Major Article

Use of PfHRP2-only RDTs rapidly select for PfHRP2-negative parasites with serious implications for malaria case management and control

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Running title: HRP2 RDTs select HRP2-negative parasites

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Summary

Most rapid diagnostic tests (RDTs) for falciparum malaria detect histidine-rich protein 2 (PfHRP2). This modelling study demonstrates that newly introduced PfHRP2-negative parasites can be readily transmitted, causing increases in prevalence and transmission, when PfHRP2-only RDTs are used for diagnosis.

Footnotes

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Abstract

Background

Rapid diagnostic tests (RDTs) are an important tool for malaria diagnosis, with most utilising antibodies against histidine-rich protein 2 (PfHRP2). Reports of Plasmodium falciparum lacking this protein are increasing, creating a problem for diagnosis of falciparum malaria in locations without quality microscopy.

Methods

An agent-based stochastic simulation model of P.falciparum transmission was used to investigate the selective pressure exerted on parasite populations by use of RDTs for diagnosis of symptomatic cases. The model considered parasites with normal, reduced or no PfHRP2, and diagnosis using PfHRP2-only or combination RDTs.

Results

Use of PfHRP2-only RDTs in communities where a PfHRP2-negative parasite was introduced during the simulation resulted in transmission of the parasite in over 80% of cases, compared to less than 30% for normal or PfHRP2-reduced parasites. Using PfHRP2-only RDTs in the presence of PfHRP2-negative parasites caused an increase in prevalence, reduced RDT positivity within symptomatic patients, but no change in number of antimalarial treatments due to false negative RDT results. Diagnosis with PfHRP2/Pf-pLDH combination RDTs didn’t select for PfHRP2-negative parasites.
Conclusions

Use of PfHRP2-only RDTs is sufficient to select \textit{P. falciparum} parasites lacking this protein, thus posing a significant public health problem which could be moderated by using PfHRP2/Pf-pLDH combination RDTs.

**Keywords**: malaria, rapid diagnostic test, HRP2, mathematical model, falciparum

Introduction

Accurate diagnosis of malaria, a disease infecting over 200 million people annually, is fundamental for its control [1]. As malaria incidence falls due to ongoing investment toward elimination, correct diagnosis and case management of non-malaria fever cases will become even more important [2]. Community-based early diagnosis and treatment services have been deployed in numerous locations, with free access to reliable rapid diagnostic tests (RDTs) and effective treatment for all clinical malaria cases in a region being key to their success [3].

The World Health Organisation (WHO) recommends parasitological confirmation prior to treatment for malaria with quality-assured microscopy or RDTs [4]. Since this recommendation was published RDT sales have increased more than six-fold, with an associated reduction in prescribing or purchasing of antimalarial drugs [1, 5]. A meta-analysis of data from community surveys showed RDTs to have detection capability comparable with, and often better than, routine microscopy [6]. RDTs with increased sensitivity are also being developed for elimination efforts where focal mass screening and treatment are being considered.

The majority of commercially available RDTs that diagnose \textit{Plasmodium falciparum} target histidine-rich protein 2 (PfHRP2) [7]. Performance testing of RDTs revealed PfHRP2 to be a more sensitive antigen for detecting \textit{P. falciparum} than other antigens such as plasmodium lactate dehydrogenase
P. falciparum also produce histidine-rich protein 3 (PfHRP3), an antigen with a high degree of similarity to PfHRP2. Neither PfHRP2 nor PfHRP3 are essential for P. falciparum survival or transmission, with parasites lacking one or both of the antigen-coding genes detected in field isolates. PfHRP2/3-negative parasites were first reported in Peru [9], and have now been confirmed to exist in multiple locations within South America, and India, at high (>20%) prevalence [10, 11]. Parasites lacking either or both PfHRP2/3 have also been reported in Mali, Senegal and Ghana [10].

