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The Role and Mechanism of Hypnozoite Formation by Plasmodium Vivax

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ABSTRACT

Background: Due to its unusual nature, *P. vivax's* latent liver stages, known as hypnozoites, have proven a burden after transmission by female anopheles mosquitoes to human hosts. New treatments are still required, despite global efforts to eliminate malaria, as resistance never stops. This review shows how there is still sizable information gap about *P. vivax* hypnozoite.
Methodology: A systematic search of published *Plasmodium vivax* papers. Digital repositories such as Scopus, Science Direct, Web of Science, Google Scholar, and PubMed were utilized.
Result: The activation, development, and production of the hypnozoite latent stage are still unclear. The host's microenvironment influences hypnozoite development either before or after hepatocyte infection, or both. This suggests that hypnozoite fate is likely partially influenced during the sporozoite stage. Hypnozoite laboratory model advancement may help in the investigation.
Conclusion: The advancement of sc RNAseq can aid in the investigation of molecular ideas and the construction of advanced laboratory models for the detection of hypnozoites.

Key word: *P. Vivax*, hypnozoite, Single cell RNA sequencing technology (scRNA seq), Brady sporozoites, Malaria, sporozoites.

1. INTRODUCTION

There are about a hundred species of Plasmodium, and they can infect a wide range of animals, such as birds, reptiles, and mammals. People can contract malaria from four different species of the genus Plasmodium: *P. vivax, P. falciparum, P. malariae*, and *P. ovale*. Female Anopheles mosquitoes are the main transmitters of the malaria parasites that infect human hosts. Anopheles vectors of human disease include thirty to forty different species (Ghosh and Rahi, 2019). Aside from variations in their definitive hosts (invertebrate mosquito vectors) and intermediate hosts (mammals), these four species differ from one another in terms of clinical manifestations, transmission dynamics, the capacity to elicit immune responses in hosts, and patterns of resistance to antimalarial drugs. (Chaturvedi et al., 2020). *P. vivax* and *P. falciparum* species of Plasmodium are quite prevalent in India.
Despite considerable multisectoral and international efforts to manage and eradicate it, malaria remains one of the deadliest infectious diseases in the world, particularly in the tropics. The World Malaria Report 2021 estimates that there are 247 million cases of malaria worldwide. *Plasmodium vivax* is the cause of 4.5 million cases, or 2% of the anticipated cases. Over the past 20 years, *P. vivax* and *Plasmodium falciparum* malaria have been reported at the same rates in India; however, in recent years, vivax

malaria rates have been inconsistent, ranging from 38% in 2017 to 52% in 2018, 57% in 2019, and 59% in 2020. The incidence of cases did not change in 2020 and 2021 (WHO, 2022).

With the highest malaria case burden in Southeast Asia, India has made impressive progress in recent years to lower the disease's prevalence. India has set a goal to eradicate malaria by 2027, which is three years sooner than the 2024 global deadline. Since an earlier revision to the Indian national malaria treatment policy in 2013, the World Health Organization (WHO) has released guidelines on new treatment options for the control and elimination of malaria. India accounted for over 79% of all malaria cases globally in 2021. About 40% of the cases in the area were responsible for *P. vivax*. Of the 0.55 million cases recorded in the SEARO area, India accounted for 29% of the burden. To accomplish the 2030 eradication goals, there are still numerous technical and operational hurdles (WHO, 2022). In 2022, the footprint of malaria in India will have significantly diminished in recent years. 65% of the 173,975 malaria cases nationwide in 2022 were found in four states: West Bengal (23%), Chhattisgarh (17%), Odisha (14%), and Jharkhand (11%). 57% of cases are due to *Plasmodium falciparum*. In states that are closer to eliminating malaria, especially in urban areas and in West Bengal, *Plasmodium vivax* predominates (WHO, 2022).

P. vivax is primarily found in Asia, Latin America, and some regions of Africa. *Plasmodium vivax* and *Plasmodium falciparum* both cause malaria in humans; however, they show different biological differences in every stage of their cycle. *Plasmodium vivax* invades young red blood cells, or reticulocytes, but *Plasmodium falciparum* does not exhibit tropism towards a specific age of red blood cells. Additionally, there are variations in the duration of gametocyte development and lifetime. Compared to other malaria parasites, *P. falciparum* has the longest maturation period, taking 9 to 12 days to complete five stages of growth. It also retains its infectious potential for several days. However, after the first infection, *Plasmodium vivax* develops dormant stages or hypnozoites that lead to relapse*; P. falciparum* does not have these phases. For these reasons, the focus of this review is *Plasmodium vivax*.

