Title: Controlled human malaria infection (CHMI) outcomes in Kenyan adults associated with prior history of malaria exposure and anti-schizont antibody response

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Abstract

Humans acquire natural immunity to malaria following repeated exposure to the parasite. Field-based studies of natural exposure are used to identify variations in host immunity in order to identify correlates of protection against infection and disease. Here we report an analysis of controlled human malaria infection (CHMI) with *Plasmodium falciparum* sporozoites NF54 strain (Sanaria® PfSPZ Challenge) in 142 adults from Kenya. Our aim was to identify *in vivo* outcomes among participants that most closely reflected naturally acquired immunity. Using anti-schizont antibody responses and location of residence as proxy markers of immunity, we show that time to endpoint (requirement of anti-malarial treatment) correlated more closely with anti-schizont antibodies and with location of residence than other parasite parameters such as growth rate or mean parasite density. In observational field-based studies in children, 0.8% of the variability in malaria outcome can be explained by anti-schizont antibodies. Here, dichotomized anti-schizont antibodies explained 17% of the variability. Thus, the CHMI model is highly effective in identifying variations in naturally acquired immunity to malaria.

Introduction

Plasmodium falciparum malaria remains a pressing global health emergency. Encouraging progress in its control has been made in some areas of Africa[1], but elimination does not appear realistic in many areas. The current lead vaccine candidate, RTS,S, is based on the circumsporozoite protein (CSP) and delivers ~50% protection against clinical manifestations of *P. falciparum* disease in children[2]. Higher vaccine efficacy against clinical manifestations might be achievable through inducing immune responses against antigens from the asexual blood-stages[3]. The clinical development pathway for any one candidate vaccine is expensive and lengthy. None of the blood-stage candidate vaccines subjected to field trials have progressed to Phase III trials[4,5].

The need to understand and interrogate naturally acquired immunity to malaria is fundamental to antigen selection and vaccine design. A common approach is to use immuno-epidemiological studies in malaria endemic regions, where immunological responses from cross-sectional surveys of children are linked with the risk of subsequent malaria episodes[6–10]. A limitation of this approach has been the reliance on uncontrolled natural but heterogenous exposure to malaria[7,8,11] as well as exposure to genetically diverse parasites[12] in the field.

Controlled Human Malaria Infection (CHMI) studies have the potential to accelerate the selection of antigens for vaccine development by controlling for malaria exposure, including parasite strain, as well as level of infectious dose. For ethical reasons, CHMI requires adult volunteers rather than children. In endemic areas immunity is acquired with age and adults usually have high levels of immunity to the consequences of infection[13]. Nevertheless, even among adults' levels of immunity may be variable. We have recently described clinical outcomes and safety of CHMI in Kenyan adults after infection with cryopreserved viable, aseptic, and purified *Plasmodium falciparum* sporozoites (PfSPZ Challenge) at a dose of 3,200 injected by syringe[14]. We showed that using CHMI in this population of 142 pre-exposed adults, 26 (18.3%) had febrile symptoms and were treated; 30 (21.1%) reached ≥500 parasites/μl and were treated; 53 (37.3%) had parasitaemia without meeting thresholds for treatment and; whilst 33 (23.2%) remained qPCR negative (in a subset of volunteers, some of those qPCR negative between days 8 and 10 post-infection had low parasitaemia in comparison to two other qPCR methods)[14]. These findings are consistent with other CHMI studies in volunteers from endemic areas[15,16]. However, the outcomes of CHMI that are most strongly associated with naturally acquired immunity have not yet been determined. Furthermore, categorizations into multi-level descriptive outcomes do not maximize analytical

power for correlates of immunity, and either a binary classification or a continuous variable would be analytically optimal.

We therefore conducted the analysis presented here using anti-schizont antibody responses and location of residence as surrogates of immunity. We examined various parameters from the patterns of parasite growth during CHMI. Our aim was to determine which parameters most closely associated with these two surrogates of immunity, and to identify whether any were more discriminatory of host immunity than the standard immuno-epidemiological studies conducted in the field.

Results

Anti-schizont antibody responses for the volunteers enrolled in the study. Data from 142 volunteers were included in the analysis as previously described[14]. The median age of the volunteers was 28 years old (range 18-45) and 30% were female. Antibody responses to schizont extract were measured for all the volunteers at screening (Supplementary Fig 1). Volunteers from Kilifi North had significantly lower anti-schizont antibodies (median of 896.2 Antibody units (AU), 95%CI 566 to 1,473) compared with volunteers from Kilifi South (median of 9,238AU, 95%CI 6,399 to 12,324, $p<0.00001$) and Ahero (median of 4,666AU, 95%CI 965.6 to 28,702, $p<0.00054$) but volunteers from Kilifi South and Ahero had similar antibody levels ($p=0.085$). For further analysis, volunteers from Kilifi North (N=34) were considered to be residents of an area of "low transmission" whilst volunteers from Kilifi South (N=93) were combined with Ahero volunteers (N=15) and considered to be residents of an area of "high transmission".

qPCR categorized outcomes in relation to location and antibody response. We had previously observed four distinct outcomes based on parasite growth as measured by qPCR following CHMI being: parasite growth by qPCR meeting the threshold criteria for malaria diagnosis (≥500 parasites/μl) either: (a) with fever (i.e. "treated febrile"); (b) without fever but reaching a parasite density requiring treatment (i.e. "treated non-febrile"); (c) with parasites detected by qPCR but not at a parasite density meeting the threshold criteria for treatment (i.e. "PCR positive untreated"); or (d) parasites not identified by qPCR throughout monitoring (i.e. "PCR negative") (Supplementary Fig. 2). Volunteers who were "treated febrile" had lowest anti-schizont antibodies and were least likely to be residents of the high transmission areas (Table 1, supplementary figure 3). The "treated non-febrile" group had intermediate levels of anti-schizont antibodies and intermediate likelihood of being residents of the high transmission area. The "untreated PCR positive" group and then "PCR negative" group had high levels of anti-schizont antibodies and were both very likely to be residents of the high transmission areas.