The presence of PfHRP2-negative parasites presents a significant problem for the diagnosis of malaria by RDT, especially for Africa where P. falciparum is predominant and PfHRP2-detecting Pf-only RDTs are used. PfHRP2/3-negative parasites can emerge by local parasites undergoing a deletion event, or they can be introduced. Both scenarios have occurred in South America [12-14]. Although the force of selection is unclear, parasites lacking PfHRP2/3 must have advantage under selection to become prevalent and a public health problem, as observed in Peru [9, 15].

This study investigated whether RDT usage alone is sufficient to select parasites lacking PfHRP2 in communities using a mathematical model. The model tracks the life course of parasites to determine the likelihood of establishment in the community, and monitors subsequent effects on malaria morbidity and transmission within the community.

Methods

An agent-based simulation model encompassing both the within-host dynamics and transmission of P. falciparum was used to investigate the spread of an introduced parasite into a hypothetical village of 400 people (Figure 1). The model is based on the previously reported work of Gatton and colleagues [16], with modification to how diagnosis and treatment of symptomatic infections occurs. Full model details are contained in the Supplementary Materials.
Treatment seeking and diagnosis

It is assumed that either 50% or 80% of individuals who develop a fever due to malaria seek treatment. The time to treatment for each individual is drawn from an empirical distribution parameterised such that 75% of individuals seeking treatment do so within 3 days of onset of fever (Supplemental Materials) [17, 18]. Diagnosis by RDT is performed on the treatment day. Two RDT types are considered; an RDT that only detects PfHRP2 (RDT1) and a combination RDT which targets both PfHRP2 and Pf specific-pLDH (RDT2).

Within the model the PfHRP2 and Pf-pLDH antigen levels in each host are tracked daily. Antigen levels are expressed as ‘equivalent parasites’ with antigen decay considered to be a first-order process with half-lives of 3.67 days and 1.84 days for PfHRP2 and pLDH, respectively [19, 20]. The probability of an RDT being positive is related to the simulated antigen level in the host, with the probability being informed by the patterns observed for RDTs meeting the WHO procurement criteria [7] (Supplementary table 1).

The RDT is the sole diagnostic included in the model with only RDT-positive cases receiving antimalarial treatment. This reflects the situation in many remote and hard-to-reach communities where community health workers are engaged to diagnose and treat malaria cases.

Drug dynamics following treatment

Two artemisinin-combination therapies (ACTs), artesunate-mefloquine and artemether-lumefantrine (Coartem), were simulated to capture partner drugs with long and short half-lives. Partner drug pharmacokinetics were adopted from Simpson et al for mefloquine (MQ) and Stepniewska et al for lumefantrine (LF) [21, 22]. Throughout the manuscript the ACTs are identified by the partner drug.
Parasite survival following treatment was modelled assuming an additive effect between artemisinin and partner drug, and that asexual and early stage gametocytes were equally susceptible to the ACTs (Supplementary Methods). Assuming the net parasite replication rate is approximately 12 parasites per 48 hours, the prophylactic protection against new blood stage infections provided by these ACTs following treatment is 35 and 17 days for MQ and LF, respectively.

Simulation process and modelled scenarios

The model was coded in Fortran, compiled using the Intel® Fortran Compiler and executed using the QUT high performance computing platform which includes a Silicon Graphics International Corp. (SGI) Altix XE Computational Cluster. For each modelled scenario a baseline simulation (Phase 1) was conducted to establish transmission within the community and allow acquired immunity (clone specific antibody-mediated) to develop as individuals are repeatedly exposed to *P. falciparum* infection. Summary parameters including the EIR, number of treatments, and prevalence were monitored over the final 2,000 days of the Phase 1 simulation to characterise the baseline transmission level. Ten independent simulations were then conducted for a further 700 days, using the end point of the Phase 1 simulation as the starting point, to ensure the baseline conditions were maintained. In all cases there was no significant change in the trajectory of the summary parameters over this period, indicating the transmission within the village was at equilibrium.

