [40].

In Southeast Asia parasites, the K10 insertion has been observed in prevalences rangingfrom 9.4% in India to 72% in Myanmar. However, no association between the presence of K10 insertion and in vitro *P. vivax* resistance to CQ was shown [27,41,42]. In fact, unlike what happens with *P. falciparum*, polymorphisms in *pvcrt* do not seem to be good molecular markers for monitoring CQR [38,40].

Regarding the *pvmdr1* gene, in patients carrying parasites with the T958M mutation (92%), no recrudescence or progression to severe malaria resulting in hospitalizations and deaths was identified through research into the search platforms. This finding is supported by other studies which have shown that the T958M mutation allele is a majority in parasite populations in endemic areas of Brazil, French Guiana, Asia, Pakistan, Afghanistan, Sri Lanka, Nepal, Sudan, São Tomé, and Ecuador, and that its presence is not associated with CQR [18,21,38,40,41,43–46].

The double mutation haplotype with amino acid changes in Y976F and F1076L, in particular, has already been cited as a possible marker of resistance to CQ [33,46]. However, this haplotype can also be found in regions with no reported cases of CQR, making its association with drug resistance uncertain and, consequently, its applicability as a marker of chemoresistance of *P. vivax* to CQ unprovable [38,46].

In the present study, no haplotypes with a double mutation profile (T958M + F1076L or Y976F + F1076L) were found in Roraima, Venezuela, or Guyana. Double mutants havenot been previously identified in Roraima, although they have been found in patients from the state of Amazonas who responded well to treatment with CQ [38].

Parasites from French Guiana may have double T958M/F1076L and triple T958M/Y976F/F1076L mutated haplotypes in the *pvmdr1* gene [44]. However, the as- sociation between clinical response to the drug and/or in vitro susceptibility

has never been demonstrated.

These data seem to suggest that the presence of these mutations in the *pvcrt-o* and *pvmdr1* genes is due to the remarkable genetic diversity of *P. vivax*, and that these polymor- phisms have no implications for the phenotype of CQR parasites. Indeed, identifying these haplotypes in *P. vivax* parasite populations circulating in the gold mining individuals in regions representative of a scenario of high transmission could be an important database for analyzing these alleles over time.

Over two decades of research using molecular markers orthologous to *P. falciparum* into the resistance of *P. vivax* to antimalarials, the findings have shown no or a weak relationship with resistance to CQ, potentially leading to false conclusions that could impact national policy for the treatment of the disease [16,22].

There are marked differences in the topologies and number of SNPs in the *crt-o* and *mdr1* genes between *P. vivax* and *P. falciparum*, which reinforces the idea that other genes may be involved in the CQR phenotype in *P. vivax*. Therefore, understanding the molecular mechanisms of antimalarial resistance in *P. vivax* and investigating candidate genes to monitor CQR through ex vivo assays and sequencing could help identify genes other than these *P. falciparum* orthologs [22,38].

Despite the comparable selection pressure from the massive use of CQ, *P. vivax* CQR wasonly reported in 1989, whereas in *P. falciparum* it has been evident since the late 1950s. This can be explained by the differences in genetic determinants and molecular mechanisms of CQR in *P. falciparum* and *P. vivax* parasites [47,48] beyond the lower parasite biomass, the gametocytes production at the beginning of the infection, and the recurrence of hepatic hypnozoites. Theseconditions allow the parasite to be transmitted before the start of treatment or after the concen-tration of the drug has decreased. Furthermore, it has been suggested that the use of PQ may have the potential to reduce the transmission of CQR parasites [49].

Understanding the evolutionary and population dynamics of antimalarial resistance in *P. vivax* will be crucial for strengthening molecular surveillance, both to identify when these alleles arise and to understand how they move through and between populations [22,50]. This is probably especially true in the state of Roraima, where the dynamics of migratory flows and mining activities make malaria elimination an even more challenging goal.

Similar to Brazil, French Guiana is experiencing intense gold mining and human migration between countries in the Guiana Shield. This raises concerns about the spread of CQR *P. vivax* isolates, making surveillance and detection of resistant parasites critical [44].Due to the remote mines' geographical location, when miners are suspected to be infected with *Plasmodium*, mainly due to the presence of fevers and chills, they buy from the clandestine market the antimalarial Artecom® (Dihydroartemisinin–Piperaquine/DHA-PQP) and take one dose/tablet of the drug, just to relieve symptoms for a fast return to the gold mines, and do not undergo complete treatment, highlighting the importance of molecular antimalarial resistance surveillance in these areas. This antimalarial, of knownlow quality, is unregistered in Brazil and illegally enters the country through the borders of
Suriname, Guyana, and French Guiana [28,36,51].

