Case-control studies of the effectiveness of the RTS,S/AS01 malaria vaccine against hospital admission with severe malaria, and associations with safety signals: statistical report

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Contents

Summary 4
Objectives 5
Methods 5
Cases: 5
Controls: 6
Vaccination status and information on potential confounders: 6
Data quality control: 6
Statistical methods: 6
Results 8
Effectiveness of the first three doses against severe malaria 8
Table 1: Characteristics of severe malaria cases and controls 9
Table 2: Incremental and cumulative effectiveness of 1,2 and 3 doses of RTSS up to the age scheduled for dose 4 10
Figure 1: Time since receipt of dose 3 among severe malaria cases and controls 11
Figure 2: Effectiveness of 3 doses of RTS,S against severe malaria, in relation to time since the 3rd dose (with 95% confidence intervals). 12
Table 3: Effectiveness of 3 doses against severe malaria, in relation to time since the 3rd dose 12
Effectiveness of four doses against severe malaria, and the incremental effectiveness of the fourth dose 13
Table 4: Effectiveness of 4 doses, and incremental effectiveness of the 4th dose (cases eligible to have received dose 4) 13
Figure 3: Time since receipt of dose 4 (months) among severe malaria cases (A) and controls (B) in discordant matched sets included in analyses, and the age distribution of the cases (C). 14
Figure 4: Distribution of time since dose 3 amongst cases (A) and controls (B) who had received 4 doses: 14
Figure 5: Effectiveness of 4 doses of RTS,S in relation to time since the fourth dose. 15
Meningitis 16
Cerebral malaria 16
Mortality 16
Discussion 17
References 19
Acknowledgments 20
Annex 21
A1. Average effectiveness of RTSS against severe malaria, including all cases, showing associations for the covariates. 21

Table A1: Odds ratios for the incremental effect of each additional dose, and for the cumulative effect of 1,2,3 and 4 doses, for severe malaria, including all cases. 21

A2: Meningitis 22

Table A2: Characteristics of meningitis cases and controls 22

Table A3: Odds ratios for the association of RTS,S with meningitis. 23

A3: Cerebral malaria 24

Table A4: Characteristics of cerebral malaria cases and controls 24

Table A5A: Odds ratios for the association of RTS,S with cerebral malaria 25

Table A5B: Effectiveness against cerebral malaria and other forms of severe malaria 26

A4: Mortality 27

Table A6: Characteristics of cases and controls, mortality study 27

Table A7: Odds ratios for the association of RTSS with mortality (any cause other than injury)..... 28

A5: Efficacy estimates for clinical malaria from the phase 3 trial, by since dose3 and since dose4, in sites with moderate/low transmission intensity and sites with high transmission intensity 29

Table A8: Sites in the phase 3 trial ordered by incidence of clinical malaria in the control group: 29

Figure A1: Efficacy of 3 doses (R3C versus C3C) against clinical malaria in the phase 3 trial, during the period from 0.5 to 6 months after dose 3, from 6 to 18 months after dose 3, and from 18 months after dose 3 until study end..... 30

Figure A2: Efficacy of 4 doses (R3R versus C3C) against clinical malaria in the phase 3 trial, during the period from 0.5 to 6 months after dose 4, and from 6 to 18 months after dose 4. 30

Figure A3: Incremental efficacy of dose 4 against clinical malaria in the phase 3 trial, during the period from 0.5 to 6 months after dose 4, and from 6 to 18 months after dose 4. 31

A6: Distribution of severe malaria cases by age (y-axis) and time (x-axis), showing eligibility for dose 3. Children were eligible for dose 3 from 7 months of age (Malawi) and 9 months (Kenya and Ghana). 32

A7: Distribution of severe malaria cases by age (y-axis) and time (x-axis), showing eligibility for dose 3. Children were eligible for dose 4 from 22months of age (Malawi), 24months (Kenya), and in Ghana from 24months until Feb 20, 2023 when the age for dose4 was reduced to 18months. 33

A8: Characteristics that an unmeasured confounder would have to have to explain the observed sustained effects of three doses of RTS,S against severe malaria 34

Summary

Case-control studies were undertaken during and shortly after the Malaria Vaccine Pilot Evaluation, to determine the incremental benefit of 4 doses of RTS,S vaccine compared to 3 doses, when implemented through routine childhood immunization programmes, to assess evidence for any rebound effect in children who received only 3 doses, and to estimate associations with safety signals that were observed in the phase3 trial. Four neighbourhood controls born within 1 month of the case were recruited for each case. Caregivers of each child were visited at home to collect information on vaccination history and potential confounders, dates of vaccination with RTS,S and other vaccines were copied from the child's health booklet or vaccination record, or from clinic registers. Records were photographed for verification later. The index date for vaccination status in the controls was the date of the case event (for safety signals), or 14 days before the event (for estimating effectiveness). Conditional logistic regression was used to estimate odds ratios adjusted for covariates.

A total of 1546 cases of severe malaria were recruited, with 6151 controls. The average effectiveness of three doses of RTS,S, during the period until the fourth dose was due, was 56% (95%CI 40%,67%), an interval of up to about 15 months. Among children who did not go on to receive the fourth dose, in children from about 2 to 4 years of age, the average effectiveness of three doses was 35% (95%CI 15%,50%).

Among children who did receive the fourth dose, the average effectiveness of four doses against severe malaria was 54% (95%CI 39%, 66%). The incremental effectiveness of dose 4, compared to three doses, was 30% (95%CI 9.3%,45%).

The effectiveness of four doses was greatest within 6 months of receiving dose 4, 67% (95%CI 43%,81%), declining to an average of 55% (95%CI 31%,71%) during the next 12 months.

The protection associated with three doses of RTS,S persisted beyond the age when children were due to receive the fourth dose. There was no evidence of a rebound effect. The fourth dose provided additional protection lasting for at least 18 months.

We found no evidence that receipt of RTS,S was associated with excess risk of meningitis, or of cerebral malaria (RTS,S was equally effective against cerebral malaria as for other forms of severe malaria), and no evidence that the gender modified the effect of RTS,S on mortality.

Objectives

The objectives of the case-control studies were to determine the incremental benefit of 4 doses of RTS,S vaccine compared to 3 doses, when implemented through routine childhood immunization programmes, and to determine the association of the RTS,S vaccine with the safety signals that were observed in the Phase 3 trial of RTS,S.

The final results of the cluster-randomized Malaria Vaccine Pilot Evaluation (MVPE) showed that RTS,S introduction was associated with significant reductions in mortality in boys and in girls, and in the number of patients admitted to hospital with severe malaria, among children who would have been eligible to have received RTS,S (whether they received it or not), with no evidence of excess cases of meningitis or of cerebral malaria [1,2]. An important strength of this evaluation was the use of randomization to reduce bias due to confounding. However, it was not possible to estimate the level of personal protection provided by RTS,S doses, or to measure directly if there was any excess risk of severe malaria from the age when children should receive the 4th dose, among children who received 3 doses of RTS,S but did not go on to receive their 4th dose (the so-called rebound effect). Measuring these effects was the main purpose of the case control studies.

We also planned to determine whether receipt of RTS,S was associated with the risk of meningitis or of cerebral malaria, to complement the impact estimates for these outcomes from the cluster-randomized evaluation. A case control study of deaths of any cause excluding injury was also planned, with the specific objective to estimate the interaction between receipt of RTS,S and gender, powered to be able to detect interaction of the magnitude observed in the phase 3 trial.

Methods

Cases:

Cases were of three types: patients admitted to MVPE sentinel hospitals with probable or confirmed meningitis, patients with severe malaria (including cerebral malaria), and children who died of any cause excluding injury reported through the MVPE community mortality surveillance. Cases were eligible for inclusion if, at the time of the event, they were resident in sentinel hospital catchment areas in an area where RTS,S was being implemented, were of an age to be eligible to have received RTSS, and were aged under 60 months (see Annex A6 for the precise definitions of eligibility: at the start of case-control recruitment, cases aged 6-30 months were eligible in Ghana, 6-37 months in Kenya, and 5-29 months in Malawi; by the end of recruitment, children aged 6-59 months (Ghana, Kenya) or 5-59 months (Malawi) were eligible). The cases for the mortality study were identified through the community mortality surveillance, recruited from the catchment areas of the sentinel hospitals. The case definitions for severe malaria and for meningitis were those defined in the MVPE protocol (using the 'broad' definition for cerebral malaria, which did not insist on lumbar puncture being performed to exclude meningitis). Cases recruited in hospital were to be visited at home shortly after discharge from hospital, to collect information about vaccinations and other data. For the mortality study, the data were collected during the visit for verbal autopsy. In practice many cases (and their matched controls) were visited later than this. Consent from parents or guardians was sought in hospital or at the home visit.

Controls:

Four controls per case were recruited, individually matched to cases, born within one month of the date of birth of the case. After completing the home visit to the case, controls were recruited from the same neighbourhood, by going systematically house to house after moving about 100m away from the case's home. On average, teams visited about 80 households to find 4 controls. In each household, all children under 5 were listed, caregivers were asked about date of birth and the child's health record, if available, was consulted to check date of birth. Possession of a health record was not a condition for recruitment. Children with date of birth within a month of the date of birth of the case were noted, consent was sought from the parents or guardian, and children with consent were recruited and the same information collected as for cases.

Vaccination status and information on potential confounders:

Caregivers of case and control children were asked about the child's vaccine history using an adapted version of the recall questionnaire in the WHO 2018 immunization coverage survey manual. The vaccine details on the child's home-based record were transcribed and photos taken to permit verification later. Caregivers were asked about the child's ITN use the night before the visit (surviving cases and controls), and before the event (all cases), and household socio-demographic data (household assets, caregiver education level, and other details) were also collected. For cases and controls without a home-based record, attempts were mqade to find the child's record in registers in the local immunization clinic, and the data were transcribed and (Ghana and Kenya only) a photograph taken to permit verification later.

As part of MVPE hospital surveillance, vaccination details were obtained in hospital. These were used only in Malawi and only during the initial months of the case control study, when field teams did not collect vaccine data from cases during home visits, if those data had already been collected in hospital.

