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Chapter

Increased Trends of *P. vivax* in Sub-Saharan Africa: What Does it Mean for Malaria Elimination?

Mary Aigbiremo Oboh, Mamadou Ndiath, Olumide Ajibola, Kolapo Oyebola and Alfred Amambua-Ngwa

Abstract

Plasmodium vivax being the most geographically spread *Plasmodium* species is considered sparsely distributed in sub-Saharan Africa (sSA) while *P. falciparum* is the most prevalent species in this region. Thus, control strategies in sSA have been disproportionately targeted towards falciparum malaria. Nevertheless, with the use of more sensitive malaria diagnostic platforms, there are more reports of *P. vivax* and other non-falciparum malaria in sSA. In addition, *P. vivax* is presumed benign, however there are new findings of severe cases recorded from *P. vivax* single or mixed infection with other *Plasmodium* species. Besides, the extended dormant period (lasting for weeks or months) is a challenge for achieving effective cure for vivax infections. Although, chloroquine has been proscribed for treatment *P. falciparum*, it still remains the drug of choice for *P. vivax* in most Asian countries where it is predominant. In sSA, artemisinin combination-based therapies (ACTs) are used for treatment of falciparum malaria and, it is probable that the use of ACT could be enhancing adaptive selection for *P. vivax* in the face of its increasing prevalence in the population. Hence, understanding epidemiological and biological factors, and data that could be contributing to the observed steady increase in *P. vivax* prevalence in sSA is important. In this chapter, we discuss the mechanisms for invasion of red blood cells, trends in increasing prevalence of vivax malaria, diagnostic tools, and the public health implications of *P. vivax* and *P. falciparum* co-endemicity in Africa.

Keywords: *Plasmodium vivax*, *Plasmodium falciparum*, sub-Saharan African, Malaria elimination, Duffy negative antigen

1. Introduction

In sub-Saharan Africa (sSA), *Plasmodium falciparum* is responsible for most of the human malaria infection and less frequently, malaria could be due to *P. ovale*, *P. malariae* and *P. vivax* [1]. Significant gains observed in massive reduction in global malaria cases and mortalities in the early 2000s have been largely due to strategic malaria interventions [1]. Nevertheless, signals of reversal in gains are beginning to emerge in most African countries [1]. This stall in progress has been attributed to increased prevalence of non-falciparum malaria parasites on one hand and the development of resistance by both the parasite and vector to drugs and insecticides

respectively [2–4]. Thus, refocusing control strategies to include all circulating species of *Plasmodium* in sSA aimed at reducing the burden of these non-falciparum parasites will greatly contribute to malaria control progress.

P. vivax is particularly interesting as regards its mechanism of invasion, reproduction, virulence and development. Understanding the biology of vivax parasites is crucial for developing strategies aimed at controlling malaria. Regrettably, vivax malaria poses a huge challenge for in vitro analysis to adapt in cell culture, in order to shed more light on invasion patterns, virulence, growth, and reproduction. For example, *P. vivax* has high predisposition for immature red blood cells-RBCs (reticulocytes) which is not readily available [5]. Another drawback as regards vivax control is the use of primaquine (PQ) to achieve hypnozoites clearance, which causes haemolytic anaemia in glucose 6 phosphate dehydrogenase (G6PD) deficient individuals [6, 7]. In sSA with very limited resources for ascertaining the G6PD status of individuals, this presents a practical challenge as individuals are either treated without their G6PD status known or remain untreated. The implication of the latter is the prolonged carriage of hypnozoites capable of relapsing weeks or months later. It has also been found that individuals with specific cytochrome P450 gene polymorphic alleles such as the CYP2D6 do not efficiently metabolise PQ and eventually miss out in the clearance of dormant liver parasites [8].

Overcoming vivax resistance to chloroquine [9], which is also a major drawback in vivax control is looking promising, with a single dose of tafenoquine seemingly independent of CYP2D6-impairement is effective in the prevention of vivax relapse [10, 11].

Evolution is shaping the biological interaction between vivax parasites and the human host [12, 13]. For instance, there is new evidence counteracting the former notion of *P. vivax* emergence in Asia from vivax-like macaque parasites [14, 15]. Recent evolutionary genomic analyses have postulated that vivax most probably originated in Africa [16]. Selection for Duffy-negative mutations could have been due to the high circulation of *P. vivax* in ancient Africans [12] which led to the eventual disappearance of vivax malaria in most parts of Africa [17]. There is need to carry out a comprehensive characterisation of the Duffy-independent vivax strains in West [18, 19] and East Africa [20] in order to understand the genetic similarities and phylogeny between the strains and, relationship with emerging zoonotic non-human primate (NHP) malaria.