2. MATERIALS AND METHOD

We thoroughly reviewed published and digital literature to investigate the therapeutic qualities of *Plasmodium vivax*. To find papers on *Plasmodium vivax*, search digital repositories like Scopus ScienceDirect, Web of Science, Google Scholar, and PubMed. Relevant terms such as hypnozoite, sporozoite of *Plasmodium vivax* splenomegaly and polymerase chain reaction were employed to refine the search.

2.1. PLASMODIUM VIVAX

Plasmodium vivax, the parasite that causes malaria in humans, is the most common parasite in the world. Due in large part to P. vivax's distinct biology and challenging treatment, worldwide efforts to lower the prevalence of malaria have not been as successful in reducing *P. vivax* as they have been in reducing P. falciparum. *P. vivax* has become the prevalent malaria parasite throughout Asia-Pacific and South America as a result, resulting in up to 14 million clinical cases annually and being considered an imminent hurdle to the eradication of malaria (Nadia and Lu, 2022). The parasite *Plasmodium vivax* uses a vector to carry out its functions. An Anopheles mosquito infects a human host by injecting sporozoites during a bite. These sporozoites travel to the liver through the bloodstream, passing through skin cells and arterial endothelium. Once in the liver, sporozoites infect hepatocytes and establish a parasitophorous vacuole, progressing through various stages. The trophozoite can either transition into exo-erythrocytic schizogony, producing exo-erythrocytic merozoites, or develop into a latent hypnozoite (a non-replicating, latent form of the Plasmodium parasite that can appear inside liver cells following sporozoite invasion into the host). Hypnozoites later

become active, leading to further schizogony (a type of asexual reproduction occurring in single-celled organisms wherein the parent splits into multiple progeny cells) and relapses.

Thus, hypnozoites serve as a reservoir for seeding an infection at the blood stage without the need for mosquito inoculation of fresh sporozoites. The mechanism governs the development, hibernation, and activation of hypnozoites. They can remain inactive for months or even for years. *P.vivax* - infected mosquitoes can produce several clinical attacks from hypnozoites.

Because Plasmodium vivax has a lower propensity than Plasmodium falciparum to cause severe illness in individual clinical episodes, it is characterized as "benign tertian malaria" and causes malaria. In endemic settings, chronic or recurring infections can cause severe anemia and malnutrition, particularly in young children.

2.2. Life cycle of Plasmodium vivax

P. vivax undergoes a convoluted lifespan, including over ten stages of cellular growth, during which it invades at least four different cell types in two different hosts. During a blood meal, a female Anopheles mosquito carrying the malaria virus injects sporozoites into the human host. After arriving in the liver, the sporozoites mature into schizonts that rupture and release merozoites. The malaria parasite can hibernate for several months in the liver as the hypnozoite, a distinct stage. Weeks or months after the original infection, hypnozoites in the human liver might reawaken, leading to many clinical relapses and additional transmission. Once released into the circulation, merozoites ensnare themselves in reticulocytes, or immature red blood cells. They then develop into schizonts, which burst to spread the infection more quickly. About 48 hours after infection, the asexual erythrocytic stage, sometimes known as the blood stage, gives rise to the malaria symptoms. The blood stage results in the removal of both infected and uninfected red blood cells, which can lead to a potentially fatal anemia. infection at the sexual stage. Additionally, merozoites differentiate into gametocytes, the infectious part of the sexual stage. Because *P. vivax* malaria can manufacture gametocytes prior to the onset of clinical signs, transmission may occur before the host becomes ill or receives therapy (Mueller et al., 2009).

The onset of clinical signs and symptoms which may occur during malaria caused by Plasmodium vivax can give rise to serious illnesses. Some of which includes-

2.3. Febrile Paroxysm

Characteristic signs of vivax malaria include severe chills and tertian fever. Moreover, the bursting of red blood cells by schizonts causes the synthesis of several inflammatory cytokines, which in turn causes fever and myalgia. The fever is irregular at first, but eventually almost simultaneous red cell rupture results in the classic cyclic fever. There are three stages to a typical febrile malarial paroxysm. The first phase, referred to as the "cold stage," is characterized by stiffness and a feeling of coldness. Followed by high temperature of 40–41°C symptoms include fever, malaise, headache, nausea, vomiting, myalgias, and sometimes seizures, particularly in younger patients. The stage of perspiration, during which the fever breaks, concludes the paroxysm. Although infrequently seen, the length of febrile episodes has historically been linked to certain species. This means that *P. falciparum*, *P. vivax,* and *P. ovale* induce malarial paroxysm every 48 hours ("tertian" fever), whereas *P. malariae* causes it every 72 hours ("quartan" fever) (Drysdale et al., 2022).