WHO recommends DHA-PQP for the treatment of CQ-resistant *P. vivax* cases. How- ever, no clinical trial has been reported to assess its efficacy in the Americas. An open randomized clinical trial of Eurartesim® vs. CQ and Primaquine (PQ) for the radical cureof vivax malaria in Manaus, Brazil, was recently concluded [52]

In relation to *P. vivax* recurrence between 5 and 60 days, in Brazil, the treatment guide- lines recommend artemether/lumefantrine or artesunate/mefloquine for 3 days to clearasexual parasites and PQ for 14 days to eliminate hypnozoites and prevent relapse, a treat- ment termed radical cure [30]. However, nonadherence to the primaquine regimen harms the effectiveness of the treatment. Tafenoquine (TFQ) is a longer-acting 8-aminoquinoline.It has about a 15-day half-life with a single-dose treatment. Thus, it can replace PQ for facilitating patient adherence and radical cure, avoiding the chances of *P. vivax* relapse and serving as a new ally in the search for the elimination of malaria [53].

In Brazil, TFQ was incorporated into the SUS in 2023, along with G6PD deficiency tests to be prescribed only to people over 16 years old and with at least 70% G6PD ac- tivity [54]. In Roraima, in April 2024, the Special Indigenous Health District Yanomami (DSEI-Yanomami) started implementing TFQ due to the greater difficulty in adhering to long-term PQ treatment among Indigenous people.

A possible limitation that could lead to incorrect conclusions is related to the treat- ment protocol because all the patients received not only CQ but also a PQ radical curative regimen, which would suggest that PQ would have eliminated resistant CQ parasites and,therefore, the presence of CQR parasitic populations could not be ruled out. However, CQ,with or without PQ, produces a rapid parasite clearance in sensitive parasites. In contrast,drugs with later-stage specificity, such as PQ, give slow parasite clearance rates [55]. Al- though there is some in vitro evidence of synergy between PQ and CQ against *P. falciparum* schizonts [56], there is no evidence of synergy between these two drugs against *P. vivax* asexual blood stages [16].

5. Conclusions

The results of this study reinforce the continuous use of CQ in Roraima and also corroborate that mutations in the *pvcrt-o* and *pvmdr-1* genes have no predictive potentialof the CQR *P. vivax* phenotype in Brazilian endemic areas. The molecular mechanisms of antimalarial resistance in *P. vivax* and the difference in the evolutionary dynamics of the *P. vivax* and *P. falciparum* populations suggest that molecular markers associated with *P. vivax* chemoresistance to CQ may lie beyond the *P. falciparum* orthologues.

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7. DISCUSSÃO INTEGRADORA

A partir dos resultados obtidos nesta pesquisa, pode-se considerar que os casos importados da Venezuela e o aumento do garimpo na área indígena Yanomami foram os principais fatores que contribuíram para o aumento dos casos de malária em Roraima, além do aumento na proporção da infecção de *P. falciparum*, refletindo no aumento das infecções mistas, internações e óbitos. O fluxo de pessoas nas áreas de fronteira e nos garimpos exigem o fortalecimento da vigilância nessas áreas, mas também de diagnóstico e tratamento oportunos.

Os garimpos em área indígena são considerados ilegais, porém os garimpeiros deveriam ser vistos sob a ótica de trabalhadores suscetíveis à malária e que ajudam a perpetuação da doença, principalmente para a população dos indígenas Yanomami. Nesse contexto é importante o desenvolvimento de estratégias de prevenção e educação em saúde nas quais os garimpeiros se reconheçam como agentes participativos na eliminação da malária. Igualmente importante é dar acesso ao diagnóstico e tratamento oportunos, como a estratégia de autodiagnóstico seguido de tratamento, visando a redução da transmissão e consequente diminuição no número de casos e na morbidade da doença.

Os garimpos estão localizados em áreas isoladas da floresta na Terra Indígena Yanomami, nos municípios de Amajarí, Alto Alegre, Mucajaí, Iracema e Caracaraí. Mais de 50% dos garimpos estão localizados ao longo do rio Uraricoera. Nessas localidades os garimpeiros não possuem acesso ao diagnóstico e tratamento da malária pela rede do Sistema Único de Saúde. A maioria dos garimpeiros se desloca para a capital de Roraima, Boa Vista, em busca de diagnóstico e tratamento para a doença, embora relatem a automedicação com o “remédio do garimpo”, que geralmente são medicamentos de qualidade duvidosa e usados em subdoses. Tal comportamento pode facilitar a seleção de parasitos resistentes aos antimaláricos. Assim, torna-se importante fortalecer a vigilância molecular para rastrear a quimiorresistência dos parasitos.

