



Figures and figure supplements

Impact of seasonal variations in *Plasmodium falciparum* malaria transmission on the surveillance of *pfhrp2* gene deletions

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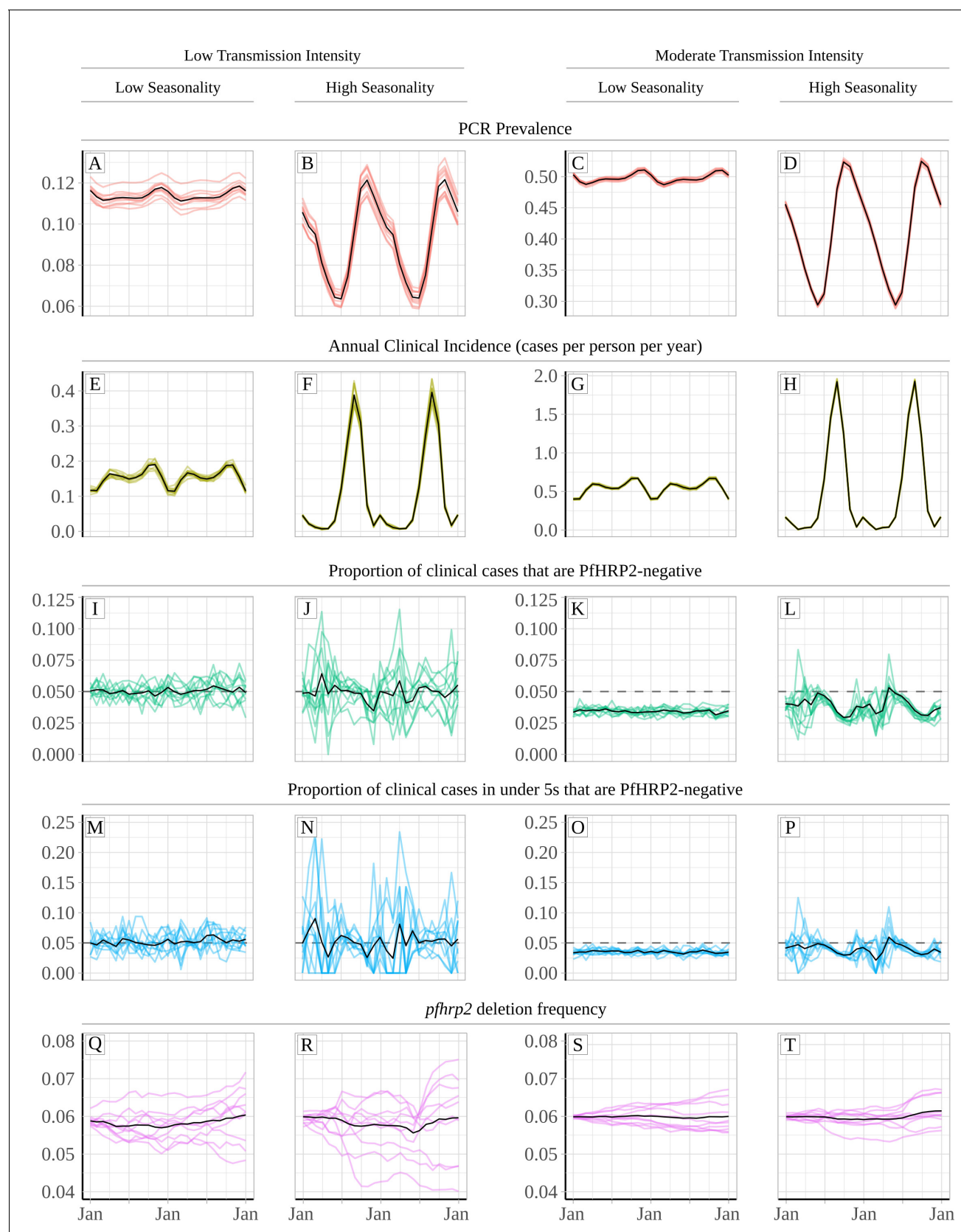


Figure 1. Relationship between seasonality, transmission intensity and proportion of clinical cases that are infected with only *pfhrp2*-deleted parasites. Graphs show in (A – D) and (E – H) the model predicted PCR prevalence and annual clinical incidence respectively at both a low and a moderate transmission intensity. Figure 1 continued on next page

