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Commentary

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Human *Plasmodium vivax* mosquito experimental transmission

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Plasmodium vivax bench research greatly lags behind *Plasmodium falciparum* because of an inability to culture in vitro. A century ago, intentionally inducing a malaria infection was a strategy commonly used to cure late-stage syphilis. These controlled human malaria infections were used with expertise and persisted to the end of World War II. While controlled malaria liver-stage infection has been achieved for both *P. vivax* and *P. falciparum*, controlled human transmission to mosquitoes falls short for both species. In this issue of the *JCI*, Collins et al. present groundbreaking work that establishes a system to transmit *P. vivax* gametocytes from humans to mosquitoes. The authors injected a unique human isolate of *P. vivax* that reached high gametocyte density within weeks. This study provides a technical advance that will facilitate the study and eradication of the human parasite *P. vivax*.

The problem of *P. vivax* malaria

Widespread attention to malaria in sub-Saharan Africa caused by *Plasmodium falciparum* obscures the importance of malaria in India, southeast Asia, and in the Americas caused by *Plasmodium vivax*. In the Americas, 75% of the malaria cases result from *P. vivax*, whereas 53% of the global burden of *P. vivax* is in Southeast Asia, with India alone representing 47% of the global *P. vivax* cases. In 2018, WHO reported 7.5 million *P. vivax* clinical cases (95% CI: 5.9 to 9.3 million), in contrast to 228 million *P. falciparum* cases (1). However, other reports estimate that the *P. vivax* burden is closer to 15 to 20 million, with subclinical cases pushing those numbers even higher (2–4). Globally, *P. vivax* cases are increasing in countries where *P. falciparum* cases are decreasing, and some countries have only *P. vivax* (1). In Africa, a substantial number of *P. vivax* cases occur in Eastern countries bordering the Indian Ocean, whereas *P. falciparum* is reported to make up 100% of reported cases, and *P. vivax* is present at only very low levels, in largely Duffy-negative parts of Africa (3).

P. vivax infections are also serious and sometimes lethal (5, 6), and are more genetically (7) and geographically diverse (8, 9) than those of *P. falciparum*. Since the 1970s, research and knowledge about *P. vivax* have lagged far behind what is known about *P. falciparum* (5). A milestone for the *P. falciparum* culture system was established by Trager and Jensen in 1976 (10), but was never established robustly for *P. vivax*. Although isolated reports documented long duration and continuous culture of *P. vivax* using stem cells or human reticulocytes, the resultant parasitemia was too low for reproducible drug inhibition experiments, basic biochemistry, or ability to produce viable gametocytes (sexual stages) infectious for mosquitoes (11–14). In contrast, the bench culture system for *P. falciparum* allows multiple mosquitoes to feed at once through a blood-filled membrane and achieves a nearly 100% infection rate.

Past and present controlled human malaria infections

Blood-stage–induced human malaria infections have been brought back to life for research purposes after a long dormancy following World War II. Julius Wagner-Jauregg was awarded the 1927 Nobel Prize in Physiology or Medicine for discovering that high malarial fevers cured patients suffering from dementia-associated, end-stage syphilis. Malaria therapy was lethal in up to 15% of patients, especially when *P. falciparum* was used. *P. vivax* was preferred to greatly reduce risk of death. Malaria therapy spread throughout the world, requiring mosquitoes to propagate the parasite instead of direct blood transmission from patient to patient. In the 1930s to 1960s, dozens of isolates (strains) were used in the United States and Europe for malariotherapy. While the differing isolates induced variable fever cycles, relapse rates and duration, and rates of infection to mosquitoes (15, 16), archived studies from the United States show that the McCoy and St. Elizabeth *P. vivax* strains were most commonly used with success (16).

Mark Boyd looked at 46 patients who were infected with the *P. vivax* McCoy strain, and identified patients as poor or good infectors. Mosquito infection rates were 50% with detectable gametocytes by blood film (17). Boyd and Kitchen directly compared *P. falciparum* and *P. vivax* gametocytes, noting that individual *P. vivax* gametocytes transmit to *Anopheles quadrimaculatus* about ten times more effectively when compared with the *P. falciparum* gametocytes. Submicrosco-

pic (blood film negative) *P. vivax* gametocytes infected 30% of mosquitoes, whereas submicroscopic *P. falciparum* gametocytes or even larger amounts (up to 25 gametocytes/μL) infected zero mosquitoes. In the range of 26 to 100 gametocytes/μL, *P. vivax* infection rates were 50%, whereas that range in *P. falciparum* resulted in low mosquito infection rates (only 10%) (18).