Os resultados da caracterização da diversidade de genes do *P. falciparum* e do *P. vivax* associados a quimiorresistência desses parasitos à artemisinina e à cloroquina, mostraram que a artemisinina ainda pode ser utilizada como primeira linha para o tratamento do *P. falciparum* e que as mutações nos genes *pvcrt-o* e *pvmdr-1* não possuem potencial preditivo para o fenótipo de resistência do *P. vivax* à cloroquina.

Como desdobramentos e novas direções a seguir a partir dos resultados deste estudo propõe-se expandir as nossas investigações para outros domínios do gene *pfk13* e outros genes como o *pfCoronin* no *P. falciparum*.

A parceria entre UFRR e Fiocruz/RJ foi fundamental e contribuiu para a formação de formadores, fortalecendo o grupo de pesquisa formado por pesquisadores e estudantes locais

desenvolvendo projetos de pesquisa na área da malariologia em Roraima, um estado que possui características desafiadoras para o controle de malária. Além disso, a cooperação entre a vigilância em saúde local com o grupo acadêmico de pesquisa, promoveu a cooperação entre o serviço e a academia, o que resultou no crescimento integrado de ambos.

8. CONCLUSÕES

As principais conclusões serão apresentadas seguindo o que foi descrito como proposta de investigação nos objetivos específicos da pesquisa:

Objetivo 1: Descrever o perfil epidemiológico da malária no estado de Roraima

O aumento da malária importada da Venezuela, a partir de 2016, e o aumento do garimpo na Terra Indígena Yanomami, a partir de 2019, impactaram no recrudescimento na malária em indígenas das Áreas de São Marcos e Yanomami, respectivamente. As pessoas que adoecem por malária no estado de Roraima são principalmente homens, jovens, da raça parda, que adoecem desenvolvendo atividade de garimpagem.

Objetivo 2: Caracterizar a diversidade de genes *P. vivax* e do *P. falciparum* associados a quimiorresistência desses parasitos a cloroquina e a artemisinina

Apesar hábito de automedicação e uso de subdoses dos garimpos, não foram identificadas mutações relacionadas com resistência à artemisinina no gene *pfk13*, fármaco que continua eficaz como de primeira linha para o tratamento do *P. falciparum*. Por outro lado, as mutações nos genes *pvcrt-o* e *pvmdr-1* não possuem potencial preditivo para resistência do *P. vivax* à cloroquina, sendo necessário identificar outros marcadores para a vigilância molecular de resistência nesse parasito.

Objetivo 3. Fornecer dados para o Ministério da Saúde quanto ao perfil de quimiorresistência dos plasmódios.

Os resultados parciais e final do presente estudo são informados para o Ministério da Saúde através de relatórios e reuniões de pesquisa. E os artigos publicados são para ampla divulgação.

9. TRABALHOS CORRELATOS

Durante o processo do Doutorado foram desenvolvidas as seguintes atividades e trabalhos correlatos e que representaram relevância para o fortalecimento deste trabalho.

- Apresentação do Projeto de Tese no XXIV Seminário Laveran & Deane sobre Malária, realizado no período de 30 de setembro a 04 de outubro de 2019 na Ilha de Itacuruça/RJ (Anexo 2). Este encontro tem o objetivo de reunir alunos de doutorado e mestrado de laboratórios de todo o Brasil com pesquisadores do Instituto Oswaldo Cruz e de outras instituições nacionais e estrangeiras, para discutir seus projetos e resultados de teses em andamento centradas na malária.

- Apresentação do trabalho intitulado: “Gold miners increase malaria transmission in indigenous areas of Roraima”, na XVI Reunião de Pesquisa em Malária, realizada no período de 25 a 28 de abril de 2022 na cidade do Rio de Janeiro/RJ (Anexo 3). Tal trabalho é derivado do primeiro capítulo da presente Tese e foi premiado com menção honrosa na modalidade poster neste evento considerado o o mais tradicional do país sobre pesquisa em malaria.

- Participação de Congresso: DE AGUIAR BARROS, JACQUELINE; GRANJA, FABIANA ; PEQUENO, PEDRO ; MARCHESINI, PAOLA ; FERREIRA-DA-CRUZ, M. F . Gold miners shoot-up malaria transmission in indigenous territories of Roraima state, Brazil. In: 17th world congress on public health, 2023, Roma. 17th world congress on public health, 2023.