Those who were qPCR positive untreated could further be examined in sub-groups by dividing them into those who were positive either early, late, or throughout the period of qPCR monitoring. We did not identify any significant differences in anti-schizont antibodies or location of residence for these additional sub-groups (Supplementary Table 1).

Table 1 qPCR outcome in relation to anti-schizont antibody responses

Outcome	N	Anti-schizont antibody concentration (AU)[a]	Proportion resident in high transmission areas[b]
Treated febrile	26	794.3 (501.2 to 1,230.3)	0.31 (0.12 to 0.49)
Treated non-febrile	30	3,311.3 (1,584.9 to 7,079.5)	0.56 (0.38 to 0.75)
Untreated PCR (+)	53	8,709.6 (6,165.9 to 12,589.3)	0.98 (0.93 to 1.0)
PCR (-)	33	15,848.9 (8,912.5 to 31,622.8)	0.93 (0.86 to 1.0)

PCR (+) and PCR (-) refer to volunteers who were PCR positive and negative respectively; N is the total number of volunteers in each outcome category. [a]Median antibody responses with 95%CI in parenthesis. [b]Proportion with 95%CI in parenthesis. N number of volunteers in the analysis.

qPCR parameter associations with location and antibody response. We examined various parameters that described the qPCR results per individual volunteer (Table 2). The strongest non-parametric correlates of location of residence (i.e. residence at high vs low transmission intensity) or anti-schizont antibodies were: time to reaching a threshold of 250 parasites/μl; time to treatment; and the categorization of treatment versus no treatment (Table 2). Other parameters that were strong correlates of location of residence or of anti-schizont antibodies were highly cross correlated with each other (Supplementary Fig. 4) and we did not identify a second independent predictor using parametric analyses after adjusting for time to treatment (Supplementary Table 2). We preferred the parameter time to treatment for further analysis over the use of time to a threshold of 250 parasites/μl since some volunteers were treated at lower parasite densities leading to missing data for time to 250 parasites/μl.

Table 2 Non-parametric analysis of qPCR parameters with anti-schizont antibody responses and location

Parameter	Rho anti-schizont antibody	*p* value anti-schizont antibody	Rho location	*p* value location	N
Inoculum[a]	-0.25	**0.003**	-0.08	0.32	142
Time to treatment	0.56	**3.60e-13**	0.64	**1.85e-17**	142
Treated vs untreated	-0.54	**2.47e-12**	-0.59	**6.59e-15**	142
Mean parasite density	-0.53	**1.26e-11**	-0.44	**6.07e-08**	142
Proportion of days with parasite growth[b]	-0.54	**3.40e-12**	-0.43	**7.34e-08**	142
Proportion of days with parasite growth[c]	-0.52	**2.93e-11**	-0.41	**4.18e-07**	142
Proportion of days with declining parasite numbers	-0.34	**0.00004**	-0.26	**0.002**	142
Days of longest consecutive parasite growth	-0.5	**1.63e-10**	-0.39	**1.00e-06**	142
Median point of days with parasite growth	0.26	**0.01**	0.39	**0.00008**	96
Maximum days of consecutive decline	-0.12	0.24	-0.01	0.88	104
Median day of decline	0.02	0.82	0.05	0.61	104
Gradient	-0.45	**2.15e-08**	-0.47	**3.47e-09**	142
Variability[d]	0.19	0.06	0.06	0.53	96
Time to threshold of parasites (1/μl)	0.33	**0.00006**	0.27	**0.001**	142
Time to threshold of parasites (5/μl)	0.45	**2.83e-08**	0.49	**4.82e-10**	142
Time to threshold of parasites (50/μl)	0.54	**3.18e-12**	0.6	**2.36e-15**	142
Time to threshold of parasites (250/μl)	0.62	**9.34e-14**	0.62	**9.69e-14**	118
Time to threshold of parasites (500/μl)	0.54	**1.37e-09**	0.56	**1.71e-10**	111
Time to threshold of parasites (1,000/μl)	0.51	**1.33e-08**	0.59	**1.28e-11**	108

[a]Peak at days considered are from days 8.5 to 10 post-infection; [b]analysis of smoothed data; [c]analysis of raw data; [d]represents the summed/average day to day increase or decrease. N number of volunteers in the analysis. Analysis uses Spearman's rank-order correlation.

Survival Models. We developed a multivariable Cox regression model of time to treatment, finding both anti-schizont antibodies and location of residence to be strong independent predictors of outcome (Table 3). The presence of parasites at screening and plasma lumefantrine drug concentrations were weak predictors of outcome in univariable analysis (Table 3), but not in multivariable analysis (Multivariable 1, Table 3). Parasites at screening and lumefantrine drug concentrations were both confounded by location of residence (r=0.20, p=0.016 and r=0.30, p=0.0003 for associations with location of residence, respectively). The year of enrolment in the trial (cohort year), anti-malarial drug concentration, age, and gender were not significant predictors of outcome. In the final model (Multivariable 2), the two independent predictors were residence (i.e. at high vs low transmission) and anti-schizont antibody concentration, explaining 35% of the variability in outcome on logistic regression (Table 3, and Fig. 2).

