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Utilizing direct skin feeding assays for development of vaccines that interrupt malaria transmission: A systematic review of methods and case study

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Abstract

Shifting the malaria priorities from a paradigm of control and elimination to a goal of global eradication calls for renewed attention to the interruption of malaria transmission. Sustained progress toward eradication will require both improved understanding of infectious reservoirs and efficient development of novel transmission-blocking interventions, such as rapidly acting and highly efficacious therapeutics and vaccines. Here, we review the direct skin feeding assay (DSF), which has been proposed as a valuable tool for measuring the *in natura* transmission of malaria parasites from human hosts to mosquito vectors across heterogeneous populations. To capture the methodological breadth of this assay's use, we first systematically review and qualitatively synthesize previously published investigations using DSFs to study malaria transmission in humans. Then, using a recent Phase 1 trial in Mali of the Pfs25H-EPA/Alhydrogel® vaccine candidate (NCT01867463) designed to interrupt *Plasmodium falciparum* transmission as a case

study, we describe the potential opportunities and current limitations of utilizing the endpoints measured by DSF in making early clinical decisions for individually randomized transmission-interrupting intervention candidates. Using simulations based on the data collected in the clinical trial, we demonstrate that the capacity of the DSF to serve as an evaluative tool is limited by the statistical power constraints of the "effective sample size" (i.e. the number of subjects that are capable of transmitting at the time of feeding). Altogether, our findings suggest DSFs have great potential utility for assessing the public health impacts of emerging antimalarial tools, but additional research is needed to address issues of scalability and to establish correlation with community-wide clinical endpoints as well as complementary in vitro measures, such as standard membrane feeding assays.

Keywords

Transmission-blocking vaccine; Malaria; Trial design; Direct skin feed; Vaccine activity

1. Introduction

Progress toward global malaria eradication will be expedited through the careful integration of established malaria elimination strategies with novel transmission-interrupting interventions operating across the host, parasite, and vector levels [1, 2]. As highly cost-effective public health tools with lasting effects on populations, "vaccines that interrupt malaria transmission" (VIMTs) are likely to be central to future elimination efforts [1]. VIMTs include classical transmission-blocking vaccines that can arrest the sporogonic development of the parasite inside the mosquito as well as highly effective pre-erythrocytic vaccines and blood-stage vaccines that may decrease the size of the infectious reservoir by reducing gametocyte carriage. VIMTs in development will achieve the "minimally acceptable target" of expected efficacy if the vaccine, in combination with existing malaria elimination tools, maintains R_e (i.e. the number of subsequent infections arising from each infected human in a population made up of both susceptible and non-susceptible hosts) below one for at least one year in a previously malaria-endemic region [1]. Although the concept of transmission-interrupting interventions is not new (e.g. indoor residual spraying is a highly effective method for decreasing transmission by shortening mosquito lifespans and reducing population densities), the individual-level randomization that is possible with VIMT candidates creates demand for new analytical frameworks. Barring completion of costly and complex cluster randomized controlled trials that evaluate measures such as the entomological inoculation rate or clinical incidence of malaria within communities [3], robust functional assays that provide potential surrogate measures of individuals' infectivity to mosquitoes have been proposed as the best available approaches for evaluating early clinical Go/No Go decisions for VIMT candidates in the research pipeline.

At present, standard membrane feeding assays (SMFAs), which have been qualified according to the International Conference on Harmonisation guidelines, are considered the gold standard for evaluating the transmission-blocking potential of test antibodies *ex vivo* [4, 5]. The SMFAs, which evaluate the functionality of test antibodies by offering cultured *Plasmodium* gametocytes combined with fractionated serum or purified immunoglobulin to

laboratory-reared, uninfected mosquitoes through an artificial membrane, have been used to rank the activity of transmission-blocking vaccine candidates [4, 6, 7]. Whereas SMFAs specifically measure antibody activity in relation to reduction in gametocyte transmission to mosquitoes, broadly defined VIMTs may also incorporate pre-erythrocytic or blood-stage vaccines that require study of human infection. Direct membrane and skin feeding assays can enable investigators to evaluate both reductions in malaria infections and the resulting interruption in transmission of parasites from human blood to mosquitoes. The direct membrane feeding assay (DMFA) is similar in design to the SMFA except that gametocytes are derived from the venous blood of infected individuals and better reflect the diversity of field populations [8]. First described by R.C. Muirhead-Thomson in 1957 as a method to test "the malarial infectivity of the human subjects in a direct manner," the direct skin feeding assay (DSF) measures the prevalence and magnitude of infection in laboratory-reared mosquitoes that are contained in cups and allowed to feed directly on the skin of human volunteers [9].