- Participação de Congresso: ILLEGAL MINING INCREASES MALARIA TRANSMISSION IN INDIGENOUS AREAS OF RORAIMA. cujos autores: Jacqueline de Aguiar Barros, Fabiana Granja, Pedro Pequeno, Paola Marchesini, Maria de Fátima Ferreira da Cruz, M F, foi submetido na modalidade E-pôster, na 57ª Edição do Congresso da Sociedade Brasileira de Medicina Tropical – MEDTROP 2022, realizado no período de 13 a 16 de novembro de 2022, no Hangar Centro de Convenções e Feiras da Amazônia na cidade de Belém, PARÁ (Anexo 4).

- Participação como coautora do artigo publicado na Revista Pathogens, classificação Qualis A3 em Biodiversidade, no dia 12 de maio de 2023, com título: ***“****Plasmodium falciparum Chloroquine-pfcrt* Resistant Haplotypes in Brazilian Endemic Areas Four Decades after CQ Withdrawn***”.*** Disponível em: https://doi.org/10.3390/pathogens12050731

- Participação como coautora do artigo publicado na Revista Biomedicines, classificação Qualis A2 em Biotecnologia, no dia 09 de janeiro de 2024, com título: *“Are pvcrt-o and pvmdr1 Gene Mutations Associated with Plasmodium vivax Chloroquine-Resistant Parasites?”.* Disponível em: https://www.mdpi.com/2227-9059/12/1/141

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APÊNDICES

APÊNDICE 1

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

REGISTRO N° **DATA DA COLETA:**

Você está sendo convidado a participar da pesquisa "Diversidade e caracterização molecular de genes associados a resistência aos fármacos antimaláricos em populações de *Plasmodium falciparum* e de *P. vivax* no estado de Roraima" sob a responsabilidade das pesquisadoras: **Fabiana Granja, Jacqueline de Aguiar Barros** e **Maria de Fátima Ferreira da Cruz** e sua participação não é obrigatória. A qualquer momento você pode desistir de participar e poderá sair da pesquisa sem nenhum prejuízo para você ou para as pesquisadoras.

O objetivo deste estudo é aumentar o conhecimento sobre a malária, doença que lhe aflige nesse momento. Sua participação nesta pesquisa será doar sangue antes e/ou durante o seu tratamento. O tratamento será igual ao normalmente usado para os casos de malária. Os resultados desse estudo não irão te beneficiar diretamente, mas poderão, no futuro, beneficiar para que outras pessoas não adoeçam e na melhoria do tratamento da malária.

Será coletado sangue usando agulha, seringa e tudo de coleta, perfurando-se a pele até alcançar a veia do braço, a fim de colher 5 ml de sangue. O principal risco na sua participação será um leve desconforto, como dor no local da punção, e, raramente, pode levar ao aparecimento de uma mancha roxa ao redor da picada, causada pelo extravasamento de pequena quantidade de sangue (hematoma). A retirada do sangue poderá ser feita por um médico, enfermeiro, farmacêutico ou biólogo da equipe de investigadores. Todos os cuidados apropriados serão tomados, como o uso de seringa, tubo de coleta e gaze descartáveis assim como álcool para limpeza local.

Serão incluídos nesta pesquisa pessoas maiores de 18 anos e com diagnóstico positivo para malária através de gota espessa, esfregaço sanguíneo ou teste rápido.

As informações desta pesquisa serão confidenciais e garantimos que somente o pesquisador saberá sobre sua participação.

Você receberá uma via deste termo com o telefone e o endereço institucional do pesquisador principal e do Comitê de Ética em Pesquisa (CEP) e poderá tirar suas dúvidas sobre o projeto e sua participação, agora ou a qualquer momento.

Você poderá ir ao CEP para eventuais dúvidas e esclarecimentos quanto aos procedimentos éticos da pesquisa, através do endereço e contato: Bloco da PRPPG-UFRR, última sala do corredor em forma de T à esquerda (o prédio da PRPPG fica localizado atrás da Reitoria e ao lado da Diretoria de Administração e Recursos Humanos - DARH) Av. Cap. Ene Garcez, 2413 – Aeroporto (Campus do Paricarana) CEP: 69.310-000 - Boa Vista – RR; E-mail: coep@ufrr.br; Telefone: (95) 3621-3112 Ramal 26.

Caso tenha alguma dúvida, você poderá entrar em contato conosco, sempre que achar necessário, através do telefone do pesquisador responsável, Jacqueline Barros, número (95) 98113-8023, no endereço Av. Capitão Ene Garcez, nº 2413, Bairro Aeroporto, CEP: 69.310-000, Boa Vista/RR, Laboratório de Biologia Molecular (LaBMol), Centro de Estudos da Biodiversidade (CBio), Universidade (UFRR), E-mail: barros.jacqueline@gmail.com.

