Trends in Parasitology

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Review

# African *Plasmodium vivax* malaria improbably rare or benign

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**The overwhelming dominance of Duffy blood group negativity among most people living in sub-Saharan Africa has been considered the basis of their protection from endemic *Plasmodium vivax* malaria. New evidence demonstrates widespread transmission of *P. vivax* in Duffy-negative Africa, though currently of unknown distribution, magnitude, or consequences. Other new evidence from outside of Africa demonstrates marked tropisms of *P. vivax* for extravascular tissues of bone marrow and spleen. Those establish states of proliferative infection with low-grade or undetectable parasitemia of peripheral blood causing acute and chronic disease. This review examines the plausibility of those infectious processes also operating in Duffy-negative Africans and causing harm of unrecognized origin.**

## Highlights

*Plasmodium vivax* transmission occurs in Duffy-negative sub-Saharan Africa at low prevalence and density of parasitemia, but conventional inference to that epidemiology and pathophysiology may underestimate its presence and harm.

The strict requirement for CD71$^{+}$ erythroid cells by *P. vivax* drives marked tropism for extravascular tissue invasion and infiltration.

Infection of erythropoietic tissues of the bone marrow, spleen, and liver by *P. vivax* may cause severe anemia, thrombocytopenia, and hepatosplenomegaly without patent parasitemia.

Parasitemia as a marker of *P. vivax* biomass and prevalence should be acknowledged as inadequate and dangerously misleading; optimized and validated alternative diagnostics for *P. vivax* are needed for the vitally important and incomplete work of estimating its burdens of infection, illness, and death.

## An insidious infection

The disparate biology of the two dominant species of the plasmodia naturally infecting humans, *Plasmodium vivax* and *Plasmodium falciparum*, underpins their distinct epidemiology and pathogenesis, clinical course, and consequences. Recent findings emphasize the importance of understanding these distinctions in avoiding dangerous underestimation of the harm done by infection with *P. vivax*. The dichotomization of these species as the causes of benign versus malignant malarias persisted through the 20th century but is demonstrably fallacious [1–3]. That canon rested on the presumption of a strict preference by *P. vivax* for circulating reticulocytes self-limiting the expansion of pathogenic **biomass** (see Glossary), whereas promiscuous invasion of any red blood cell (RBC) by *P. falciparum* results in fulminant **parasitemia**. However, severe illness with *P. vivax* infection occurs and consistently does so at lower grades of parasitemia than those of *P. falciparum* malaria [4–6]. Multiple lines of new evidence corroborate the hypothesized operation of **tropisms** for **extravascular** spaces of deep organs yielding an unobserved pathogenic biomass [7]. We begin to appreciate that the prevalence and density of **peripheral blood** parasitemia in *P. vivax* infection very probably underestimates its presence and harm [8]. This review examines the plausibility of that principle also operating in Duffy-negative sub-Saharan Africa.

## Duffy factor and *P. vivax* epidemiology

Endemic transmission of the four *Plasmodium* species of humans occurs predominantly across tropical latitudes, from sporadically or low levels to seasonally or continuously high incidence. *P. vivax* exhibits comparatively great adaptive capacities to a wider range of habitats and climatic conditions. Endemic transmission ranges from Vanuatu in the far southeast, the Korean Peninsula to the northeast, southern Brazil in the southwest, and northern Mexico at the northwestern global reach (Figure 1) [9]. Across much of sub-Saharan Africa, however, *P. vivax* transmission appears virtually absent. That scarcity is made all the more conspicuous by the very character of the region, that is, that supporting an abundance of the notoriously efficient mosquito vectors of the *Anopheles gambiae* species complex and the most intense widespread transmission, especially of *P. falciparum*. The African vectors are capable of transmitting *P. vivax*

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and do so routinely in Madagascar, South Sudan, and on the Horn of Africa. As for the rest of the continent, malaria has been successfully eliminated from the north, and the dominance of inherited human Duffy blood group negativity, especially in western, central, and parts of eastern Africa [10], has been firmly considered the basis of the near absence of endemic *P. vivax* transmission.

The Duffy antigen receptor for chemokines (DARC or FY for Fy glycoprotein) normally occurs on the surface of human RBCs. The *DARC* gene (*ACKR1*) occurs predominantly as two common alleles (among many), *FY*01* and *FY*02*, expressing $Fy^a$ and $Fy^b$ antigens, yielding phenotypes Fy(a+b–), Fy(a–b+) and Fy(a+b+). The phenotype Fy(a–b–) in Africans occurs with homozygosity for the FY*02 allele carrying a point mutation that prevents $Fy^b$ expression only in RBCs [11]. Duffy binding protein (PvDBP) is one of many erythrocyte-binding ligands of *P. vivax*. Others include the reticulocyte-binding proteins, one of which, PvRBP2b, binds to transferrin receptor 1 (also called CD71) [12]. Both PvDBP and PvRBP2b and their cogent receptors appear to be essential to efficient *P. vivax* invasion of RBCs. The molecular pathways to invasion are not fully understood, including those of Duffy-negative RBCs [13,14]. The certainty of *P. vivax* infecting Duffy-negative patients is recent and carries nuanced but very significant implications.

In a prophetically titled 2007 paper in this journal [15], Rosenberg described awareness of the absence of *P. vivax* in much of sub-Saharan Africa as dawning only with the slow acceptance of *Plasmodium ovale* (described by Stephens in 1922) as a legitimate species. Under the microscope, stained *P. vivax* and *P. ovale* present essentially similar morphologies; ameboid trophozoites infecting enlarged RBCs with Schüffner's stippling could be attributed to either species and rule out both *P. falciparum* and *Plasmodium malariae*. The idea of genetic refractoriness to infection by *P. vivax* in Africans found support in findings from experimental infections of them during the 1940s and 1950s [16–18]. The seminal report by Miller *et al.* [19] in 1976 linking Duffy factor to *P. vivax* invasion of RBCs offered an elegant genetic and molecular explanation for the scarcity of the species in much of Africa. Further demonstrations of uninfectable Duffy-negative individuals soon followed [20,21]. During the ensuing decades, microscopically confirmed endemic *P. vivax* appeared only where Duffy negativity was either rare (northern and southwestern Africa), less frequent (the Horn), or present but admixed with Duffy-positive people of Asian heritage (Madagascar). This exclusivity seemed to affirm the notion of a continent otherwise impenetrable to endemic *P. vivax* transmission. Since 2006, however, growing numbers of reports using more sensitive and specific nucleic acid diagnostics have challenged that idea.

## Endemic transmission of *P. vivax* occurs in Duffy-negative Africa

### Early evidence

Garnham described an outbreak of *P. vivax* in Kenya as early as 1943 (cited in [22]). In 1999, Rubio *et al.* [23] reported four indigenous cases of *P. vivax* in residents of Equatorial Guinea, all of them of mixed-race parentage. Confirmation of *P. vivax* despite Duffy negativity requires microscopic and nucleic acid/antigen testing confirmation of both in the individuals involved. Ryan *et al.* [22], in 2006, reported 32 anophelines infected by *P. vivax* among 4901 collected in western Kenya. They surveyed 31 children at the same location for parasitemia, finding 9 infected by *P. vivax,* all of whom proved Duffy-negative. However, a 2008 [24] survey of 2588 samples from 9 African nations using PCR detection methods found only one positive sample result for *P. vivax* from Sao Tome. In 2009, Culleton *et al.* [25] assessed seropositivity for *P. vivax* among 409 blood samples from residents of the Democratic Republic of the Congo (DRC), finding 13% to be positive. These reports attracted little attention until Menard *et al.* [26] convincingly reported, in 2010, a prevalence of *P. vivax* of 8.8% among 476 confirmed Duffy-negative residents of Madagascar. In 2011, Mendes *et al.* [27] surveyed 995 people and 820 anophelines