While membrane feeding assays will continue to serve as valuable and well-controlled tools for assessing the blockade of transmission, methods that examine host-vector interactions through direct skin feeds may offer greater scope for investigating the activity of candidate VIMTs on *in natura* transmission. Overall, there is a high concordance between the direct membrane and direct skin feeding methods, and DSFs offer additional biological advantages. An analysis of 241 paired transmission experiments demonstrated a significant positive correlation (Spearman's rho of 0.36, $p<0.0001$) between the proportion of mosquitoes infected using DMFAs versus DSFs [8]. However, DSFs offer 2-fold higher efficiency in terms of the proportion mosquitoes infected than DMFAs and are also more robust to human error [8, 10, 11]. In DMFAs, failure to maintain blood samples at a constant temperature of 37°C could lead to the premature activation of gametocytes and interfere with gametocyte infectiousness and successful parasitic reproduction [12, 13]. Further, DSFs better mimic *in vivo* feeding conditions than DMFAs. By design, membrane feeding assays eliminate differences in host attractiveness to mosquitoes and are also incapable of simulating the immune response and gametocyte densities and dynamics that occur in host microvasculature [14]. Therefore, we ask: Could DSFs be used as a primary evaluative tool for early clinical development of vaccines designed to interrupt malaria transmission?

To answer this, we first systematically reviewed published literature in order to understand the scope of research questions and experimental approaches used by scientists who have employed DSFs to measure malaria transmission. Then, using a recent Phase 1 trial in Mali of the Pfs25H-EPA/Alhydrogel® transmission-blocking vaccine candidate as a case study, we outline our experiences in a pilot investigation into the use of DSFs for early phase clinical trials and provide estimates for the numbers of participants and feeding assays that will provide sufficient power to estimate VIMT activity by DSF.

2. Direct skin feeding assays to measure malaria transmission: A systematic review

2.1. Methods

Using PubMed, Web of Science, and EMBASE, we identified studies that used direct feeding assays to estimate the transmission of *Plasmodium* parasites from human hosts to mosquito vectors. We searched the databases using keywords related to the assay, parasite, and vector and scanned reference lists for additional relevant articles (Table 1). We screened records published after 1 January 1985 and before 24 July 2015 and applied no language restrictions; studies using non-human subjects were excluded. Study level characteristics were extracted using a standardized data extraction form to collect information on: geographic location, number of participants, age range, target *Plasmodium* and *Anopheline* species, number of mosquitoes per cup, number of cups, mosquito age, length and location of feed, repeats of feed, and reported measures of human-to-mosquito transmission, including oocyst prevalence (i.e. the proportion of mosquitoes with at least one oocyst), oocyst intensity (i.e. the number of oocysts per mosquito), and the relative prevalence of gametocytemia in the study sample.

2.2. Results

Our database search retrieved 213 records (Figure 1); 7 additional records were identified by reference list review. We excluded 129 duplicate records and 60 records by abstract. After carefully reviewing the full-text articles for the remaining 31 records, 11 publications were selected for inclusion in the systematic review (Table 2). The 11 studies measuring malaria transmission in human populations with DSF were undertaken in 7 countries from 4 continents. The investigations were designed: (i) to compare direct and membrane feeding assays [10, 15–19], (ii) to characterize infectious reservoirs [10, 11, 17, 20–22], and/or (iii) to investigate transmission-interrupting activity [17, 18, 23]. The studies employed six *Anopheline* species/ sibling species and primarily targeted *Plasmodium falciparum* and *vivax*; however, infections by *P. malariae* and *P. ovale* were also considered in five studies [10, 11, 16, 21, 22]. Study samples, which included between 15 and 685 participants, represented a wide range of ages (0.8 to 77 years); 7 of the studies restricted the cohorts to individuals who were gametocyte positive at the time of feeding. With the exception of the study by Alves, et al. (2005) [20], which incorporated polymerase chain reaction (PCR) to identify *Plasmodium* carriers with low parasitemias, gametocytemia was identified microscopically in the included studies. There was great variability in the direct feeding procedures. Between 1 to 3 cups, containing 20 to 100 starving mosquitoes aged 3 to 6 days were applied to the calves, thighs, and arms of the study participants for between 10 and 30 minutes. Measures of oocyst prevalence and intensity were commonly reported. Differences between study groups (e.g. age categories) were most frequently evaluated using non-parametric approaches [11, 15–17, 19, 21, 22]; however, several studies – and, notably, two investigating transmission-interrupting activity – reported qualitative differences alone [18, 20, 23]. Diallo, et al. (2008) compared mean oocyst prevalence with a student's t-test [10]. Although Beavogui, et al. (2010) reported completing repeated feeds on the same participants over time, oocyst prevalence was less than one percent, and neither the

longitudinal results nor statistical adjustment for intra-individual correlations were reported [23]. Currently, there is no precedent for utilizing DSFs to quantitatively evaluate the activity of candidate VIMTs in a clinical trial.

3. Direct skin feeding assays to evaluate VIMT candidates: A case study

Pfs25, a leading transmission-blocking vaccine candidate, is a highly conserved *P. falciparum* surface antigen expressed during the zygote, ookinete, and young oocyst stages of the parasitic lifecycle in the mosquito midgut [24, 25]. When chemically conjugated with the outer membrane protein complex of *Neisseria meningitidis* and a mutant, detoxified protein from *Pseudomonas aeruginosa* known as ExoProtein A (EPA), Pfs25 has been demonstrated to elicit sustained antibody responses in mice and guinea pigs as well as transmission-reducing and blocking activity in an *ex vivo* SMFA [26, 27]. For an earlier US-based Phase 1 clinical trial in healthy adults beginning in 2011, the Pfs25H-EPA vaccine was formulated with the aluminum-based adjuvant Alhydrogel® and was shown to be both safe and immunogenic; results from SMFAs are consistent with transmission-reducing activity (Talaat, et al., submitted).