Data quality control:

Images of vaccine records were checked, and the database updated if necessary, when there were inconsistent dates for multi-dose vaccines, when there were atypical patterns of vaccination (doses for different vaccines out of the expected normal sequence, and records where later doses appear complete but earlier doses are missing); records where doses were indicated as having been received but dates of vaccination are missing; and discrepancies between vaccine records from different sources. This report (Sep 2, 2025) is based on final data after this process was completed in all three countries and includes data on the second dose of measles vaccine, which was not captured in Kenya originally and were obtained from the images of home-based records. During this process data on RTS,S vaccine doses were re-entered and checked. For all meningitis cases and controls only, independent re-entry of vaccine data from images of Home Based Records was completed, compared with the original data capture, and discrepancies resolved.

Statistical methods:

Details of sample size calculations are described in the protocol [3] and analysis plan [4]. Analysis was limited to cases and controls with documented vaccine history from a home-based record (HBR) or immunization register. Only vaccine doses with a date were included. Doses of multi-dose vaccines were sorted on date and renumbered if necessary. One source of

vaccine data is used for a particular child, in the following order: HBR reviewed at home, HBR reviewed in hospital, vaccine clinic register. Field teams were asked to note if a dose was indicated by a sticker or a tick mark, and then to record the date of the dose. Children with any RTS,S doses marked as given, but without a date, were excluded from analysis.

For hospital outcomes, children with replacement HBRs in Kenya and Malawi were excluded from analysis. In Ghana, it was not recorded if the card was a replacement but replacements were reported to be uncommon. For the mortality study, children with replacement HBRs have been excluded in Kenya only, as it was not systematically documented whether the card was a replacement in Ghana and Malawi.

The index date for vaccination status in the controls is the date of the event (where the question relates to safety), or 14 days before the event (where the purpose is to estimate effectiveness). Conditional logistic regression was used to estimate odds ratios, unadjusted and adjusted for covariates. We also present in the tables the unadjusted odds ratios calculated using the subset of records that were non-missing for the covariates. The odds ratios (OR) are estimates of (are interpreted as) incidence rate ratios, and vaccine effectiveness expressed as a percentage is calculated as 100x(1-OR).

For analysis of severe malaria including cerebral malaria, we adjusted for ITN use, caregiver education, household wealth ranking, and child gender. For analysis of meningitis, we also adjusted for receipt of pentavalent, pneumococcal, and men-A vaccines. For analysis of all-cause mortality, we adjusted for ITN use, caregiver education, household wealth ranking, and child gender, and receipt of other vaccines. However, we were not able to adjust for receipt of the second dose of measles vaccine, as this was not captured in Kenya. These data are being entered from the images of HBRs, and results for mortality will then be updated including receipt of the second dose of measles in the adjustment.

Vaccine effects are presented in two ways: as the incremental effectiveness of each dose, adjusted for the previous doses; and comparing children who received 1,2,3 or 4 doses with unvaccinated children (the cumulative effects). Analyses are for all countries combined.

For severe malaria, we present several types of analysis:

estimates of the effectiveness of 1,2 and 3 doses up until the age scheduled for dose 4;

estimates of the effectiveness of 1,2 and 3 doses from the age scheduled for dose 4, in children who did not receive the fourth dose;

estimates of the effectiveness of the fourth dose.

We also present the effectiveness of three doses in successive time periods after the third dose: within 6 months of the third dose, 6-18 months after the third dose, and 18 months and more after the third dose. And we present effectiveness of the fourth dose in the same time intervals after the fourth dose.

For meningitis, cerebral malaria, and for mortality, analysis is for the whole study period. Additional analyses compare effectiveness against cerebral malaria and other forms of severe malaria.

Results

A total of 1546 cases of severe malaria were recruited, with 6151 controls, of these 1370 (89%) of cases and 5698 (93%) of controls had a HBR. Among cases, 93 (7%) of HBRs were replacements and among controls, 447 (8%), these were excluded from analysis. 1271/1546 (82%) and 4736/6151 (77%) matched controls were included in analyses. Table 1 shows the characteristics of these cases and controls. Most children slept under an ITN (84% of cases and 89% of controls). As the analysis is matched, only matched sets which are discordant for RTS,S status contribute to the analyses, this is not reflected in Table 1.

Effectiveness of the first three doses against severe malaria

During the period until children were eligible to receive the fourth dose, the average effectiveness of three doses of RTS,S against severe malaria was 56% (95%CI 40%,67%), Table 2. This represents an interval of up to 14.5 months. Among children who did not go on to receive the fourth dose, the effectiveness of three doses from the age when they were eligible to receive the fourth dose was 35% (95%CI 15%,50%) (Table 4). In the latter analysis, the cases were aged about 2-4yrs, and amongst the controls who had received three doses, most (78%) received the third dose between 12 and 36 months previously (Figure 1).

These estimates, and all the estimates for severe malaria, were adjusted for the sex of the child, ITN use at the time of the home visit, caregiver's household's wealth ranking, and caregivers highest level of education. The effects of these covariates are shown in the Annex (Table A1). The risk of severe malaria was lower in girls, and lower risk was also associated with higher levels of caregiver education, socio-economic status, and among children reported to use an ITN at the time of the home visit.

The effectiveness of three doses was greatest within 6 months of receiving dose 3 (56%, 95%CI 35%,71%), decreasing to 37% (95%CI 15%, 53%) over the following 12 months, and 41% (95%CI 14%, 59%) in the period 18 months or more after receiving the dose 3, in children who did not receive a fourth dose (Figure 2).

There was no evidence that one dose was effective. In the earlier period, the data were consistent with there being some limited protection from 2 doses, but as few children had received only one or two doses, there was wide uncertainty around these effects.

Table 1: Characteristics of severe malaria cases and controls

	Controls	Cases
Number recruited	6,151	1,546
Number (%) with a vaccine record (HBR or clinic register)	5,698 (93%)	1,370 (89%)
Number excluding replacement HBR's	5,251	1,277
Number in matched sets that were retained	4,736 (77%)	1,271 (82%)
Gender		
Boys	2,322 (49%)	715 (56%)
Girls	2,404 (51%)	556 (44%)
Missing	10 (0.2%)	0 (0%)
Vaccine source		
HBR at home visit	4,172 (88%)	896 (70%)
HBR in hospital	0 (0.0%)	233 (18%)
Clinic register	564 (12%)	142 (11%)
RTS,S doses		
0	1,017 (21.5%)	341 (26.8%)
1	355 (7.5%)	138 (10.9%)
2	434 (9.2%)	139 (10.9%)
3	2,025 (42.8%)	470 (37.0%)
4	905 (19.1%)	183 (14.4%)
Mean age, months	24.8	24.7
Caregiver's household's wealth ranking		
Low	1,584 (33.4%)	468 (36.8%)
Middle	1,547 (32.7%)	443 (34.9%)
High	1,605 (33.9%)	360 (28.3%)
Caregiver's highest level of education		
None or informal	231 (4.9%)	80 (6.3%)
Primary	2,989 (63.1%)	890 (70.0%)
Secondary	1,244 (26.3%)	257 (20.2%)
Tertiary	271 (5.7%)	44 (3.5%)
Missing	1 (0.02%)	0 (0.0%)
Bednet use, at the time of the home visit		
No	515 (10.9%)	183 (14.4%)
Yes	4,213 (89.0%)	1,065 (83.8%)
Missing	8 (0.2%)	23 (1.8%)

Table 2: Incremental and cumulative effectiveness of 1,2 and 3 doses of RTSS up to the age scheduled for dose 4

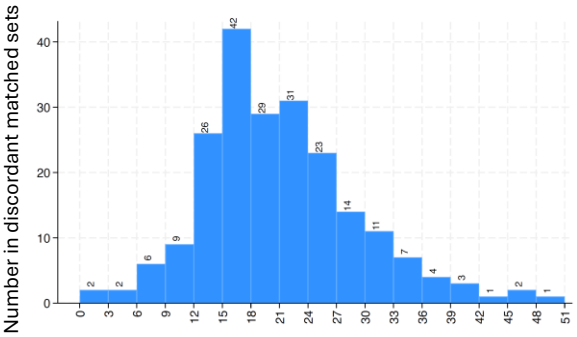
	Crude OR (95%CI)	Crude OR* (95%CI)	Adjusted OR# (95%CI)	% effectiveness (95%CI)
The period from 0.5m after the dose until the age scheduled for dose 4:				
RTSS dose number	**Incremental effect of each dose**			
0	1	1	1	
1	1.22 [0.88, 1.71]	1.26 [0.89, 1.77]	1.24 [0.88, 1.76]	-24% (-76%,12%)
2	0.62 [0.43, 0.89]	0.62 [0.42, 0.90]	0.64 [0.44, 0.93]	36% (6.8%,56%)
3	0.56 [0.40, 0.78]	0.56 [0.40, 0.78]	0.56 [0.40, 0.78]	44% (22%,60%)
Number of doses	**Cumulative effect**			
0	1 (Reference)	1 (Reference)	1 (Reference)	
1	1.22 [0.88, 1.71]	1.26 [0.89, 1.77]	1.24 [0.88, 1.76]	-24% (-76%,12%)
2	0.75 [0.53, 1.07]	0.78 [0.54, 1.11]	0.79 [0.55, 1.14]	21% (-14%,45%)
3	0.42 [0.31, 0.56]	0.43 [0.32, 0.59]	0.44 [0.33, 0.60]	56% (40%,67%)

*unadjusted, calculated on the subset of data non-missing for covariates #adjusted for child sex, ITN use reported at home visit, caregiver highest level of education, household wealth ranking.

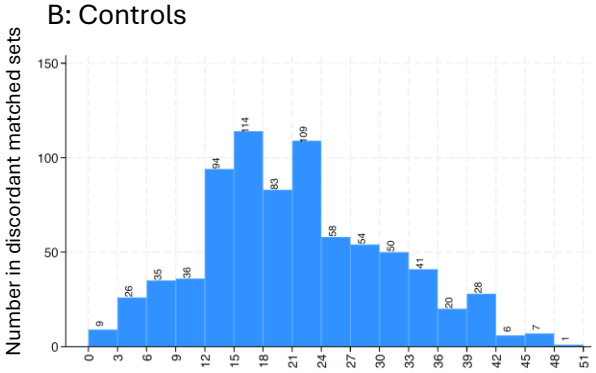
Figure 1: Time since receipt of dose 3 among severe malaria cases and controls

The distribution of time since dose 3 (in months) is shown for cases (A) and controls (B) in discordant matched sets included in analyses of the effectiveness of 3 doses from the time when children were eligible for the fourth dose, in children who did not receive the fourth dose, and the age distribution of the cases (C).