## Glossary

**Biomass:** the sum of parasite matter in the entire human host, typically that which is active and pathogenic; its magnitude is a correlate of disease severity.
**Dyserythropoiesis:** defective development of erythroid cells within erythropoietic tissues.
**Erythropoiesis:** production of RBCs occurring in the bone marrow and other organs, such as the spleen and liver, under conditions of disease.
**Extramedullary erythropoiesis:** also called 'stress erythropoiesis,' the formation of erythropoietic tissue beyond the bone marrow (in the tissues of spleen, liver, and other organs) in response to compromised RBC production in the bone marrow cavity due to a wide variety of disease conditions.
**Extravascular:** any anatomic location outside the sinuses of the vascular system.
**Gametocytemia:** gametocytes demonstrable in peripheral blood.
**Inefficient erythropoiesis:** destruction of developing erythroid cells within erythropoietic tissues.
**Malaria, hepatic latent:** inactive infection of hepatic tissues by clinically silent hypnozoites of the plasmodia.
**Malaria, latent sexual:** infection of dermal microvasculature by clinically silent infectious gametocytes of the plasmodia.
**Malaria, patent:** acute febrile illness attended by active plasmodial parasites demonstrated in peripheral blood.
**Malaria, prepatent:** early active infection of hepatic tissues or blood by plasmodia before onset of patent malaria.
**Malaria, subpatent:** active infection of peripheral blood by plasmodial parasites occurring in numbers detectable by PCR testing but not by microscopy or immunochromatographic rapid tests.
**Malaria, tenebrous:** active infection of the human host with acute or chronic illness and transmission to mosquitoes in the absence of patent parasitemia of peripheral blood.
**Parasitemia:** the population of parasites circulating in peripheral blood; may be asexual, sexual, or both.
**Peripheral blood:** the blood circulating throughout the body within the vascular sinuses; in the malaria context, typically inferring that sampled by venipuncture or lancet fingerstick.

from Angola and Equatorial Guinea, finding a total of 15 Duffy-negative people and 24 anophelines infected by *P. vivax*. Case reports of *P. vivax* in Duffy-negative patients came from Mauritania and Ethiopia [28,29]. Collectively, these early reports triggered awareness of a potential *P. vivax* threat in sub-Saharan Africa, and more surveys followed.

**Tropism:** the turning of all or part of an organism in a particular direction in response to an external stimulus; in malaria, the affinity for or movement to anatomic locations in the human host where specific stages of the parasite gather for successful development.

## Emergent evidence

In 2014, Mbenda and Das [30] surveyed 485 native Cameroonians with acute febrile illness, finding 8 PCR-positive sample results for *P. vivax*, all Duffy-negative. Fru-Cho *et al.* [31], in the same year and nation, reported 6 *P. vivax* cases in Duffy-negative adults among 269 examined. In 2015, Lo *et al.* [32] performed PCR surveys of 416 clinical samples from Duffy-mixed populations in Ethiopia, finding 4 Duffy-negative individuals positive for *P. vivax*. In 2016, Poirier *et al.* [33] reported a survey of 1234 asymptomatic residents of Benin using microscopy, serological diagnostics, and PCR. Though none were positive by microscopy, 22–29% (depending on antigen probe) were positive for antibodies to *P. vivax*, and 15% (13 of 84) were positive by PCR, all of those being Duffy-negative. In 2017, Niangaly *et al.* [34] described 25 Duffy-negative Malian children as being PCR-positive for *P. vivax*. Russo *et al.*[35] performed PCR surveys of 484 blood samples from febrile patients at a hospital in Cameroon, reporting a prevalence of *P. vivax* of 5.6%, all cases of which proved Duffy-negative. In 2017, Niang *et al.* [36] described highly prevalent (53%) sero-positivity for *P. vivax* in southeastern Senegal, confirming a subset of those as positive by PCR. Oboh *et al.* [37], in 2018, conducted a survey at a hospital in southwestern Nigeria, finding a 1.6% positivity rate for *P. vivax*, all Duffy-negative. In 2018, Kavunga-Membo *et al.* [38] surveyed 408 acutely ill children in the DRC, finding 8 coinfected by *P. falciparum* and *P. vivax*. In Senegal, Niang *et al.* [39] followed 48 Duffy-negative children semiannually for 2 years, collecting 192 samples; 8% were PCR-positive for *P. vivax*. The exhaustive analyses of Twohig *et al.* [40] captured much of the published evidence of *P. vivax* in Africa up to 2019 against a backdrop of the geographic distribution of the frequency of Duffy negativity.

The study by Twohig *et al.* [40] also assembled and analyzed hundreds of instances of *P. vivax* acquired by travelers repatriated from Africa. In viewing those travelers as sentinels of infection incidence where acquired, it may be understood that *P. vivax*, though broadly incident, appears in minority to the dominating incidence of *P. falciparum*, where Duffy negativity predominates. Proportionate endemic prevalence of the two species, however, may not be inferred from

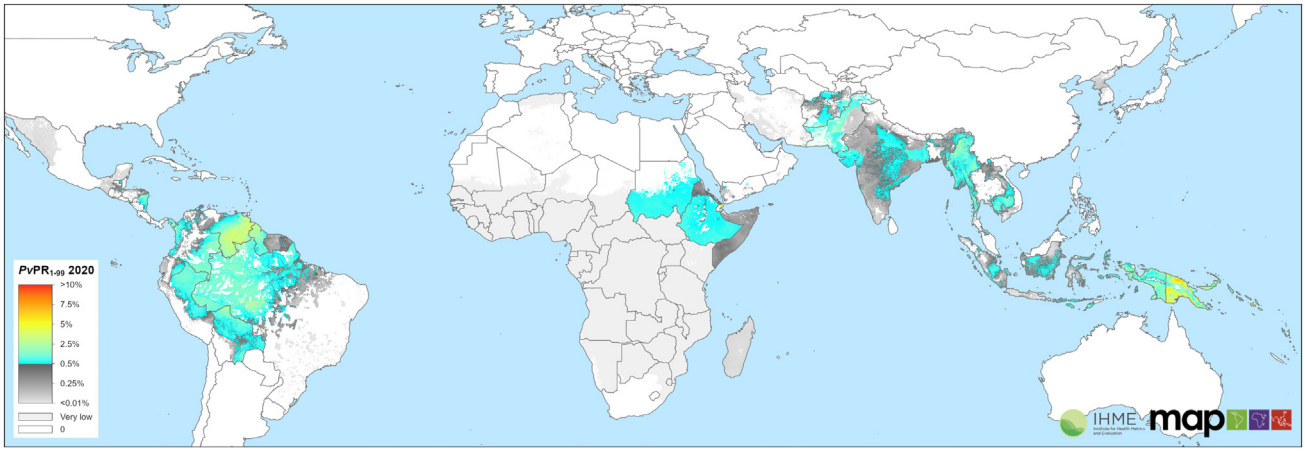


Figure 1. Global distribution of *Plasmodium vivax* prevalence during 2020. Map modeled using reported patent infections, with permission of the Malaria Atlas Project (https://malariaatlas.org/trends/region/MAP/GLOBAL).

those data, because relatively low incidence may occur despite high prevalence of infections of long duration. That may be especially true of *P. vivax* with its durable **hepatic latency** that accounts for 80% of acute attacks in endemic zones [41]. Visitors to Duffy-negative Africa consistently acquire *P. vivax* infections and demonstrate sustained local transmission.

### New evidence

In 2019, Albsheer *et al.* [42] found that 17% of *P. vivax* infections in Sudan occurred in Duffy-negative subjects. In 2021, Kepple *et al.* [43] examined the genetic character of *P. vivax* from 107 Duffy-negative and 305 Duffy-positive residents of Sudan or Ethiopia, finding no evidence of separation between those parasite populations, consistent with ongoing transmission of infections to and from the Duffy-negative human pool. Lo *et al.* [44] surveyed 1215 febrile patients in Sudan and Botswana, where the prevalence of Duffy negativity among *P. vivax* cases was 9.2% and 88%, respectively. In 2021, Motshoge *et al.* [45] surveyed 1614 healthy schoolchildren in Botswana, finding a prevalence of parasitemia by PCR of 12.7% for both *P. vivax* and *P. falciparum*. Also reporting in 2021, Brazeau *et al.* [46] described a PCR survey of more than 17 000 adults sampled in a national demographic health survey during 2014 in the DRC, finding an overall *P. vivax* prevalence of 2.97%. Cases from Namibia have recently been confirmed [47]. Dongho *et al.* [48] reported 177 of 500 febrile outpatients positive for *P. vivax* at Dschang, Cameroon, but the infection was infrequent (3 of 501) at two other locations. A PCR survey of 952 residents of Ghana proved that they were wholly negative for *P. vivax* [49]. In 2022, Wilairatana *et al.* [50] reported a systematic review and meta-analysis of the prevalence and risk of *P. vivax* in Duffy-negative individuals.

### Summation of evidence

The evidence from surveys of people for parasitemia, anophelines for infection, and limited serological testing, along with travel-associated malaria, consistently point to widespread endemic transmission of *P. vivax* with dominating Duffy negativity. Those parasitemias appear relatively infrequent and very low-grade, but we may nonetheless confidently surmise that the parasite somehow effectively navigates the Duffy-negative human host by successfully invading RBCs and placing infectious gametocytes within reach of feeding anophelines often enough for sustained transmission (Box 1). How the parasite does this, how frequently, and with what clinical consequences may be currently unknown, but relevant biological and clinical evidence plausibly inform tentative and testable answers to those important questions.