_____

Pesquisador

Declaro que entendi os objetivos, riscos e benefícios de minha participação na pesquisa e concordo em participar.

_____

Participante da Pesquisa

APÊNDICE 2

FORMULARIO DE CONSENTIMIENTO INFORMADO

REGISTRO N°: **FECHA DE RECOGIDA:**

Está siendo invitado a participar en la investigación "Diversidad y caracterización molecular de genes asociados con la resistencia a los medicamentos antipalúdicos en las poblaciones de *Plasmodium falciparum* y *P. vivax* en Roraima" bajo la responsabilidad de los investigadores: Fabiana Granja y Jacqueline de Aguiar Barros e no se requiere su participación. En cualquier momento, puede dejar de participar, sin causar daño a usted ni a los investigadores.

El objetivo de este estudio es aumentar el conocimiento sobre la malaria, una enfermedad que la afecta en este momento. Su participación en esta investigación será donar sangre antes y/o durante su tratamiento. El tratamiento será el mismo que se usa comúnmente para los casos de malaria. Los resultados de este estudio no lo beneficiarán directamente, pero pueden beneficiarse en el futuro para que otras personas no se enfermen y mejoren el tratamiento de la malaria.

La sangre se recolectará con una aguja, una jeringa y toda la recolección, perforando la piel hasta llegar a la vena del brazo para recolectar 5 ml de sangre. El principal riesgo en su participación será una leve molestia, como dolor en el sitio de punción, y rara vez puede provocar la aparición de una mancha púrpura alrededor de la picadura causada por la fuga de sangre pequeña (moretones). La sangre puede ser tomada por un médico, enfermera, farmacéutico o biólogo del equipo de investigación. Se tomarán todas las precauciones apropiadas, como el uso de jeringas desechables, tubos de recolección y gasas, así como alcohol para la limpieza local.

Se incluirá en esta investigación a personas mayores de 18 años y con un diagnóstico positivo de malaria a través de gota gruesa, frotis de sangre o prueba rápida.

As informações desta pesquisa serão confidenciais e garantimos que somente o pesquisador saberá sobre sua participação.

Recibirá una copia de este termino con el número de teléfono y la dirección institucional del investigador principal y el Comité de Ética de Investigación (CEP) y podrá responder sus preguntas sobre el proyecto y su participación, ahora o en cualquier momento.

Puede dirigirse al CEP para cualquier pregunta y aclaración con respecto a los procedimientos éticos de la investigación, a través de la dirección y el contacto: Bloque PRPPG-UFRR, última sala del corredor em forma de T a la izquierda (el edificio PRPPG se encuentra detrás de la Rectoría y junto a la Dirección de Administración y Recursos Humanos - DARH) Av. Cap. Ene Garcez, 2413 - Aeropuerto (Campus do Paricarana) Código postal: 69.310-000 - Boa Vista - RR; Correo electrónico: coep@ufrr.br; Teléfono: (95) 3621-3112 Extensión 26.

Si tiene alguna pregunta, puede contactarnos, cuando lo considere necesario, a través del teléfono de la investigadora responsable, Jacqueline Barros, (95) 98113-8023, en Av. Capitão Ene Garcez, 2413, Bairro Aeroporto, CEP: 69.310-000, Boa Vista / RR, Laboratorio de Biología Molecular (LaBMol), Centro de Estudios de Biodiversidad (CBio), Universidad (UFRR), Correo electrónico: barros.jacqueline@gmail.com.

_____

Investigador

Declaro que entiendo los objetivos, riesgos y beneficios de mi participación en la investigación y acepto participar.

_____

Participante de encuesta

APÊNDICE 3

DIVERSIDADE DE GENES ASSOCIADOS A RESISTÊNCIA AOS ANTIMALÁRICOS EM POPULAÇÕES DE *PLASMODIUM FALCIPARUM* E DE *PLASMODIUM VIVAX* NO ESTADO DE RORAIMA.