Figure 1 continued

transmission intensity. In (I – L) and (M – P) the proportion of clinical cases only infected with *pfhrp2*-negative parasites is shown for both the whole population and in children under 5 years old, respectively. Lastly, graphs (Q – T) show the population allele frequency of *pfhrp2* gene deletions, which was set equal to 6% at the beginning of each simulation. 10 simulation realisations are shown in each graph, with the mean shown with by the black line. Lastly, the 5% threshold for switching RDT provided by the WHO is shown with the dashed horizontal line in plots (I – P).

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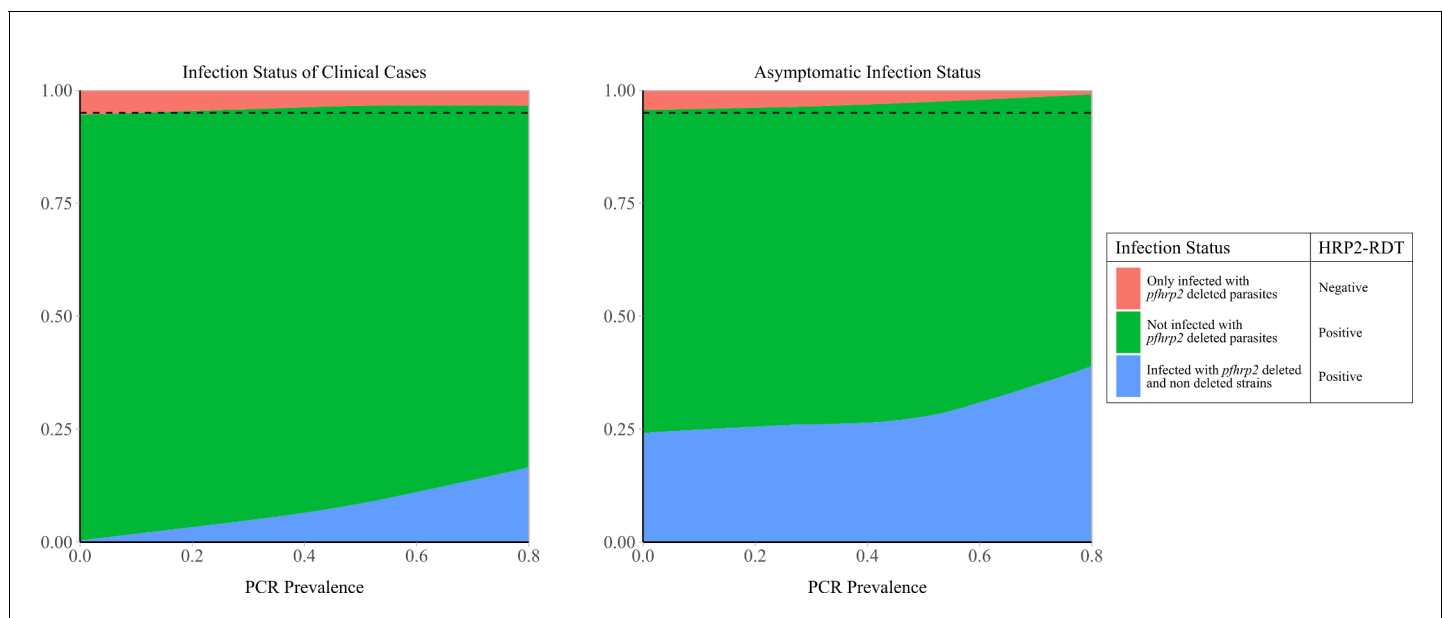


Figure 1—figure supplement 1. Model predicted relationship between clonality of infection in asymptomatic and clinical cases against prevalence of malaria. The graphs show the proportion of (A) clinical cases of malaria and (B) asymptomatic individuals that are infected with only *pfhrp2*-deleted parasites, not infected with *pfhrp2*-deleted parasites or polyclonally infected (infected with both *pfhrp2*-deleted parasites and wild-type parasites). These proportions are shown with respect to increasing prevalence of malaria, identifying the increased proportion of individuals that are polyclonally infected at high transmission intensities. For both plots, the population frequency of *pfhrp2* gene deletions in the population is 5%, which is shown by the dashed black line. Consequently, at very low PCR prevalence, when the majority of infections contain only one infecting strain and the proportion of polyclonal infections tends to zero, the observed prevalence of the deletions will tend to the population frequency of 5%.