3.1. Study objectives

From 2013 to 2015, a double-blind, comparator-controlled Phase 1 clinical trial of Pfs25H-EPA/Alhydrogel® vaccine was conducted in Bancoumana, Mali in a sample of healthy, malaria-exposed volunteers aged 18 to 45 years (Figure 2). The primary objective was to assess the safety and reactogenicity of the Pfs25H-EPA vaccine, and the results will be published separately. As a secondary objective, DSFs were performed to compare human-to-mosquito transmission between the study arms and to explore the effectiveness of the assay in terms of mosquito feeding behaviors and survival. This case study focuses on the results of DSFs that were performed following the fourth vaccination and will discuss how the findings of this feasibility study can inform future use of DSFs for evaluation of VIMTs.

3.2. Ethical approval and informed consent

The protocol used for the case study was approved by the the Faculty of Medicine, Pharmacy, and Oto-Stomatology (FMPOS) ethical committee of the University of Bamako, Mali, with Federal Wide Assurance #00001769, the NIH institutional review board, and the Mali national regulatory authority and was conducted under FDA IND 14781. First, community permission was obtained from village elders, family heads, and community associations. Then, before enrollment, individuals were provided with a written informed consent form, which was literally translated into French, orally translated into local languages, and independently verified for understanding by a witness external to the study team. No subject was permitted to undergo more than 12 DSFs within a 12 month study period. Volunteers were carefully monitored for adverse events. Prompt care was provided to sick participants per standard of care, including treatment of symptomatic malaria in accordance with Malian Ministry of Health protocols. Blood smear results were evaluated during regular study visits and unscheduled study visits as clinically indicated.

3.3. Study sample

Of the 100 individuals in the high dose group (receiving either 47 μg Pfs25H-EPA/Alhydrogel®; n=50 or Euvax/ Hepatitis B followed by Menactra in year 2), 82 volunteers (including 42 Pfs25H-EPA/Alhydrogel® and 40 comparators) completed four vaccinations (reasons for dropout included: 10 consent withdrawals, 4 losses to follow-up, 2 pregnancies, 1 relocation, and 1 incarceration). Of the 82 vaccinated individuals, 79 volunteers (including 41 Pfs25H-EPA/Alhydrogel® and 38 comparators) completed ≥ 1 DSF following the fourth vaccination for a total of 467 DSFs completed (reasons for dropout included: 2 consent withdrawals and 1 adverse event).

3.4. DSF procedure

To replicate *in natura* conditions, laboratory colonies of *Anopheles coluzzii* were established from wild gravid females and reared under controlled conditions in order to minimize microbiota contamination and the introduction of adventitious pathogens; mosquitoes from the 105th to 109th generations were used for the DSF experiments. In the insectary, mosquitoes were maintained on fish food and water in a standardized environment with temperature of 25±2° C, humidity of 70±10%, and photoperiodicity of 12 hours. For breeding, female mosquitoes were fed on human blood donated by consented individuals queried and monitored for fever or other illnesses. All samples of donated human blood that were used for colony maintenance and random samples of mosquitoes from the colony were regularly monitored by PCR for evidence of Rift Valley Fever. At the age of 3 to 6 days, mosquitoes were collected and transferred to small paper cups. Trained project nurses applied two cups, containing 30 female mosquitoes each, for 15 minutes directly to the skin of the calves of all participants. The gametocyte status of participants at the time of feeding was quantified by microscopic examination of Giemsa-stained bloodsmear until 1000 white blood cells had been counted. Duplicate bloodsmears were prepared and read, and in cases of discordant results (i.e., one positive, one negative), a third bloodsmear was evaluated to draw a final inference. Study participants were provided with anti-histamine cream following exposure. Engorged mosquitoes were maintained in the feeding cups for an additional 7–8 days. Experienced laboratory technicians dissected surviving mosquitoes in saline solution. Midguts were stained with mercurochrome, and oocysts were counted by microscopy.

3.5. Endpoints of the DSFs

Table 3 summarizes the endpoints produced by the DSFs. 3% (n=14 experiments) of the DSFs in the case study resulted in at least one positive mosquito infection (i.e. a feed where ≥ 1 mosquito that survived to dissection had an oocyst). Notably, 5 of these 14 experiments were performed on volunteers who were gametocyte-negative by microscopy at the time of feeding.

At each administration, the DSFs produced three population-wide measures of transmission: oocyst prevalence, oocyst intensity, and host infectiousness. Oocyst prevalence was low; overall, 0.4% of the 17,128 dissected, engorged mosquitoes had ≥ 1 oocyst. Further, oocyst prevalence was approximately 15% within the subgroup of DSF experiments where at least one mosquito became infected (n=14 experiments, n=560 dissected mosquitoes). Although

the median oocyst intensity was 0 overall, the median oocyst intensity was 1 (range: 1 to 7) within the 83 infected mosquitoes. Overall, 14% of the 79 volunteers infected at least one mosquito over the duration of follow-up.