A: Cases

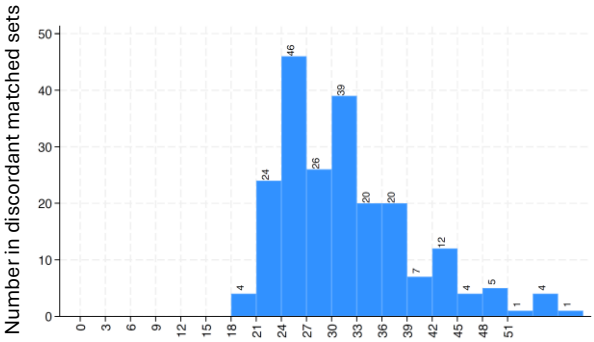


B: Controls



Time (months) since receipt of dose 3

C: Age distribution of the cases who were eligible for but did not receive the fourth dose:



Age (months) of severe malaria cases

Figure 2: Effectiveness of 3 doses of RTS,S against severe malaria, in relation to time since the 3rd dose (with 95% confidence intervals).

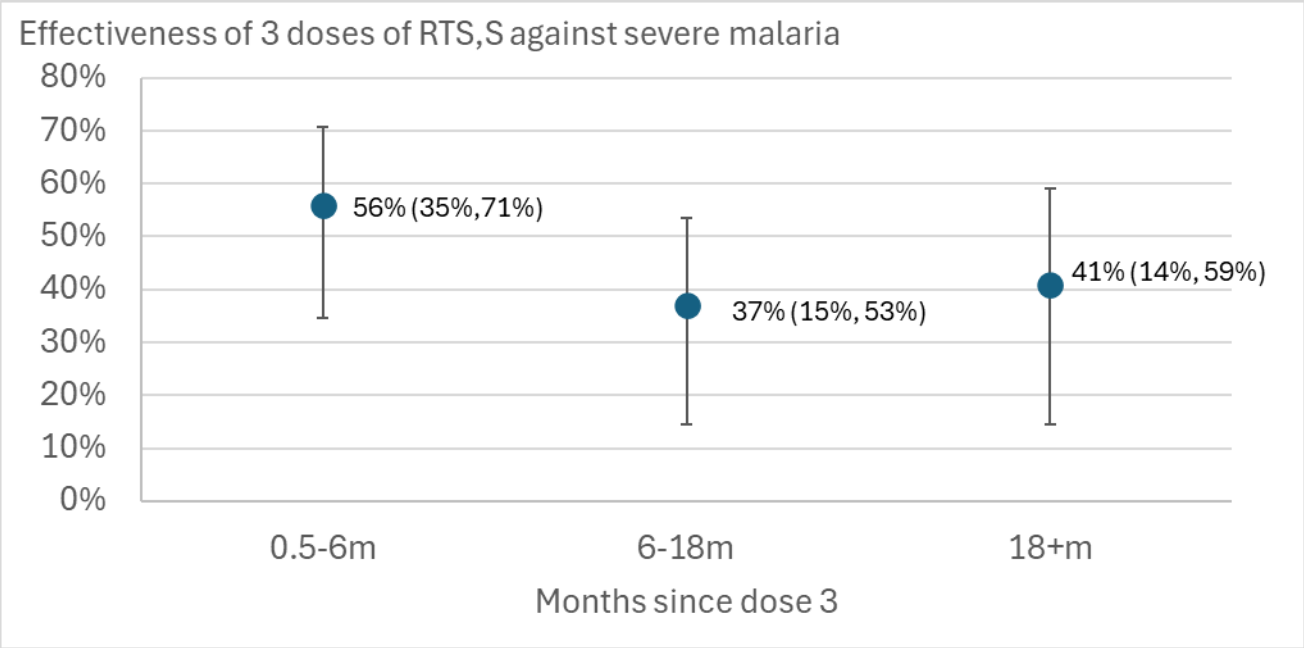


Table 3: Effectiveness of 3 doses against severe malaria, in relation to time since the 3rd dose

	Adjusted OR# (95%CI)	**% effectiveness (95%CI)**
Unvaccinated	1 (Reference)	
Received exactly 3 doses: time since dose 3:		
0.5 - <6m	0.44 [0.29, 0.66]	56% (35%,71%)
6 - <18m	0.63 [0.47, 0.85]	37% (15%,53%)
18+m	0.59 [0.41, 0.86]	41% (14%,59%)

#adjusted for child sex, ITN use reported at home visit, caregiver highest level of education, household wealth ranking.

Effectiveness of four doses against severe malaria, and the incremental effectiveness of the fourth dose

The average effectiveness of four doses against severe malaria was 54% (95%CI 39%, 66%), Table 4. Most of the cases in this analysis were 2-4years old and, among the controls, most (92%) who had received dose 4 had received it in the previous 2 years (Figure 3). The incremental effectiveness of dose 4, compared to three doses, was 30% (95%CI 9.3%,45%). In this analysis, amongst the controls who had received 4 doses, most (83%) had received their third dose 12-36 months previously (Figure 4). The distribution of time since dose 3 was similar amongst controls who had not received dose 4.

Effectiveness of four doses was greatest within 6 months of receiving dose 4, 67% (95%CI 43%,81%), declining to an average of 55% (95%CI 31%,71%) during the next 12 months. Protection of 4 doses lasted at least 18 months. There was limited data for the period beyond 18 months after receiving dose 4, there was no evidence of continuing protection in this period but the confidence interval was wide (Table 4, Figure 5).

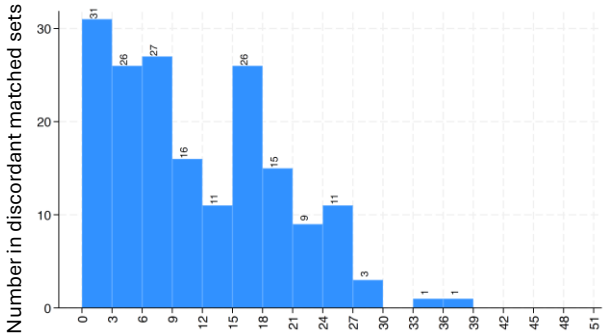
Table 4: Effectiveness of 4 doses, and incremental effectiveness of the 4th dose (cases eligible to have received dose 4)

	Crude OR (95%CI)	**Crude OR* (95%CI)**	**Adjusted OR# (95%CI)**	**% effectiveness (95%CI)**
Incremental effectiveness:				
0	1	1	1	
1	0.99 [0.64, 1.53]	0.92 [0.59, 1.43]	0.95 [0.61, 1.49]	5% (-49%, 39%)
2	1.27 [0.76, 2.13]	1.34 [0.80, 2.26]	1.32 [0.78, 2.24]	-32% (-124%, 22%)
3	0.50 [0.34, 0.73]	0.50 [0.34, 0.74]	0.51 [0.35, 0.76]	49% (24%, 65%)
4	0.66 [0.52, 0.85]	0.66 [0.52, 0.85]	0.70 [0.55, 0.91]	30% (9.3%, 45%)
Cumulative effectiveness:				
0	1 (Reference)	1 (Reference)	1 (Reference)	
1	0.99 [0.64, 1.53]	0.92 [0.59, 1.43]	0.95 [0.61, 1.49]	5% (-49%, 39%)
2	1.26 [0.85, 1.88]	1.24 [0.83, 1.85]	1.26 [0.84, 1.90]	-26% (-90%, 16%)
3	0.63 [0.48, 0.82]	0.62 [0.48, 0.81]	0.65 [0.50, 0.85]	35% (15%, 50%)
4	0.42 [0.32, 0.55]	0.41 [0.31, 0.55]	0.46 [0.34, 0.61]	54% (39%, 66%)

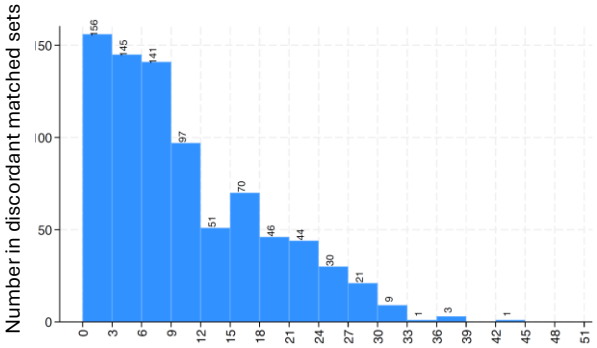
*unadjusted, calculated on the subset of data non-missing for covariates #adjusted for child sex, ITN use reported at home visit, caregiver highest level of education, household wealth ranking.

Figure 3: Time since receipt of dose 4 (months) among severe malaria cases (A) and controls (B) in discordant matched sets included in analyses, and the age distribution of the cases (C).

A: Cases

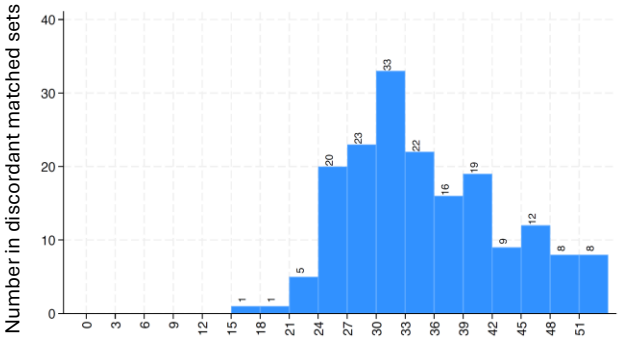


B: Controls



Time (months) since receipt of dose 4

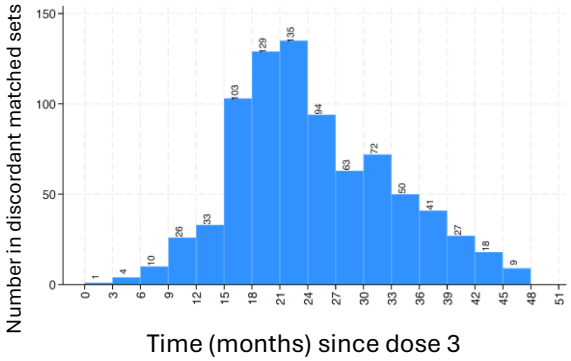
C: Age distribution of cases eligible to have received dose 4