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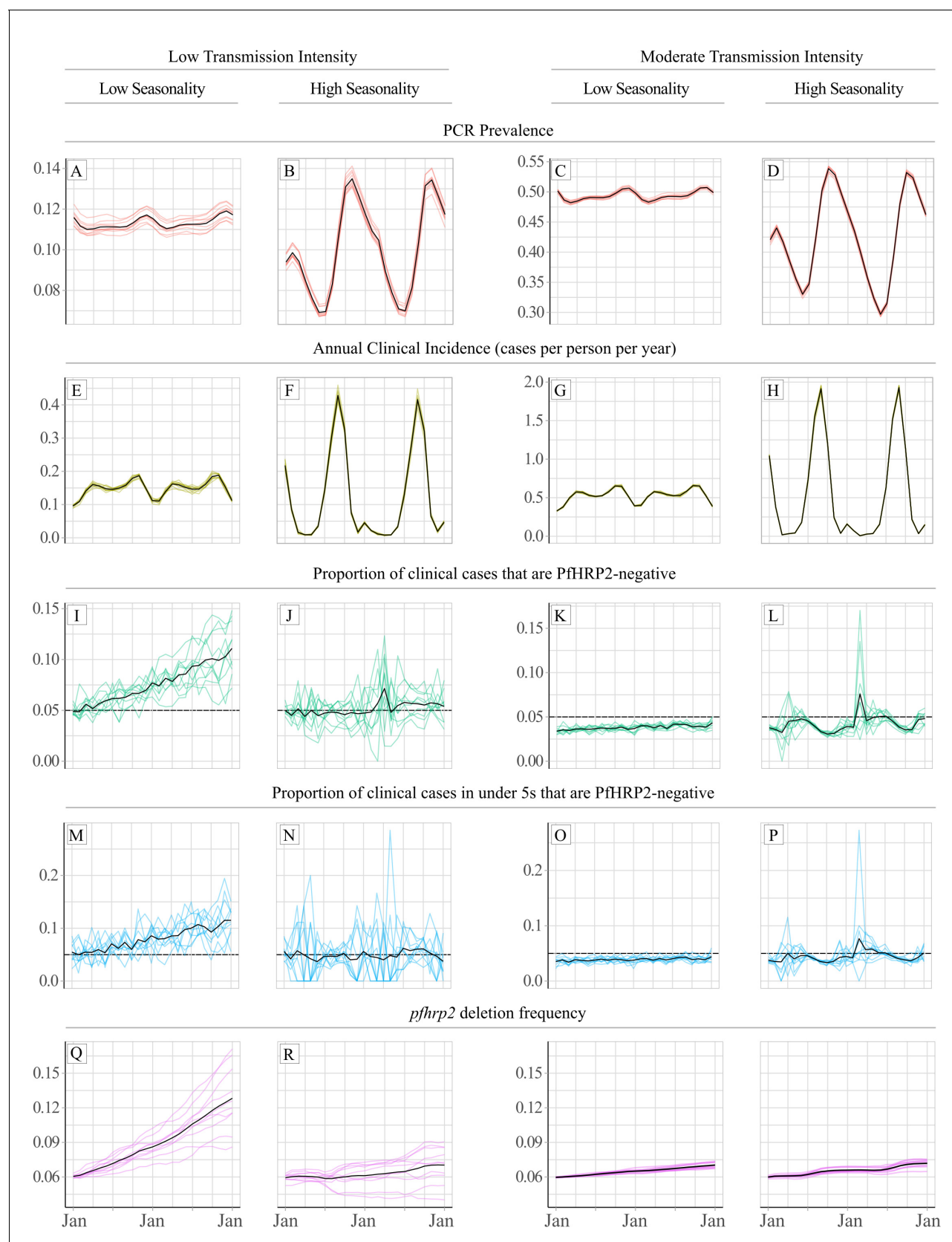


