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Modern controlled human malaria infection (CHMI) clinical trials have almost entirely focussed on *Plasmodium falciparum*, providing a highly informative means to investigate host–pathogen interactions as well as assess potential new prophylactic and therapeutic interventions. However, in recent years, there has been renewed interest in *Plasmodium vivax*, with CHMI models developed by groups in Colombia, the USA, and Australia. This review summarizes the published experiences, and examines the advantages and disadvantages of the different models that initiate infection either by mosquito bite or using a blood-stage inoculum. As for *P. falciparum*, CHMI studies with *P. vivax* will provide a platform for early proof-of-concept testing of drugs and vaccines, accelerating the development of novel interventions.

**Controlled Human Malaria Infection**

CHMI with *Plasmodium falciparum* is an established method for evaluating new candidate vaccines and antimalarial drugs in early-phase proof-of-concept clinical trials. The controlled nature of these studies enables trials to be undertaken with small numbers of volunteers with power to investigate efficacy against malaria using a variety of defined end-points, thereby accelerating development of antimalarial drugs [1,2] and vaccines [3]. CHMI can be initiated by the traditional mosquito-bite method (still frequently used), by the injection of cryopreserved sporozoites, or by an inoculum of blood-stage parasites, so-called induced blood stage malaria (IBSM) [3–10]. *P. falciparum* strains other than the reference clone 3D7 and its parental strain NF54 are now being tested, including the 7G8 laboratory isolate and the Cambodian clone NF135.C10 [11,12]. Genetically attenuated parasites that arrest development during the liver stage of infection have now been tested in humans [13]. Most of these studies have been carried out in nonendemic settings, but more recently they have also taken place in endemic countries, in particular through the use of cryopreserved sporozoites [14,15].

By contrast, modern CHMI with *Plasmodium vivax* has been less utilized, with only a small handful of studies reported in the last few years. In only two of the studies published to date has efficacy of immunization been assessed (Table 1).

There is an extensive history of deliberate infection with *P. vivax* – most notably in malarotherapy, which was carried out for the treatment of neurosyphilis almost a century ago. The Austrian psychiatrist Julius Wagner-Jauregg later received a Nobel Prize for his work with this therapy [16], and the practice was widely adopted as the only effective treatment available at the time. Malarotherapy provided a wealth of information about *P. vivax* infection, which has been reviewed previously [17]. Deliberate infection with *P. vivax* was also conducted in the USA
from the 1940s to the 1970s in prisoners involved in the Malaria Research Project at the Illinois State Penitentiary. The studies mainly examined compounds for their potential use as antimalarials [18]. Similar studies were also carried out at the United States Penitentiary, Atlanta, and in both programs the Chesson strain of malaria was used because it was noted to be more likely to relapse and have a shorter latency period than previously utilized strains, meaning that compounds could be assessed more rapidly [18,19]. Key discoveries of the biology of \( P. \) vivax were made during this period including, for example, the association between Duffy negativity and resistance to \( P. \) vivax infection [20]. This review focuses on the more recent trials using \( P. \) vivax CHMI rather than these early studies and treatment programs.

\textbf{P. vivax Studies Using Sporozoite (Mosquito-Bite) CHMI}

Following on from the studies of \( P. \) vivax infection conducted in Illinois, CHMI experiments were carried out to see if prior exposure to irradiated mosquitoes could confer protection by immunization. Rieckmann et al. [21] reported no protection against CHMI in three participants previously exposed to \( P. \) vivax-infected irradiated mosquitoes on four occasions at intervals of 2–4 weeks (total of <200 mosquitoes).

Three \( P. \) vivax CHMI studies assessing the ability to ‘immunize’ with X-irradiated sporozoites also took place in Maryland, USA, during the 1970s. Following immunization, challenge infection was initiated by periodic exposure to the bites of nonirradiated infected mosquitoes in three volunteers in separate experiments, and blood films were taken at least daily for all volunteers.