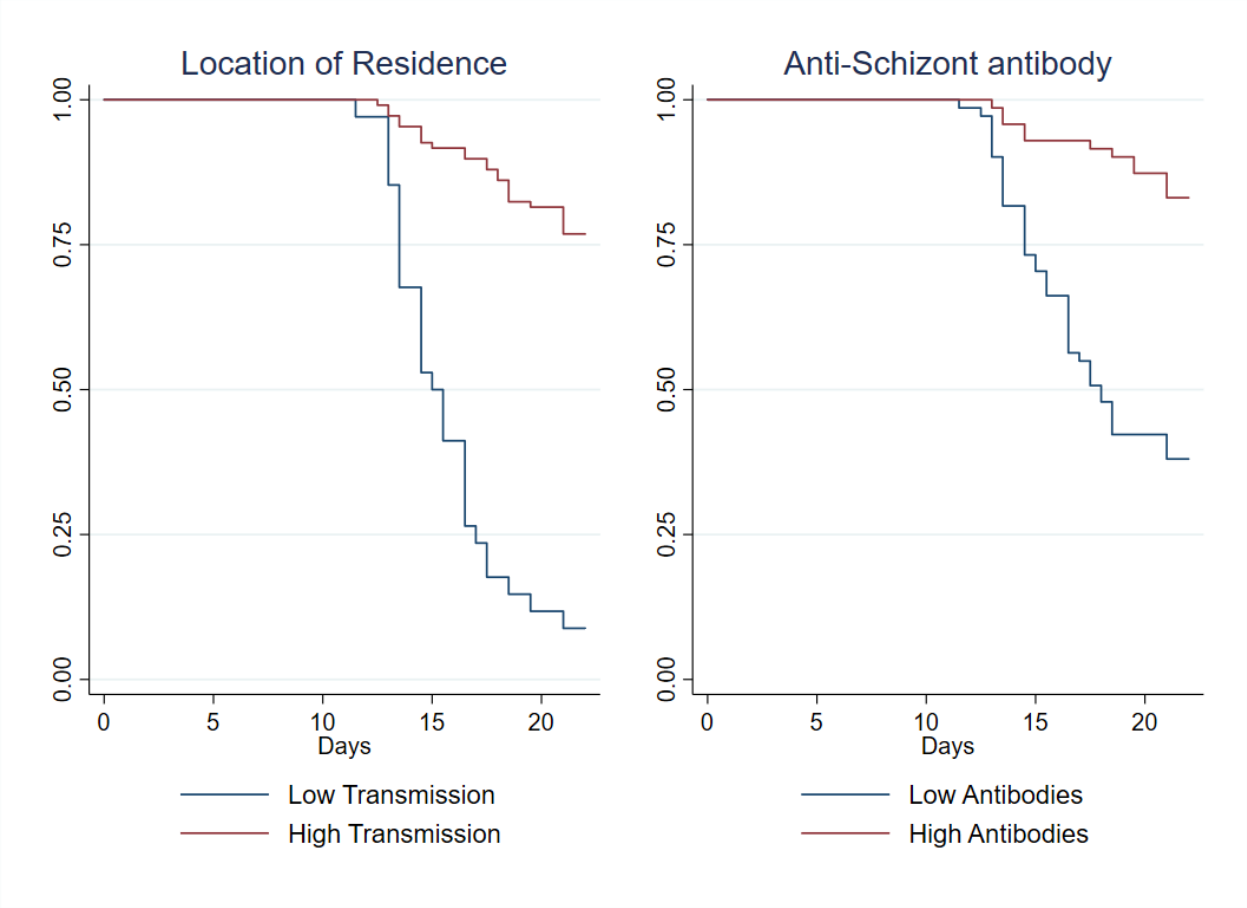

Fig. 2 Time to treatment survival analysis for location of residence (left panel) and anti-schizont antibody response (right panel).

Table 3 Cox Regression analysis of time to treatment

	Univariable			Multivariable 1			Multivariable 2		
Variable	HR	95% CI	*p* value	HR	95% CI	*p*	HR	95% CI	*p*
Cohort (i.e. one cohort per year)									
2016	1								
2017	0.74	0.42, 1.32	0.31			.			.
2018	0.87	0.51, 1.50	0.62			.			.
Age (years)	1	0.97, 1.04	0.82			.			.
Residence at low transmission[a]	1			1			1		
Residence at high transmission[a]	0.11	0.06, 0.19	**1×10^{-14}**	0.22	0.11, 0 .44	**0.00003**	0.20	0.10, 0.40	**5×10^{-6}**
Sulfadoxine[b]	1.21	0.78, 1.88	0.4			.			.
Lumefantrine[b]	0.55	0.32, 0.96	**0.04**	0.78	0.45, 1.34	0.37	.		.
Anti-Schizont	0.23	0.14, 0.36	**2×10^{-10}**	0.51	0.30, 0.88	**0.02**	0.44	0.27, 0.74	**0.0016**
qPCR (+) at screening	0.17	0.05, 0.55	**0.003**	0.39	0.11, 1.36	0.14	.		.
Sex (Male)	1.1	0.62, 1.94	0.75			.			.