Although, non-human primates (NHPs) and human vivax species are considered non-interbreedable (allopatric) [12], it is possible that recombination events between vivax parasites of apes and human lineages have occurred to favour the adaptation of currently circulating vivax strains [21]. This hypothesis is being supported by binding studies of recombinant orthologues; reticulocyte binding protein 2- RBP2 and RBP3, to gorillas, humans and chimpanzees erythrocytes' that displayed no evidence of host specificity to invasions of red cells [12]. Moreover, there has been detection of ape-like *P. vivax* in a European returnee from Africa [21], while human *P. vivax* has been identified in monkeys from the wild in South America [22]. To infer genetic epidemiology of *P. vivax* and relationship with other closely related species, population genetic studies is necessary. This has been employed in *P. falciparum* to demonstrate the origin, complexity, evolutionary history, changes in population size and relatedness between parasite populations.

2. Mechanism of invasion of *P. vivax* malaria to Duffy antigen receptor on red blood cells

Duffy antigen receptor for chemokines (DARC) gene is the fourth erythrocyte gene associated with resistance to *Plasmodium* species [23]. Polymorphisms in other

genes associated with malaria are specifically accompanied by severity or protection against falciparum malaria. DARC confers protection against vivax malaria [24]. DARC has a 36–46 kDa glycoprotein major sub-unit and is made up of 338 amino acids (AAs) that is organised into seven transmembrane domain [25] with an extracellular epitope at the N-terminal that mediates *P. vivax* invasion of RBCs. DARC, a non-specific receptor is expressed on erythrocytes and employed by *P. vivax* merozoites in invasion of RBCs [26]. It is located on the long arm of chromosome 1 (1.q22–1.q23) and possesses two exons [27]. A mutation from a thymine to a cytosine at the GATA-box upstream of the promoter region (33rd position) results in the non-expression of DARC on RBCs, giving rise to the *FYO** allele also known as the Duffy negative phenotype [28].

The *FYO** allele is prevalent in sSA as well as in African-Americans but rare in other racial groups lacking black admixtures [27]. For instance, all 1000 individuals screened in a study in the Gambia were Duffy negative [29]; likewise more than 80% of the participants of a study carried out in northern Nigeria were Duffy-negative [30]. Similarly, another report among pregnant women in Nigeria confirmed a 9:1 Duffy negative to positive ratio [31]. Thus, the null expression of the Duffy gene and reduced prevalence of *P. vivax* in sSA suggests an interplay of adaptive features being employed by RBC overtime. Consequently, the rare observation of *FYA* and *FYB* alleles in this part of the world [32, 33] substantiates the argument of the selective adaptation of the Duffy gene on RBC and its exertion in sSA.

There are several reports of vivax malaria in hitherto low risk regions [34–37] in sSA. The first *Plasmodium* invasion molecules identified was the Duffy binding proteins (DBP) otherwise referred to as, erythrocyte-binding-like (EBL) proteins. These proteins are characteristically homogenous in their cysteine-rich Duffy-binding-like (DBL) domains. The EBL protein of *P. falciparum* and *P. knowlesi* are able to use different receptors in invading the RBC due to variation in EBL gene numbers [38]. Therefore, it is likely that, *P. vivax* having phylogenetic similarity to *P. knowlesi* might have developed diverse EBL proteins also capable of binding to other receptors in the RBCs [2]. The high genome diversity in *P. vivax* [39, 40] provides a plausible explanation that supports and augments its capability of developing alternative invasion pathways through other receptors [41]. An alternate hypothesis is that, *P. vivax* is more endemic in north Africa as well as the Afro-Asiatic populations found in Sudan, Somalia [42, 43] and Ethiopia [20, 44] where there are higher populations of Duffy positive individuals. In this case, the Duffy positive carriers are reservoirs of infection to Duffy negative individuals. Despite the two assumptions, the precise route of transmission of *P. vivax* to/from Africa has not been fully established.

3. Increasing trends of *P. vivax* in sub-Saharan Africa and potential selection for Duffy-negative individuals

Previous reports on vivax epidemiology are now under scrutiny due to the use of more sensitive detection tools [37, 45–51]. *P. vivax* has been detected in south-eastern Senegal: a region sharing border with Mali, Guinea Conakry and The Gambia. In addition, the detection of *Anopheles* vector capable of transmitting vivax in Kenya [52], as well as identification of vivax in mosquito in a study carried out in Angola and Equatorial Guinea [35] points to active transmission of vivax malaria in this sub-region. In a case report, a pregnant Nigerian residing in Italy visited Nigeria briefly and was diagnosed with vivax infection [53], with no further investigation as to the source of infection. Whether these infections were transmitted from Duffy positive individuals serving as reservoir to the negative individuals was not determined.