Age (months) of severe malaria cases

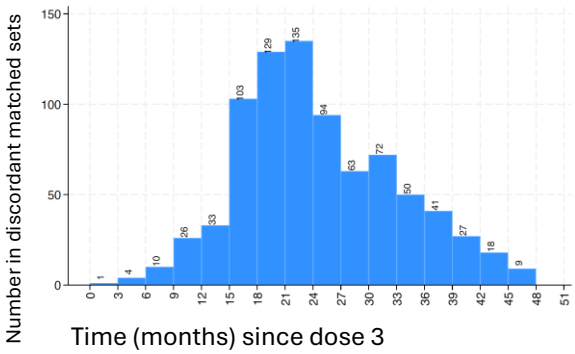
Figure 4: Distribution of time since dose 3 amongst cases (A) and controls (B) who had received 4 doses:

A: Cases



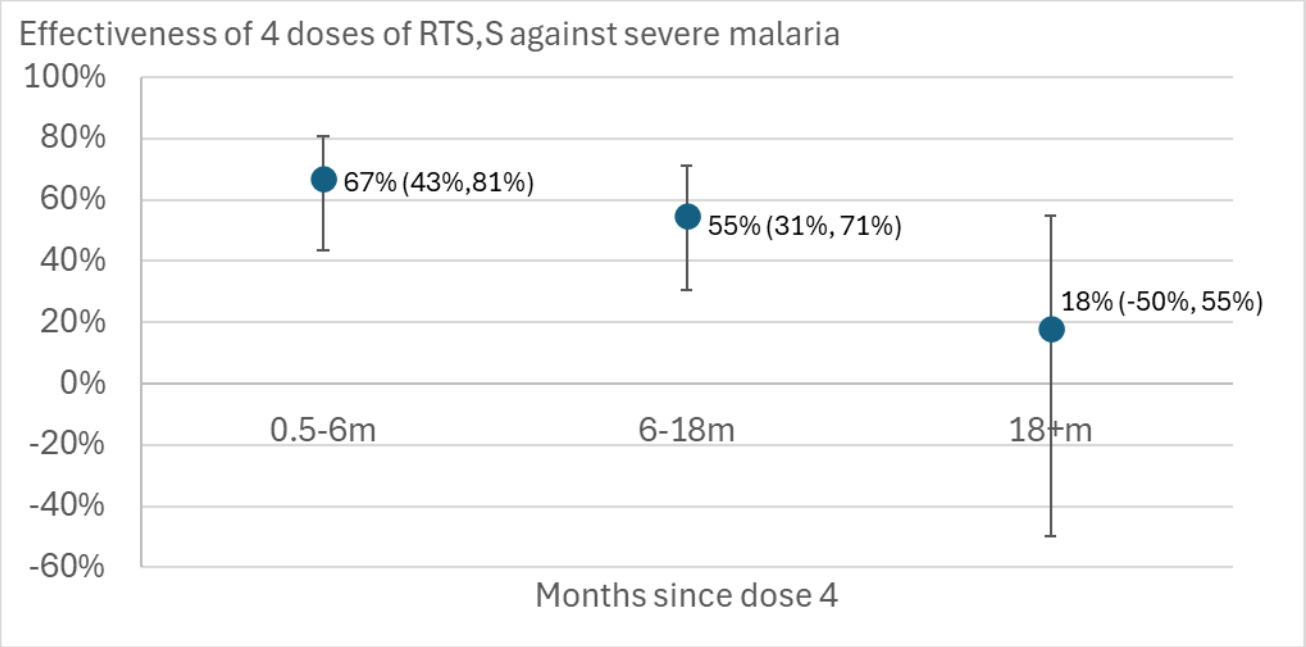
Time (months) since dose 3

B: Controls



Time (months) since dose 3

Figure 5: Effectiveness of 4 doses of RTS,S in relation to time since the fourth dose.



Meningitis

A total of 57 cases of probable or confirmed meningitis, and 226 controls, were recruited, 53(93%) of cases and 209 (93%) of controls had an HBR, 4 cases and 17 controls who had replacement HBRs were excluded, leaving 49 cases and 179 matched controls which were included in analyses (Annex, Table A2). Of these, 38/49 (77.6%) cases and 134/179 (74.9%) controls had received 1 or more doses of RTS,S. There was no evidence that meningitis was associated with RTS,S vaccination, adjusted odds ratio 0.96 (95%CI 0.38,2.4), (Annex, Table A3).

Cerebral malaria

A total of 182 cases meeting the criteria for cerebral malaria were recruited, and 727 controls, 146 (80%) cases and 539 (74%) controls had documented vaccine history (replacement HBR excluded) and were included in analyses, and of these 94/146 (64%) of cases and 426/539 (79%) of controls had received 1 or more doses of RTS,S (Annex, Table A4). There was no evidence that RTS,S was associated with an excess risk of cerebral malaria (Annex, Table A5A). The estimates of effectiveness of three doses of RTS,S against cerebral malaria were 73% (18%,91%) during the period until children were eligible for dose 4, and 58% (20%,78%)59% (20%,79%) after this time in children who did not receive the 4th dose, and the effectiveness of four doses was 78% (54%,89%) 77% (50%,89%), however tests of interaction did not indicate that effectiveness against cerebral malaria differed from other forms of severe malaria, with respect to three doses of RTS,S and four doses of RTS,S (Annex, Table A5B).

Mortality

A total of 1144 cases were recruited, 855 (75%) with a vaccine record were included in analyses, 491 boys and 364 girls. There were 4569 controls, 3167 (69%) with a vaccine record and included in analyses, 1615 boys and 1552 girls. The crude odds ratios were 0.58 (95%CI 0.46,0.74) in boys and 0.68 (95%CI 0.52, 0.89) in girls, interaction parameter (relative odds ratio girls:boys) was 1.16 (95%CI 0.82, 1.63), p=0.394, and when adjusted for caregiver education, household wealth ranking, gender, receipt of first dose and of second dose of measles vaccine, 1.24 (95%CI 0.87,1.77) p=0.224.

Discussion

This study showed that the protection against severe malaria associated with three doses of RTS,S persisted beyond the age when children were due to receive the fourth dose. There was no evidence of a rebound effect. The fourth dose provided significant additional protection lasting for at least 18 months. There was no evidence of protection in children who received only one or two doses but the data did not exclude some benefit of two doses. We found no evidence that receipt of RTS,S was associated with an excess risk of meningitis, or of cerebral malaria. RTS,S was as effective against cerebral malaria as other forms of severe malaria. And there was no evidence that gender modified the effect of RTS,S on mortality.

The study took advantage of strengthened surveillance for severe malaria and meningitis in MVPE sentinel hospitals, and used the same case definitions as the MVPE. Documented vaccine histories were obtained from home-based records or clinic registers and images of vaccine records were used for data quality control. Over 1500 cases of severe malaria were recruited in three countries, our results are therefore based on a much larger sample of severe cases than in the phase 3 trial.

Controls were closely matched to cases on date of birth and vaccine status was considered at the time of the event, so matching controls for calendar time and age, and controls were matched on neighbourhood of residence (and hence on distance to care, to vaccination, and to hospital, as well as local malaria transmission intensity). Information on other potential confounders, socio-economic status of the household, caregiver education, and use of ITNs, and receipt of other vaccines[1], was collected for cases and controls. Analyses were restricted to children (cases and controls) who had a home-based vaccine record or a record of vaccination in a clinic register, and therefore this restriction controls for access to vaccination (all children with a record are likely to have received at least one vaccine).

Limitations include the lower availability of vaccine records for cases than controls, especially for the mortality study. Efforts were made to trace records in immunization registers but as a consequence there was a greater reliance on clinic registers for cases than controls. Some home visits were several weeks after the event, ITN use and other potential confounders could have changed, and control selection was limited to surviving children. ITN use prior to the event could not be determined reliably, we used ITN use at the time of the visit as a proxy but cases might have changed their ITN use after the illness. We did not adjust for ITN use in the mortality study.

There is likely to be residual confounding, but in the severe malaria analyses, to explain the effects we see, an unmeasured confounder would need to be strongly associated with the risk of severe malaria admission, and with the uptake of RTSS, after adjusting for neighbourhood (distance to care, distance to vaccination, distance to hospital, local malaria transmission intensity), the child's age and gender, time, household socio-economic status, and caregiver's level of formal education, which may be unlikely. In the severe malaria analyses, adjustments for covariates made small changes to odds ratios for the effects of RTS,S, suggesting that the close neighbourhood matching accounted for effects of socio-economic status and education, which are often strongly associated with malaria risk.

[1] Data on receipt of the second dose of measles vaccine was not included in the data collected in Kenya, but these data are being captured from the images of vaccine records, analyses will then be updated adjusting for receipt of measles vaccine in the second year of life.

A further point supporting our findings for severe malaria is that they are broadly consistent with the efficacy against clinical malaria observed in the phase 3 trial in the moderate and low transmission sites in the trial. The overall results from the phase 3 trial (all sites combined) are dominated by sites with very high incidence but re-analysis of the phase 3 data (Annex, A5) showed that in the sites with moderate/low transmission, the efficacy of 3 doses and of 4 doses waned less rapidly than in the high transmission sites, and efficacy of 3 doses persisted beyond the age for dose 4 (i.e. past 18 months after dose 3 in the trial). Efficacy of four doses against clinical malaria was sustained over a period up to 18 months after dose 4, as we observed for severe malaria in our study.

In addition, our estimates of the incremental efficacy of the fourth dose with respect to severe malaria are also consistent with the incremental efficacy of dose 4 against clinical malaria in the phase 3 trial. During the 12 months following dose 4 in the phase 3 trial (all sites combined) the incremental efficacy provided by the fourth dose was 25.6% (95%CI 18.2%-32.3%). The incremental efficacy did not differ by transmission intensity. Our estimate of the incremental effectiveness of the 4th dose was 30% (95%CI 9.3%,45%).

Residual confounding may be more of a concern in the mortality study where, since we include mortality due to any cause (apart from injury), any factor that influences child survival is a potential confounder. However, our main focus was the interaction by gender, which may be less influenced by confounding than the main effects.

Our findings for severe malaria are also broadly consistent with the impact observed against severe malaria in the cluster-randomized evaluation. They add to those results by providing estimates of the protective effectiveness of three doses of RTS,S against severe malaria, including cerebral malaria, and the incremental effectiveness of the fourth dose, suggesting less rapid waning of effects than was previously observed for clinical malaria in high transmission sites in the phase 3 trial.