Figure 1—figure supplement 2. Impact of a selective advantage for *pfhrp2*-deleted parasites on the relationship between seasonality, transmission intensity and proportion of clinical cases that are infected with only *pfhrp2*-deleted parasites. Graphs show in A – D and E – H, the model predicted

Figure 1—figure supplement 2 continued on next page

Figure 1—figure supplement 2 continued

PCR prevalence and annual clinical incidence respectively at both a low and a moderate transmission intensity. In I – L and M – P the proportion of clinical cases only infected with *pfhrp2*-negative parasites is shown for both the whole population and in children under 5 years old, respectively. Lastly, graphs Q – T show the population allele frequency of *pfhrp2* gene deletions, which was set equal to 6% at the beginning of each simulation before increasing due to selection. 10 simulation realisations are shown in each graph, with the mean shown with by the black line. Lastly, the 5% threshold for switching RDT provided by the WHO is shown with the dashed horizontal line in plots I – P.

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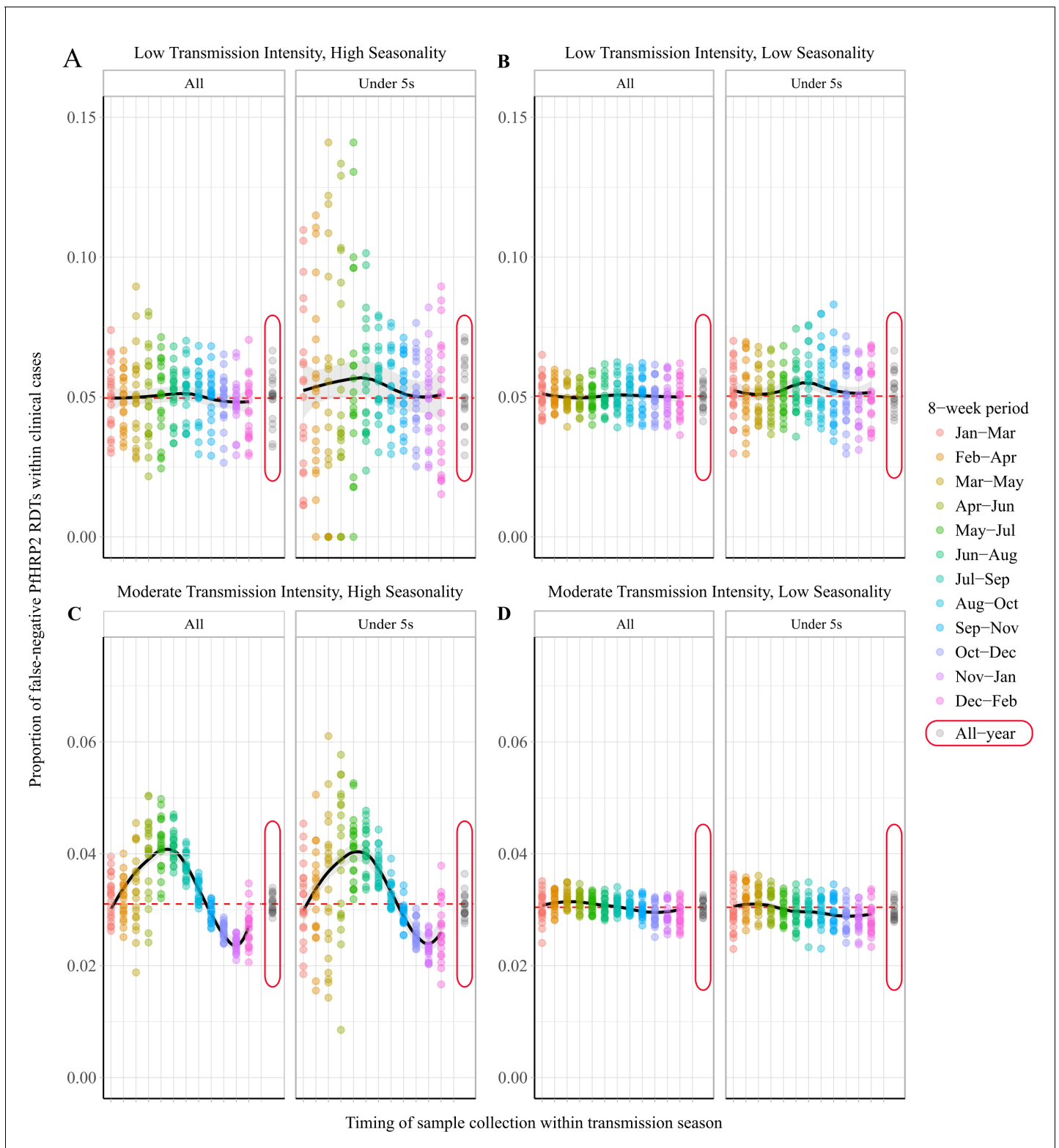