[a]Residence of volunteers; [b]Log transformed concentration values used; PCR (+) volunteers, qPCR positive at screening

We compared the predictive strength of anti-schizont antibodies in the CHMI model with previous cohort studies based on natural exposure in the field[8], to determine whether the CHMI model would advance the field in examining for correlates of infection. In order to make comparisons across models, we used dichotomized anti-schizont antibody levels above and below the median for each study so as to have a bi-level comparison in each setting that was not dependent on the different range of antibody levels. We compared the pseudo R^2 in logistic regression to determine the variability in outcome explained by antibody levels in each setting. In CHMI, the odds ratio (OR) of requiring treatment based on anti-schizont antibody levels above the median was OR=0.12 (95%CI 0.06 to 0.27, $p=2\times10^{-7}$) and explained 17% of the variability. In the previously reported cohort of 121 children between the ages of 1 and 8 years found to be parasite positive at baseline, having anti-schizont antibodies above the median level was associated with OR=0.64 (95%CI 0.29 to 1.4, p=0.26) for febrile malaria, explaining 0.8% of the variability in outcome.

Survival plots from the CHMI study showed a clear distinction in time to treatment by anti-schizont antibody responses (Fig. 3, left panel), in contrast to the less clear distinction seen in field studies based on natural exposure (Fig. 3, right panel).

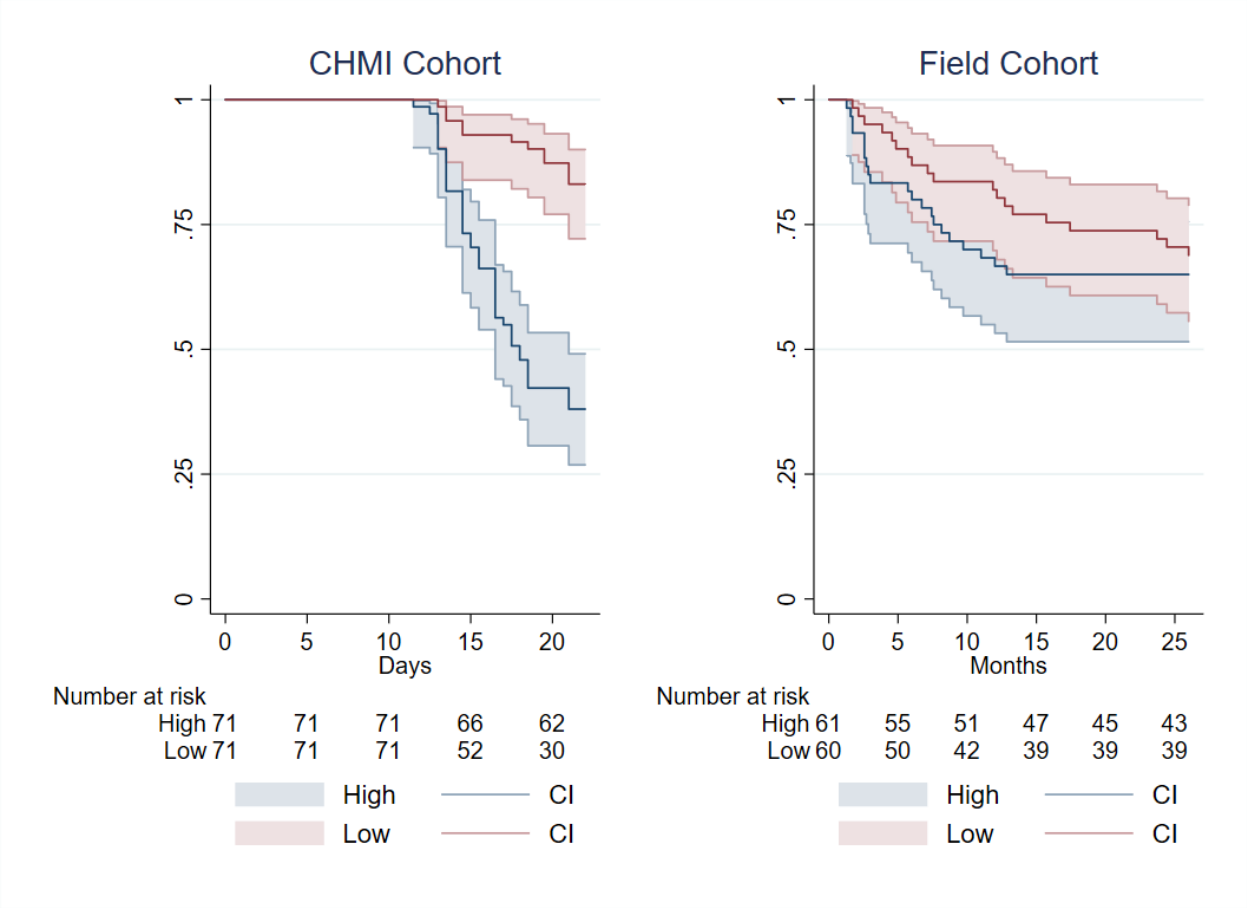

Fig. 3 Survival analysis of the CHMI cohort (left panel) and field-based cohort (right panel) in relation to requirement for treatment and anti-schizont antibody responses.

Discussion

We used serial qPCR to determine the outcomes most strongly associated with anti-schizont antibodies and with location of residence (low vs high transmission), in order to define the outcomes for CHMI in exposed adults that are most strongly associated with surrogates of immunity. We used anti-schizont antibody levels and location of residence at varying prior exposure to malaria as surrogates for immunity to malaria. We examined several potential parameters based on the qPCR monitoring done for CHMI for their association with anti-schizont antibodies and location. Time to treatment and time to 250 parasites/µl were strongly associated with anti-schizont antibodies and with location. We preferred time to treatment rather than time to 250 parasites/µl, as the former included the full set of volunteer data and avoids the potential bias of missing data from volunteers who were treated before reaching 250 parasites/µl.