References

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Annex

A1. Average effectiveness of RTSS against severe malaria, including all cases, showing associations for the covariates.

Table A1: Odds ratios for the incremental effect of each additional dose, and for the cumulative effect of 1,2,3 and 4 doses, for severe malaria, including all cases.

	Crude OR (95%CI)	Crude OR* (95%CI)	Adjusted OR# (95%CI)	% effectiveness (95%CI)
RTSS dose number (incremental effect of each additional dose)				
0	1	1	1	
1	1.19 [0.91,1.54]	1.17 [0.90,1.53]	1.17 [0.90,1.54]	-17%(-54%,10%)
2	0.80 [0.60,1.08]	0.82 [0.61,1.11]	0.83 [0.61,1.12]	17%(-12%,39%)
3	0.56 [0.44, 0.71]	0.56 [0.44,0.72]	0.57 [0.45,0.74]	43%(26%,55%)
4	0.72 [0.57, 0.91]	0.73 [0.58,0.92]	0.76 [0.60,0.96]	24%(4%,40%)
Number of RTSS doses (cumulative effect compared to unvaccinated)				
0	1 (Reference)	1 (Reference)	1 (Reference)	
1	1.19 [0.91,1.5]	1.17 [0.90,1.53]	1.17 [0.90,1.54]	-17%(-54%,10%)
2	0.95 [0.74,1.24]	0.96 [0.74,1.25]	0.97 [0.74,1.27]	3%(-27%,26%)
3	0.53 [0.44,0.65]	0.54 [0.44,0.66]	0.56 [0.46, 0.68]	44%(32%,54%)
4	0.38 [0.30,0.49]	0.39 [0.31,0.50]	0.42 [0.33, 0.54]	58%(46%,67%)
Caregiver's household's wealth ranking				
Low	1	1	1	
Middle	0.94 [0.80, 1.10]	0.92 [0.78, 1.08]	0.94 [0.80, 1.10]	
High	0.64 [0.54, 0.78]	0.65 [0.54, 0.78]	0.71 [0.59, 0.86]	
Child's ITN use at the time of the home visit				
Non-user	1	1	1	
User	0.66 [0.54, 0.81]	0.66 [0.54, 0.81]	0.75 [0.61, 0.92]	
Caregiver's highest level of education				
None or informal	1	1	1	
Primary	0.85 [0.63, 1.15]	0.83 [0.62, 1.13]	0.93 [0.68, 1.27]	
Secondary or tertiary	0.54 [0.39, 0.75]	0.54 [0.39, 0.75]	0.65 [0.47, 0.91]	
Child's gender				
Boy	1	1	1	
Girl	0.74 [0.66, 0.85]	0.75 [0.66, 0.85]	0.75 [0.66, 0.85]	

*unadjusted, calculated on subset of data non-missing for covariates #adjusted for the variables in the table.

A2: Meningitis

Table A2: Characteristics of meningitis cases and controls

	Controls	**Cases**
Number recruited	227	57
Number (%) with record (HBR or clinic)	210 (92.5%)	53 (93.0%)
Excluding replacement HBRs	193	49
Number retained	179 (78.9%)	49 (86.0%)
Vaccine source		
HBR at home visit	151 (84.4%)	33 (67.3%)
HBR in hospital	0 (0.0%)	7 (14.3%)
Clinic register	28 (15.6%)	9 (18.4%)
RTS,S doses		
0	45 (25.7%)	11 (22.4%)
1	10 (5.6%)	8 (16.3%)
2	18 (10.1%)	5 (10.2%)
3	79 (44.1%)	17 (34.7%)
4	26 (14.5%)	8 (16.3%)
Mean age, months	21.4	21.3
Household wealth ranking		
1	40 (22.3%)	15 (30.6%)
2	68 (38.0%)	14 (28.6%)
3	71 (39.7%)	20 (40.8%)
Caregiver's highest level of education		
None or informal	12 (6.7%)	4 (8.2%)
Primary	107 (59.8%)	27 (55.1%)
Secondary	47 (26.3%)	11 (22.4%)
Tertiary	13 (7.3%)	7 (14.3%)
ITN use at time of visit		
No	15 (8.4%)	6 (12.2%)
Yes	163 (91.1%)	43 (87.8%)
Missing	1 (0.6%)	0 (0.0%)
Penta doses		
0	6 (3.4%)	2 (4.1%)
1	8 (4.5%)	1 (2.0%)
2	6 (3.4%)	0 (0.0%)
3	159 (88.8%)	46 (93.9%)
PCV doses		
0	6 (3.4%)	2 (4.1%)
1	8 (4.5%)	2 (4.1%)
2	7 (3.9%)	1 (2.0%)
3	158 (88.3%)	44 (89.8%)
Men. A received		
	156 (87.2%)	46 (93.9%)
	23 (12.8%)	3 (6.1%)

Table A3: Odds ratios for the association of RTS,S with meningitis.

		Crude OR (95%CI)	**Crude OR* (95%CI)**	**Adjusted OR# (95%CI)**
RTSS	0 doses	1 (Reference)	1 (Reference)	1 (Reference)
	1 or more doses	1.13 (0.47,2.7)	1.13 (0.47,2.7)	0.96 (0.38,2.4)
Pentavalent vaccine	0 doses	1 (Reference)	1 (Reference)	1 (reference)
	1 or 2 doses	0.16 (0.01,2.3)	0.16 (0.01,2.3)	0.15 (0.01,2.4)
	3 doses	0.78 (0.12,5.0)	0.78 (0.12,5.0)	0.69 (0.11,4.6)
Men-A	0	1 (Reference)	1 (Reference)	1 (reference)
	1	1.00 (0.11,9.0)	1.00 (0.11,8.9)	1.12 (0.12,10.3)
Wealth ranking	Low	1 (Reference)	1 (Reference)	1 (reference)
	Middle	0.55 (0.23,1.3)	0.55 (0.23,1.3)	0.56 (0.22,1.4)
	High	0.72 (0.30,1.7)	0.73 (0.30,1.8)	0.77 (0.30,2.0)
Caregiver highest level of education	None or informal	1 (Reference)	1 (Reference)	1 (reference)
	Primary	0.64 (0.15,2.7)	0.64 (0.15,2.7)	0.73 (0.17,3.1)
	Secondary or Tertiary	0.76 (0.16,3.6)	0.78 (0.17,3.7)	0.80 (0.17,3.8)
Gender	Boy	1 (Reference)	1 (Reference)	1 (reference)
	Girl	0.91 (0.47,1.7)	0.89 (0.46,1.7)	0.95 (0.47,1.9)

*unadjusted, calculated on subset of data non-missing for covariates #adjusted for the variables in the table.

A3: Cerebral malaria

Table A4: Characteristics of cerebral malaria cases and controls

	Controls	Cases
Number recruited	727	182
Number (%) with a record (HBR or clinic)	669 (92%)	158 (87%)
Number excluding replacement HBRs	620	147
Number retained	539 (74%)	146 (80%)
Gender		
Boys	277 (51%)	89 (61%)
Girls	259 (48%)	57 (39%)
Missing	3 (0.6%)	0 (0%)
Vaccine source		
HBR at home visit	448 (83%)	109 (75%)
HBR in hospital	0 (0.0%)	24 (16%)
Clinic register	91 (17%)	13 (9.0%)
RTS,S doses		
0	113 (21%)	52 (36%)
1	37 (6.9%)	16 (11%)
2	43 (8.0%)	11 (7.5%)
3	216 (40%)	45 (31%)
4	130 (24%)	22 (15%)
Mean age, months	28.6	28.3
Household wealth ranking		
Low	186 (35%)	56 (38%)
Middle	157 (29%)	57 (39%)
High	196 (36%)	33 (23%)
Caregiver's highest level of education		
None or informal	41 (7.6%)	15 (10%)
Primary	356 (66%)	104 (71%)
Secondary	122 (23%)	27 (19%)
Tertiary	20 (3.7%)	0 (0.0%)
Bednet use at the time of the visit		
No	73 (14%)	26 (18%)
Yes	464 (86%)	111 (76%)
Missing	2 (0.4%)	9 (6.2%)

Table A5A: Odds ratios for the association of RTS,S with cerebral malaria

	Crude OR (95%CI)	**Crude OR* (95%CI)**	**Adjusted OR# (95%CI)**
No RTS,S	1 (Reference)	1 (Reference)	1 (Reference)
Any doses of RTS,S	0.35 [0.22, 0.56]	0.35 [0.21, 0.56]	0.39 [0.24, 0.65]
Household wealth ranking			
Low	1 (Reference)	1 (Reference)	1 (Reference)
Middle	1.15 [0.73,1.82]	1.05 [0.66, 1.68]	1.03 [0.63, 1.68]
High	0.51 [0.29, 0.88]	0.51 [0.29, 0.89]	0.50 [0.28, 0.90]
ITN use at time of visit			
No	1 (Reference)	1 (Reference)	1 (Reference)
Yes	0.59 [0.34, 1.04]	0.59 [0.34,1.04]	0.76 [0.42, 1.37]
Caregiver education			
None or informal	1 (Reference)	1 (Reference)	1 (Reference)
Primary	0.62 [0.28, 1.38]	0.58 [0.26,1.29]	0.64 [0.27, 1.49]
Secondary or tertiary	0.39 [0.16, 0.97]	0.37 [0.15, 0.92]	0.50 [0.19, 1.31]
Child gender			
Boy	1 (Reference)	1 (Reference)	1 (Reference)
Girl	0.71 [0.48, 1.05]	0.72 [0.48, 1.08]	0.75 [0.49, 1.14]

*unadjusted, calculated on subset of data non-missing for covariates #adjusted for the variables in the table.

Table A5B: Effectiveness against cerebral malaria and other forms of severe malaria

Although point estimates of effectiveness of RTS,S against cerebral malaria were higher than for other forms of severe malaria, during the period until children were eligible for the fourth dose, and during the period following eligibility for dose 4, tests of interaction indicated no evidence of a difference in effectiveness (p-value 0.197 and 0.295, respectively).