QUESTIONÁRIO

1. Data da entrevista

____/_____/_____

2. Número do indivíduo para a coleta de sangue

DADOS PESSOAIS

3. Nome

_____

4. Gênero
   a. Masculino
   b. Feminino

5. Qual a sua idade? Anos

6. Qual a data do seu nascimento?
____/_____/_____

7. Raça/cor
   a. Branca
   b. Preta
   c. Amarela
   d. Parda
   e. Indígena

8. Naturalidade

9. Nacionalidade:

10. Profissão:

HISTÓRIA DE MALÁRIA

11. Principal Atividade nos últimos 15 dias:
   a. Agricultura
   b. Pecuária
   c. Doméstica
   d. Turismo
   e. Garimpagem
   f. Exploração vegetal
   g. Caça/pesca
   h. Construção de estradas/barragens
   i. Mineração
   j. Outros

_____

12. Já teve Malária antes?
   a. Sim
   b. Não

_____ Quantas?

13. Quantas Comprovadas por Laboratório?
   a. Comprovada (s) _____
   b. Nenhuma
   c. Não Lembra

14. Já foi hospitalizado com malária?
   1. Sim
   2. Não

Data: _____/_____/_____

15. Recebeu tratamento para malária vivax nos últimos 60 dias?

a. Sim b. Não

16. Recebeu tratamento para malária falciparum nos últimos 40 dias?

a. Sim b. Não

17. Fez o tratamento completo
   a. Sim
   b. Não

18. Está em tratamento?
   a. Sim
   b. Não

19. Pegou o remédio no local do Diagnóstico
   a. Sim
   b. Não

Nome da Unidade de Saúde? _____

Quando terminou de tomar o medicamento? _____/_____/ _____

EXPOSIÇÃO À INFECÇÃO POR MALÁRIA

20. Localização da casa?
   a. Cidade
   b. Periferia
   c. Floresta
   d. Água Parada
   e. Garimpo.

21. Sabe como a malária é transmitida?
   a. Sim
   b. Não

Como?

_____

22. Utiliza alguma medida profilárica contra malária?
   1. Mosquiteiro
   2. Iseticida
   3. Antimaláricos
   4. Outras
   5. Nenhuma

MALÁRIA ATUAL

23. Sintomas?
   a. Febre
   b. Dor de cabeça
   c. Calafrios
   d. Náusea/Vômito
   e. Mialgia
   f. Dor abdominal
   g. Sudorese
   h. Artralgia

24. Diagnóstico
   1. P. falciparum
   2. P. vivax
   3. P. malarie
   4. Nenhuma

Parasitemia: _____

26. Data do início do tratamento:

_____/_____/_____

27. Resultado da lâmina de verificação de cura

i. Diarreia j. Dispnéia k. Nenhum Data do início dos sintomas: ___/___/_____	25. Tipo de exame a. Gota espessa b. Distensão sanguinea c. Sangue papel de filtro d. Sangue total	a. Positivo b. Negativo
PARÂMETROS LABORATÓRIO		
28. Hemograma Hemácias: _____ Hemoglobina: _____ Hematócrito: _____ VCM: _____ HCM: _____ CHCM: _____ RDW CV: _____ RDW SD: _____ Leucócitos: _____ Neutrófilos: _____ Linfócitos: _____ Monócitos: _____ Eusinófilos: _____ Basófilos: _____ Monócitos: _____ Plaquetas: _____ VPM: _____ ADP: _____ PCT: _____	29. Bioquímica ALT: _____ AST: _____ Fosfatase Alcalina: _____ GGT: _____ TAP: _____ TTPA: _____ RNI: _____ Albumina: _____ Bilirrubina total: _____ Bilirrubina Direta: _____ Bilirrubina Indireta: _____ Creatinina: _____ Uréia: _____ PCR: _____	OBSERVAÇÕES IMPORTANTES

ANEXOS

ANEXO 1

UNIVERSIDADE FEDERAL DE RORAIMA - UFRR

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Diversidade e caracterização molecular de genes associados a resistência aos fármacos antimaláricos em populações de Plasmodium falciparum e de P.vivax no estado de Roraima.

Pesquisador: Jacqueline de Aguiar Barros

Área Temática:

Versão: 2

CAAE: 24122619.6.0000.5302

Instituição Proponente: Universidade Federal de Roraima - UFR

Patrocinador Principal: Universidade Federal de Roraima - UFR

DADOS DO PARECER

Número do Parecer: 3.920.373

Apresentação do Projeto:

Projeto de Pesquisa:

Diversidade e caracterização molecular de genes associados a resistência aos fármacos antimaláricos em populações de Plasmodium falciparum e de P.vivax no estado de Roraima.