After adjusting for time to treatment, there were no other independent predictors of anti-schizont antibodies or of location of residence. We therefore developed a survival analysis based on time to treatment. The combination of location of residence and anti-schizont antibodies as a continuous variable explained 35% of the variability in time to treatment in CHMI. Since prior residence and anti-schizont antibodies only offer limited information on the true extent of host immunity, this implies that a very significant proportion of the variability in outcome in CHMI is due to host immunity.

We examined whether the analysis of CHMI for naturally acquired immunity was a significant advance over previous studies conducted in the field based on natural exposure to malaria. Adults have higher levels of immunity than children, different endpoints are used for adults participating in CHMI compared with children in field observational studies, but nevertheless these study designs both share the aim to define potential correlates of immunity. In order to make

comparisons, we used logistic regression with febrile malaria as the outcome in the field studies, and with treatment criteria as the outcome in CHMI. We used anti-schizont antibodies as the predictor variable. Levels of anti-schizont antibodies higher among adults than children, so we divided antibodies into high or low categories based on the median antibody level in each study. In the field-based observational study analysed here in a cohort of children, anti-schizont antibody responses explained less than 1% of the observed variability, but anti-schizont antibodies explained 17% of the variability in CHMI outcomes. This is not surprising given the variability in exposure to malaria seen in the field[7,8], whereas in CHMI exposure is controlled and does not vary between participants. This analysis, here, shows how, adjusting and accounting for heterogeneity of exposure and infection, and given that anti-schizont antibodies in field-based studies account for a small fraction of the variability, CHMI in an adult pre-exposed population has a larger discriminatory power to study immunity in relation to past exposure. Furthermore, in non-immune CHMI studies, a large proportion of the volunteers develop illness and require treatment at relatively low parasitaemia thresholds (between 5 to 50 parasites/μL) whilst in our study, individuals were often asymptomatic and parasite-free and this could largely be as a result of differences in responses in non-immunes with semi-immunes[18,19]. With the exception of innate factors such as sickle cell trait[15], this resistance in previously malaria exposed individuals thus might be as a result of acquired adaptive immunity which is confirmed by anti-schizont antibody responses.

We thus conclude that CHMI studies in malaria endemic areas, using a standardized inoculum, are an effective platform and powerful tool with which to study host immunity. Variability in outcomes are more closely attributable to host immunity than for field-based studies based on natural exposure. Anti-schizont antibodies are generally not considered to be mechanistically

related to immunity but rather a marker of past exposure. Anti-schizont antibodies are thus likely to be cross correlated with multiple other potential mechanisms of immunity[10,17–19]. Hence in further studies of host immunity, we would expect mechanistic correlates of immunity to be independently associated with outcome after adjusting for anti-schizont antibody. Such correlates may include antigen-specific responses and functional antibodies. Previous immuno-epidemiological studies have identified several immunological markers much more strongly associated with immunity than anti-schizont antibody[20], and these findings can now be tested using CHMI in malaria endemic areas.

Methods

Study design and population. The full protocol has previously been published[21]. Briefly, data from the CHMI-SIKA study, which was an open, un-blinded and non-randomised with all volunteers receiving an intravenous injection (direct venous inoculation (DVI)) of PfSPZ Challenge PfNF54 strain (i.e. cryopreserved, infectious sporozoites). A dose of $3.2x10^3$ PfSPZ was administered after which volunteers were monitored for blood parasitaemia by quantitative polymerase chain reaction (qPCR) to determine parasite growth. The $3.2x10^3$ PfSPZ dose was selected because this has infected 100% malaria-naïve volunteers undergoing CHMI in studies in the US and EU [22,23]. PfNF54 is African in origin and it is therefore expected that < 100% of African volunteers with well-developed naturally acquired immunity will become infected[24]. The study was conducted at the KEMRI Wellcome Trust Research Programme in Kilifi, Kenya and received ethical approval from the KEMRI Scientific and Ethics Review Unit (KEMRI//SERU/CGMR-C/029/3190) and the University of Oxford Tropical Research Ethics Committee (OxTREC 2-16).

The study was registered on ClinicalTrials.gov (NCT02739763), conducted based on good clinical practice (GCP), and under the principles of the Declaration of Helsinki.

Anti-malarial drug concentration. We excluded those with drug levels above the minimum inhibitory concentration (MIC) for lumefantrine but retained those with levels below the MIC for sulfadoxine (in absence of pyrimethamine) and with trace levels of chloroquine as described previously[14].

Anti-schizont antibody measurement. Plasma samples were tested by ELISA for the presence of human IgG against schizont extract as described previously[10,18]. In brief, *P. falciparum* 3D7 strain parasites were cultured from which trophozoites were isolated using magnetic cell separation (MACS) with >90% purity and then cultured to schizont stage. The schizont extract was prepared by spinning the culture for 5minutes at 1800rpm, diluted in carbonate bicarbonatre buffer (ration of 1:5), sonicated for 30minutes followed by three freeze thaw cycles. To run the ELISAs, the extract was used to coat high absorbance plates at an established concentration shown to have saturation of responses using plasma from hyper immune individuals and stored at 4°C overnight. The plates were washed in phosphate-buffered saline (PBS) with 0.05% Tween 20 (PBS-T) and blocked with 1% w/v dried skimmed milk powder in PBS-T. 100 microliter of each plasma sample were added to duplicate plates at a final dilution of 1/1000 in PBS-T. Plates were developed using horse radish peroxidase–conjugated antihuman IgG (DAKO) at a dilution of 1:5000 in blocking buffer with H_20_2 as substrate and OPD (Sigma) as the colorimetric indicator. Tests were repeated if duplicate optical density (OD) values for an individual plasma sample varied by more than a factor of 1.5. A pool of serum samples from an area in Africa where malaria is highly endemic

was titrated on each plate and acted both as a positive control and provided values for a standard curve for converting optical density (OD) readings into concentrations (Antibody units, AU), minimizing inter-plate and inter-day variations. previously

Location of residence. Volunteers were recruited from differing malaria endemic regions in Kenya: Ahero in Western Kenya (moderate to high transmission region); Kilifi North on the Kenyan Coast (low to no malaria transmission); and Kilifi South (moderate transmission region)[1,25]. In this analysis volunteers from Ahero and Kilifi South were combined as resident at "high transmission" intensity and Kilifi North taken as resident at "low transmission" intensity.