The assays also provided some insight into the exploratory objectives of host attractiveness, feed intensity, and mosquito survival. Overall, we found that 100% of individuals successfully fed mosquitoes during the primary follow-up period. In total, 99% of the 467 DSF experiments resulted in any engorged mosquitoes. Within the 461 DSF experiments where at least one mosquito fed, the median feed intensity was 95% (range: 33 to 100%) and the median percentage of engorged mosquitoes that survived to dissection was 68% (range: 13 to 95%).

3.6. Estimating vaccine activity

Oocyst prevalence was selected as the primary endpoint to estimate the vaccine activity. Oocyst prevalence was selected over other DSF endpoints (i.e. oocyst intensity, host infectiousness) to reflect the goal of the VIMT to reduce the number of infected mosquitoes that may infect another human host. To evaluate the transmission-blocking activity of the vaccine, the primary analysis used a binomial GEE regression to estimate a population-averaged odds ratio, comparing the odds of a mosquito having ≥ 1 oocyst across the study arms while accounting for the repeated nature of the DSF measurements (i.e. up to 6 repeats). As the oocyst prevalence was very low (i.e. 0.4% of mosquitoes had ≥ 1 oocyst), the odds of a mosquito having ≥ 1 oocyst provided a robust approximation for the oocyst prevalence within each of the study arms. As a sensitivity analysis, a zero-inflated negative binomial mixed-effects model that allowed for volunteer-specific random effects and provided an offset for DSF experiment-specific mosquito survival was used to compare the per DSF experiment difference in the oocyst prevalence across the study arms while accounting for the repeated nature of the DSF measurements (i.e. up to 6 repeats), the overdispersion of the data, and the high number of zeroes (i.e. in 99.6% of the data). The results of the subsidiary analysis did not differ materially from those of the primary analysis. The secondary analysis on oocyst intensity (i.e. averaged across all engorged, dissected mosquitoes in a given DSF experiment) used a zero-inflated linear mixed-effects model that allowed for volunteer-specific random effects to investigate the transmission-reducing activity of the vaccine. The tertiary analysis on host infectiousness used a Fisher's exact test to compare the number of infectious volunteers across the study arms.

3.7. Sample size requirements: lessons learned

In this case study, there was insufficient power to adequately evaluate differences between the study arms in terms of any of these DSF endpoints due to the low level of host infectiousness. To explore different approaches for optimizing the statistical power for future experiments, we first simulated data from a beta-binomial model using parameters based on the control arm data from this case study and assuming an individual's infectivity remains constant over time, a constant waning of immune duration based on a 6 month half-life, and a fixed zero inflation level at the observed 84% or an optimistic test level of 75% (i.e. reflecting the percentage of control arm volunteers who never transmitted malaria to a mosquito during all DSF repeats). Then, we used a binomial generalized estimating equation

with a two-sided alpha of 0.05 for the vaccine indicator to estimate the numbers of volunteers, repeats, and mosquitoes that would be necessary to detect an 80% transmission-blocking activity by a VIMT candidate using oocyst prevalence values measured by DSF.

As shown in Table 4, the current case study with 38 and 41 participants per study arm and 6 feeds with 2 cups containing a total of 60 mosquitoes had an estimated 12% statistical power to detect a transmission-blocking activity of 80% in a binomial GEE model holding the zero inflation constant at the observed rate of 84%. Increasing the number of subjects per study arm up to 100 would increase the statistical power to 40%. Alternatively, doubling the number of feed repeats to 12 would similarly increase the statistical power to 38%, assuming 50 subjects per arm. In contrast, doubling the number of mosquitoes applied (i.e. while using 4 cups instead of 2) would have a minimal impact on statistical power. Increasing both the sample size to 100 subjects per arm and doubling the number of DSF repeats to 12 would raise the statistical power to 80%, a more acceptable threshold for clinical trials; however, further studies are needed to determine whether increasing the number of feeds per volunteer will increase study attrition. Table 4 also presents statistical power under the same model for a 75% zero inflation level (i.e. where 75% of the participants never transmit during all DSFs). With this assumption, increasing the number of subjects per study arm up to 100 would increase the statistical power to 95% and doubling the number of feed repeats to 12 in a study with 50 subjects per arm would increase the statistical power to 80%. These results underscore the idea that the capacity of the DSF to serve as an evaluative tool for candidate VIMTs is limited by the "effective sample size" (i.e. the number of subjects that are capable of transmitting at the time of feeding). Assuming similar rates of drop-out during immunizations and that the percentage of participants who transmitted during DSFs remained at 16% (i.e. a zero inflation of 84%), these simulations suggest we would need a total number 220 participants (i.e. with 110 subjects per arm) to achieve the effective sample size needed to measure a transmission-blocking activity of 80% using the oocyst prevalence measurements for the current case study scenario.