Responsável Principal:

Jacqueline de Aguiar Barros

Grandes Áreas do Conhecimento (CNPq)

Grande Área 2. Ciências Biológicas

Grande Área 4. Ciências da Saúde

Propósito Principal do Estudo (OMS)

Saúde Coletiva / Saúde Pública

Desenho:

Para realizar um levantamento do perfil da morbimortalidade da infecção pelo P. vivax e P. falciparum no estado de Roraima, será um estudo do tipo descritivo-exploratório de corte transversal, pautado em dados secundários. As fontes para a coleta de tais dados serão a partir do

Endereço: Av. Cap. Ene Garcez, nº 2413, UFRR, Campus Paricarana, Bloco PRPPG/UFRR, Sala CEP/UFRR.
Bairro: Aeroporto **CEP:** 69.310-000
UF: RR **Município:** BOA VISTA
Telefone: (95)3621-3112 **Fax:** (95)3621-3112 **E-mail:** coep@ufrr.br

UNIVERSIDADE FEDERAL DE RORAIMA - UFRR

Continuação do Parecer: 3.920.373

Cronograma	CRONOGRAMA.pdf	24/02/2020 18:13:35	Jacqueline de Aguiar Barros	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	24/02/2020 18:12:34	Jacqueline de Aguiar Barros	Aceito
Orçamento	ORCAMENTO.pdf	02/09/2019 13:17:54	Jacqueline de Aguiar Barros	Aceito
Folha de Rosto	FOLHADEROSTOCOEP.pdf	02/09/2019 13:09:58	Jacqueline de Aguiar Barros	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BOA VISTA, 17 de Março de 2020

Assinado por:
Fernanda Ax Wilhelm
(Coordenador(a))

Endereço: Av. Cap. Ene Garcez, nº 2413, UFRR, Campus Paricarana, Bloco PRPPG/UFRR, Sala CEP/UFRR.
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XXIV SEMINÁRIO
LAVERAN & DEANE SOBRE MALÁRIA

Certificado

Certificamos que Jacqueline de Aguiar Barros participou, na qualidade de estudante de pós-graduação, apresentando o projeto: Genetic diversity of *Plasmodium falciparum* and *Plasmodium vivax* populations associated with chemoresistance in Roraima State durante o XXIV Seminário Laveran & Deane sobre Malária, promovido pelo Laboratório de Pesquisa em Malária, Instituto Oswaldo Cruz e Centro de Pesquisa, Diagnóstico e Treinamento em Malária (CPD-Mal), Fiocruz, no período de 30 de setembro a 04 de outubro de 2019.

Itacuruçá, 04 de outubro de 2019

Cláudio Tadeu Daniel-Ribeiro
Coordenador/Organizador
Seminários Laveran & Deane

Maria de Fátima Ferreira da Cruz
Organizadora
Seminários Laveran & Deane

José Paulo Gagliardi Leite
Diretor do Instituto Oswaldo Cruz/Fiocruz

Realização e Organização: IOC

Financiamento:

Apoio: FAPERJ

Bio-Manguinhos

ANEXO 2

XVI REUNIÃO NACIONAL DE
PESQUISA EM MALÁRIA
25 a 28 de Abril de 2022
Rio de Janeiro

Certificado

Certificamos que

Jacqueline de Aguiar Barros, Fabiana Granja, Pedro Pequeno, Paola Marchesini, Maria de Fátima Ferreira da Cruz

foram premiados com a menção honrosa, pelo trabalho **"GOLD MINERS INCREASE MALARIA TRANSMISSION IN INDIGENOUS AREAS OF RORAIMA"**, na modalidade poster, durante a "XVI Reunião Nacional de Pesquisa em Malária", realizada no Rio de Janeiro, no período de 25 à 28 de Abril de 2022

Rio de Janeiro, 28 de abril de 2022

Dr. Maria de Fátima Ferreira da Cruz
Presidente do Comitê Científico

Dr Leonardo João de Moura Carvalho
Presidente da XVI RNPM

ANEXO 3

57º CONGRESSO DA SBMT • Belém | Pará | Brasil
MEDTROP
13a16.novembro.2022

CERTIFICADO

Certificamos que o trabalho

ILLEGAL MINING INCREASES MALARIA TRANSMISSION IN INDIGENOUS AREAS OF RORAIMA

cujos autores: **Jacqueline de Aguiar Barros, Fabiana Fabiana Granja, Pedro Pequeno, Paola Marchesini, Maria de Fátima Ferreira da Cruz, M F**, foi submetido na modalidade **E-pôster**, na **57ª Edição do Congresso da Sociedade Brasileira de Medicina Tropical – MEDTROP 2022**, realizado no período de **13 a 16 de novembro de 2022**, no Hangar Centro de Convenções e Feiras da Amazônia na cidade de Belém, PARÁ.

Belém/PA, 16 de novembro de 2022.