Parasite detection by qPCR. For parasite detection, venous blood samples were collected twice every day from days 8 to 15 of CHMI and then once every day from days 16 to 22 of CHMI for qPCR analysis by detection of the 18S ribosomal RNA *P. falciparum* gene. A sensitive high-volume qPCR assay was used in real time for detection where 500 µl of whole venous blood were used for automated DNA extraction and purification (QIAsymphony machine, Qiagen, Germany) according to the manufacturer's instructions. From this, 100 µl of DNA was eluted from which 13.5µl was used to amplify the 18S ribosomal RNA gene by qPCR in triplicates in a TaqMan assay using primers and probes previously described on an Applied Biosystems 7500 real-time PCR system by Applied Biosystems 7500 quantification software. Non-template control was used as a negative control (in triplicate wells) with parasite quantification against known cultured parasite standards comprising of 6 serial dilutions of extracted DNA also run in triplicates. Therefore 40.5 µl of 100 µl of elute were assayed, corresponding to extracted material from 202.5 µl of whole blood.

The final qPCR result per sample was taken as the geometric mean of the three replicate assays. The cultured parasite standards were produced in 3 different batches. We re-ran selected samples from each CHMI cohort against a final set of standards, including the WHO external quantified quality control sample, to normalize quantities against the WHO standard.

Statistical analysis. PCR results are presented as the geometric mean of three replicate assays at each timepoint. Time to treatment was the number of days between challenge and the treatment decision taken either because: (a) the volunteer reached the pre-assigned threshold of 500 *falciparum* parasites/µl by qPCR; (b) they had developed febrile symptoms and clinicians had treated them at lower parasite density; or (c) they reached the end of the study without reaching parasite density threshold or having symptoms. Other parameters to describe the outcomes were derived from qPCR results as follows: (1) the time to particular parasite density thresholds, where volunteers not reaching those thresholds were described as missing data; (2) the "proportion of days growing" where any consecutive increase in parasite density is considered a "day growing" (this was calculated from raw data, then also from smoothed data taking the moving average over 2 days); (3) the mean parasite density as a geometric mean, excluding timepoints after treatment; (4) the maximum number of days of continuous consecutive growth; (5) the gradient of growth from a best-linear-fit of the period defined in (4); (6) the median number of days since challenge for the days of parasite growth as defined in (2); (7) the converse of (2), (4), and (5); (8) for days of decline in parasite density rather than growth; (9) the "inoculum" defined as the peak parasite density observed between days 8.5 and 10 after challenge and; (10) the "variability" calculated as the summed day-to-day variation in parasite density.

Kruskal-Wallis tests with multiple comparisons were used to compare anti-schizont antibodies by location and qPCR outcome and Spearman's rank correlation was used to explore correlations between anti-schizont antibody, location, and qPCR parameters.

Survival models were developed using Cox regression in three stages; (a) univariable analysis of all potential independent predictors; (b) multivariable analysis including significant predictors from (a); second multivariable analysis retaining only significant predictors from (b). The variability in outcome explained by anti-schizont antibodies was calculated using pseudo r^2. To compare the CHMI cohort with a previous field study of children, the antibody levels were divided into two groups (above and below the median), and analysis of the child cohort was restricted to the asymptomatically infected group where the protective effect of anti-schizont antibodies had been shown to be most evident[7,9,10].

Data availability

Data will be made available including data dictionaries after de-identification of volunteers. The data will be available to researchers who submit requests to dgc@kemri-wellcome.org to gain access to the data following a signed data access agreement. The study protocol, informed consent forms, and all other associated documents have been previously published.

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Acknowledgments

This work was supported by a Wellcome Trust grant (107499). We would like to thank all the study volunteers who participated in the CHMI-SIKA study and contributed data to this analysis. We are also very thankful to the larger study teams in Kilifi and Ahero specifically all the fieldworkers and health community workers who recruited volunteers; data entry clerks; clinical, pharmacy, and laboratory teams; and the collaborating manufacturing, quality systems, regulatory, pharmaceutical operations and clinical teams at Sanaria Inc. without whom this work would not have been possible. This manuscript is published with permission and/or approval of the Director KEMRI.

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Contributions

M.C.K and P.B designed the study and wrote the first draft of the manuscript. M.C.K., P.B., and E.O. analysed the data. D.K, J.M.N., Z.L., Jo.M., K.S.M., and Dw.O., performed molecular assays for parasite detection and quantification. M.C.K., R.K., and J.T. performed assays for anti-schizont antibody detection. M.H., P.N., J.M., O.N., Fr.O., A.I.A., Si.K., Sa.K., C.K., Mo.M, Je.M., Mi.M., Jd.M., Ke.M., I.N., D.O. J.O., .J.S., J.W., and T.N.W. contributed to data collection. B.K.L.S., P.F.B., Y.A., S.L.H., E.R.J., and T.L.R. contributed to sporozoite preparation and manufacture.