4. Future Research

While DSFs have great potential as tools to quantify the elicitation of transmission-blocking activity by candidate VIMTs at the individual level, additional research is needed to address the scalability and clinical relevance of these assays.

Questions of scale must first consider how one can adapt and extend successful practices to reach an expanding pool of people in varied geographies. The results of the systematic review suggest that DSFs offer flexibility in terms of the regionally specific *Anopheline-Plasmodium* pairings and acceptability across diverse cultural and demographic contexts. Extra consideration will be needed before administration of the DSF to special populations, including pregnant women and young children under five, to whom the assay is typically not administered. In addition, as the DSF is a rough facsimile of true transmission, further research is needed to test whether assay design features, such as the use of multigenerational, laboratory-reared mosquitoes, containment to feeding cups, could limit the relevance of the assay results to field conditions [28]. Moreover, the active follow-up of

participants could modify the natural progression of the disease and potentially alter the density of gametocytes available for transmission.

Scaling up DSFs for the evaluation of VIMTs may also be limited by the low throughput nature of the assay. The logistics of maintaining a mosquito colony and conducting feeding assays are considerable, and manually dissecting mosquito midguts for oocysts is a labor-intensive procedure. In the pilot investigation of the case study, DSFs were repeatedly administered to all participants regardless of gametocyte status over the course of the transmission season with the aim of capturing the infectivity of the entire study sample, including individuals with submicroscopic infections. While gametocyte-negative hosts did contribute substantively to the absolute numbers of DSF experiments with transmission, the relative proportion of DSFs experiments on gametocyte-negative volunteers versus those in gametocyte-positive volunteers that resulted in transmission was low. Therefore, there may be value in further investigation into whether greater efficiencies could be achieved through a two-stage assessment that incorporates information from both gametocyte screening and the DSF endpoints of gametocyte-positive individuals. Alternatively, the effective sample size could be increased by targeting early stage VIMT trials to regions with exceptionally high transmission or to subgroups of the population that act as the main infectious reservoirs [16, 29, 30]. Still in development, immunochemistry may enable the quantification of parasites from a given cup as a group and, potentially, at an earlier point in the gametocyte to oocyst transition [31], and nucleic acid-based methods for oocyst detection in pooled mosquito samples might similarly increase automation and throughput.

Although the case study DSF experiments generally performed well in terms of host attractiveness and feeding intensity, experimental design parameters could also be further optimized to increase the numbers of successful feeds and infections in order to improve the sensitivity of the DSF. For example, much remains unknown about the somatic distribution of the gametocytes within the human microvasculature, and the specific placement of the paper cups on the participant's body may alter results. It has also been previously established that the number of mosquitoes in a container is important for competition; further investigations are required to identify the optimal number of mosquitoes per cup for maximizing mosquito survivorship [5, 8]. Ultimately, standardization of the DSF and its associated analytical methods are needed to reduce variation and improve reproducibility for its use in support of regulatory submissions of emerging antimalarial tools.

From a clinical perspective, additional research is needed to validate the correlation between the individual DSF endpoints and a community-level effect, such as the incidence of malaria infection or clinical disease. In lieu of a large, cluster-randomized trial that simultaneously undertakes DSFs and clinical follow-up, smaller scale experiments using known transmission-blocking agents, such as primaquine, may provide partial validation of the DSF. In addition, mathematical models may facilitate estimation of the VIMT candidate's total effectiveness in a community, including both the direct effects on the vaccinated population as well as the herd effects on the remainder of the population, in terms of transmission. Commendably, Smith, et al., (2011) have developed a quantitative framework, based on the Ross-McDonald method, to explore the effects of malaria vaccines on the R_e [32]. In the model, vaccine efficacy is defined as "the probability that an infectious host

transmits when a mosquito feeds on it," which could as a proxy for "the reduction of transmission in the community" called for by the malERA target product profile [32].

5. Conclusions

Sustained progress toward malaria control, elimination, and eventual eradication will require both improved understanding of infectious reservoirs and efficient development of novel transmission-blocking interventions, such as vaccines that interrupt malaria transmission. Here we demonstrate that DSFs, which measure the infectiousness of individuals living in diverse settings using naturally occurring parasite strains under controlled conditions that simulate in natura transmission, have great potential utility for assessing emerging antimalarial tools, but require considerable logistics and hence will most likely be applied when evaluating the most promising new candidates or strategies for malaria elimination.

Acknowledgments

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213 records identified through database searching on 24 July 2015.
PubMed (N=64)
Web of Science (N=72)
EMBASE (N=77)

7 additional records identified through reference list screening

91 records after duplicates removed.

91 abstracts screened.

60 records excluded.

31 full-text articles assessed for eligibility.