## *P. vivax* exhibits tropisms for extravascular tissue invasion and infiltration

Varied physical and behavioral features of *P. vivax* distinguish it from *P. falciparum* in important ways (Table 1). The most conspicuous may be the vigorous ameboid motility of *P. vivax* trophozoites versus immotile *P. falciparum*. RBCs infected by *P. vivax* become more flexible than uninfected RBCs, whereas those of *P. falciparum* become more rigid [51]. We routinely see evidence of motility and mutability in stained thin blood films of RBCs containing mature ameboid trophozoites of *P. vivax*, whereas those infected by *P. falciparum* invariably show unaltered sizes and shapes (Figure 2). The molecules and energy devoted to motility must offer some advantage but seem futile in an organism passively flowing within the vascular sinuses. Invasive access to extravascular niches hospitable to its proliferation may explain motility in *P. vivax*.

Tropism for **erythropoietic** tissues may be inferred by the highly fastidious character of *P. vivax* invasion of RBCs. In 2015, Malleret *et al.* [52] described *ex vivo* the exquisite preference of *P. vivax* merozoites for the most immature reticulocytes still expressing CD71. That molecule is scarcely expressed beyond stage I (of V) of reticulocyte development but occurs on most erythroid cells within erythropoietic tissues while being exceedingly rare in peripheral blood (<0.02% of RBC)

[52–54]. As CD71 is the cogent receptor for PvRBP2b, and because that binding is necessary to RBC invasion [12], *P. vivax* infection is thus narrowed to the youngest reticulocytes and their erythroid precursors [52,53]. Preliminary evidence suggests expression of Duffy factor in erythroid precursor cells of genotypically Duffy-negative people [55]. If confirmed, that finding would be of conspicuous importance to *P. vivax* as a biological problem in sub-Saharan Africa.

Through repeated cycles of asexual schizogony unfettered by scarcity of receptive host cells, *P. vivax* may infiltrate extravascular tissues harboring concentrations of immature reticulocytes and their precursors. Using splenectomized rhesus macaques infected by *P. vivax*, Obaldia *et al.* [56] described the extravascular spaces of bone marrow (parenchyma) as a major reservoir of that experimental infectious biomass. The extravascular spaces of the livers (sinusoids) of those animals also proved heavily infected, but not those of the lungs. Both liver and spleen, but not the lungs, are common sites of **extramedullary** or **stress erythropoiesis** in humans [57]. That condition is induced by infection in some murine malaria models [58]. Extravascular invasion and proliferation by *P. vivax* may thus occur not only in bone marrow but also in any tissue where extramedullary erythropoiesis may be occurring.

Key clinical evidence reviewed in the following sections points to the biomass of *P. vivax* (but not *P. falciparum*) weighted away from peripheral blood and toward extravascular compartments of bone marrow and other tissues where immature reticulocytes may form or gather (Figure 2). The strict preference for these cells by *P. vivax* may limit expansion of pathogenic biomass in

Box 1. Paradox of stable transmission with weakness of parasitemia

Endemic vivax malaria transmission in Duffy-negative Africa occurs with infrequent and uniformly very low-grade parasitemia. However, numerous experiments of anophelines exposed to human blood harboring varying levels of parasitemia/**gametocytemia** consistently show vanishing probability of mosquito infection as parasite loads slip below microscopic patency [96]. How can those experimental observations be reconciled with natural *P. vivax* transmission in Duffy-negative Africa occurring with such consistently overt weakness of peripheral blood infection?

The unnatural character of the experimental feeding of mosquitoes on infected humans may be involved in that reconciliation. Laboratory mosquitoes are typically given daytime access to either a relatively small anatomic site of a human volunteer or a sample of venous blood. If the physical and temporal distribution of infectious gametocytes in the vasculature of the dermis across the human surface is random, along with where and when the mosquito chooses to feed, then efficiency of infection of mosquitoes in the experiment may reliably estimate that occurring in nature. However, if both prove nonrandom in ways that make mosquito infection more probable, the experiment may underestimate the success rate of natural infection (Figure I). Garnham and Powers [97], for example, described mosquito feeds on *P. cynomolgi*–infected macaques as showing 'much greater production of oocytes after midnight as compared with midday feedings.'

The extraordinary report of Emami *et al.* [98] describes strikingly nonrandom feeding behaviors in laboratory anophelines driven by specific volatile organic attractants derived from human blood exposed to a specific molecule of parasite origin. Other evidence suggests sequestration of gametocytes in the microcapillaries of the upper dermis [93,99]. If both phenomena occur in nature, the relatively great longevity of gametocytes may infer an enduring and efficient infectiousness to mosquitoes independently of gametocytemia in peripheral blood at the time of feeding. Indeed, Gouagna *et al.* [95] infected mosquitoes with *P. falciparum* after feeding on 44 of 57 aparasitemic subjects, whereas only 8 of them infected mosquitoes via contemporaneous venous blood samples. Those investigators allowed the experimental mosquitoes to feed at night on sleeping subjects with unimpeded access to exposed anatomic sites. This approach would have allowed the natural operation of any nonrandom behaviors by parasites or mosquitoes. A durable **latent sexual reservoir** of accumulated gametocytes accessible by anophelines drawn to their locations plausibly explains efficient transmission despite infrequent and very low-grade parasitemia of peripheral blood.

Finally, in contrast to the consistency of correlation between gametocytemia and experimental mosquito infection in *P. falciparum* [96], McKenzie *et al.* [100] analyzed 221 human subjects experimentally infected by *P. vivax* and summarized, 'The presence or absence of detected gametocytes [in peripheral blood] did not determine the success or failure of a [mosquito] feed, and level of parasitemia did not either.' The processes leading to infection of feeding anophelines may be fundamentally distinct between these species.

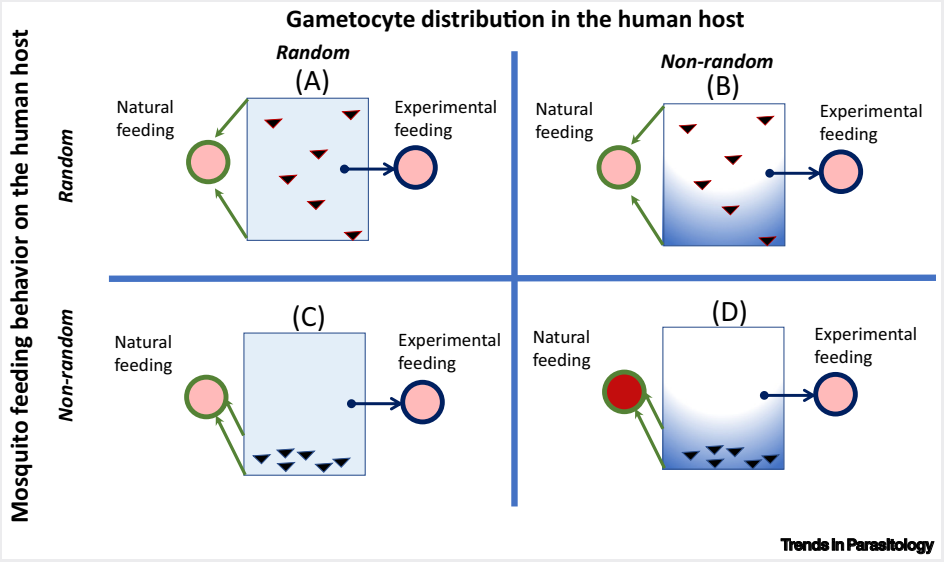


Figure I. Illustration of hypothetical random (A) and nonrandom distribution (B) of gametocytes across the surface of the human host (shading within rectangle), along with random (C) and nonrandom (D) feeding behaviors by anopheline mosquitoes (triangles within rectangles represent natural mosquito feed sites). Whether in the random condition of both (A) or in natural mosquito feeding only (C), the efficiency of infection in experimental feeds on skin (represented by shading within blue circles to right from a single point of the human surface, blue arrow) may reliably estimate that of natural mosquito infection (shading within green circles). The same outcome is predicted in the nonrandom condition of gametocyte distribution with random mosquito feeding (B), but in the nonrandom condition of both (D), the efficiency of natural infection would exceed that of experimental infection.

peripheral blood, but not elsewhere. This phenomenon underpins the hypothesis of an unseen pathogenic biomass beyond the vascular sinuses causing severe illness despite low-grade parasitemia [7].