<table>
<thead>
<tr>
<th>Trial site</th>
<th>Number of volunteers</th>
<th>Pre-patent period (days)*</th>
<th>Number of infected mosquitoes OR infective inoculum</th>
<th>Number of volunteers with patent parasitemia</th>
<th>Refs</th>
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<tbody>
<tr>
<td><strong>Sporozoite (mosquito-bite) CHMI studies</strong></td>
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<tr>
<td>Cali, Columbia</td>
<td>18</td>
<td>9–13</td>
<td>2–10</td>
<td>17/18(^{a})</td>
<td>[26]</td>
</tr>
<tr>
<td>Cali, Columbia</td>
<td>17 Duffy positive 5 Duffy negative</td>
<td>9–16</td>
<td>2–4</td>
<td>17/17 (Duffy positive) 0/5 (Duffy negative)</td>
<td>[27]</td>
</tr>
<tr>
<td>Cali, Columbia</td>
<td>7 malaria-naïve 9 semi-immune</td>
<td>11–13</td>
<td>2–4</td>
<td>16/16(^{a})</td>
<td>[28]</td>
</tr>
<tr>
<td>Cali, Columbia</td>
<td>12 Duffy-positive vaccinees 2 Duffy-positive controls 5 Duffy-negative controls</td>
<td>12–13</td>
<td>2–4</td>
<td>7/12 vaccinees 2/2 Duffy-positive controls 0/5 Duffy-negative controls</td>
<td>[31]</td>
</tr>
<tr>
<td>WRAIR, USA</td>
<td>27 vaccinees 6 infectivity controls</td>
<td>10–13 10–11</td>
<td>5</td>
<td>27/27 vaccinees 6/6 controls</td>
<td>[32]</td>
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<td><strong>Blood-stage CHMI studies (IBSM)</strong></td>
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<tr>
<td>QIMRB, Australia</td>
<td>2</td>
<td>8–9</td>
<td>13 000 genome equivalents</td>
<td>2/2</td>
<td>[35]</td>
</tr>
<tr>
<td>QIMRB, Australia</td>
<td>6</td>
<td>8–9</td>
<td>31 786 (± 11 947) as determined by qPCR (± 15 ± 5 viable ( P. ) vivax parasites)</td>
<td>8/6</td>
<td>[37]</td>
</tr>
</tbody>
</table>

\(^{a}\)The pre-patent period refers to the period before malaria diagnosis which was made by blood film in sporozoite (mosquito-bite) studies and qPCR in the blood-stage studies.

\(^{b}\)One volunteer did not develop parasitemia; the authors of the study suggested that this may have been due to surreptitious self-administration of antimalarial medication, but this was not proven.

\(^{c}\)One volunteer developed parasitemia detectable by qPCR but cleared it spontaneously within 4 days.
to assess outcome; between 6 and 14 infected nonirradiated mosquitoes were used for the infection challenges. These experiments in Maryland demonstrated that CHMI with both *P. falciparum* and *P. vivax* could be successfully carried out, but the studies were very small. In these studies, exposure to *P. falciparum* did not confer protection against subsequent CHMI with Chesson strain *P. vivax* [22,23]. However, exposure to X-irradiated Chesson strain and El Salvador strain *P. vivax* was able to confer protection against *P. vivax* CHMI following some of the ‘immunization phases’, but not all [22–24]. It was also noted that, unlike the Chesson strain, the newly isolated El Salvador *P. vivax* strain demonstrated long latency. In summary, the pre-patent period (time before development of blood film-detectable parasitemia) for control volunteers in the three experiments ranged between 9 and 16 days. These experiments also demonstrated that protection against *P. vivax* infection could be achieved for a short duration (3–5 months), but required exposure to hundreds of X-irradiated mosquitoes. Recent data emerging from the field of *P. falciparum* sporozoite immunization indicate that the magnitude of protection, longevity of protection, and the ability to protect against heterologous strains requires increasing doses of sporozoites [25]. Future CHMI studies with *P. falciparum* and *P. vivax* may build on these historical experiments to assess whether the same is true for *P. vivax* and even cross-species protection.