Cumulative effectiveness of RTS,S doses, children not eligible for dose 4			
RTS,S doses	Non-cerebral forms of severe malaria	Cerebral malaria	Severe malaria (all forms)
0	1 (Reference)	1 (Reference)	1 (Reference)
1	1.23 [0.87,1.74]	2.69 [0.63, 11.5]	1.24 [0.88, 1.76]
2	0.80 [0.56,1.14]	0.63 [0.16, 2.52]	0.79 [0.55, 1.14]
3	0.45 [0.34, 0.61]	0.27 [0.09, 0.82]	0.44 [0.33, 0.60]
Effectiveness of 3 doses	55% (39%,67%)	73% (18%,91%)	56%(40%,67%)
	Interaction test: chi-sq 4.68 (3df), p=0.1969		
Cumulative effectiveness of RTS,S doses, children eligible for dose 4			
0	1 (Reference)	1 (Reference)	1 (Reference)
1	1.08 [0.67, 1.76]	0.62 [0.19,1.97]	0.95 [0.61, 1.49]
2	1.47 [0.95, 2.29]	0.55 [0.17,1.79]	1.26 [0.84, 1.90]
3	0.70 [0.52, 0.93]	0.42 [0.22, 0.80]	0.65 [0.50, 0.85]
4	0.51 [0.37, 0.69]	0.22 [0.11, 0.46]	0.46 [0.34, 0.61]
Effectiveness of 3 doses	30% (7%,48%)	58% (20%,78%)	35% (15%,50%)
Effectiveness of 4 doses	49% (31%,63%)	78% (54%,89%)	54% (39%,66%)
	Interaction test: chi-sq 4.9 (4df), p=0.2945		

Adjusted odds ratios (adjusted for child sex, ITN use, caregiver education, household wealth ranking) with 95% confidence intervals.

A4: Mortality

Table A6: Characteristics of cases and controls, mortality study

	Controls	Cases
Number recruited	4569	1144
Number (%) with vaccine record	4178 (91%)	866 (76%)
Number in retained matched sets	3167 (69%)	855 (75%)
Gender		
Boys	1,615 (51%)	491 (57%)
Girls	1,552 (49%)	364 (43%)
Vaccine source		
HBR at home visit	2,790 (88%)	565 (66%)
HBR at earlier VA	0 (0.0%)	11 (1.3%)
Clinic register	375 (12%)	279 (33%)
RTS,S doses		
0	746 (24%)	269 (32%)
1	286 (9.0%)	90 (11%)
2	369 (12%)	105 (12%)
3	1,293 (41%)	322 (38%)
4	473 (15%)	69 (8.1%)
Mean age, months	21.5	21.9
Wealth ranking of household		
Low	987 (31%)	309 (36%)
Middle	1,112 (35%)	316 (37%)
High	1,068 (34%)	230 (27%)
Caregiver's education		
None or informal	321 (10%)	112 (13%)
Primary	2,094 (66%)	618 (72%)
Secondary	655 (21%)	106 (12%)
Tertiary	97 (3.1%)	17 (2.0%)
Missing	0 (0.0%)	2 (0.2%)

ITN use has not been used due to the difficulty of reliably determining ITN use before death.

Table A7: Odds ratios for the association of RTSS with mortality (any cause other than injury)

	Unadjusted OR (95%CI)	Unadjusted OR* (95%CI)	Adjusted OR# (95% CI)
Receipt of RTSS in boys			
0 doses	1 (Reference)	1 (Reference)	1 (Reference)
1 or more doses	0.58 [0.46,0.74]	0.58 [0.45, 0.73]	0.72 [0.56, 0.92]
Receipt of RTSS in girls			
0 doses	1 (Reference)	1 (Reference)	1 (Reference)
1 or more doses	0.68 [0.52, 0.89]	0.68 [0.52, 0.90]	0.90 [0.67, 1.19]
Interaction by gender (any RTSS, girls:boys)	1.16 [0.82, 1.63] p = 0.394	1.19 [0.84, 1.67] p = 0.327	1.24 [0.87, 1.77] p = 0.224
Gender			
Boy	1 (Reference)	1 (Reference)	1 (Reference)
Girl	0.77 [0.66, 0.90]	0.77 [0.66, 0.90]	0.79 [0.67, 0.92]
Other vaccines			
MCV1			
No	1 (Reference)	1 (Reference)	1 (Reference)
Yes	0.65 [0.52, 0.80]	0.65 [0.52, 0.81]	0.71 [0.67, 0.92]
MCV2			
No	1 (Reference)	1 (Reference)	1 (Reference)
Yes	0.24 [0.18, 0.31]	0.24 [0.18, 0.31]	0.38 [0.29, 0.49]
Wealth ranking of caregiver's household			
Low	1 (Reference)	1 (Reference)	1 (Reference)
Middle	0.89 [0.74, 1.08]	0.90 [0.74, 1.09]	0.95 [0.78, 1.15]
High	0.64 [0.51, 0.79]	0.65 [0.52, 0.81]	0.75 [0.60, 0.94]
Highest level of education of caregiver			
None or informal	1 (Reference)	1 (Reference)	1 (Reference)
Primary	0.78 [0.59, 1.03]	0.77 [0.58, 1.02]	0.84 [0.63, 1.12]
Secondary or tertiary	0.40 [0.29, 0.56]	0.39 [0.28, 0.55]	0.49 [0.34, 0.68]

*unadjusted, calculated on subset of data non-missing for covariates #adjusted for the variables in the table.

A5: Efficacy estimates for clinical malaria from the phase 3 trial, by since dose3 and since dose4, in sites with moderate/low transmission intensity and sites with high transmission intensity

Data from the phase 3 trial[2] were reanalysed to estimate efficacy against clinical malaria in trial sites which had similar transmission intensity to the sites used for the case control study. Phase3 trial sites were ranked according to the incidence of clinical malaria in the control group, and grouped into two strata, high transmission, and moderate/low transmission (Table A8). Vaccine efficacy of three doses of RTS,S/AS01 against clinical malaria was estimated for three time periods: 0.5 to 6months post dose 3, 6 to 18 months post dose 3, and from 18 months post dose 3 to study end (median 46 months after dose 3). Vaccine efficacy of four doses, and the incremental efficacy of dose 4, was estimated for the periods 0.5 to 6months post dose 4, and 6 to 18 months post dose 4. Analysis was limited to children who received 4 doses of vaccine (RTSS or control). Malaria was defined as in the phase 3 trial article (fever with parasite density ≥5000/μL, or severe malaria). To avoid double counting, repeat episodes within 14 days of a previous episode were ignored (but unlike the phase 3 publication we did not deduct person time after each episode). Efficacy was estimated using Cox regression, with centre as a fixed effect, with robust standard error to account for the presence of multiple episodes per child, in each transmission intensity stratum. A test of interaction compared efficacy between strata, in each time period.

Table A8: Sites in the phase 3 trial ordered by incidence of clinical malaria in the control group:

Site	Rate/child/year, overall (control group)	Rate/child/year during 12months post dose 3 (control group)
High transmission:		
Kenya (Siaya)	2.81	5.45
Burkina Faso (Nanoro)	2.40	3.92
Kenya (Kombewa)	1.53	3.07
Ghana (Kintampo)	1.50	2.92
Ghana (Agogo)	0.93	1.94
Moderate/low transmission:		
Tanzania (Bagamoyo)	0.31	0.46
Malawi (Lilongwe)	0.24	0.35
Gabon (Lambarene)	0.20	0.39
Mozambique (Manhica)	0.20	
Tanzania (Korogwe)	0.10	0.11
Kenya (Kilifi)	0.08	0.07

[2] RTS,S Clinical Trials Partnership (2015) Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. Lancet 386: 31–45.

We thank www.clinicalstudydatarequest.com and GlaxoSmithKline for providing access to the anonymized trial data.

Figure A1: Efficacy of 3 doses (R3C versus C3C) against clinical malaria in the phase 3 trial, during the period from 0.5 to 6 months after dose 3, from 6 to 18 months after dose 3, and from 18 months after dose 3 until study end.

The efficacy and 95% confidence interval is shown for each transmission intensity stratum. P-values are from the test of interaction (difference in efficacy between the transmission intensity strata).

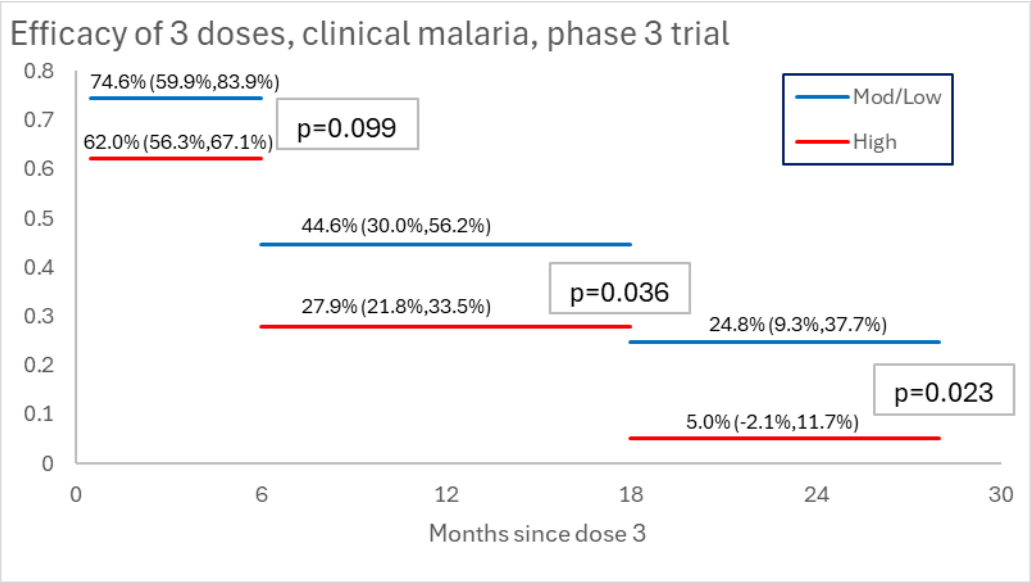


Figure A2: Efficacy of 4 doses (R3R versus C3C) against clinical malaria in the phase 3 trial, during the period from 0.5 to 6 months after dose 4, and from 6 to 18 months after dose 4.

The efficacy and 95% confidence interval is shown for each transmission intensity stratum. P-values are from the test of interaction (difference in efficacy between the transmission intensity strata).