20 full-text articles excluded.
2 full-text articles not available
3 non-DSF mosquito assay
1 non-*Plasmodium* parasite
10 non-human subjects
4 review articles

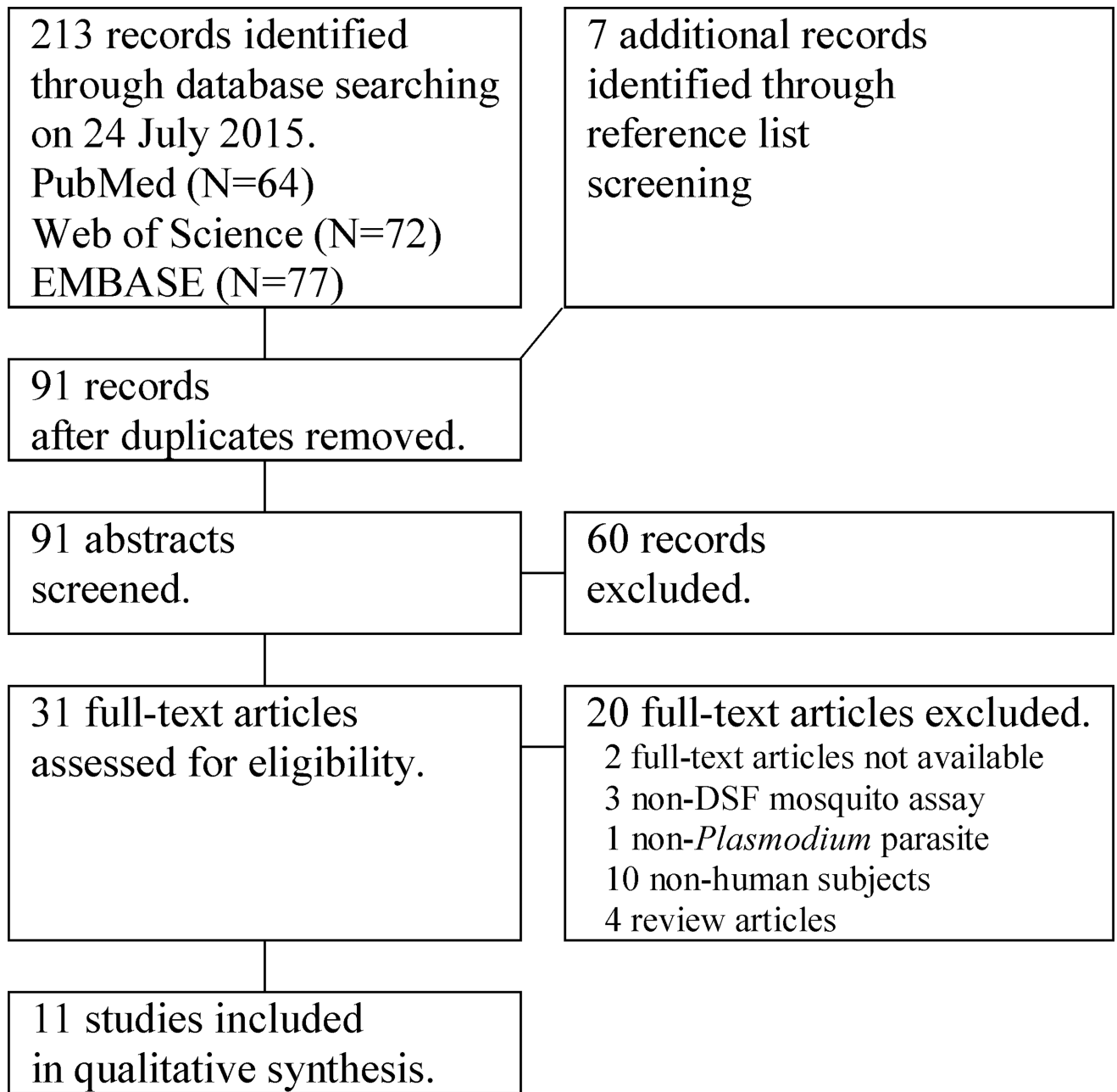11 studies included in qualitative synthesis.