## Pathogenic biomass of *P. vivax* is predominantly extravascular

### 'Hidden' reservoir

Investigations using measurements of plasmodial or *P. vivax* lactate dehydrogenase (pLDH or PvLDH) as a marker of parasite biomass have been compared with peripheral parasitemia in patients with acute *P. vivax* malaria. The 2015 study by Barber *et al.* [59] examined patients having uncomplicated ($n$ = 53) or severe vivax malaria ($n$ = 9), along with the same in falciparum malaria ($n$ = 109 and 22, respectively). They examined peripheral parasitemia, pLDH, PvLDH, and numerous markers of systemic disease processes, concluding that peripheral parasitemia underestimated pathogenic biomass, especially in severe disease. As explained by Silva-Filho *et al.* [60], the ratio of pLDH to peripheral parasitemia was sixfold higher in severe than in nonsevere *P. vivax*–infected patients, whereas those values in *P. falciparum*–infected patients were similar (a ratio of 1.4). These findings are consistent with a substantial hidden biomass occurring in patients infected by *P. vivax* that may not occur in those infected by *P. falciparum*. Fonseca *et al.* [61] studied infections of macaques by *Plasmodium cynomolgi* (a model for *P. vivax* in humans) and reconciled computational models of parasitemia by invoking a substantial extravascular compartment of infection.

Silva-Filho *et al.* [62] studied their own patient population in exploring the relationship between illness with *P. vivax* relative to peripheral parasitemia and total parasite biomass. They recruited 79 patients infected by *P. vivax*, categorizing them as 'Vivax$^{low}$' or 'Vivax$^{high}$' according to the

Table 1. Key biological distinctions between *Plasmodium vivax* and *Plasmodium falciparum*

| Characteristic | *Plasmodium vivax* | *Plasmodium falciparum* |
|---|---|---|
| Motility | Yes | No |
| RBC mutability | Yes | No |
| RBC enlargement | Yes | No |
| Flexibility of infected RBC | Increased | Decreased |
| RBC invasion | Erythroid precursors and the most immature reticulocytes only | Any erythroid cell |
| Bone marrow habitation | All stages | Gametocytes only |
| Spleen habitation | All stages except gametocytes | All stages |
| Splenic tropism | Active sequestration | Passive sequestration |
| Multigene variant family/protein<br>Target host cells | *vir*/VIR14<br>Spleen fibroblasts | *var*/EMP1<br>Vascular endothelium |
| Severe disease with low-grade parasitemia | Ordinary | Exceptional |
| Fulminant hyperparasitemia | Exceptional | Ordinary |
| Plasma pLDH/parasitemia ratio with severe/nonsevere malaria | 6 | 1 |
| Hepatic latency | Yes | No |
| Coemergence of asexual and sexual blood forms | Yes | No |
| Gametocytemia not a correlate of infectiousness to mosquitoes | Yes | No |

Abbreviations: pLDH, *Plasmodium vivax* lactate dehydrogenase; RBC, red blood cell.

pLDH-estimated total parasite biomass without regard to parasitemia density. High association was observed with Vivax$^{high}$ and markers of endothelial cell activation, thrombocytopenia, and lymphopenia severity. They concluded biomass better correlated with illness than parasitemia and pointed to the consistency of these findings with an extravascular reservoir of disease-causing parasites.

## Splenic reservoir

Enlargement of the spleen, sometimes dangerously, is a well-known and common sign of chronic malaria. Almost two decades ago, del Portillo *et al.* [63] reviewed *P. vivax* and human spleen biology and hypothesized a molecular tropism (parasite VIR-mediated adhesion) for this organ. In 2012, Siqueira *et al.* [64] reported trauma-associated rupture of a spleen heavily infected by trophozoites of *P. vivax* in a 19-year-old Brazilian man. Recent evidence strongly supports the hypothesis of VIR-mediated splenic tropism, including that reported in 2020 by Fernandez-Becerra *et al.* [65] describing *P. vivax* adhesion to spleen (but not lung) fibroblasts involving parasite VIR14 and host ICAM1 proteins. In the same year, that group also reported that plasma-derived extracellular vesicles from *P. vivax* patients signal spleen fibroblasts and enhance VIR14-ICAM1 cytoadherence [66].

In 2021, Kho *et al.* [67] reported an extraordinary study of 22 living spleens surgically removed (for indications unrelated to acute malaria) at Timika, Papua, in eastern Indonesia. Just one of those spleens did not contain living forms of *P. falciparum* or *P. vivax*. Among the 21 thus infected, 12 were negative by peripheral blood microscopy (5 of 13 *P. falciparum*, 6 of 7 *P. vivax*, 1 mixed infection), and 3 (all *P. falciparum*) by PCR examination of the same. The investigators estimated that living *P. vivax* in those spleens was 3590 times more concentrated than in peripheral blood

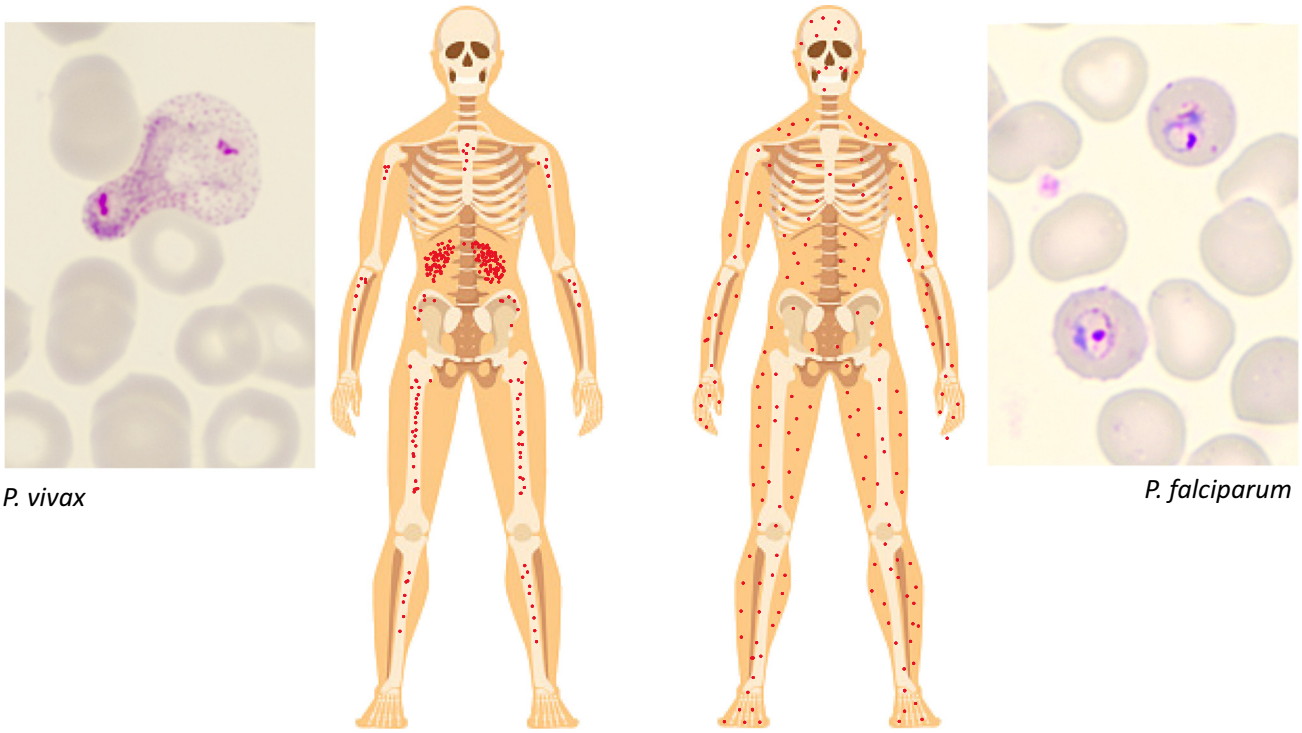


Figure 2. Dominant anatomic sites of schizogony in *Plasmodium vivax* versus *Plasmodium falciparum*.

For a Figure360 author presentation of Figure 2, see the figure legend at https://doi.org/10.1016/j.pt.2022.05.006.

Trophozoites of *P. vivax* and *P. falciparum* in Giemsa-stained thin blood films taken from acutely ill residents of Sumba in southeastern Indonesia, illustrating the conspicuous deformability of red blood cells (RBCs) infected by *P. vivax* and the motility needed to do so versus the absence of both in *P. falciparum* (specimens courtesy of Claus Bogh of the Sumba Foundation at Sumba, Indonesia, and photomicrographs by Lenny Ekawati at the Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia; reprinted with permission). The human figures illustrate hypothesized dominating anatomic sites of blood schizogony in humans infected by *P. vivax* or *P. falciparum* . In *P. vivax*, these are in the accessible extravascular spaces of bone marrow and those of the spleen and liver, where extramedullary erythropoiesis may occur, providing the $CD71^+$ immature reticulocytes required for *P. vivax* propagation. The scarcity of those host cells in peripheral circulation limits capacity for parasitemia. In *P. falciparum*, promiscuous invasion of any RBC makes that entire organ system hospitable to propagation (and fulminant parasitemia).

and accounted for an estimated 98.7% of parasite biomass. Trophozoites and schizonts of *P. vivax* colocated with concentrations of $CD71^+$ immature reticulocytes in the cords and sinuses of the red pulp. The density of adherent $CD71^+$ immature reticulocytes was greater in spleens infected by *P. vivax* than in those infected by *P. falciparum*, suggesting recruitment or extramedullary erythropoiesis. The remarkable near absence of coemergent *P. vivax* gametocytes in those spleens suggests immediate clearance from that site and, apparently, from peripheral circulation as well – sequestration of those in the dermal microvasculature (Box 1) may plausibly explain those absences.