Dr. Pedro Vasconcelos
Presidente da Comissão Científica do Medtrop 2022

Dr. Julio Croda
Presidente da SBMT | Presidente do Medtrop 2022

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ANEXO 4

ANEXO 5

WFPHA World Federation of Public Health Associations

17TH WORLD CONGRESS ON PUBLIC HEALTH 2023 May 2-6 ROME ITALY

c/o Institute of Global Health - University of Geneva, Campus Biotech - G6 - Chemin des Mines 9, 1202 Geneva - Switzerland - www.wfpha.org

Geneva, 05 February 2023

To whom it may concern

This is to confirm that *Prof Maria de Fatima Ferreira da Cruz* has been invited to attend the World Congress on Public Health, which will be held from May 2 to 6, 2023 in Rome - Italy, for presenting her abstract entitled "Gold miners shoot-up malaria transmission in indigenous areas of Roraima, Brazil".

Please, do not hesitate to contact me if you need further information.

Yours sincerely,

Maria Mata
WFPHA Administrative Manager
Maria.mata@wfpha.org

ANEXO 6

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SPRINGER NATURE

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Journal Name:	Malaria Journal	(the 'Journal')
Manuscript Number:	0108dc2b-6dc2-4dac-8bf0-0e7103eebf2e	
Proposed Title of Article:	Gold miners augment malaria transmission in indigenous territories of Roraima state, Brazil	(the 'Article')
Author(s) [Please list all named Authors]:	Maria de Fátima Ferreira da Cruz, Jacqueline de Aguiar Barros, Fabiana Granja, Pedro Pequeno, Paola Marchesini	(the 'Author')
Corresponding Author Name:	Maria de Fátima Ferreira da Cruz	

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ANEXO 7

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Sistema de Avaliação e Gerenciamento de Artigos
Cadernos de Saúde Pública / *Reports in Public Health*

Prezado(a) Dr(a). Jacqueline de Aguiar Barros:

Comunicamos que o artigo de sua autoria, intitulado "A snapshot of a representative Brazilian state of illegal mining in indigenous areas during the era of malaria elimination", foi aprovado quanto ao seu mérito científico.

Segue em anexo o documento de aprovação.

Atenciosamente,
Leandro Carvalho
Editor(a) Assistente.

Rio de Janeiro, 29 de março de 2024.

Ilmo(a) Sr(a). Jacqueline de Aguiar Barros:

Em nome do Conselho Editorial de Cadernos de Saúde Pública, comunicamos que o artigo de sua autoria, em colaboração com Fabiana Granja, Daniel da Silva e Silva, Arthur Camurça Citó, Cassio Peterka, Maria de Fátima Ferreira-da-Cruz, intitulado "Um retrato de um estado brasileiro representativo da mineração ilegal em áreas indígenas durante a era da eliminação da malária", foi aprovado quanto ao seu mérito científico.

A conclusão do processo editorial de seu artigo dependerá da avaliação técnico-editorial com vistas a detectar dúvidas de formatação, referências bibliográficas, figuras e/ou tabelas. Comunicação nesse sentido lhe será enviada oportunamente.

Atenciosamente,
Profª. Marilia Sá Carvalho
Profª. Luciana Correia Alves
Profª. Luciana Dias de Lima
Co-editoras-chefe

ANEXO 8

International Journal of
Environmental Research and Public Health

an Open Access Journal by MDPI

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CERTIFICATE OF PUBLICATION

The certificate of publication for the article titled:

Molecular Surveillance of Artemisinin-Resistant *Plasmodium falciparum* Parasites in Mining Areas of the Roraima Indigenous Territory in Brazil

Authored by:

Jacqueline de Aguiar-Barros; Fabiana Granja; Rebecca de Abreu-Fernandes; Lucas Tavares de Queiroz; Daniel da Silva e Silva; Arthur Camurça Citó; Natália Ketrin Almeida-de-Oliveira Mocelin; Cláudio Tadeu Daniel-Ribeiro; Maria de Fátima Ferreira-da-Cruz

Published in:

Int. J. Environ. Res. Public Health **2024**, Volume 21, Issue 6, 679

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ANEXO 9

microorganisms

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CERTIFICATE OF PUBLICATION

The certificate of publication for the article titled:

Investigation of Mutations in the *crt-o* and *mdr1* Genes of *Plasmodium vivax* for the Molecular Surveillance of Chloroquine Resistance in Parasites from Gold Mining Areas in Roraima, Brazil

Authored by:

Jacqueline de Aguiar Barros; Fabiana Granja; Rebecca de Abreu-Fernandes; Lucas Tavares de Queiroz; Daniel da Silva e Silva; Arthur Camurça Citó; Natália Ketrin Almeida-de-Oliveira Mocelin; Cláudio Tadeu Daniel-Ribeiro; Maria de Fátima Ferreira-da-Cruz

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Microorganisms **2024**, Volume 12, Issue 8, 1680

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