More recent mosquito-bite CHMI trials have taken place in Cali, Colombia. The first of these involved 18 healthy volunteers exposed to the bites of two to ten infected *Anopheles albimanus* mosquitoes [26]. *P. vivax* infection was established in mosquito lots fed on blood from 15 patients presenting to outpatient clinics at the Immunology Institute in Cali and Buenaventura with *P. vivax* infection. Four mosquito lots had to be discarded due to coinfection with *P. falciparum*, hepatitis B, or hepatitis C in the donor blood. A mosquito lot that demonstrated a sporozoite rate of 97% the day before CHMI was selected for use in the trial. Seventeen of the 18 volunteers developed *P. vivax* malaria, confirmed by thick blood smear, with a pre-patent period between 9 and 13 days. At diagnosis, volunteers were treated with standard *P. vivax* therapy consisting of chloroquine (600 mg initially, followed by 450 mg 24 and 48 h later) to clear blood-stage infection and primaquine (30 mg/day for 14 days) to achieve radical cure. Levels of parasitemia ranged from 75 to 420 parasites/µL; parasites were cleared within 48 h of starting treatment in all infected volunteers. There were no serious adverse events (SAEs) in this trial, but seven volunteers required fluid therapy due to nausea and vomiting, and five developed blurred vision lasting 2–3 days after treatment initiation. Authors speculated that the volunteer who did not develop malaria had surreptitiously taken antimalarial medication, but this was never confirmed.

The second *P. vivax* CHMI trial was carried out by the same group in Colombia, aiming to demonstrate the reproducibility of this method of infection using three different *A. albimanus* mosquito lots fed on blood from three *P. vivax*-infected donors [27]. Seventeen Duffy-positive individuals and five Duffy-negative controls were enrolled into the study. Participants were randomly assigned to three groups (with six Duffy-positive individuals in two of the groups and five in the third group), and exposed to the bites of two to four infected mosquitoes. The Duffy-negative controls were assigned across the three groups, with two controls in the first two groups and one in the third. All Duffy-positive participants (and none of the Duffy-negative participants) developed blood-stage malaria. The pre-patent period ranged from 9 to 16 days, and was different in the first group, with a median of 14 days, as opposed to a median of 10 days in the second two groups.

A third *P. vivax* CHMI trial was carried out in Cali but this time among both ‘semi-immune’ (previously-exposed; n = 9) and malaria-naïve adult volunteers (n = 7) [28]. Previous exposure was confirmed by clinical history and a positive indirect fluorescent antibody test against *P. vivax* blood stages. A mosquito lot from one of six *P. vivax*-infected patients was used for all volunteers, who were each exposed to bites from two to four infected mosquitoes. The pre-patent periods
ranged between 11 and 13 days, with no significant difference between the malaria-naïve and ‘semi-immune’ volunteers. Symptoms were significantly worse among the malaria-naïve subjects but there were no SAEs. One malaria-naïve volunteer developed parasitemia detectable by real-time quantitative polymerase chain reaction (RT-qPCR) by day 9, but cleared the parasitemia spontaneously in 4 days. An antimalarial drug screen was negative; the authors speculated that this phenomenon may be due to Duffy antigen polymorphism. Another malaria-naïve volunteer presented 3 months after treatment with a P. vivax infection after visiting an endemic area. However, the investigators were unable to determine whether this episode of malaria was due to reinfection or relapse, as the volunteer had visited the same endemic area that the CHMI strain had come from.

A subsequent analysis of differences in gene expression between malaria-naïve and ‘semi-immune’ volunteers demonstrated significant changes in gene expression at the time of malaria diagnosis, particularly in the naïve volunteers, with downregulation of multiple genes related to innate immunity, inflammation, and neutrophil abundance [29]. Antibody profiling was also undertaken, using a custom protein microarray. This demonstrated increased responses in the semi-immune participants compared with naïve individuals before CHMI, although responses in both groups were higher than those from US controls. Following CHMI, antibody responses increased on day 45 and had declined to near baseline by day 145. Volunteers who experienced fever were found to have significantly higher responses to the P. vivax antigens merozoite surface protein 3 (MSP3), MSP4, MSP5, and MSP10 at the day 45 time-point [30].