Phase 2 modelling started at the end of the Phase 1 simulations for a parallel series of scenarios (Supplementary figure 2). On Day 50 of each Phase 2 simulation the key parasite was introduced in a randomly selected host. It is assumed that this person was infected by the bite of an infectious mosquito outside of the village, returning with a liver-stage infection. The characteristics of this initial host such as age, multiplicity of infection (MOI) and whether (and when) treatment was sought were stored for later analysis. Three types of key parasites were investigated:
• ‘normal’ parasites that fully express PfHRP2,

• ‘HRP2-negative’ parasites which express no PfHRP2, and

• ‘reduced HRP2’ parasites which produce PfHRP2 at a level equivalent to 25% of the normal parasite.

In total, four different parasite/RDT scenarios were simulated from the same starting conditions (Supplementary figure 2), with 50 simulations of each scenario being conducted. Each simulation stopped when the key parasite, introduced at Day 50, became extinct or the end of the simulation time was reached. The simulation time was 700 days as this was sufficient to investigate whether the parasite spread within the community.

The above simulation process was applied to 16 combinations of transmission settings and mosquito feeding/survival characteristics, resulting in a total of 3,200 simulations (50 simulations x 4 parasite/RDT scenarios x 4 transmission settings x 4 mosquito feeding/survival characteristics) for each treatment regime. Impact of malaria control interventions such as long-lasting impregnated bed nets or indoor residual spraying were captured in the model, along with inherent mosquito behaviours, by two mosquito parameters; the proportion of mosquitoes feeding on humans \( (H) \) and the probability of mosquitoes surviving the feeding cycle \( (F) \). To reflect different vector characteristics and levels of control two values for \( H \) (0.5 and 0.75) and \( F \) (0.5 and 0.65) were simulated producing four mosquito feeding/survival combinations.

Four transmission settings, classified using results from the end of Phase 1, were:

1. Epidemic malaria. The village is malaria-free except where parasite introductions occur, sometimes leading to outbreaks that persist for several hundred days, but eventually die-out under the simulation conditions.

2. Low endemic. Continuous transmission occurs at low level with individuals having approximately one infection per year.
3. Moderate-low endemic. Continuous transmission occurs with individuals having approximately 2-3 infections per year.

4. Moderate endemic. Continuous transmission occurs with individuals having approximately 4-5 infections per year.

The entire simulation procedure was replicated for the three different treatment regimens; artesunate-MQ with 50% treatment seeking rate (MQ(50%)), artemether-LF with 50% treatment seeking rate (LF(50%)), and artesunate-MQ with 80% treatment seeking rate (MQ(80%)).

Statistical analysis

Data processing and statistical analysis was conducted using SPSS Version 23 (IBM Corporation, Release 23.0.0.0) and MATLAB (The MathWorks Inc., version R2015b). Summary variables calculated from simulation output included: total number of infections caused by the key parasite, time to extinction for the key parasite, prevalence of asexual infection in community and proportion of infections attributed to the key parasite, entomological inoculation rate (EIR), number of symptomatic individuals who sought treatment and were tested using an RDT, number of positive RDT tests and number of treatments administered within the community. RDT positivity was calculated as (number of positive RDTs)/(number of symptomatic individuals seeking treatment)×100.

Success of the introduction of the key parasites was measured by i) the proportion of simulations where the key parasite was transmitted to at least one new host, and ii) the parasite extinction time. The first of these variables was used as an indicator of the potential for successful introduction, recognising that stochastic die-out occurs in a proportion of simulations where the key parasite spreads beyond the initial host. For this reason the analysis also considered the parasite extinction time. Kaplan-Meier survival analysis using the Log Rank test was used to compare the distribution of extinction times, while Cox Proportional Hazards models were developed to investigate factors
associated with extinction time. Binary logistic regression was used to identify factors associated with the spread of the introduced parasite beyond the initial host.