M.C.K., P.B., P.C.B., K.M., S.L.H., F.N., B.O., and Fa.O conceived the study. P.B., P.C.B., K.M., S.L.H., F.N., B.O., and Fa.O acquired the funding for the study. M.C.K. and P.B. led the study team. All authors contributed to interpretation of the analyses and revised the draft manuscript.

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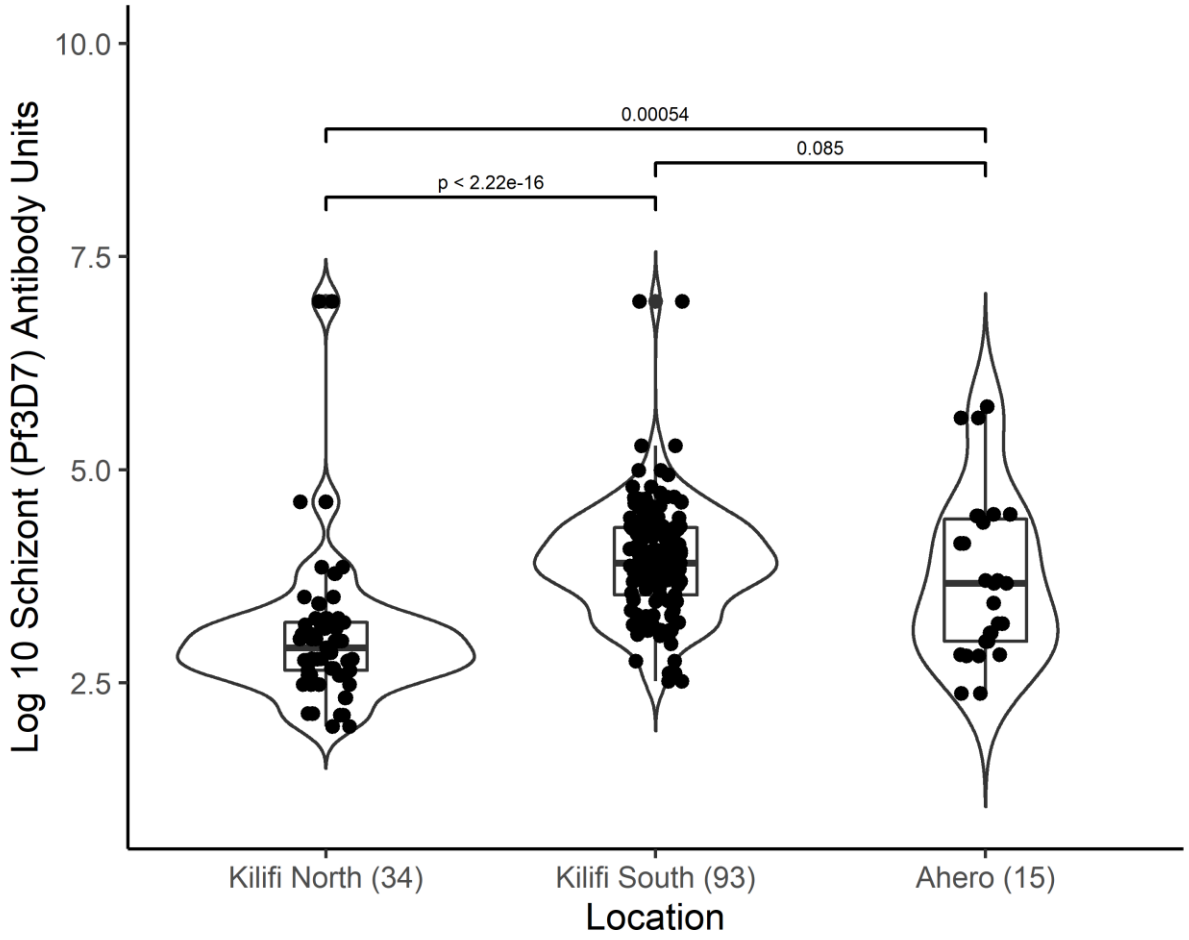
Ethical declarations