The most recent CHMI trial from the Cali group [31] involved the use of radiation-attenuated sporozoites in Duffy-positive and Duffy-negative healthy adult volunteers, delivered by mosquito bite. Mosquitoes were infected as described above for the other trials conducted by this group. Sporozoites were attenuated by exposing the mosquitoes to 150 ± 10 cGy of gamma irradiation. Twelve Duffy-positive participants and five Duffy-negative controls completed the immunization phase with exposure to P. vivax-infected irradiated A. albimanus mosquitoes followed by CHMI. Two Duffy-positive controls also completed the trial; these participants were exposed to nonirradiated, noninfected mosquitoes. Seven immunizations were carried out for each volunteer on weeks 0, 8, 12, 23, 48, 51, and 56, with a mean of 65 infectious bites for each immunization. Two weeks after the final immunization, participants were treated with chloroquine and primaquine to clear any malaria infections that may have developed during the immunization phase. Plasma levels of chloroquine and primaquine were checked prior to CHMI to ensure drug clearance. CHMI was carried out at week 64 using two to four P. vivax-infected mosquito bites, and participants were monitored daily with thick blood films from day 6. There were no reported SAEs related to immunization, although one volunteer developed severe elevation of hepatic transaminases (>10 times the upper limit of normal [x ULN]) with associated abdominal pain and vomiting following CHMI, with no alternative cause found. These symptoms resolved spontaneously. The protective efficacy of the immunization schedule was 42% (five out of twelve Duffy-positive participants protected). In volunteers who developed malaria, the mean pre-patent period until thick blood film positivity was 12.8 days. Interestingly, all of the volunteers protected in this trial were female [31].

Only one other CHMI trial assessing a P. vivax vaccine has been published to date. The VMP001/AS01B vaccine was tested in healthy malaria-naïve adults at the Walter Reed Army Institute of Research (WRAIR) in the USA [32]. VMP001 is a soluble recombinant protein vaccine [33], encoding the P. vivax circumsporozoite protein (PvCSP), administered with the AS01B adjuvant (GlaxoSmithKline). The vaccine was administered to 30 volunteers in three cohorts (10 in each) at doses of 15 μg, 30 μg, and 60 μg, given three times at a 4-week interval between the first and second dose; the third dose was given 8 (15 μg cohort), 6 (30 μg cohort), or 4 (60 μg cohort) weeks after the second. Twenty-nine volunteers completed the vaccination phase, with
validate of based determine both intravenously screening parasites modeling (The qPCR presence fantrine detectable been at currently proceeding. The mean dose of parasites (± SD) administered to the six subjects in this study was 31 786 (± 11 947) parasites as determined by qPCR testing of the inoculum, whilst linear regression modelling of the in vivo qPCR parasite growth data estimated the starting dose of viable parasites to be a mean ± SEM of 15 ± 5 parasites. The kinetics of the parasitemia (determined
by qPCR) were similar in all volunteers with first detection on day 8 in four subjects and day 9 in two subjects. Peak parasitemia occurred on day 14 (the day of antimalarial treatment) with a median of 31 parasites/μL. The parasite multiplication rate (PMR), assuming a 48-hour lifecycle, was 9.9 (95% CI: 7.7–12.4). The clinical course was also similar in all volunteers, with the mean onset of symptoms occurring on day 12.2 (range 11–13).

There were no SAEs reported in this study; most of the adverse events were mild expected symptoms of malaria. However, four of the six subjects demonstrated significant elevations of hepatocellular enzymes (>5 x ULN), although asymptomatic and not associated with significant elevations in bilirubin (Hy’s law was not met) (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf). The specific cause of the elevated hepatocellular enzymes was not conclusively determined, but extensive additional investigations, including viral serologies (hepatitis viruses, herpes viruses, alphaviruses and flaviviruses), other biomarkers (creatinine kinase, paracetamol levels), and liver ultrasound did not demonstrate any significant abnormalities. The inoculum (and donor) had been screened to exclude the presence of blood-borne pathogens other than malaria to a greater level than required by the local blood service. All subjects were treated with artemether–lumefantrine on study day 14 with a corresponding rapid decline in parasitemia and clinical symptoms.

There was no evidence of reactions to the donor’s blood, that is, no transfusion reactions and no red cell alloantibodies detected, and no evidence of transmitted infection (other than malaria). Whilst the initial *P. vivax* isolate demonstrated a favourable profile in two studies, it was obtained from a blood group A donor. Therefore, to reduce the probability of alloantibody generation and to optimize the probability of infection, it is required that recipients are also blood group A (and Duffy positive). This significantly limits the number of eligible volunteers. To overcome this limitation, and as a result of an ongoing cryobanking project, a second *P. vivax* isolate was obtained from a blood group O patient who returned to Brisbane, Australia, having acquired *P. vivax* infection in India. This inoculum has since been used in two further studies (ACTRN12614000930684 and ACTRN12616000174482), but results have not yet been published.