**Results**

*Parasite transmission*

A randomly selected host introduces the key parasite into the population (Supplementary table 3). Key parasites were only transmitted to new hosts in 26.5%, 25.3% and 25.9% of simulations for normal key parasites, HRP2-reduced key parasites in a community using RDT1, and HRP2-negative key parasites in a community using RDT2, respectively. In contrast, transmission to at least one new host occurred in 79.7% of simulations when RDT1 was used and the key parasite was HRP2-negative (Supplementary figure 3).

Binary logistic regression analysis of results for the normal, reduced and HRP2-negative (RDT2) key parasite introductions revealed that receiving treatment and MOI of the initial host were significant factors for predicting transmission of the key parasite to at least one new host (p<0.001), while treatment regime and transmission level were not (p>0.1). Host age was also a significant factor for simulations with $F=0.5$ (p=0.001, OR 1.010, 95% CI 1.004 – 1.016). Compared to receiving treatment within 1-3 days of first fever, the odds of transmission in simulations with $F=0.5$ increased 10.79 fold (95% CI: 6.32 – 18.41) and 129.90 fold (95% CI: 81.43 – 207.23) if treatment was received 4-6 days post-fever, or no treatment was received, respectively. These odds ratios were 6.21 (95% CI 3.74 – 10.31) and 60.87 (95% CI 40.13 – 92.32) for simulations with $F=0.65$. The odds of transmission were significantly lower when the initial host had MOI>1 compared to MOI=1; OR=0.519 (95% CI 0.425 – 0.634) for simulations with $F=0.5$; OR=0.619 (95% CI 0.512 – 0.748) for simulations with $F=0.65$. 
Treatment was a significant factor for the transmission of HRP2-negative key parasites for the scenario where RDT1 was the diagnostic tool (p<0.001); odds ratios were greater than 150 when no treatment was received compared to receiving treatment for all simulation sets. It should be noted that few treatments were received in these scenarios, typically occurring when the host had MOI>1. MOI was a significant factor in simulations where 50% of symptomatic hosts seek treatment (p<0.001); odds ratio for MOI>1 were 0.544 (95% CI 0.342 – 0.865) when F=0.5 and 0.650 (95% CI 0.452 – 0.935) when F=0.65. When the treatment regime involved 80% of symptomatic hosts seeking treatment, MOI was not a significant factor (p>0.1), being replaced by transmission level: epidemic and low endemic settings had 0.211 (95% CI 0.125 – 0.392) the odds of transmitting the HRP2-negative parasite to at least one new host, compared to the moderate-low and moderate settings (p<0.001). The predicted proportion of key parasites transmitted to at least one new host are provided in Supplementary table 4.

Cox regression indicated that the hazard of parasite extinction was not significantly influenced by the proportion of mosquitoes feeding on humans (H) (p>0.09), or the partner drug (p=0.792). However a variety of other factors were found to influence the hazard of extinction (Table 1). The largest differences in the parasite survival time in the community were seen for the HRP2-negative parasites when RDT1 was used as the sole diagnostic; the median survival time was >650 days compared to 90 days for the other parasite/RDT combinations (Figure 2).

**Impact of introduction of HRP2-negative parasite on parasite population**

Linear mixed effects modelling using an autoregressive covariance structure indicated that time after introduction, transmission level and treatment regimen all influenced the percentage of asexual infections in the population caused by the HRP2-negative parasite (p<0.001) (Figure 3). In all transmission settings the proportion of infections attributed to the HRP2-negative parasite increased...
over time. Some reversal of this trend was observed in simulations of the 80% treatment regime in the moderate-low and moderate transmission settings after approximately 400 days. This is likely due to the complex relationship between acquired clinical immunity and multi-clone infections in hosts in these simulation scenarios.

Clinical impact of introduction of HRP-negative parasite

Figure 4 illustrates a pattern of reducing RDT positivity over time as the HRP2-negative parasite spreads through communities with a treatment seeking rate of 50%. When the treatment seeking rate is 80% the RDT positivity rate decreases rapidly during the first 300 days following the introduction, after which the RDT positivity increases for the moderate-low and moderate transmission settings (Figure 4), reflecting a reduction of HRP2-negative parasites causing symptomatic episodes as acquired immunity develops. In contrast, the number of treatments administered after the introduction of the HRP2-negative parasite remained relatively constant over time, for all transmission settings (Figure 5). The relative stability in the number of treatments appears to be coincidental as the increase in transmission caused by lack of treatment is offset by the falling RDT positivity rate.