Figure 1. Study flow diagram for the literature-based systematic review
The figure is based on the 2009 PRISMA flow diagram template (available from http://www.prisma-statement.org/statement.htm).
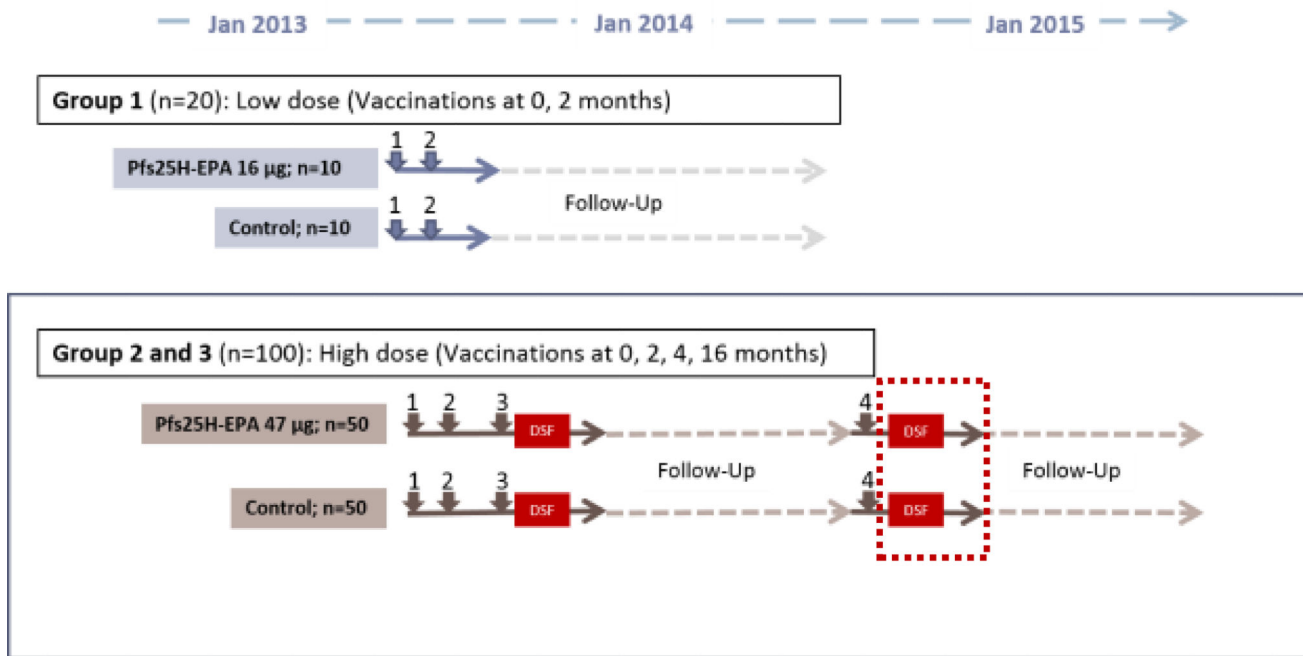
Figure 2. Simplified study schema for NIAID Protocol 13-I-N109 trial (clinicaltrials.gov NCT01867463), a Phase 1 study of the Pfs25H-EPA/Alhydrogel® malaria transmission blocking vaccine started in May 2013 at the Malaria Research and Training Center in Bancoumana, Mali

The dashed red rectangle indicates the 6 week period of follow-up contributing to the case study data. For the trial, 120 health adults were enrolled in a staggered, dose-escalating manner. Volunteers were individually randomized 1:1 to receive either the study vaccine or the comparator vaccine(s), Euvax B (LG Life Sciences, Seoul, Korea), a recombinant hepatitis B vaccine, and Menactra® (Sanofi Pasteur, Lyon, France), a conjugated, polysaccharide vaccine against *Neisseria meningitides* serogroups A, C, Y, and W-135 (Figure 2). Twenty subjects (Group 1) received a low dose (16 μg Pfs25H-EPA/ Alhydrogel®; N=10 or Euvax/Hepatitis B; N=10) and 100 subjects (Groups 2 and 3) received a high dose (47 μg Pfs25H-EPA/Alhydrogel®; n=50 or Euvax/ Hepatitis B followed by Menactra in year 2; n=50). Vaccinations in the low dose group were scheduled at 0 and 2 months with 12 months of safety follow-up. Vaccinations in the high dose group were scheduled at 0, 2, 4, and 16 months. The third vaccination was given in September 2013, and during the following six months, DSFs were completed if a subject was found to be parasite/ gametocyte positive in order to establish assay methodologies (these data are not included in the transmission-blocking activity estimates). Starting two weeks after the fourth vaccination in September 2014, DSFs were performed weekly for six weeks and at least once on all enrolled volunteers (n=79). As DSFs were repeatedly administered to all participants regardless of gametocyte status over the course of the transmission season, these assays were designed to capture the infectivity of the entire study sample, including individuals with submicroscopic infections.

Table 1

Search strategy for the literature-based systematic review.

Database	Search Terms
PubMed	(direct feed* [tiab] or direct skin feed* [tiab] or feeding assay [tiab] or feed assay [tiab]) & (Vaccine [MeSH] or Malaria Vaccines [MesH] or Malaria [MeSH] or Malaria, falciparum [MeSH] or malaria [tiab] or Culicidae [MeSH] or mosquito* [tiab] or oocysts [MesH] or oocyst* [tiab])
Web of Science	((("direct feed*" OR "direct skin feed*" OR "feed assay" OR "feeding assay") AND (vaccine* OR malaria OR falciparum OR Culicidae OR mosquito* OR oocyst*)))
EMBASE	'direct feed' OR 'direct feeding' OR 'direct skin feed' OR 'direct skin feeding' OR 'feed assay' OR 'feeding assay' AND ('vaccine'/exp OR 'vaccine' OR vaccine* OR 'malaria vaccine'/exp OR 'malaria vaccine' OR falciparum OR 'culicidae' OR 'culicidae'/exp OR culicidae OR mosquito* OR oocyst*) AND ([article]/lim OR [article in press]/lim OR [editorial]/lim OR [erratum]/lim OR [letter]/lim OR [note]/lim OR [review]/lim OR [short survey]/lim)

Table 2

Description of 11 studies using direct feeding assays to measure the transmission of malaria from human to mosquito

