Underdiagnosed *Plasmodium vivax* infections and increased *pvmdr1* gene amplification in the Brazil-Venezuela-Guyana tri-border region

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In areas of co-circulation of *Plasmodium vivax* and *P. falciparum*, diagnostic limitations may favor the persistence of undetected infections and influence the dynamics of drug resistance. This study investigated the underreporting of *P. vivax* and *pvmdr1* copy number variation across different epidemiological settings in the Brazilian Amazon. In this context, underdiagnosis of *P. vivax* in co-endemic settings may shape the persistence of parasite populations with increased *pvmdr1* copy number. Samples from malaria-positive patients diagnosed by microscopy were analyzed by quantitative PCR (qPCR) for molecular species identification in Roraima (n = 500; 2016–2024), Acre (n = 84; 2014–2015), and Rondônia (n = 105; 2023). Variation in *pvmdr1* copy number was determined by qPCR using β-tubulin as the reference gene. The *pvmdr1* gene encodes a multidrug resistance transporter, and increases in its copy number have been associated with reduced susceptibility to antimalarial drugs, particularly mefloquine and lumefantrine, which are widely used in malaria treatment. In Roraima, approximately 20% of samples initially classified as mono-infections were reclassified as mixed infections by qPCR, with *P. vivax* being more frequently underdetected than *P. falciparum*. Underreporting of *P. vivax* increased from 2020 onward, paralleling a rise in the proportion of *P. falciparum* cases. Analysis of *pvmdr1* revealed marked geographic heterogeneity: all samples from Acre and 99% from Rondônia showed a single gene copy, whereas 61% of isolates from Roraima exhibited gene amplification. Temporally, a progressive increase in the frequency of multiple copies was observed in Roraima after 2020, exceeding 70% in 2023–2024. These findings suggest that underdiagnosis of *P. vivax* in border areas may contribute to the selection and maintenance of parasite populations with *pvmdr1* amplification, highlighting the importance of molecular surveillance and improved diagnostic strategies in co-endemic settings.

Keywords: *P. vivax*, *P. falciparum*, mixed infections, *pvmdr1*, underdiagnosis

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