Impact of introduction of HRP2-negative parasite on transmission and infection

Two indicators were used to assess transmission within the community after the introduction of the HRP2-negative parasite; 1) EIR and 2) prevalence of infection with >100 parasites/µL. The patterns observed in both indicators were similar; prevalence and EIR increased approximately 300 days after the introduction of the HRP2-negative parasite in communities where the treatment seeking rates is 50%, and slightly earlier for the MQ(80%) scenario (Figure 6, supplementary figure 4). In line with patterns for other indicators, a reversal in this trend occurred after 400 days in MQ(80%) simulations in the moderate-low and moderate transmission scenarios.
Discussion

Parasites lacking PfHRP2 have been detected in several regions of the world, most recently Africa and India [11, 23]. These parasites present a threat to the correct diagnosis of malaria using PfHRP2-based RDTs, potentially resulting in a false negative parasitological diagnosis which would deprive the patient of prompt treatment [4].

The results of this modelling study are the first to demonstrate that a newly introduced parasite that produces no PfHRP2 can rapidly spread through a community if a PfHRP2-only RDT is used as the sole diagnostic tool to inform treatment. However, this is not the case when parasites produce a reduced quantity of PfHRP2, equivalent to 25% of normal, or when a combination PfHRP2/Pf-pLDH RDT is used (Figure 2). The reduced level of PfHRP2 production by parasites was sufficient in the model to return positive PfHRP2-detecting RDT results in most symptomatic individuals due to the low limit of detection of the test, typically high parasitemia and longevity of circulating PfHRP2.

There is uncertainty about whether PfHRP2-detecting RDTs cross-react with PfHRP3. Evidence suggests that some monoclonal antibodies raised against PfHRP2 cross-react with PfHRP3 [24], but only very limited evidence suggesting that PfHRP2-negative/PfHRP3-positive parasites are positive by certain RDTs [9]. However, such parasites are rarely observed, with parasites more likely to lack either pfhrp3 or both pfhrp2 and pfhrp3 [25, 26]. Additional studies are required to investigate whether different brands of PfHRP2-detecting RDTs react with PfHRP2-negative/PfHRP3-positive parasites. If cross-reactivity does occur then the modelling results for parasites with reduced levels of PfHRP2 suggest that PfHRP2-negative/PfHRP3-positive parasites will not be selected for by the use of PfHRP2-detecting RDTs.

The findings from this study indicate that the introduction of PfHRP2-negative parasites will potentially have a severe impact with increased parasite prevalence, morbidity and transmission, and although
not explicit in the model, mortality. These changes may occur despite continuation of other control activities such as use of long-lasting impregnated nets and IRS, and are not unexpected considering PfHRP2-negative parasites evade a key control measure, namely treatment of symptomatic patients. The model results showed that the initial host being infected by more than one parasite was a significant factor in predicting whether the PfHRP2-negative parasite was transmitted, with the proportion of individuals having multi-clone infections increasing with transmission intensity. We hypothesise that these multi-clone infections explain the result where emergence of PfHRP2-negative parasites was slower with increasing transmission intensity. This is because patients co-infected with both PfHRP2-negative and PfHRP2-positive parasites have an increased chance of returning a positive PfHRP2-detecting RDT result due to the antigen produced by the PfHRP2-positive parasite, thus resulting in treatment and removal of both PfHRP2-negative and PfHRP2-positive parasites. Therefore lower transmission settings create the ideal situation for the emergence of drug sensitive PfHRP2-negative parasites. This hypothesis does not hold if the PfHRP2-negative parasite is drug resistant, and further work is required to better understand the dynamics in this situation.