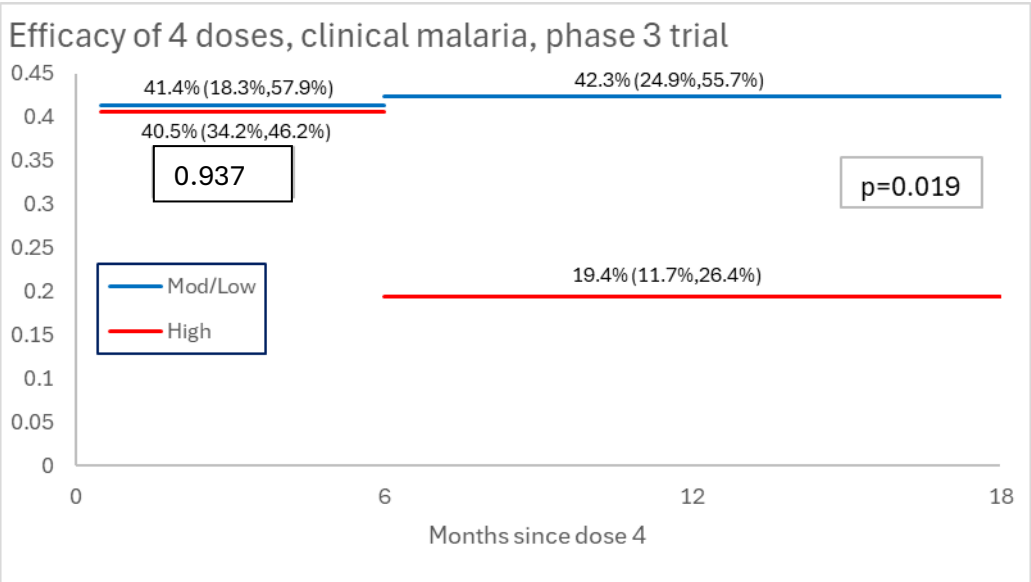
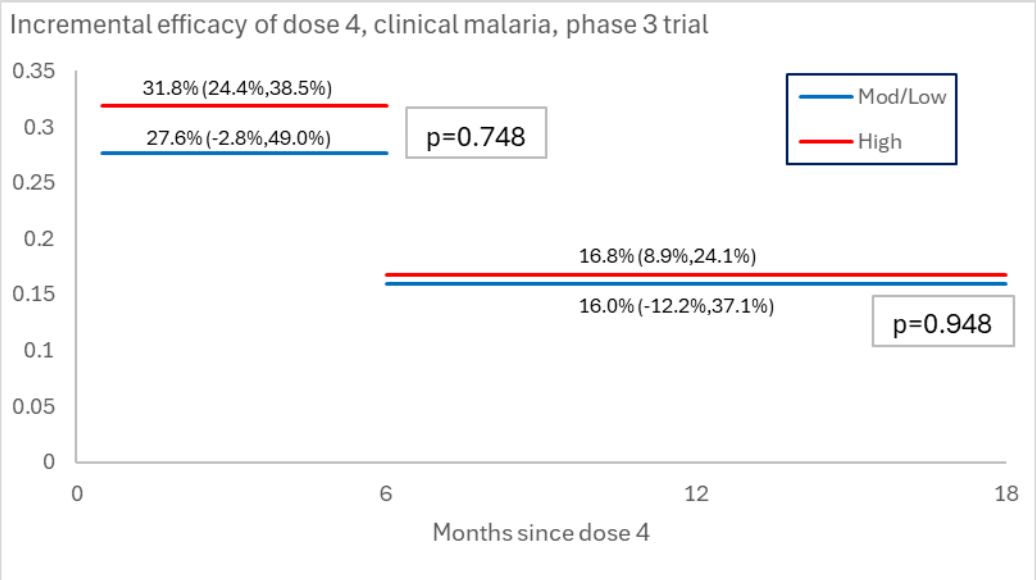
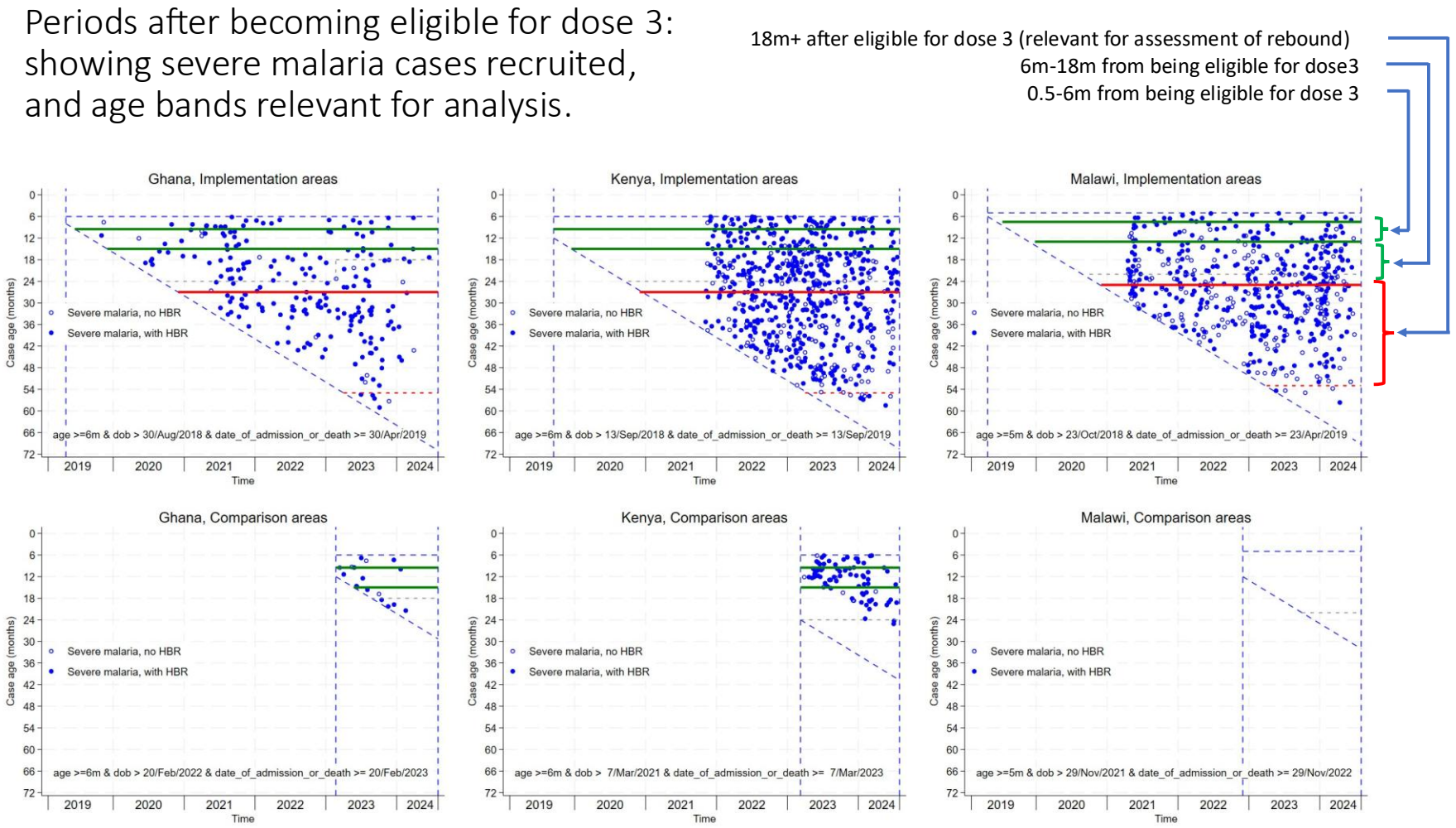


Figure A3: Incremental efficacy of dose 4 against clinical malaria in the phase 3 trial, during the period from 0.5 to 6 months after dose 4, and from 6 to 18 months after dose 4.

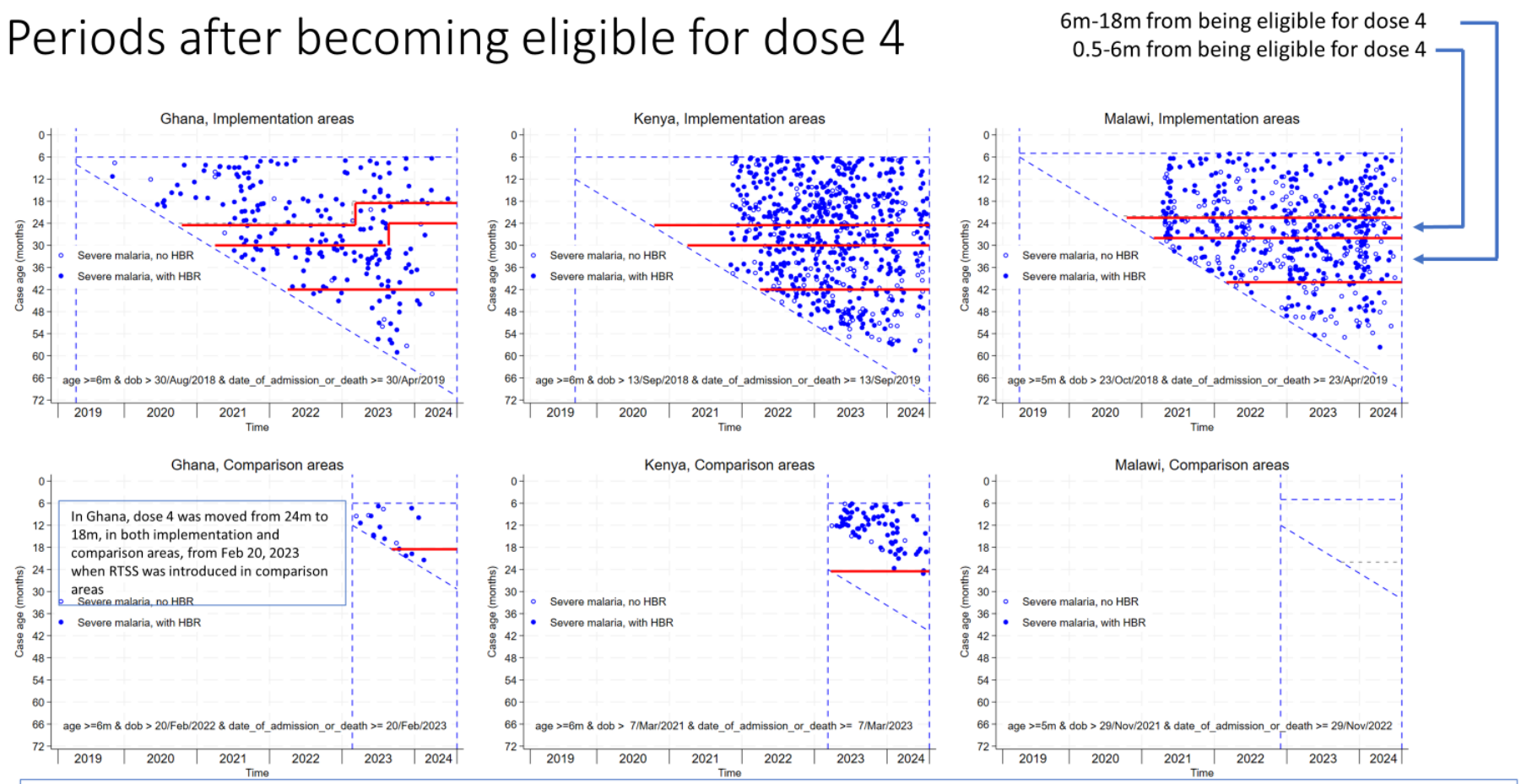
The incremental efficacy and 95% confidence interval is shown for each transmission intensity stratum. P-values are from the test of interaction (difference in efficacy between the transmission intensity strata).