The expertise of malariotherapy was utilized for malaria drug and vaccine research during World War II when thousands of drugs, including chloroquine and primaquine, were screened in animals and humans. After WWII, human malaria studies ended, with research on malaria waning during the eradication efforts in the 1950s.

Modern day investigators first recreated human liver-stage infections by allowing *P. falciparum*–infected mosquitoes to bite subjects then testing the efficacy of liver-stage drugs and vaccines. As soon as blood stages appeared, the infections were cured, long before viable gametocytes achieved levels high enough to infect mosquitoes. A few studies used *P. vivax* liver-stage infection to look at relapse of dormant *P. vivax*.

Recently, a team of Australians led by James McCarthy repioneered infected human blood-stage *P. falciparum* infections based on careful screening for other human and animal pathogens and cryopreservation of large volumes of blood from an infected patient donor to look at drug and vaccine efficacy. Because of *P. falciparum* lethal outcome and the known delay of sexual-stage gametocyte appearance about a week after microscopic asexual stages, the ability to produce a *P. falciparum* model for human infection of mosquitoes is still a hurdle (19, 20).

The Australian investigators partially overcame the *P. falciparum* hurdle by using intermediate piperaquine doses to decrease asexual stages while preserving gametocytes. In the study, most subjects infected mosquitoes that had fed at any time over three weeks in *P. falciparum* blood-stage infection (21). In a follow-up study, combined drug regimens to cure asexual stages while preserving sexual stage gametocytes controlled malaria infection that was initiated by sporozoites; scarcely any mosquitoes became infected. In another study, researchers evaluated a

malaria drug candidate (dihydroorotate dehydrogenase inhibitor) and noted that a dose of less than 1 gametocyte/μL failed to infect the thousand mosquitoes tested (22). This *P. falciparum* human model is not robust!

In the first induced blood-stage infection reported with *P. vivax* acquired from the Solomon Islands in 2013, two subjects were followed without mosquito feeds. Both sustained gametocyte levels of over 1 gametocyte/μL for two to three days before treatment on day 14 (23). In work published in 2016 for six volunteers, the initial *P. vivax* inoculum from the same Solomon Islands isolate was about six parasites per person. Later, the gametocyte level was less than 1 gametocyte/μL at peak parasitemia, and infections to mosquitoes were scored by direct visualization of oocysts, which was subject to interpretation and gave mixed results.

In this issue of the *JCI*, Collins, McCarthy, and colleagues used a new *P. vivax* isolate from India (HMP013) that was inoculated at the higher density of 564 parasites per person, which is almost 100 times more than the six parasites per person in the previous 2016 cohort (24). The peak gametocyte density was 47 gametocytes/μL in this study, shown by a revised PCR assay, yielding more than 300 times the gametocytes than with the Solomon Island isolate. Notably, 70% of the participants were susceptible to infected mosquitoes at peak gametocytemia. The researchers obtained pooled blood from participants and using a centrifugation gradient, enriched it ten-fold for gametocytes. The mosquito infection rate increased to 97%, similar to that obtained for *P. falciparum* , when mosquitos were fed gametocyte-cultured blood through a membrane feeding assay. (24).

How do these infection rates compare with the rates of mosquitoes that fed on people with naturally acquired *P. falciparum* and *P. vivax* in recent years, as well as to those obtained from mosquito feeds on malariotherapy for syphilis studies or WWII research studies? Children with subclinical and clinical *P. falciparum* malaria present with fewer than 10 gametocytes/μL of blood, which can infect 0.1% to 5% of mosquitoes. When there is a higher density of gametocytes, in the range of 100 to 1000 gametocytes/μL, the amount of mosquitoes that become infect-

ed jumps to 10% to 20% (25). More than 1000 gametocytes/μL is rare in human subjects. Notably, *P. vivax* mosquitoes fed on gametocytemic patients yielded an infection rate close to 5% (24).

Conclusions

For malaria therapy, doctors infecting mosquitoes was a means to continue propagation of the isolate for future syphilis patients. For modern day malariologists, natural infections are a means for testing the critical human-to-mosquito bottleneck with drugs or vaccines. The most recent study by Collins and colleagues from the McCarthy team marks an important advance in the quest to study and defeat the nefarious human parasite *P. vivax* (24).

Acknowledgments

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