The model showed that in simulations with higher transmission levels where 80% of symptomatic hosts seek treatment the trend for decreased RDT positivity and increased transmission was reversed after approximately 400 days. We hypothesise that this reversal is due to the complex interaction between clinical immunity acquired after repeated exposure to the PfHRP2-negative parasite and hosts having multi-clone infections. When the PfHRP2-negative parasite is introduced it is antigenically distinct, causing symptomatic infections that produce false negative results on PfHRP2-detecting RDTs which go untreated and spread, leading to increased transmission and repeated exposure for individuals within the community. Subsequently, individuals develop immunity specific for the PfHRP2-negative parasite which suppress the parasite density leading to fewer clinical symptoms, and treatment seeking. Simultaneously, other PfHRP2-positive parasites circulate in the population; therefore as clinical immunity against the PfHRP2-negative parasite develops, the bulk of symptomatic episodes return to being caused by PfHRP2-positive parasites, as either monoclonal or multi-clonal
infections containing the PfHRP2-negative parasite, with multi-clone infections dominating. This leads to an increase in the RDT positivity rate, resulting in clearance of parasites from the host and a reduction in the reservoir and subsequent transmission of PfHRP2-negative parasites.

One of the challenges currently facing public health practitioners is how to best determine whether HRP2-negative parasites are present in an area. It is important not to jeopardise the effective treatment of patients, whilst also retaining the most widely available, accessible and sensitive diagnostic tests. The modelling results illustrate that, on a backdrop of decreasing malaria transmission, it may be difficult to detect the early stages of emergence of these parasites. This calls for vigilant reporting and investigation by National Malaria Control Programme (NMCP) staff of reported suspect malaria infections which repeatedly return negative results using PfHRP2-detecting RDTs against quality microscopy or pLDH-based RDTs.

As with all modelling studies, the results are sensitive to the assumptions made and these are clearly outlined in the Supplementary Materials. The model is specific to P. falciparum and does not consider the situation where P. vivax may also be present. The model output would normally be validated using field data, however presently no sufficiently detailed data exists on the emergence of PfHRP2-negative parasites to perform this validation. However, the patterns observed in the results appear consistent and reasonable based on known epidemiological relationships between malaria disease and transmission.

This study does not consider the impact of seasonality. Further studies are needed; however it is expected that the timing of the introduction of the pfhrp2-negative parasite relative to the malaria season would significantly impact the likelihood of the parasite becoming established in the community, as would the length of the transmission season, and the transmission level during the low season.
The results of this study demonstrate that there is a high potential for pfhrp2-negative parasites to spread through a community when detection of *P. falciparum* malaria is dependent solely on PfHRP2. The potential impact in terms of increased transmission and untreated malaria infections highlights the potential public health threat that these parasites present, supporting recent efforts by WHO to engage with countries to increase surveillance for these parasites.

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**Conflicts of Interests**

The authors have no conflicts of interests.
References


Figure 1: Conceptual model of the stochastic simulation model illustrating how mosquitoes and humans interact and the flow between different infection states.

Figure 2: Kaplan-Meier survival curves for parasite survival time for four transmission settings; epidemic (top left), low endemic (top right), moderate-low endemic (bottom left) and moderate (bottom right). RDT1 is a PfHRP2-detecting Pf-only RDT.

Figure 3. Simulation trajectories for the proportion of all asexual infections caused by the HRP2-negative parasite in a village where a PfHRP2-detecting Pf-only RDT is used as the diagnostic test. The trajectory of the baseline simulation is displayed for the 200 days prior to the introduction of the HRP2-negative parasite, after which the results of each simulation are displayed. Trajectories are coloured according to the values of the $H$ and $F$ parameters: black: $H=0.5$, $F=0.5$; blue: $H=0.5$, $F=0.65$, green: $H=0.75$, $F=0.5$; red: $H=0.75$, $F=0.65$. MQ(50%): 50% of symptomatic people seek treatment and treatment is artesunate-mefloquine; LF(50%): 50% of symptomatic people seek treatment and treatment is artemether-lumefantrine.