## Bone marrow reservoir

The finding of *P. vivax* in bone marrow has been reported over many decades, either by autopsy, aspiration, biopsy, or acute infection following marrow transplant. That living forms inhabit those tissues is certain, but exactly where they reside and in what relative proportion of pathogenic biomass had not been explored until recently.

Some of those reports detail malaria-like illnesses in patients without parasitemia. In 2006, a man repatriated to Germany from Sudan developed a chronic recurring febrile illness, but the results of

repeated examinations of peripheral blood by microscopy, rapid diagnostic testing, PCR, and serological testing for plasmodia proved negative over several days [68]. Exhaustive diagnostic work-up along with ineffective presumptive antibiotic therapy followed. PCR examination of bone marrow biopsy ultimately revealed both *P. vivax* and *P. malariae* infections. Illness resolved with antimalarial therapy. In 2008, Lacerda *et al.* [69] described a patient experiencing a febrile illness without detectable parasitemia that resolved after 2 months without diagnosis or treatment. The patient presented afebrile but with marked splenomegaly and severe thrombocytopenia. Examinations of peripheral blood for plasmodia remained negative. Schizonts of *P. vivax* were ultimately demonstrated in bone marrow biopsy, and the patient recovered with antimalarial therapy. In 2009, Ru *et al.* [70] demonstrated *P. vivax* infecting erythroblasts in the marrow of two patients with anemia without parasitemia. O'Donnell *et al.* [71] described a 14-year-old girl in the UK developing acute *P. vivax* malaria 40 days after receiving an unrelated donor bone marrow transplant. The donor had traveled to Papua New Guinea and acquired *P. vivax* malaria but had been twice treated and remained healthy in the 11 months before marrow donation. In a series of 108 bone marrow aspirates from patients in India with fever of unknown cause and having anemia, thrombocytopenia, or pancytopenia, 47 showed trophozoites of *P. vivax* [72]. Jandial *et al.* [73] similarly screened 76 patients, finding 4 infected by *P. vivax*. Infection of bone marrow without parasitemia occurs and may often do so in febrile patients also presenting with hepatosplenomegaly and anemia, thrombocytopenia, or pancytopenia.

Instances of infection of marrow have also been reported in patients positive for *P. vivax* in peripheral blood. A survey of 25 867 bone marrow aspirates in Karachi, Pakistan, showed evidence of *P. vivax* in 12 patients, all of those except one also being positive in peripheral blood [74]. In 2017, Baro *et al*. [75] reported gametocytes of *P. vivax* in the bone marrow of a patient with acute *P. vivax* with parasitemia. In 2020, Brito *et al*. [76] examined the bone marrow of seven patients with acute *P. vivax* malaria, finding all to be positive for those parasites, along with **dyserythropoiesis** and **inefficient erythropoiesis**. Salazar Alvarez *et al.* [77] reported *P. vivax* gametocyte adhesion to bone marrow endothelial cells. Infection of bone marrow in *P. falciparum* malaria occurs but appears narrowly restricted to immature gametocytes. Kho *et al.* [67] estimated bone marrow as a minor compartment of *P. vivax* biomass in their subjects.

## Hematologic pathology and disease in vivax malaria

Vivax malaria as an infection of blood and erythropoietic tissues provokes a variety of hematologic pathologies dominated by often profound anemia and thrombocytopenia. Collins *et al.* [78] analyzed anemia in 98 patients with neurosyphilis with induced and untreated *P. vivax* malaria, documenting an exponential decay in hemoglobin to an average nadir of 20% below baseline at 4–5 weeks after infection. After 11 weeks, hemoglobin levels remained 12% below baseline. The *P. vivax* course of a patient with neurosyphilis detailed by Kitchen [4] also reached a hemoglobin nadir at 4 weeks, but at 58% below baseline (at 5.0 g/dL). The review of anemia in *P. vivax* by Douglas *et al.* [79] characterized the problem as 'a common and frequently severe consequence of vivax infection.' They also pointed out the substantial evidence indicating an exacerbation of anemia in patients infected by both *P. falciparum* and *P. vivax*, especially in young children. In another study, Douglas *et al.* [80] identified anemia as the most common cause of death attributable to infection by *P. vivax* at a hospital in eastern Indonesia.

Thrombocytopenia is also common and often severe, along with leukopenia and neutropenia, sometimes manifesting as pancytopenia [80–84]. Lampah *et al.* [85] found both anemia and thrombocytopenia as risk factors for death with hospitalization due to acute malaria (any species) in eastern Indonesia; those had adjusted odds ratios of 4.9 and 2.8, respectively, whereas in patients experiencing both, that ratio was 13.8 ($P < .001$). The marrow may show normoblastic

and megablastic hyperplasia, dyserythropoiesis, inefficient erythropoiesis, and evidence of secondary hemophagocytic lymphohistiocytosis in some cases [71–76,83–86].

The large longitudinal cohort studies by Patriani *et al.* [87] and Dini *et al.* [88] suggest serious consequences to health with repeated attacks by *P. vivax*. In both studies, the authors pointed to renewed attacks before hematologic recovery as a likely explanation for the observed higher risk of hospitalization and all-cause mortality in the year after primary diagnosis of *P. vivax* versus *P. falciparum*. Onset of serious illness with vivax malaria, especially that not prevented with antirelapse therapy, appears to be a prolonged and insidious infectious process very often exacerbated by malnutrition, other endemic infections, and limited access to health care. Attribution of this morbidity and mortality to malaria is unlikely with current diagnosis and reporting systems based almost entirely on patent acute malaria.

## Implications for African *P. vivax*

We may confidently surmise that anatomic sites of active erythropoiesis offer niches where expansion of pathogenic *P. vivax* biomass may occur. In Duffy-positive patients, these infectious processes are associated with serious disease states dominated by severe anemia and thrombocytopenia, but respiratory, hepatic, renal, and circulatory dysfunctions also occur [1–3,6,7,82]. We cannot now know if Duffy negativity somehow mitigates this virulence, but a presumption of benign character may be wanting in wariness and sagacity. Likewise, we cannot now know the scale of prevalence of *P. vivax* infection in Duffy-negative Africa. Nearly all the evidence for that derived from conventional examinations of peripheral blood; few considered the likelihood of active deeper infections with very low-grade or no parasitemia such as those reviewed here. The limited surveys of *P. vivax* seropositivity from Africa ranged from 13% to 53% prevalence, which is suggestive of more broadly prevalent infections than may be inferred from that of detectable parasitemia. If *P. vivax* indeed infects very many Africans, the clinical consequences may remain unnoticed without diagnostics suited to a parasitism seated predominantly in extravascular anatomic sites.

## Terminology for active malaria without patent parasitemia

Malaria is understood to describe illness caused by infecting plasmodia, but the term encompasses multiple species, states of infection, and disease conditions. At the root of all those malarias is almost always definitive demonstration of the parasite in a sample of peripheral blood (i.e., **patent malaria**). Negativity for parasitemia leads us to accept the absence of malaria as a cause of acute illness, though we also accept that some malarias may nonetheless be present and sometimes responsible for chronic states of illness (**subpatent**) or later acute attacks (subpatent, **prepatent**, or **latent**).

The evidence reviewed here indicates that humans infected by *P. vivax* may often be infected and ill but without patent parasitemia. The term ‘occult’ has been applied to malaria without parasitemia, but recent authoritative glossaries of malaria terminology do not recognize it [89,90]. The same word describes supernatural phenomena and may be poorly suited to a natural state of infection. The term ‘cryptic’ has also been used but refers to a case of malaria of unknown origin [90]. No current standard terminology describes active malaria in patients without parasitemia, perhaps in part because it is presumed to be a relatively rare and inconsequential phenomenon in both public health and clinical contexts. That presumption may no longer be tenable.