The development of gametocytemia in volunteers undergoing CHMI has implications for carrying out such studies in areas of the world where *Anopheles* mosquito vectors are present and capable of supporting transmission. Of note, *A. albimanus* mosquitoes fed on blood from infected volunteers between day 7 post-CHMI and the day of diagnosis (days 11–13) in the third trial carried out in Cali, Columbia [28], did not develop oocysts over the next 7 days. This was despite the presence of parasites, likely to be gametocytes, being identified in the circulation as early as day 7 by qPCR for two sexual stage-specific molecular markers, *pvs25* and *pvs16*. Direct (skin) feeding was carried out as well as via a membrane feeding assay. In contrast, parasites obtained from submicroscopic asymptomatic and naturally infected individuals were able to infect mosquitoes [38]. This difference is thought to be due to insufficient maturation of gametocytes at the point of diagnosis in the early CHMI infections.

The second blood-stage CHMI trial in Brisbane showed more promise for assessment of transmission [37]. In this trial, detection of *pvs25* transcripts was used as a marker of gametocytemia. Expected kinetics relative to the parasitemia were observed, with peak *pvs25* detection occurring immediately prior to antimalarial treatment (day 14), with a median of 4.90 × 10^6 transcripts/mL (range from 3.96 × 10^4 to 2.37 × 10^6). The potential for transmission to mosquito vectors was assessed by direct (skin) and membrane mosquito feeding for 2 to 3 days prior to antimalarial therapy. A total of 16 direct feeding assays and 32 membrane feeding assays were conducted. A total of 1801 *Anopheles stephensi* mosquitoes were dissected for the detection of oocysts, and a low prevalence of mosquito infection was observed
(1.8%; n = 32/1801 mosquitoes). Moreover, unlike *P. falciparum*, *P. vivax* gametocytes are susceptible to asexual-stage antimalarial treatment so any gametocytes should also be cleared following initiation of standard therapy [39].

In line with these experiences, a recently developed simulation model (using data from a previously developed model of red blood cell invasion and information from six published time series of parasitemia from neurosyphilis patients treated with *P. vivax*) further supports these findings. Data suggest that a density of 116 gametocytes/μL is required for there to be at least one male and one female gametocyte in a 1 μL blood meal, and this model predicts that this threshold will first be reached in an infected naive host at 7.9 days post patency on average (95% CI 5–10) [40]. Overall, these data suggest that transmission efficiency during standard CHMI protocols would likely be very low.

**Comparison of CHMI Models**

Modern CHMI studies have proved extremely useful in early-phase testing of *P. falciparum* vaccines [3,6,41–46] and drugs [1,2,47,48]. The development of modern CHMI approaches for *P. vivax* is less well established, but has the potential to provide a useful and cost-effective means of early-phase analysis of antimalarial drugs and vaccines. To date, the only methods trialled in humans are CHMI by mosquito bite or by intravenous inoculation of parasitized red blood cells. Cryopreserved sporozoites, as developed by Sanaria, are another potential method for CHMI, but unlike *P. falciparum* cryopreserved sporozoites (PRSPZ Challenge), have not yet been used in any human trials (http://projectreporter.nih.gov/project_info_description.cfm?aid=8446961).

There are advantages and limitations to both available CHMI models (see Outstanding Questions). Mosquito-bite CHMI most closely resembles natural infection but requires fresh gametocytes from an infected patient due to the unavailability of long-term *P. vivax* culture. This requires at least part of the trial to be undertaken in an endemic setting to screen and enrol patients, with appropriate entomological facilities established to produce an infected mosquito lot. Subsequently, the mosquito lot can be used in trials in the same location, or transported to nonendemic areas. Ensuring the successful production of infected mosquitoes, in conjunction with recruitment of volunteers who may receive an intervention such as a vaccine, poses significant logistical challenges, especially if the timing of the vaccination and subsequent CHMI are critical. Moreover, a different isolate of *P. vivax* will inevitably be used for every trial, meaning that CHMI assessment of vaccines is almost certainly to be with a heterologous strain to that used in the vaccine; that the parasites may have different susceptibility to antimalarial treatment between strains; and these differences will be unknown at the time of CHMI. As seen in the trials carried out in Cali, different strains are likely to have different pre-patent periods which can limit comparability between trials [27]. The use of sporozoites for CHMI also necessitates a liver stage of infection, with a high risk of hypnozoite formation and potential relapse. This requires participants to be screened for glucose-6-phosphate dehydrogenase (G6PD) deficiency in order to avoid hemolysis induced by primaquine, and now also requires assessment of the volunteers’ ability to metabolize primaquine to maximize safety. Volunteers with poor or intermediate metabolizer CYP2D6 phenotypes should not be enrolled for such studies in the future.