2.4. Anemia

Anaemia caused by P. vivax is caused by splenic sequestration, bone marrow suppression, and schizont destruction of red blood cells. Dyserythropoiesis, parasitized erythrocyte destruction, and nonparasitized erythrocyte destruction are the three main causes of anemia in malaria. In acute vivax malaria, unlike falciparum malaria, parasite

levels greater than 2% infected erythrocytes are rare. One of the things that has been suggested to be inhibiting P. falciparum from experiencing unrestricted parasite multiplication is P. vivax's inclination to infiltrate younger erythrocytes. Conversely, the degree of anemia may be comparable or worse. Collins hypothesized that the destruction of reticulocytes during production could be the cause of this (Collins et al.,2003). There is a decrease in uninfected red cell survival following a malaria bout. The degree of anemia caused by *P. vivax* infections in malaria endemic areas varies depending on the intensity of transmission, patient age, and consequently the degree of acquired immunity, the frequency of strain relapses, and the antimalarial treatment chosen. For example, drugs that are more slowly eliminated can suppress the first relapse and give time for hematological recovery, but treatment failure due to resistance will impede recovery.

2.5. Renal Failure

The diagnosis of acute renal failure in severe malaria is made using either blood urea nitrogen (BUN) > 56 mg/dL or creatinine > 3 mg/dL. Severe malaria raises the risk of renal impairment by causing damage to the interstitial area, tubules, and glomeruli. Red blood cells obstructing the renal vasculature cause acute tubular necrosis. Furthermore, aggravating pre-renal kidney damage includes hypovolemia and shock. When complement is activated, immune complex deposition can lead to glomerulonephritis. The term interstitial nephritis is also mentioned. Declining renal function exacerbates acidosis (Weiland, 2023).

The kidneys' glomeruli, tubules, and proximal tubules are especially linked. RBC clumping lowers circulation and damages the kidneys by causing hemodynamic instability due to the interaction between the affected RBCs and the capillary endothelium. Hemodynamic instability is the term for abnormal blood pressure that prevents the kidneys from receiving the right amount of blood. Hemodynamic instability has the potential to produce abrupt tubular necrosis, which results in damaged renal tubule cells and rapid kidney failure. Normally, tubule cells aid the kidneys in blood filtration. Glomerulonephritis, or inflammation of the glomeruli, is another possibility. Glomerulonephritis is a disorder of the glomeruli, or blood vessels, of the kidney. An essential function of the glomeruli is the elimination of surplus fluid and waste products from the circulation. RBCs can be seen clumping as they pass through the glomeruli; this has an impact on the kidney's filtration system. As a result of reduced filtration, urine is not effectively filtered, and the kidneys gradually lose their capacity to eliminate waste. The incapacity of the body to filter pee causes inflammation and fatigue. Antimalarials, hydroelectric adjustments, fluid replacement, and dialysis are treatments used to treat glomerulonephritis accompanied by a malaria diagnosis.

2.6. Cerebral malaria

Cerebral malaria is common and most severe in plasmodium falciparum infection, but rarely it is seen as a complication in plasmodium vivax. Cerebral malaria (CM), a widespread, symmetrical, potentially reversible encephalopathy brought on by parasite sequestration in brain vasculature, is clinically characterized as less than 11 points on the Glasgow Coma Scale. Although disruption of the blood-brain barrier may exist to some extent, parasites cannot cross it (Conroy et al., 2023). Imaging studies frequently show brain edema. When retinal hemorrhages and patchy retinal whitening indicate the presence of malarial retinopathy, the diagnostic sensitivity and specificity of CM are raised by 90% and 95%, respectively. If a lumbar puncture is done, raised opening pressure and nonspecific cerebrospinal fluid analysis are seen in CM. Clinicians must rule out meningitis and meningoencephalitis because of similar clinical presentations when contemplating CM. A typical neurological complication in survivors includes blindness, ataxia, hemiplegia,

epilepsy, and long-term cognitive impairments. (Weiland, 2023).