The adjective ‘tenebrous’ derives from the Latin word for ‘darkness’ and in English language describes a thing of obscure character. **Tenebrous malaria** may be that which is active,

disease-causing, and transmissible to mosquitoes (Box 1), but occurs without patent parasitemia and is thus obscured. Confirmation of tenebrous malaria by some means other than invasive aspirate, biopsy, or surgery should be accepted as a research priority.

## Rethinking diagnosis and clinical research

The critical questions for research (see Outstanding questions) cut across several disciplines, but the specific problem of the diagnosis of tenebrous malaria lies at the core of these. If harm is being done by extravascular biomass independently of parasitemia, attribution will require affirmation of that presence and, ideally, quantifying it. Some of the pioneering work with pLDH detailed here [58,61] offers a logical starting point for that aim; amounts of that molecule indeed correlated with harm attributable to *P. vivax* infection. However, the limited evidence thus far gathered falls short of optimized and validated. Further work on pLDH and other novel approaches, such as serology [91,92], microRNA diagnostics, or breath analysis, across anode arrays by machine learning/artificial intelligence [93] should be explored. Furthermore, a simple modification to conventional fingerstick by lancet may also be explored, that is, sampling the dermal vasculature no deeper than the reach of a feeding anopheline (~500 μm) for gametocytes [94]. Preliminary work in a mesoendemic area of Sumatra, Indonesia, by the author and his colleagues using this approach (on skin above and behind the ankle, using a lancet device designed for neonatal patients) indeed yielded gametocytes visible in thin blood films. Some laboratories could use experimental mosquito feeds for xenodiagnosis by detection of oocyst development [95] (Box 1) or, more easily and quickly, by immediate quantitative PCR assay of engorged mosquitoes. The abundance of gametocytes in skin may correlate with the scale of the proliferative biomass in deep organs generating them.

Validated diagnosis of tenebrous malaria would enable multiple avenues of research of vitally important questions involving the prevalence of infection and the incidence and character of associated morbidity. The ability of *P. vivax* to provoke anemia and thrombocytopenia associated with serious illness and death, especially in young children coinfected by *P. falciparum*, logically informs the exploration of possible morbidity in Duffy-negative Africans. Demonstrating that harm as a correlate of infection and biomass, along with resolution of illness with antimalarial chemotherapies administered in randomized controlled fashion may test the hypothesis of *P. vivax* being an unrecognized agent of harm in Duffy-negative patients. The administration of hypnozoiticidal therapies with prolonged follow-up may prove especially useful in that regard and carries no firm requirement for validated diagnostics; that is, resolution of chronic hematologic pathologies relative to placebo controls among cohorts of presumptively treated subjects would implicate a latent hypnozoite reservoir in establishing a proliferative extravascular biomass as a source of disease. Such evidence would likely inform the very difficult question of 8-aminoquinoline antirelapse therapies (at doses that are dangerous to the many patients deficient in glucose-6-phosphate dehydrogenase) for much of sub-Saharan Africa. Strategizing use of these therapies on that continent would demand firm evidence of substantial clinical benefit.

Forward motion on these research fronts will require acknowledgment of the probability of *P. vivax* in Duffy-negative Africa and elsewhere being neither rare, nor benign, nor nearing declared global elimination goals. Reliance on parasitemia as a definitive diagnosis of malaria – dating to Laveran's original observation of the plasmodia nearly 150 years ago – has perhaps deceived us in the specific instance of *P. vivax* presence and harm. The perspective of a deep parasitism that appears to fleetingly transit the peripheral blood rather than residing in it logically frames the weighty research questions before us.

## Concluding remarks

Available evidence demonstrates that Duffy negativity does not prevent infection by *P. vivax* and that stable transmission occurs at very many sites across sub-Saharan Africa. Those facts, taken with the large body of evidence consistent with tenebrous states of deep *P. vivax* infection, supports the biological, epidemiological, and clinical plausibility of prevalent *P. vivax* infection through sub-Saharan Africa causing unattributed morbidity. Specific molecular, cellular, physical, and behavioral characteristics of *P. vivax* not shared with *P. falciparum* drive the marked tropisms for extravascular spaces of bone marrow and spleen (and probably the liver and elsewhere) associated with $CD71^{+}$-enriched erythropoietic tissues or otherwise gathered immature reticulocytes. The pathogenic biomass of vivax malaria appears overwhelmingly seated in those spaces rather than the vascular sinuses. The peripheral blood window through which we have always viewed malaria distribution, prevalence, and clinical consequences may be suitable for *P. falciparum* but is improbably adequate to the deeper infection caused by *P. vivax*. Exploring those basic features of this parasitism – especially in Duffy-negative Africans in whom tropism away from peripheral blood may be more pronounced – will require accepting the urgent necessity of discovering, optimizing, and validating diagnostics suited to this singular biology (see Outstanding questions). Until such methodologies are broadly applied, the burdens of infection, illness, and death due to vivax malaria globally may remain obscured.

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### Declaration of interests

The author declares no competing interests.

### References

1. Im, J.H. *et al.* (2017) Severe *Plasmodium vivax* infection in Korea. *Malar. J.* 16, 51
2. Siqueira, A.M. *et al.* (2015) Characterization of *Plasmodium vivax*-associated admissions to reference hospitals in Brazil and India. *BMC Med.* 13, 57
3. Quispe, A.M. *et al.* (2014) *Plasmodium vivax* hospitalizations in a mononendemic malaria region: severe vivax malaria? *Am. J. Trop. Med. Hyg.* 91, 11–17
4. Kitchen, S.F. (1949) Chapters 40–43. In *Malariology: A Comprehensive Survey of All Aspects of This Group of Diseases From a Global Standpoint: Vol. II* (Boyd, M.F., ed.), pp. 966–1045, W.B. Saunders
5. Collins, W.E. *et al.* (2004) A retrospective examination of reinfection of humans with *Plasmodium vivax*. *Am. J. Trop. Med. Hyg.* 70, 642–644
6. Nurleila, S. *et al.* (2012) Serious and fatal illness associated with falciparum and vivax malaria among patients admitted to hospital at West Sumba in eastern Indonesia. *Am. J. Trop. Med. Hyg.* 87, 41–49
7. Baird, J.K. (2013) Evidence and implications of mortality associated with acute *Plasmodium vivax* malaria. *Clin. Microbiol. Rev.* 26, 36–57
8. Baird, J.K. and Battle, K.E. (2021) The global burden of *Plasmodium vivax* malaria is obscure and insidious. *PLoS Med.* 18, e1003799
9. Battle, K.E. *et al.* (2019) Mapping the global endemicity and clinical burden of *Plasmodium vivax*, 2000-2017: A spatial and temporal modelling study. *Lancet* 394, 332–343
10. Howes, R.E. *et al.* (2011) The global distribution of the Duffy blood group. *Nat. Commun.* 2, 266
11. Hoher, G. *et al.* (2018) Molecular basis of the Duffy blood group system. *Blood Transfus.* 16, 93–100
12. Gruszczyk, J. *et al.* (2018) Transferrin receptor 1 is a reticulocyte-specific receptor for *Plasmodium vivax*. *Science* 359, 48–55
13. Chan, L.-J. *et al.* (2020) *Plasmodium vivax* reticulocyte binding proteins for invasion into reticulocytes. *Cell. Microbiol.* 22, e13110
14. Popovici, J. *et al.* (2020) The enigmatic mechanisms by which *Plasmodium vivax* infects Duffy-negative individuals. *PLoS Pathog.* 16, e1008258
15. Rosenberg, R. (2007) *Plasmodium vivax* in Africa: Hidden in plain sight? *Trends Parasitol.* 23, 193–196
16. Whorton, C.M. *et al.* (1947) The Chesson strain of *Plasmodium vivax* malaria. IV. Immunity. *J. Infect. Dis.* 81, 1–6
17. Young, M.D. *et al.* (1955) Experimental testing of the immunity of Negroes to *Plasmodium vivax*. *J. Parasitol.* 41, 315–318
18. Bray, R.S. (1958) The susceptibility of Liberians to the Madagascar strain of *Plasmodium vivax*. *J. Parasitol.* 44, 371–373
19. Miller, L.H. *et al.* (1976) The resistance factor to *Plasmodium vivax* in Blacks – the Duffy-blood group genotype, FyFy. *N. Engl. J. Med.* 295, 302–304
20. Spencer, H.C. *et al.* (1978) The Duffy blood group and resistance to *Plasmodium vivax* in Honduras. *Am. J. Trop. Med. Hyg.* 27, 664–670
21. Miller, L.H. *et al.* (1978) The Duffy blood group phenotype in American blacks infected with *Plasmodium vivax* in Vietnam. *Am. J. Trop. Med. Hyg.* 27, 1069–1072
22. Ryan, J.R. *et al.* (2006) Evidence for transmission of *Plasmodium vivax* among a Duffy antigen-negative population in western Kenya. *Am. J. Trop. Med. Hyg.* 75, 575–581
23. Rubio, J.M. *et al.* (1999) Semi-nested multiplex polymerase chain reaction for detection of human malaria parasites and evidence of *Plasmodium vivax* infection in Equatorial Guinea. *Am. J. Trop. Med. Hyg.* 60, 183–187

## Outstanding questions

What antigen–antibody complexes of *P. vivax* may be optimized and validated to reliably estimate the prevalence of exposure to current (IgM?) or recent (IgG?) infection by *P. vivax* in patients or populations (PvAb)?