A6: Distribution of severe malaria cases by age (y-axis) and time (x-axis), showing eligibility for dose 3. Children were eligible for dose 3 from 7 months of age (Malawi) and 9 months (Kenya and Ghana).



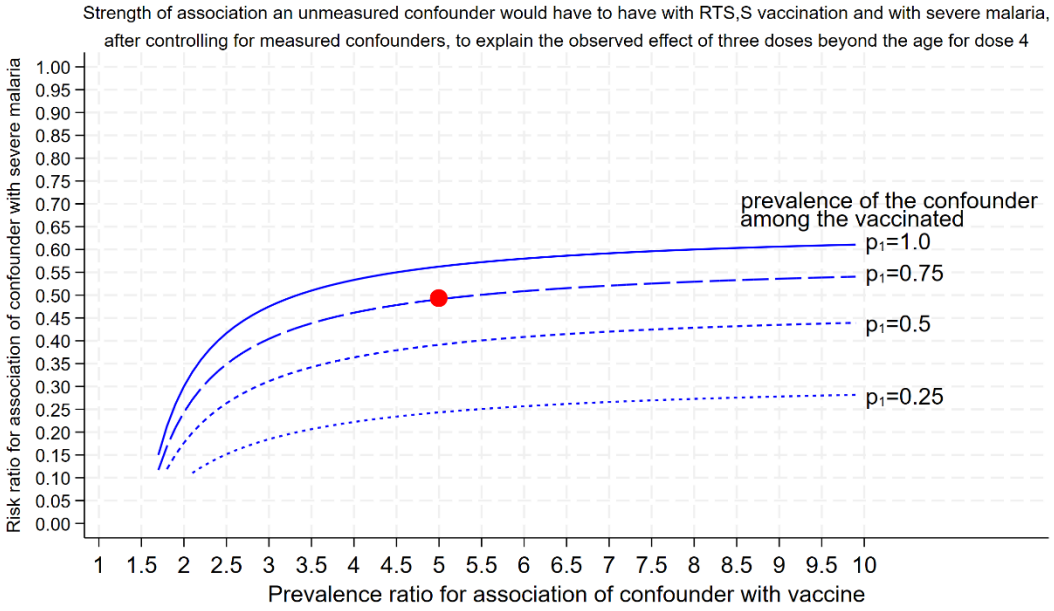
A7: Distribution of severe malaria cases by age (y-axis) and time (x-axis), showing eligibility for dose 3. Children were eligible for dose 4 from 22months of age (Malawi), 24months (Kenya), and in Ghana from 24months until Feb 20, 2023 when the age for dose4 was reduced to 18months.

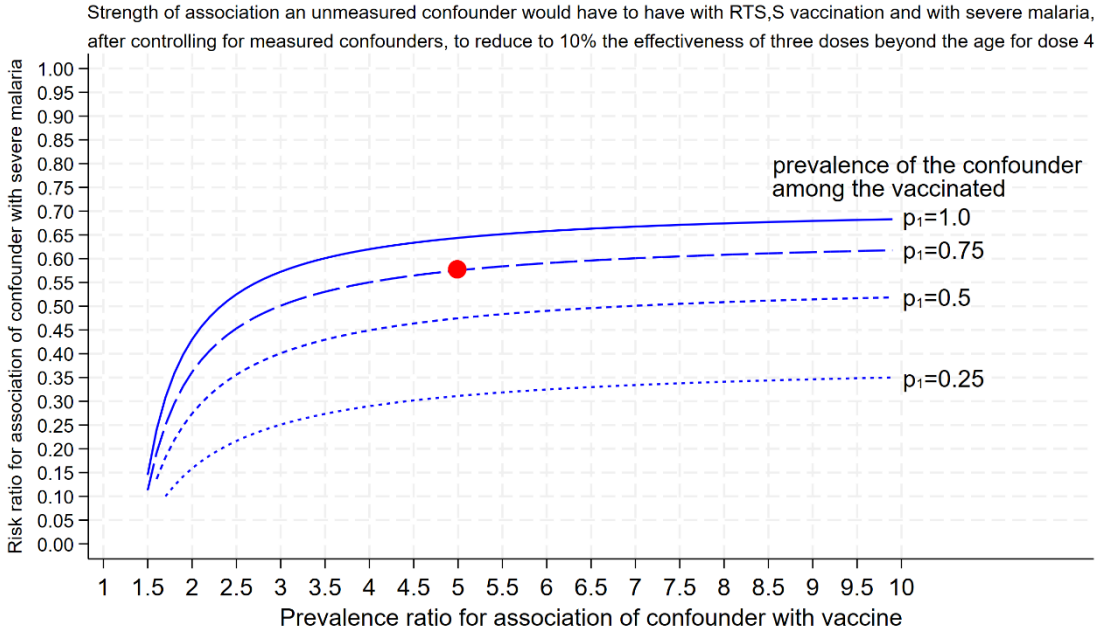


Note that children were eligible for dose 1 from 6-11m of age (5-11m in Malawi, and from 6-23m in Kenya after expansion to comparison areas), but children who get dose 1 late, say at 11m, should get dose 2 and 3 at 12m and 13m even where the schedule is 6,7,9, but these children are still due for dose 4 at the same age as children who get dose 1 at 6m. Because in theory a child in Kenya could get dose 1 at 23m, and dose 3 then at 25m, WHO suggested adding a requirement that there should be a minimum interval of 6months between dose 3 and dose 4.

A8: Characteristics that an unmeasured confounder would have to have to explain the observed sustained effects of three doses of RTS,S against severe malaria

The odds ratio for the effect of three doses of RTS,S beyond the age when children were due to receive a 4th dose, in children who did not receive the 4th dose, was 0.65 (95%CI 0.50, 0.85). The strength of association an unmeasured confounder would need to have in order to explain the observed effect can be estimated to help appreciate the plausibility that there could be such a confounder. The first figure below shows the combinations of the association of a binary confounder with the vaccine, and with disease, that would be needed to explain the observed effect. For example, a confounder that was present in 75% of vaccinated children (p_1=0.75), and 15% of unvaccinated children (p_0=0.15, prevalence ratio of p_1/p_0=5), (indicated by the red dot), i.e. a characteristic that was common and was strongly associated with vaccination, would need to be associated with more than a 50% reduction in severe malaria risk, after controlling for other confounders (age, neighbourhood, socioeconomic status, caregiver education, ITN use). The lower figure shows the combinations that would be needed for the effect, adjusted for the confounder, to be reduced to an effectiveness of 10% from the observed value of 35%.





If an unmeasured confounder occurs in a proportion p_1 of the exposed and p_0 of the unexposed, then risk ratio if we were able to adjust for this unmeasured confounder, RR_{adj}, is related to the observed risk ratio adjusted for all measured confounders, RR_{obs}, by:

$$RR_{adj} = RR_{obs}\frac{p_0 RR_{CD} + (1 - p_0)}{p_1 RR_{CD} + (1 - p_1)}$$

where RR_{CD} is the risk ratio for the association of the unmeasured confounder with the disease, and it is assumed this association is constant with respect to the exposure (no interaction between the confounder and the exposure in their effect on disease), (Fox, MacLehose and Lash, 2021).

The value of RR_{CD}, the risk ratio for the association with the disease that an unmeasured confounder would have to have, such that adjusting for this confounder would result in an adjusted risk ratio of RR_{adj}, is given by:

$$RR_{CD} = \frac{RR_{obs}(p_1 - RR_{EC}) + RR_{adj}RR_{EC}(1 - p_1)}{p_1(RR_{obs} - RR_{adj}RR_{EC})}$$

where $RR_{EC}=p_1/p_0$.

When $RR_{adj}=1$ (the unmeasured confounder completely explains the observed association),

$$RR_{CD} = \frac{RR_{obs}(p_1 - RR_{EC}) + RR_{EC}(1 - p_1)}{p_1(RR_{obs} - RR_{EC})}$$

(The E value (VanderWeele and Ding, 2017) is the special case when $p_1=1$ and $RR_{EC} = RR_{CD}$, so that

$RR_{CD}^2 - 2RR_{obs}RR_{CD} + RR_{obs} = 0$, leading to the value $RR_{CD} = RR_{obs} + \sqrt{RR_{obs}^2 - RR_{obs}}$, the strength of association of an unmeasured confounder would need to have to completely explain the observed association, if the association of the confounder with the exposure was the same magnitude ($RR_{CD}= RR_{EC}$), and the confounder was present in 100% of the exposed).

Severe malaria is a rare outcome, so the rate ratio is approximately equal to the risk ratio and so these expressions can be used to calculate bias parameters that would be needed for an unmeasured confounder to be able to explain the observed associations in our study.

[1] Fox MP, MacLehose RF and Lash TL (2021) Applying Quantitative Bias Analysis to Epidemiological Data. 2nd edition. Statistics for Biology and Health. Springer, Switzerland.

[2] VanderWeele TJ and Ding P (2017) Sensitivity analysis in observational research: introducing the E value. Ann Intern Med 167:268-74.