2.7. Splenomegaly

Among the most typical signs of malaria is splenomegaly. Nonetheless, although rare, spontaneous splenic rupture is a serious consequence that frequently results in mortality. Plasmodium vivax is most frequently linked to it, and it is primarily observed in acute infections and primary attacks.

A spontaneous splenic rupture is a rare etiology, and mortality rates can range from 15% to 70%, depending on the cause, the absence of splenic pathology, the presence of splenic adhesions, and the requirement for a normal spleen on anatomical, histological, and infectious workup. Spontaneous splenic rupture can be brought on by a number of conditions, including amyloidosis, infectious mononucleosis, rupture of a splenic aneurysm, malignancy-induced coagulopathy, bleeding issues, and anticoagulant use. Although the precise mechanism causing spontaneous splenic rupture is unknown, numerous hypotheses have been discussed in the literature. Cellular hyperplasia, venous engorgement, hyperplasia resulting in thrombosis or ischemia, vascular occlusion, and hyperplasia leading to ischemia or thrombosis, and sporadic increase in intra-abdominal pressure with coughing, laughing, sneezing, and vomiting leading to increased stress on the abnormal spleen. When all of these variables are present, the splenic capsule may rupture and generate subcapsular hematomas. Because spleen swelling is gradual and tension on the capsule is less severe during the acute phase of the illness than it does during recurring or chronic malaria infection, spontaneous splenic rupture occurs. Additionally, the previous infection's fibrous tissue hinders the growth of this complication.

Even in areas where malaria is prevalent, spontaneous splenic rupture is a rare complication. As a result, it calls for a high degree of clinical suspicion, particularly in patients who have malaria and come with hemodynamic instability and abdominal pain. Patients can be encouraged to try managing the case conservatively, but splenectomy is always necessary when conservative care has proven ineffective or is no longer appropriate (Eltayb and Hegazi., 2023)

2.8. Other complication

Aside from pulmonary edema and acidosis, other potentially fatal consequence includes hypoglycemia, hyperbilirubinemia, distributive shock, and high-grade parasitemia. Aspiration pneumonia and gram-negative sepsis are a few examples of potential side effects that clinicians should be aware of. Early identification and treatment of malaria are essential since it can progress quickly.

3. HYPNOZOITE LIFE CYCLE –

The hypnozoites have the ability to hibernate for several months or even years, and over their life cycle, they have become familiar with the human hepatocyte's salivary gland. During the feeding phase, the vector injects saliva into the host. According to White et al. (2014), the hypnozoite's interaction with a protein unique to the Anopheles genus found in the vector saliva may cause the change from dormancy to activation. Although a number of relapse triggers have been proposed, the molecular mechanisms governing hypnozoite activation remain unclear. By controlling the frequency of hypnozoite creation that takes place prior to, following, or concurrently with hepatocyte infection and the host's microenvironment, the sporozoite stage influences the destiny of hypnozoites to some extent. Liver stage schizont formation, activation, and replication lead to blood stage infection, disease recurrence, and additional transmission. Because of this, when circumstances are unfavorable for mosquito reproduction, the latent hypnozoite may wait for an opportunity to spread. To explain how the fate of hypnozoites is decided, three theories have been proposed.

Firstly, it was proposed that the infectious sporozoite undergoes pre-programming in the salivary glands and decides whether the cell will advance towards the development of sporozoite in the liver or persist for extended durations as a hypnozoite once it enters the liver (Schafer et al., 2021). This process may be governed by many factors, such as a stochastic mechanism (a random procedure that can be done to analyze a genetic regulatory mechanism) or by specific vector and host microenvironmental factors (Hagan et al., 2018). The environment surely affects physiological and behavioral changes. Dehydration, for instance, leads to increased activity and blood feeding. These elements could all have an impact on how sporozoites behave and their dedication to latent hypnozoite development (Hagan and Didion et al.,2018). Therefore, changes to the mosquito's habitat might have an effect on how sporozoites behave. Additionally, sporozoites may come into contact with various microenvironmental cues in the human host that result in a particular fate decision.

Numerous studies have shown that long-lasting insecticidal nets intended to prevent contagious mosquito bites have affected biting behavior as well as the status of sporozoite behavior and commitment to latent hypnozoite development.