Although DBP1 possess unique sequences and are highly polymorphic [53], as revealed from correlation analysis of DBP1 mutations, they do not explain the ability of *P. vivax* to infect Duffy-null Africans [54]. The high haplotype diversity of DBP1 and its flanking region in Ethiopia is predictive of multiple independent emergence of vivax variants that were not purged from the population [55]. Therefore, genome-wide comparative studies of Duffy-dependent and emerging Duffy-independent strains are critical for understanding the different forms of adaptive responses, fitness and selection operating in the parasites. The occurrence of DBP1 gene amplification in Ethiopia [55], Madagascar [56] and Cambodia [57] is implicative of adaptation to blood stage infection in the host. A substantial knowledge of the patterns of genetic variation in different geographic scales as well as genetic differences between populations permits inference of population relatedness or structural variation [58].

4. Strategies for *P. vivax* diagnosis in Africa in the era of malaria elimination

Diagnosis of *P. vivax* has relied primarily on two approaches; light microscopy and rapid diagnostic tests (non-molecular). However, modern tools (molecular based tests) such as PCR tests have been demonstrated to be useful, but has suffered widespread utilisation in African countries due to lack of expensive equipment and trained personnel to carry these complex assays.

4.1 Light microscopy (LM)

The World Health Organisation's gold standard for *P. vivax* testing is the detection of parasites in Giemsa stained blood smears by light microscopy [59]. In detecting parasitemia from blood, thick smears are more sensitive, while thin smears allow for clearer visualisation of the morphology of the parasites for accurate speciation and calculation of parasite density. It is recommended that 3 sets of thick and thin smears should be collected for analysing *P. vivax* suspected blood smears. Light microscopy has a number of benefits in African health laboratories such as low cost of operation, and ease of deployment on the field. However, in Africa, the main constraint in accurate microscopy diagnosis is the lack of trained microscopists, and epileptic power supply in most parts of the continent. One of the main challenges in microscopic diagnosis of *P. vivax* is the presence of fewer number of parasites circulating in the blood compared to *P. falciparum* infections making it more likely to report false negatives. The preference of *P. vivax* for reticulocytes which accounts for less than 1% of the fraction of erythrocytes in circulation explains the reason for lower parasite densities compared to *P. falciparum*. In addition, clinical immunity to *P. vivax* infections occur earlier and the immune response is effective in the control of parasitemia [60].

4.2 Malaria rapid diagnostic tests (mRDTs)

The use of mRDTs has become very popular and critical to controlling malaria in Africa. Malaria RDTs provide results within 15 to 20 minutes, do not require special training to interpret results and special storage facilities. The use of mRDTs has contributed significantly to reducing the burden of malaria and is the most commonly used approach in rural laboratory settings with a sensitivity as high as 99% for *P. falciparum*. Malaria RDTs that test for *P. vivax* detect the P.vivax - pLDH antigen specific to *P. vivax*, or pan-pLDH or aldolase antigens common to all *Plasmodium* species. The sensitivity of mRDTs in detecting *P. vivax,* is lower [61–63] when compared to those for falciparum malaria. Moreover, cross reactions with other disease conditions, presence of certain

immunological factors and gametocytaemia can result in false positives. They also have a limited shelf life (~24 months) and need to be kept dry and away from extreme temperatures (> 40°C). Malaria RDTs are very reliable at high parasitemia, while at lower parasitemia chances of false negatives increase [64]. A combination of RDTs with light microscopy would significantly improve *P. vivax* diagnosis in Africa.

4.3 Polymerase chain reaction (PCR)

LM has been the gold standard for malaria diagnosis, but with substantial progress in malaria control, interventions have begun to consider asymptomatic carriers as well. Hence, there is increase interests in the utilisation of molecular tools for detection of sub-microscopic infections. Molecular techniques allow investigation of 5–10 μL whole blood, which increases test sensitivity when compared to 0.025–0.0625 μL whole blood) used for LM [59].