Figure 2. Observed proportion of false-negative PfHRP2 RDTs within clinical cases during 8-week intervals. Graphs show the proportion of clinical cases yielding false-negative PfHRP2 RDTs at 8-week intervals within a transmission season for both moderate (C, D) and low (A, B) transmission settings and high (A, C) and low (B, D) seasonality. In each panel, the observed proportion *pfhrp2*-negative clinical cases is shown for the whole population and within children aged under 5 years old. Ten stochastic realisations are represented by the points in each plot, with the mean relationship throughout the transmission shown in black with a locally weighted scatterplot smoothing regression (loess). The annual average proportion of false-negative RDTs

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Figure 2 continued

due to *pfhrp2* gene deletions is shown with the horizontal dashed red line, and a sampling scheme that occurs throughout the year, with samples collected proportionally to clinical incidence, is shown with grey points circled in red.

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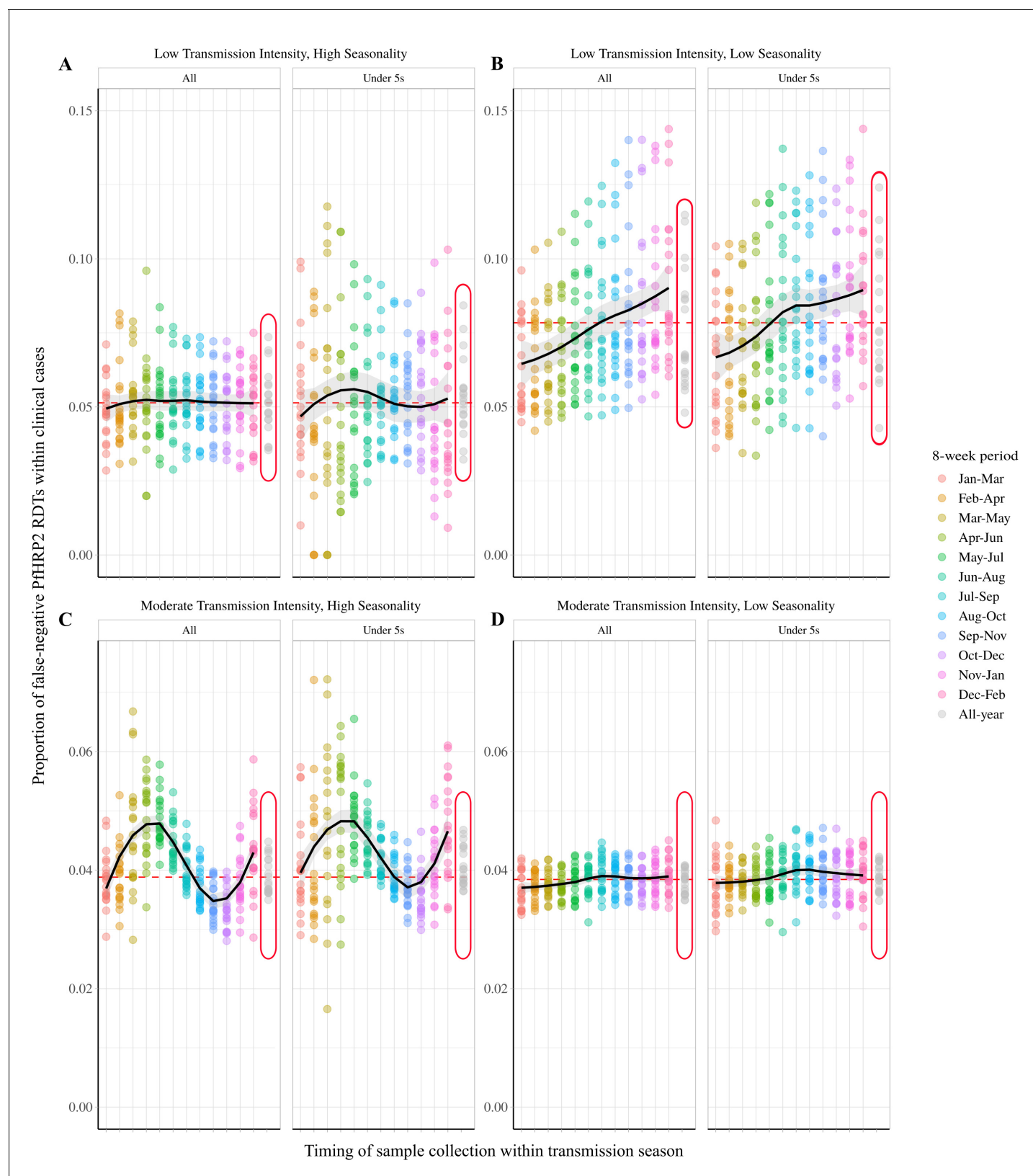