Can PvLDH or another parasite biomarker (PvBm) be optimized and validated to detect and measure *P. vivax* prevalence and biomass in the human host?

Do surgically removed spleens in endemic Duffy-negative Africa show infiltration by living *P. vivax* parasites and, if so, how heavily and frequently?

In acutely ill Duffy-negative patients experiencing a fever of unknown origin along with hepatosplenomegaly and anemia or thrombocytopenia, is PvBm or PvAb present more frequently and at higher levels than in healthy control subjects?

In cross-sectional surveys of outwardly healthy but vulnerable young Duffy-negative children, does the frequency and level of PvBm or PvAb positivity correlate with signs of hematologic/infectious disease in peripheral blood?

In longitudinal cohorts of glucose-6-phosphate dehydrogenase–normal Duffy-negative young children found to be positive for PvBm or PvAb and randomized to presumptive radical cure or blood schizonticidal therapy alone, does radical cure deliver superior health experiences in the months that follow?

Can Duffy-negative volunteers undergoing controlled human malaria infection (CHMI) with *P. vivax* sporozoites later infect anophelines without patent parasitemia/gametocytemia?

Do Duffy-negative volunteers undergoing *P. vivax* CHMI later show plasma PvBm signal without patent parasitemia?

What are the kinetics of PvBm and PvAb in Duffy-negative or -positive volunteers undergoing *P. vivax* CHMI in the weeks and months after infection?

Do nonrandom distributions of durable gametocytes across the human surface, along with nonrandom

24. Culleton, R.L. *et al.* (2008) Failure to detect *Plasmodium vivax* in West and Central Africa by PCR species typing. *Malar. J.* 7, 174
25. Culleton, R. *et al.* (2009) Evidence for the transmission of *Plasmodium vivax* in the Republic of the Congo, West Central Africa. *J. Infect. Dis.* 200, 1465–1469
26. Menard, D. *et al.* (2010) *Plasmodium vivax* clinical malaria is commonly observed in Duffy-negative Malagasy people. *Proc. Natl. Acad. Sci. U. S. A.* 107, 5967–5971
27. Mendes, C. *et al.* (2011) Duffy negative antigen is no longer a barrier to *Plasmodium vivax* – molecular evidences from the African west coast (Angola and Equatorial Guinea). *PLoS Negl. Trop. Dis.* 5, e1192
28. Wurtz, N. *et al.* (2011) Vivax malaria in Mauritania includes infection of a Duffy-negative individual. *Malar. J.* 8, 92
29. Woldearegai, T.G. *et al.* (2013) *Plasmodium vivax* malaria in Duffy-negative individuals from Ethiopia. *Trans. R. Soc. Trop. Med. Hyg.* 107, 328–331
30. Mbenda, H.G.N. and Das, A. (2014) Molecular evidence of *Plasmodium vivax* mono and mixed malaria infections in Duffy-negative native Cameroonians. *PLoS One* 9, e103262
31. Fru-Cho, J. *et al.* (2014) Molecular typing reveals substantial *Plasmodium vivax* infection in asymptomatic adults in a rural area of Cameroon. *Malar. J.* 13, 170
32. Lo, E. *et al.* (2015) Molecular epidemiology of *Plasmodium vivax* and *Plasmodium falciparum* among Duffy-positive and Duffy-negative populations in Ethiopia. *Malar. J.* 14, 84
33. Poirier, P. *et al.* (2016) The hide-and-seek of *Plasmodium vivax* in West Africa: A report from a large-scale study in Beninese asymptomatic subjects. *Malar. J.* 15, 570
34. Niangaly, A. *et al.* (2017) *Plasmodium vivax* infections over 3 years in Duffy blood group negative Malians in Bandiagara, Mali. *Am. J. Trop. Med. Hyg.* 97, 744–752
35. Russo, G. *et al.* (2017) Molecular evidence of *Plasmodium vivax* infection in Duffy negative symptomatic individuals from Dschang, West Cameroon. *Malar. J.* 16, 74
36. Niang, M. *et al.* (2017) Unexpected high circulation of *Plasmodium vivax* in asymptomatic children from Kedougou, southeastern Senegal. *Malar. J.* 16, 497
37. Oboh, M.A. *et al.* (2018) Molecular identification of *Plasmodium* species responsible for malaria reveals *Plasmodium vivax* isolates in Duffy negative individuals from southwestern Nigeria. *Malar. J.* 17, 439
38. Kavunga-Membo, H. *et al.* (2018) Molecular identification of *Plasmodium* species in symptomatic children of Democratic Republic of Congo. *Malar. J.* 17, 334
39. Niang, M. *et al.* (2018) Asymptomatic *Plasmodium vivax* infections among Duffy-negative population in Kedougou, Senegal. *Trop. Med. Health* 46, 45
40. Twohig, K.A. *et al.* (2019) Growing evidence of *Plasmodium vivax* across malaria-endemic Africa. *PLoS Negl. Trop. Dis.* 13, e000714
41. Commons, R.J. *et al.* (2020) Estimating the proportion of *Plasmodium vivax* recurrences caused by relapse: a systematic review and meta-analysis. *Am. J. Trop. Med. Hyg.* 103, 1094–1099
42. Albsheer, M.M.A. *et al.* (2019) Distribution of Duffy phenotypes among *Plasmodium vivax* infections in Sudan. *Genes (Basel)* 10, 437
43. Kepple, D. *et al.* (2021) *Plasmodium vivax* from Duffy-negative and Duffy-positive individuals share similar gene pools in East Africa. *J. Inf. Dis.* 224, 1422–1431
44. Lo, E. *et al.* (2021) Contrasting epidemiology and genetic variation of *Plasmodium vivax* infecting Duffy-negative individuals across Africa. *Int. J. Infect. Dis.* 108, 63–71
45. Motshoge, T. *et al.* (2021) Recent molecular assessment of *Plasmodium vivax* and *Plasmodium falciparum* asymptomatic infections in Botswana. *Am. J. Trop. Med. Hyg.* 104, 2159–2164
46. Brazeau, N.F. *et al.* (2021) The epidemiology of *Plasmodium vivax* among adults in the Democratic Republic of the Congo. *Nat. Commun.* 12, 4169
47. Haiyambo, D.H. *et al.* (2021) Children with *Plasmodium vivax* infection previously observed in Namibia were Duffy negative and carried a c.136G>A mutation. *BMC Infect. Dis.* 21, 856
48. Dongho, G.B.D. *et al.* (2021) *Plasmodium vivax* infections detected in large number of febrile Duffy-negative Africans in Dschang, Cameroon. *Am. J. Trop. Med. Hyg.* 104, 987–992
49. Brown, C.A. *et al.* (2021) High frequency of the Duffy-negative genotype and absence of *Plasmodium vivax* infections in Ghana. *Malar. J.* 20, 99
50. Wilairatana, P. *et al.* (2022) Prevalence and risk of *Plasmodium vivax* infection among Duffy-negative individuals: a systematic review and meta-analysis. *Sci. Rep.* 12, 3998
51. Suwanarusk, R. *et al.* (2004) The deformability of red blood cells parasitized by *Plasmodium falciparum* and *P. vivax*. *J. Infect. Dis.* 189, 190–194
52. Malleret, B. *et al.* (2015) *Plasmodium vivax*: restricted tropism and rapid remodeling of CD71-positive reticulocytes. *Blood* 125, 1314–1324
53. Ovchynnikova, E. *et al.* (2017) DARC extracellular domain remodelling in maturing reticulocytes explains *Plasmodium vivax* tropism. *Blood* 130, 1441–1444
54. Nakahata, T. and Okumura, N. (1994) Cell surface antigen expression in human erythroid progenitors: erythroid and megakaryocytic markers. *Leuk. Lymphoma* 13, 401–409
55. Dechavanne, C. *et al.* (2018) Duffy antigen expression in erythroid bone marrow precursor cells of genotypically Duffy negative individuals. *bioRxiv* Published online December 31, 2018. https://doi.org/10.1101/508481
56. Obaldia, N. *et al.* (2018) Bone marrow is a major parasite reservoir in *Plasmodium vivax* infection. *mBio* 9, e00625-18
57. Mende, N. and Laurenti, E. (2021) Hematopoietic stem and progenitor cells outside the bone marrow: Where, when, and why. *Exp. Hematol.* 104, 9–16
58. Belyaev, N.N. *et al.* (2013) Extramedullary myelopoiesis in malaria depends on mobilization of myeloid-restricted progenitors by IFN-gamma induced chemokines. *PLoS Pathog.* 9, e1003406
59. Barber, B.E. *et al.* (2015) Parasite biomass-related inflammation, endothelial activation, microvascular dysfunction and disease severity in vivax malaria. *PLoS Pathog.* 11, e1004558
60. Silva-Filho, J.L. *et al.* (2020) *Plasmodium vivax* in hematopoietic niches: Hidden and dangerous. *Trends Parasitol.* 36, 447–458
61. Fonseca, L.L. *et al.* (2018) Analysis of erythrocyte dynamics in Rhesus macaque monkeys during infection with *Plasmodium cynomolgi*. *Malar. J.* 17, 410
62. Silva-Filho, J.L. *et al.* (2021) Total parasite biomass but not peripheral parasitemia is associated with endothelial and hematological perturbations in *Plasmodium vivax* patients. *Elife* 10, e71351
63. del Portillo, H.A. *et al.* (2004) Variant genes and the spleen in *Plasmodium vivax* malaria. *Int. J. Parasitol.* 34, 1547–1554
64. Siqueira, A.M. *et al.* (2012) Spleen rupture in a case of untreated *Plasmodium vivax* infection. *PLoS Negl. Trop. Dis.* 6, e1934
65. Fernandez-Becerra, D. *et al.* (2020) *Plasmodium vivax* spleen-dependent genes encode antigens associated with cytoadhesion and clinical protection. *Proc. Natl. Acad. Sci. U. S. A.* 117, 13056–13065
66. Toda, H. *et al.* (2020) Plasma-derived extracellular vesicles from *Plasmodium vivax* patients signal spleen fibroblasts via NF-κB facilitating parasite cytoadherence. *Nat. Commun.* 11, 2761
67. Kho, S. *et al.* (2021) Evaluation of splenic accumulation and colocalization if immature reticulocytes and *Plasmodium vivax* in asymptomatic malaria: a prospective human splenectomy study. *PLoS Med.* 18, e1003632
68. Imirzalioglu, C. *et al.* (2006) Diagnosis of mixed *Plasmodium malariae* and *P. vivax* infection in a development aid volunteer by examination of bone marrow by real-time PCR. *J. Clin. Microbiol.* 44, 2307–2310
69. Lacerda, M.V.G. *et al.* (2008) Chronic *Plasmodium vivax* infection in a patient with splenomegaly and severe thrombocytopenia. *Rev. Soc. Bras. Med. Trop.* 41, 522–523
70. Ru, Y.X. *et al.* (2009) Invasion of erythroblasts by *Plasmodium vivax*: A new mechanism contributing to malarial anemia. *Ultrastruct. Pathol.* 33, 236–242
71. O'Donnell, J.O. *et al.* (1998) Donor-derived *Plasmodium vivax* infection following volunteer unrelated bone marrow transplant. *Bone Marrow Transpl.* 21, 313–314