PCR based tests such as conventional PCR and real time PCR have been used to detect *P. vivax* mostly in laboratory settings as part of research projects and not usually as routine clinical based tests. PCR based tests detect *P. vivax* with high sensitivities in diagnosing asymptomatic people particularly in samples with low parasitemia. Molecular based tests have a lower detection limit than both mRDTs and LM. The type of input material (DNA, RNA or whole blood) target gene, species detected, primer/probe composition and concentration, amplification technique (PCR or isothermal), read-out (gel electrophoresis, fluorescence detection, lateral flow) and whether it is qualitative or quantitative impact on the sensitivity of molecular detection tests. The standard molecular marker for *Plasmodium* species differentiation detection is the 18S rRNA genes [65]. In the reference genomes of *P. vivax* (Sal1 and P01), there are 3 distinct copies of 18S rRNA that are expressed at different stages [66]. Some of the Pv18S rRNA assays in use target only one of the three copies by qPCR [67]. Pv18S rRNA assays have also been used to target PV18S rRNA transcripts in addition to the genes [67, 68]. The detection of *P. vivax* in clinical samples can also be improved by targeting high multi-copy genes in PCR assays which provides improved sensitivity over low-copy Pv18S rRNA approaches. Targeting multicopy genes allows pooling samples in community surveys in areas with low parasite prevalence or in elimination settings. Pooling will also bring down costs of assays without compromising on the sensitivity. An example of a multi-copy gene that has been tested for *P. vivax* diagnosis is the non-coding subtelomeric repeat sequence Pvr 47 which has 14 copies in the *P. vivax* Sal1 reference genome. The Pvr47 single step PCR was almost as sensitive as the *P. vivax* 18S rRNA nested PCR [69]. Other targets such as vir/pir multigene family with high copy numbers similar to the var. gene family in *P. falciparum* have also been tested with limited success [70, 71]. Other molecular assays for *P. vivax* have also targeted the mitochondrial DNA in a one-step PCR reaction, loop mediated isothermal amplification or qPCR targeting cytochrome C oxidase I (*cox1*) [72–74], genus-specific PCR followed by targeting vivax specific non-coding regions between cytochrome B gene and *cox1* [75].

Loop-mediated isothermal amplification (LAMP) assays amplify single or multi-copy molecular markers in an isothermal reaction. This technique is useful at point of care settings in the field because of ease of implementation and limited infrastructure requirement. LAMP is also useful in detecting submicroscopic infections with high sensitivity [76, 77].

5. Public health implications of *P. vivax* and *P. falciparum* co-endemicity

Co-infections of *P. vivax* and *P. falciparum* are often unrecognised in sSA, likely due to the predominance of *P. falciparum* as the major infecting species in most

parts of the continent and the dogma that *P. vivax* was absent in sSA [78, 79]. With several lines of evidence, it is now being appreciated that *P. vivax* prevalence in sSA might actually be much higher than presumed [80–82]. Detection of mixed species infection with *Plasmodium* vivax, are also missed as most health care facilities use mRDTs specific for *P. falciparum* and usually do not have skilled man-power and resources to analyse all species that might be present by microscopy. In health facilities that use microscopy, misdiagnosis of co-infections of *P. falciparum* and *P. vivax* as *P. vivax* mono-infection, will lead to wrong treatment which could increase antimalarial resistance or *P. falciparum* parasitemia, leading to severe malaria. There are conflicting reports on the impact of co-infections of *P. falciparum* and *P. vivax*, some studies reported a decrease in disease severity through cross species immunity [83], while others have suggested an increased morbidity in children [84]. A recent meta-analysis also demonstrated a high prevalence of severe mixed malaria compared to *P. falciparum* mono-infection in the studies included in the meta-analysis [85]. This finding has serious implications on malaria public health control efforts and further reiterates the reason why malaria remains a leading cause of morbidity and mortality globally. Also, this emphasises the importance of improved diagnostic tools and the use of species-specific mRDTs in combination with routine microscopy or PCR where present for continuous surveillance. The possibility of mixed infections resulting in a significantly higher risk of developing severe malaria than patients with *P. falciparum* mono-infections is a grave public health risk, considering that mixed infections are underreported in sSA [86, 87]. This could be one of the reasons why treatment failure from ACT use and antimalarial drug resistance is on gradual increase on the continent. Taken together, it is important that clinical laboratories recognise the likelihood of other non-falciparum species especially vivax in their setting, considering the movement of persons (due to trade within the continent) that could carry parasites from one location to the other. Therefore, improved capacity for detection of suspected cases of mixed infections through a combination of species specific mRDTs together with microscopy is crucial for accurate treatment.

6. Conclusion

Duffy negativity is no longer a guaranteed barrier against *P. vivax* invasion, and the transmission of Duffy-independent adapted-vivax parasites will interrupt malaria control. National malaria elimination programmes should prioritise *P vivax* surveillance. Focus should also be on *P. vivax* biology especially as it concerns erythrocyte invasion in order to improve control strategies. Fine-scale epidemiological mapping is also required to elucidate the evolutionary dynamics of erythrocyte invasion by *P. vivax.*

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Conflict of interest

All authors declare that they have no competing interest.

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