Figure 2—figure supplement 1. Impact of a selective advantage for *pfhrp2*-deleted parasites on the observed proportion of false-negative PfHRP2 RDTs within clinical cases during 8-week intervals. Graphs show the proportion of clinical cases yielding false-negative PfHRP2 RDTs at 8-week intervals within a transmission season for both moderate (C, D) and low (A, B) transmission settings and high (A, C) and low (B, D) seasonality. In each panel the

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observed proportion *pfhrp2*-negative clinical cases is shown for the whole population and within children aged under 5 years old. Ten stochastic realisations are represented by the points in each plot, with the mean relationship throughout the transmission shown in black with a locally weighted scatterplot smoothing regression (loess). The annual average proportion of false-negative RDTs due to *pfhrp2* gene deletions is shown with the horizontal dashed red line, and a sampling scheme that occurs throughout the year, with samples collected proportionally to clinical incidence, is shown with grey points circled in red.

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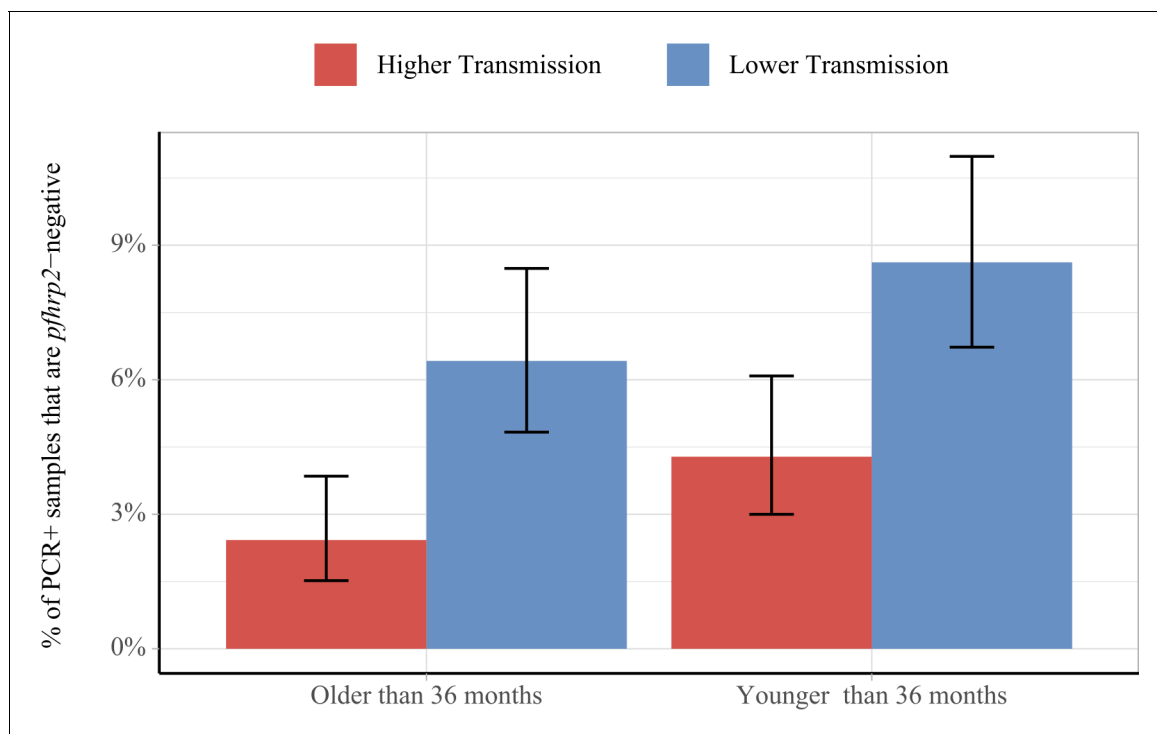
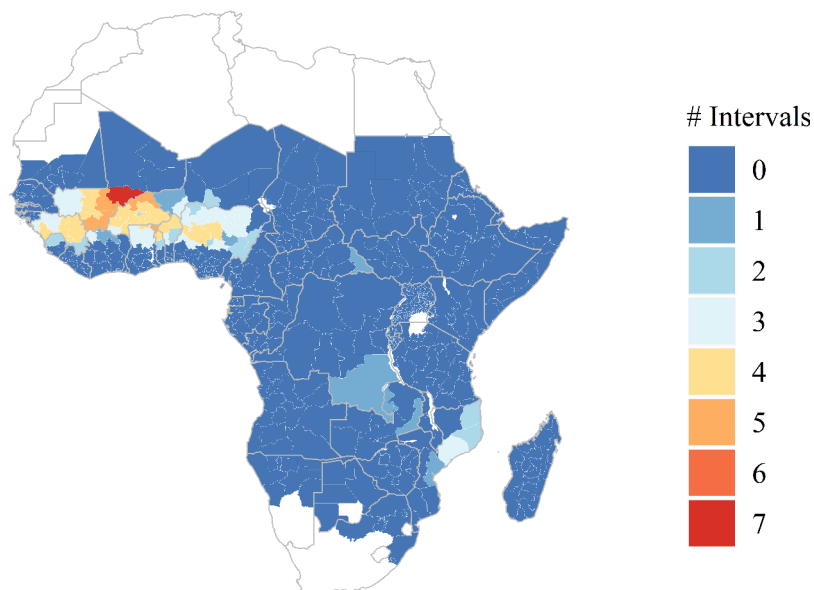


Figure 3. Impact of age and transmission intensity upon *pfhrp2* deletion in the Democratic Republic of the Congo (DRC), 2013–2014. Graphs show the percentage of PCR-positive *P. falciparum* samples taken from children under the age of 5 years from the 2013–2014 Demographic and Health Survey in DRC that are *pfhrp2*-negative. Children who are younger than the median age in the 2752 samples are grouped within the younger category. In addition, samples are classified as lower transmission if the incidence of malaria in the month prior to sample collection is lower than the median clinical incidence. The 95% binomial confidence intervals are indicated with the vertical error bars.

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a

Number of 8-week intervals in which an incorrect switch to a non PfHRP2-based RDT would have been made in more than 75% simulations

**b**

Number of 8-week intervals in which an incorrect decision to continue using PfHRP2-based RDTs would have been made in more than 75% simulations