Because there were no current biomarkers available to support this thesis, it is challenging to make an early diagnosis of hypnozoite infection in individuals.

3.1. Tachysporozoite and Bradysporozoites-

The second theory states that the formation of sporozoites is predetermined. According to this theory, there are two distinct sporozoite populations, each with a different molecular profile. One population of sporozoites, referred to as tachysporozoites, is preprogrammed to become active immediately after hepatocyte infection and to undergo schizogony. The second population goes into the latent hypnozoite stage, where they are referred to as bradysporozoites. Brady sporozoites and tachysporozoites have different transcriptional patterns. Alternatively, it is possible that the changes in transcription between brady sporozoites and tachysporozoites are epigenetic in nature and manifest only when the sporozoites penetrate hepatocytes (Ruberto et al., 2022). Molecular data, however, is insufficient to substantiate this theory.

According to the third theory, sporozoites enter a hepatocyte in an uncommitted state, undergo dedifferentiation, and then decide to produce hypnozoites or schizogony based on the host cell's milieu. ScRNA-seq analyses of human liver cells predicted significant inter-cell variations in hepatocyte metabolic activity, which is partly explained by food availability and liver division. Furthermore, hepatocyte ploidy has been connected to sporozoite infection predisposition. Because of this, as the liver stage trophozoite interacts with its host hepatocyte, a range of cues may influence its decision regarding its fate. If the hepatocyte's cellular environment is favorable, it may enter schizogony and replicate its genome; if not, it may enter latency through the formation of hypnozoites.

Current research on the first markers of liver schizogony, including DNA synthesis, nucleus division, and expression of the liver-specific protein 2 at 3 days post-hepatocyte infection, indicates that commitment to schizogony may not occur immediately (Gupta et al., 2019). Rather, it appears that during the first 24-48 hours post-infection, sporozoites may be able to sense, or at least be influenced by, the hepatocyte's intracellular environment and then react to specific circumstances or stimuli by forming a hypnozoite or schizont. This is because there seems to be sufficient time for a cell-cycle checkpoint to halt DNA synthesis as liver forms are established (Botnar et al., 2022).

These three theories are mutually exclusive. Although the fate of hypnozoites is most likely partially decided during the sporozoite stage, the frequency of hypnozoite formation is influenced by the host's microenvironment,

either before or after hepatocyte infection, or both.

Modern single-cell 'omics technologies and advanced laboratory models for liver infection will greatly benefit future studies into the mechanics of destiny decisions in P. vivax liver infection in order to reduce this complexity.

3.2. Implication of the Hypothesis

P. vivax sporozoite proteomics, which aids in the identification of early biomarkers, is made possible by the advancement of scRNA-seq technology. In a recent discovery, scientists discovered that complex P. vivax can be identified by the early biomarker microRNA 7977 (miRNA), which acts as a perfect indicator of being a short, stable, single-stranded, non-coding RNA molecule (Kaur and Sehgal et al., 2018). Extracellular vesicles (EVs) were identified to be a latent liver marker in an experiment using an in vivo model of P. vivax liver hypnozoite infections (Lopez and Verala et al., 2022). Extracellular vectors (EVs) containing parasite proteins and implicated in host-parasite interactions are released by certain malaria-infected cell types. They initially identified all P. vivax proteins compared between groups, and then they selected only those proteins detected in animals treated with MMV048 medicine in order to detect a hypnozoite biomarker. One protein (PVP01_0814300) exists from the group and was found to be essential for the parasite's dissemination and involved in membrane fusion during fertilization of sporozoite. Therefore, the removal of even a tiny portion of hypnozoites would prove advantageous to support the hypothesis that sporozoites undergo pre-programming in the salivary glands.

In addition, the development of novel single-cell RNA sequencing (scRNA-seq) technologies also permits comparisons of *P. vivax* sporozoite proteomics and bulk RNA-seq studies, which support molecular-level investigation of this concept.

Since Plasmodium species sporozoites have been shown to exhibit distinct transcriptome states according to ScRNA-seq analysis. So, the use of scRNA-seq technology offers a chance to investigate heterogeneity among *P. vivax* sporozoites and investigate the presence of unique transcriptional fingerprints that could aid in a better understanding of the sporozoite's developmental fate (Ruberto et al., 2022).