Figure 4. Simulation trajectories for the RDT positivity in the previous 50 days in a village where a PfHRP2-detecting Pf-only RDT is used as the diagnostic test and a HRP2-negative parasite was introduced in a randomly selected host. The trajectory of the baseline simulation is displayed for the 200 days prior to the introduction of the HRP2-negative parasite, after which the results of each simulation are displayed. Trajectories are coloured according to the values of the $H$ and $F$ parameters: black: $H=0.5$, $F=0.5$; blue: $H=0.5$, $F=0.65$, green: $H=0.75$, $F=0.5$; red: $H=0.75$, $F=0.65$. MQ(50%): 50% of symptomatic people seek treatment and treatment is artesunate-mefloquine; LF(50%): 50% of symptomatic people seek treatment and treatment is artemether-lumefantrine.
Figure 5. Simulation trajectories for the number of treatments in the previous 50 days in a village where a PfHRP2-detecting Pf-only RDT is used as the diagnostic test and a HRP2-negative parasite was introduced in a randomly selected host. Number of treatments is displayed on a log\textsubscript{10} scale. The trajectory of the baseline simulation is displayed for the 200 days prior to the introduction of the HRP2-negative parasite, after which the results of each simulation are displayed. Trajectories are coloured according to the values of the $H$ and $F$ parameters: black: $H=0.5$, $F=0.5$; blue: $H=0.5$, $F=0.65$, green: $H=0.75$, $F=0.5$; red: $H=0.75$, $F=0.65$. MQ(50%): 50% of symptomatic people seek treatment and treatment is artesunate-mefloquine; LF(50%): 50% of symptomatic people seek treatment and treatment is artemether-lumefantrine.

Figure 6. Simulation trajectories for the prevalence of infection (>100 parasites/µL) in a village where a PfHRP2-detecting Pf-only RDT is used as the diagnostic test and a HRP2-negative parasite was introduced in a randomly selected host. Data for prevalence of infection with >100 parasites/µL represented a census of the community taken every 50 days. The trajectory of the baseline simulation is displayed for the 200 days prior to the introduction of the HRP2-negative parasite, after which the results of each simulation are displayed. Trajectories are coloured according to the values of the $H$ and $F$ parameters: black: $H=0.5$, $F=0.5$; blue: $H=0.5$, $F=0.65$, green: $H=0.75$, $F=0.5$; red: $H=0.75$, $F=0.65$. MQ(50%): 50% of symptomatic people seek treatment and treatment is artesunate-mefloquine; LF(50%): 50% of symptomatic people seek treatment and treatment is artemether-lumefantrine.
Table 1: Results of multivariate Cox proportional hazards models for 50% and 80% treatment seeking rates

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<td>80% treatment</td>
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<td>0.905 (0.857 – 0.957)</td>
<td>&lt;0.001</td>
<td>0.913 (0.844 – 0.987)</td>
<td>0.021</td>
</tr>
</tbody>
</table>
Figure 1
Figure 3

Partner Drug (Treatment rate)

MQ(50%)  LF(50%)  MQ(80%)

% of asexual infections

0  20  40  60  80  100

Days since parasite introduced

Days since parasite introduced

Days since parasite introduced

H=0.50, F=0.50  H=0.50, F=0.65  H=0.75, F=0.50  H=0.75, F=0.65
Figure 4

Partner Drug (Treatment rate)

MQ(50%)  LF(50%)  MQ(80%)

RDT positivity

Epidemic  Endemic

Low  Moderate - low  Moderate

Transmissions

Days since parasite introduced

H=0.50, F=0.50  H=0.50, F=0.65  H=0.75, F=0.50  H=0.75, F=0.65
Figure 6

Partner Drug (Treatment rate)

MQ(50%)  LF(50%)  MQ(80%)

Prevalence of infection

Days since parasite introduced

H=0.50, F=0.50  H=0.50, F=0.65  H=0.75, F=0.50  H=0.75, F=0.65