mosquito feeding behaviors, explain infection of them in the near absence of parasitemia/gametocytemia?

72. Rastogi, N. and Rehman, N. (2018) Changes in bone marrow: A prospective study of 47 cases. *Int. J. Res. Med. Sc.* 6, 232–235
73. Jandial, R. *et al.* (2019) Different causes of pyrexia of unknown origin on bone marrow examination: An institutional experience. *J. Med. Sci. Clin. Res.* 7, 519–524
74. Shaikh, M.S. *et al.* (2021) *Plasmodium* in the bone marrow: Case series from a hospital in Pakistan, 2007-2015. *Malar. J.* 20, 254
75. Baro, B. *et al.* (2017) *Plasmodium vivax* gametocytes in the bone marrow of an acute malaria patient and changes in the erythroid miRNA profile. *PLoS Negl. Trop. Dis.* 11, e0005365
76. Brito, M.A.M. *et al.* (2022) Morphological and transcriptional changes in human bone marrow during natural *Plasmodium vivax* malaria infections. *J. Infect. Dis.* 225, 1274–1283
77. Salazar Alvarez, L.C. *et al.* (2021) *Plasmodium vivax* gametocytes adherence to bone marrow endothelial cells. *Front. Cell. Infect. Microbiol.* 11, 614985
78. Collins, W.E. *et al.* (2003) A retrospective examination of anemia during infection of humans with *Plasmodium vivax*. *Am. J. Trop. Med. Hyg.* 68, 410–412
79. Douglas, N.M. *et al.* (2012) The anaemia of *Plasmodium vivax* malaria. *Malar. J.* 11, 135
80. Niang, C. and Whittaker, M.A. (2018) Severe thrombocytopenia in patients with vivax malaria compared to falciparum malaria: a systematic review and meta-analysis. *Infect. Dis. Poverty* 7, 10
81. Douglas, N.M. *et al.* (2014) Mortality attributable to *Plasmodium vivax* malaria: A clinical audit from Papua, Indonesia. *BMC Med.* 12, 217
82. Anstey, N.M. *et al.* (2012) *Plasmodium vivax*: Clinical spectrum, risk factors and pathogenesis. *Adv. Parasitol.* 80, 151–201
83. Sung, P.S. *et al.* (2011) Hemophagocytic lymphohistocytosis associated with *Plasmodium vivax* infection: Case report and review of the literature. *Chonnam. Med. J.* 47, 173–176
84. Memon, S. *et al.* (2008) Etiological spectrum of pancytopenia base on bone marrow examination in children. *J. Coll. Physicians Surg. Pak.* 18, 163–167
85. Lampah, D.A. *et al.* (2015) Severe malarial thrombocytopenia: A risk factor for mortality in Papua, Indonesia. *J. Infect. Dis.* 211, 623–634
86. Muthu, V. *et al.* (2017) Malaria-associated haemophagocytic lymphohistiocytosis: Report of two cases and a review of literature. *Ind. J. Med. Res.* 145, 399–404
87. Patriani, D. *et al.* (2019) Early and late mortality after malaria in young children in Papua, Indonesia. *BMC Infect. Dis.* 19, 992
88. Dini, S. *et al.* (2020) The risk of morbidity and mortality following recurrent malaria in Papua, Indonesia: A retrospective cohort study. *BMC Med.* 18, 28
89. World Health Organization (WHO) (2016) *WHO malaria terminology*. WHO/HTM/GMP/2016.6
90. Centers for Disease Control and Prevention (n.d.) Malaria glossary. https://www.cdc.gov/malaria/glossary.html. Accessed 17 December 2021
91. Yeom, J.S. *et al.* (2008) Naturally acquired IgM antibody response to the C-terminal region of the merozoite surface protein 1 of *Plasmodium vivax* in Korea: use for serodiagnosis of vivax malaria. *J. Parasitol.* 94, 1410–1414
92. Longley, R.J. *et al.* (2020) Development and validation of serological markers for detecting recent *Plasmodium vivax* infection. *Nat. Med.* 26, 741–749
93. Nixon, C.P. (2016) *Plasmodium falciparum* gametocyte transit through the cutaneous microvasculature: A new target for malaria transmission blocking vaccines? *Hum. Vaccin. Immunother.* 12, 3189–3195
94. Schaber, C.L. *et al.* (2018) Breathprinting reveals malaria-associated biomarkers and mosquito attractants. *J. Infect. Dis.* 217, 1553–1560
95. Gouagna, L.C. *et al.* (2014) Comparison of field-based xenodiagnosis and direct membrane feeding assays for evaluating host infectiousness to malaria vector *Anopheles gambiae*. *Acta Trop.* 130, 131–139
96. Barry, A. *et al.* (2021) Higher gametocyte production and mosquito infectivity in chronic compared to incident *Plasmodium falciparum* infections. *Nat. Commun.* 12, 2443
97. Garnham, P.C.C. and Powers, K.G. (1974) Periodicity of infectivity of plasmodial gametocytes: the 'Hawking phenomenon'. *Int. J. Parasitol.* 4, 103–106
98. Emami, S.N. *et al.* (2017) A key malaria metabolite modulates vector blood seeking, feeding, and susceptibility to infection. *Science* 355, 1076–1080
99. Meibalan, E. and Marti, M. (2017) Biology of malaria transmission. *Cold Spring Harb. Perspect. Med.* 7, a025452
100. McKenzie, F.E. *et al.* (2002) *Plasmodium vivax* blood-stage dynamics. *J. Parasitol.* 88, 521–535