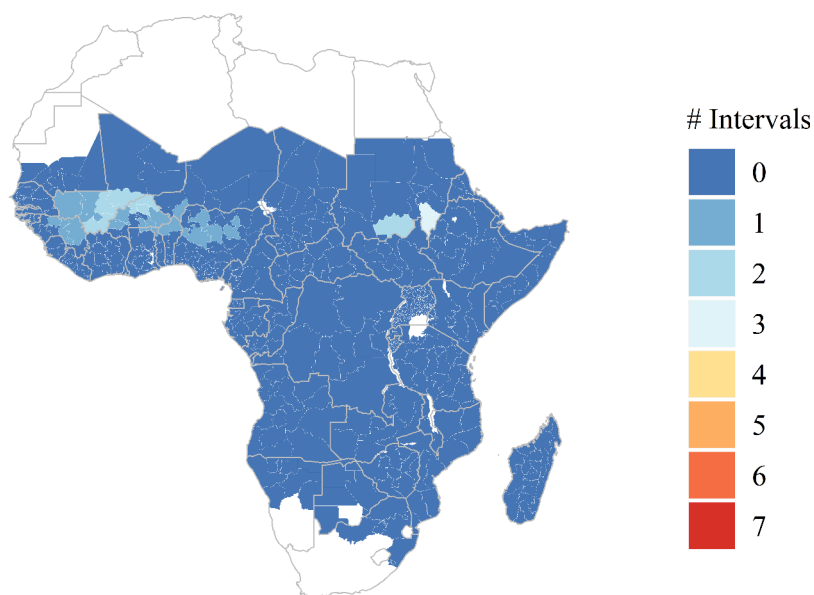


Figure 4. Predicted areas with the potential for collected estimates of the proportion of false-negative PfHRP2 RDTs due to *pfhrp2* deletions to be unrepresentative of the annual average. The maps show (A) the number of 8-week intervals at which an administrative region would prematurely swap
Figure 4 continued on next page

Figure 4 continued

to a non PfHRP2-based RDT due to overestimating the proportion of false-negative PfHRP2 RDTs due to *pfhrp2* gene deletions in more than 75% of simulations. In (A) the opposing trend is shown, with the number of 8-week intervals at which an administrative region would prematurely continue to use PfHRP2-based RDTs due to underestimating the proportion of false-negative PfHRP2 RDTs due to *pfhrp2* gene deletions in more than 75% of simulations.

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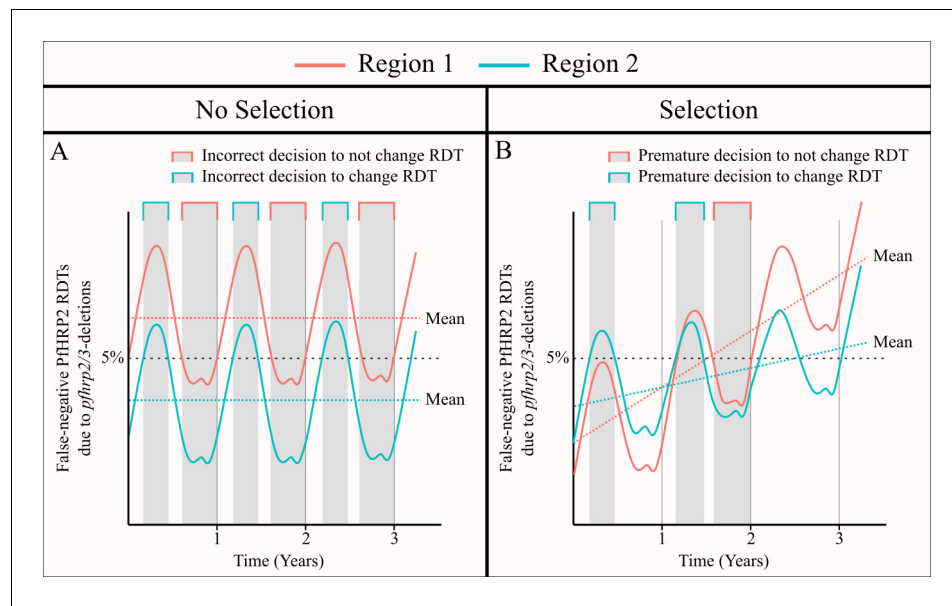


Figure 5. The impact of an assumed selective pressure for *pfhrp2/3*-deleted parasites on the decision to switch RDT. The graphs show two hypothetical scenarios with two different regions shown in red and blue for each region. In (A) there are strong seasonal dynamics but no selective pressure. The absence of a selective pressure causes that the mean proportion of false-negative RDTs due to *pfhrp2/3* deletions over a 1 year period to be constant and is shown with a horizontal dashed line. Consequently, there are time periods in which an incorrect decision to switch RDT could be made for the region in blue, and an incorrect decision to not switch RDT could be made for the region in red. In (B), there are both seasonal dynamics and a selective pressure, which results in an increasing annual mean proportion of false-negative RDTs due to *pfhrp2/3* deletions over time. As in (A), there are periods in which the observed proportion of false-negative RDTs due to *pfhrp2/3* deletions is both higher and lower than the rolling mean shown. However, decisions made in these periods are premature rather than definitively incorrect as the selection pressure would eventually cause the proportion to be greater than 5%.

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