First Author (Ref)	Location	n	Ages, years	Plasmodium spp.[a]	Anopheline spp.[a]	Direct Feeding Assay Experimental Design						Transmission Endpoints		Gametocyte Carriers	
						No. of cups	Est. mosq. per cup	Mosq. age, days	Length of feed, mins	Location of feed	Repeats of feed	Oocyst Prevalence	Oocyst Intensity	All	Some/ Uncertain
Ranawaka [18]	Sri Lanka	31	--	*P. vivax*	*A. tessellatus*	1	20	3–4	15–20	forearm	0	■	■	■	□
Graves [11]	Papua New Guinea	212[b]	All ages	*P. falciparum, P. vivax, P. malariae, P. ovale*	*A. faruati*	--	20–30	--	--	--	0	■	■	■[b]	■[b]
Toure [17]	Mali	96	2+	*P. falciparum*	*A. gambiae s.l.*	3	30	--	10–15	--	0	■	■	■	□
Bonnet [16]	Cameroon	37	5+	*P. falciparum, P. malariae, P. ovale*	*A. gambiae s.l.*	--	30	3	15	inner thigh	0	■	■	■	■
Awono-Ambene [15]	Senegal	21	7–48	*P. falciparum*	*A. arabiensis*	2	35	3	15	calves	0	■	■	□	□
Sattabongkot [19]	Thailand	285	15–63	*P. vivax*	*A. dirus*	1	100	6–8	30	forearm	0	■	■	■	□
Bonnet [21]	Cameroon	685	0.8–77	*P. falciparum, P. malariae*	*A. gambiae s.l.*	1	30	3	15	inner thigh	0	■	■	□	■
Alves [20]	Brazil	15	adult	*P. falciparum, P. vivax*	*A. darlingi*	1	20	adult	15	forearm	0	■	■	□	■[c]
Diallo [10]	Mali	372	4–18	*P. falciparum, P. malariae, P. ovale*	*A. gambiae s.l.*	2	30	3–6	10–15	legs	0	■	□	■	□
Beavogui [23]	Mali	48	6–18	*P. falciparum*	*A. gambiae s.l.*	2	45	adult	15	legs	5d	■	■	■	□
Gaye [22]	Senegal	187	5+	*P. falciparum, P. malariae*	*A. arabiensis*	1	25	3	10–15	arm	0	■	□	□	■
Case Study	Mali	79	18–45	*P. falciparum*	*A. coluzzii*	2	30	3	15	calves	6	■	■	□	■

Abbreviations: mosq., mosquitoes; no., number; Ref, reference; spp., several species.

[a] Potential target species as reported in article; we note that *P. malariae* and *P. ovale* could be more prevalent than described.

[b] The cohort comprised two parts: a community survey (n=201) and an outpatient-derived group of gametocyte carriers (n=24). 13 individuals participated in both investigations.

[c] Cohort included both untreated, asymptomatic, PCR-positive and treated, symptomatic, highly parasitemic participants.

[d] In gametocytemic participants, 5 direct feeding experiments were performed on study days 0, 7, 14, 21, and 28 following treatment with chloroquine or sulfadoxine-pyrimethamine.

Table 3

Definitions of DSF experimental endpoints.

Objective	Metric	Measure Name	Measure Definition
Primary	Transmission-blocking activity	Oocyst prevalence	Proportion of engorged, dissected mosquitoes with 1 oocyst
Secondary	Transmission-reducing activity	Oocyst intensity	Number of oocysts per engorged, dissected mosquito
Tertiary	Infectious reservoir	Host infectiousness	Proportion of volunteers infecting 1 mosquito over duration of study
Exploratory	Mosquito behavior	Host attractiveness	Proportion of volunteers feeding 1 mosquito over duration of study
		Feed intensity	Proportion of engorged mosquitoes per experiment
		Mosquito survivorship	Proportion of engorged mosquitoes that survived to dissection

Table 4

Statistical power for estimating 80% transmission-blocking activity by a VIMT candidate

	Total n	No. of subjects per study arm	Repeats of feed	No. of cups per feed	Total no. of mosquitoes per feed	Estimated statistical power to detect 80% transmission-blocking activity	
						Assuming 84% zero inflation	Assuming 75% zero inflation
Case Study	79	38/41	6	2	60	12%	N/A
Increase sample size	100	50/50	6	2	60	19%	39%
	200	100/100	6	2	60	40%	95%
	400	200/200	6	2	60	96%	100%
Increase number of repeats	100	50/50	12	2	60	38%	80%
	200	100/100	12	2	60	80%	98%
	400	200/200	12	2	60	98%	100%
Increase number of mosquitoes per experiment	100	50/50	6	4	120	19%	39%
	200	100/100	6	4	120	40%	95%
	400	200/200	6	4	120	96%	100%
Increase number of repeats and number of mosquitoes per experiment	100	50/50	12	4	120	38%	81%
	200	100/100	12	4	120	81%	98%
	400	200/200	12	4	120	98%	100%

The models assume either the observed 84% zero inflation level or a hypothetical and optimistic 75% zero inflation level, where zero inflation level indicates the percentage of volunteers who never transmitted over the duration of follow-up.