The use of IBSM/blood-stage CHMI does not mimic natural infection but has several advantages over mosquito-bite CHMI. Practical advantages include the ability to carry out CHMI studies more easily in a nonendemic setting; having access to the *P. vivax* strain genetic data before CHMI; being able to carry out multiple studies with the same strain (for which a safety database can be established); and being able to use the same inoculum size for each volunteer or even vary the dose of parasites administered if required. There are also advantages for participants with this method – the use of blood-stage parasites means that there is no liver stage of infection,
and therefore no risk of hypnozoite formation or relapse. This means that participants do not require primaquine treatment, and therefore do not require G6PD deficiency or CYP2D6 phenotype screening. Although the numbers of participants who have undergone this method of CHMI are still currently very small, all have successfully been infected with an expected rise in parasitemia detected by qPCR multiplication, unlike in two of the five reported mosquito-bite CHMI studies. The ability to give a small known inoculum directly into the bloodstream also means that the blood stage of infection can be observed for longer (than following mosquito-bite CHMI), and differences in PMR are more likely to be observed between participants and controls. A recent blood-stage P. falciparum CHMI trial in Oxford has demonstrated greater power to see a reduction in PMR with smaller numbers of volunteers as compared with sporozoite CHMI studies [36]. Blood-stage CHMI is thus particularly advantageous for the assessment of antimalarial drugs and vaccines which target the erythrocytic stage of the malaria parasite. The model is of course unable to be used for assessment of pre-erythrocytic interventions which does limit its utility, and whether either model can be optimized to robustly assess transmission-blocking interventions remains to be determined.

Concluding Remarks and Future Perspectives

The development of CHMI models for P. vivax is a vital step towards early-phase vaccine and drug testing, allowing relatively small and inexpensive Phase II efficacy trials to take place before larger field trials. The use of P. vivax CHMI is more complex than the now established P. falciparum models because of inherent differences between the parasites’ biology and, in particular, the inability to maintain P. vivax in long-term culture. Mosquito-bite CHMI currently requires access to infected patients and entomology facilities, and leads to the use of genetically variable isolates in each study. The use of cryopreserved sporozoites in the future may represent a particularly useful advancement, but this approach has yet to reach clinical testing. Other approaches may include the use of P. falciparum parasites transgenics for antigens of interest to allow for easier CHMI assessment of sporozoite or liver-stage vaccine candidates. To date only one clinical study has been reported using a genetically modified P. falciparum parasite, in this case a knockout parasite line [13]. Transgenic parasites have been developed for use in preclinical and in vitro studies against P. vivax, so there is potential for development for clinical use [49,50].

Blood-stage P. vivax CHMI remains in its infancy, but will likely be more widely used for novel vaccine and drug testing in the coming years. To date, only one vaccine targeting the P. vivax blood-stage merozoite – using the chimpanzee adenovirus serotype 63 (ChAd63) and modified vaccinia virus Ankara (MVA) viral vectors encoding the P. vivax Duffy-binding protein region II (PvDBP_RII) in a heterologous prime-boost regimen [51] – has completed Phase I clinical testing in the UK (NCT01816113) and could progress to Phase Ila efficacy testing. A second protein-based PvDBP_RII vaccine formulated in SE-GLA adjuvant has recently entered Phase I clinical trial in India (CTRI/2016/09/007289). The demonstration of a blood-stage CHMI model for vaccine testing, using P. falciparum [36], should allow for similar advances with P. vivax in the future. This model may also allow for studies of naturally acquired P. vivax immunity if trial centres are established in endemic areas, as is starting to occur now for P. falciparum in Africa [14,15]. The development of multiple banks of cryopreserved strains will also allow for vaccine testing against homologous and heterologous strains potentially originating from different geographic areas. CHMI trials can also be used to further investigate the mechanism, immunity, and host-pathogen interactions under controlled infection conditions in humans – the ultimate animal model. These endeavours should help to provide essential new tools to aid in the fight against P. vivax, as efforts continue to move towards local malaria elimination.

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