Proteomic analysis aids in the characterization of the transcriptome and histone epigenetic properties of P. vivax sporozoites, as well as proteins of blood stage parasites. In order to gain a better understanding of these early phases of infection, a recent study found that transcripts associated with functions necessary for the vertebrate host's early infection are not detectable as proteins and may be regulated through translational repression. These findings were confirmed by comparisons with recently published proteomic data for the P. vivax sporozoite (Swearingen and Lindner et al., 2017). The transcriptomes of asexual blood stages, mixed liver stages, and hypnozoite-enriched liver stages were found to have different transcription patterns from those of sporozoites. These comparisons indicate the presence of several levels of transcriptional, post-transcriptional, and post-translational regulation that are mainly lacking in replicating liver schizonts or mixed blood stages but appear active in sporozoites and, to a lesser extent, hypnozoites. (Muller et al., 2019).

Transcriptome-wide profiling, which examines the set of RNA molecules of the Plasmodium during hepatic stages, can be done using standard bulk RNA sequencing techniques. However, this approach eliminates any transcriptional differences that might exist between individual parasites by providing average transcriptional expression from either mixed (schizont and hypnozoite) or hypnozoite-only populations (Zou, 2023). Moreover, the potential impact of the parasite on host cells was disregarded, impeding the investigation of host-pathogen dynamics. These drawbacks emphasize the need for further research into the differences

between individual plasmodia and the mechanisms by which the parasite impacts liver cells. Developments in these fields might make it possible to characterize host and parasite elements crucial to the parasite's liver stage's growth.

Dr. Sangeeta N. Bhatia studied the host- and state-dependent gene expression patterns in hepatocytes and parasites using dual transcriptional profiling of P. vivax infection (Bhatia, 2022). This group employed a bioengineered human microliver platform to cultivate patient-derived P. vivax in order to create a single-cell liver atlas of relapsing human malaria by scRNA-seq analysis. The research described the early, latent, mid, and late phases of the infection as well as the distinctive characteristics of sexually committed P. vivax subpopulations in the liver. It was previously believed that these were exclusive to erythrocytic infections (Silva and Bhatia et al., 2022). These disadvantages do, however, emphasize the necessity for further knowledge on the differences between certain parasites and the parasite's mechanisms of hepatocyte modification. Taken together, Sc RNA sequencing technology is a useful tool for the malaria community as it sheds light on the mechanisms affecting the pathogenicity and developmental trajectory of P. vivax sporozoites.

Emphasizing hypnozoites is particularly difficult because most antimalarial medications do not affect them, and a liver sample is required for identification. The biochemistry underpinning the formation of hypnozoites is also poorly understood, and it is unclear when a schizont decides to become a hypnozoite. There remains a substantial information gap about P. vivax, according to a new theory proposed in a study. (Ruberto et al., 2022) presented three theories. Each region has significantly different rates of relapses and their frequency. Prevalence of early recurrence (>80%) is higher in tropical regions, with further relapses occurring every 3.4–5.6 weeks. The incidence of relapse is reduced in temperate and some subtropical locations, where there are longer incubation or latency periods (8–12 months) between the initial sickness and relapse. (White, 2011). Yet, every hypnozoite activation event has the potential to result in a fresh blood-stage infection, known as a relapse. So, even after all current infections have been eradicated from a community, the hypnozoite reservoir can restart transmission there. (Shanks and White, 2013)

4.PRESENT AND FUTURE STUDIES: CONCLUSION REMARKS

Following P. vivax mosquito transmission, hepatocytes generate hypnozoites, which can activate weeks to years after the initial infection and result in a relapsed blood stage infection. At this point, a number of theories govern the hypnozoite lifespan as well, making it possible to evaluate how the hypnozoites develop within their host. scATAC-seq and scRNA-seq are crucial for evaluating transcriptional and epigenetic modifications in sporozoites during hepatocyte entry and hypnozoite development. Using these approaches to analyze the infected host cells, will reveal changes required for long-term hypnozoite occupancy, making it easier to evaluate the hypotheses. Further investigation will also be facilitated by enhanced in vitro hepatocyte culture platforms and liver-humanized mouse models for experimental validation of theories regulating molecular mechanisms regulating hypnozoite production, persistence, and activation.

However, the primary issue that needs to be addressed in order to start additional research and evaluation of the hypnozoite stage is the difficulty of growing P. vivax sporozoites in a laboratory setting.

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