Competing interests

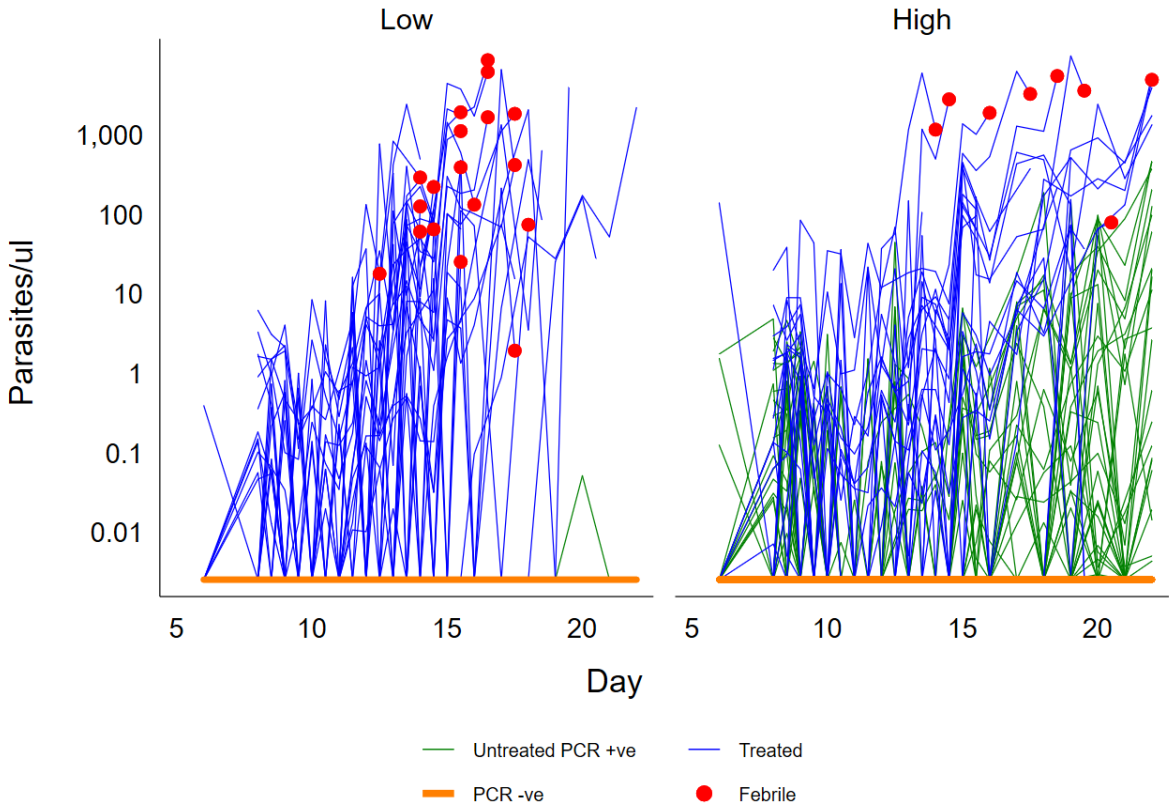
B. K. L. S., Y. A., P. F. B., S. L. H, E.R.J., TR. are salaried, full-time employees of Sanaria Inc., the manufacturer of Sanaria PfSPZ Challenge. Thus, all authors associated with Sanaria Inc have potential conflicts of interest. All other authors declare no competing interests.

Supplementary information

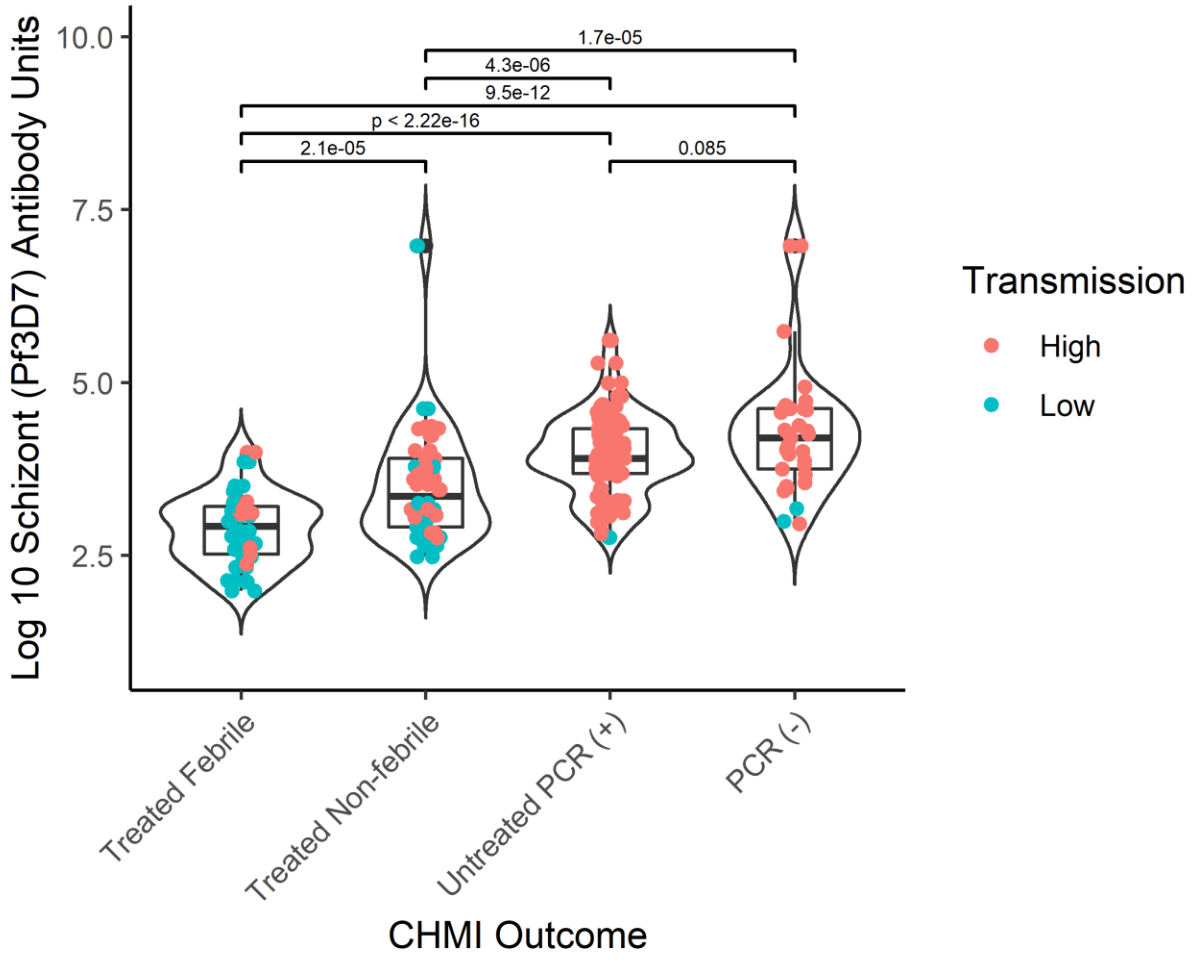
Supplementary Figures and Tables



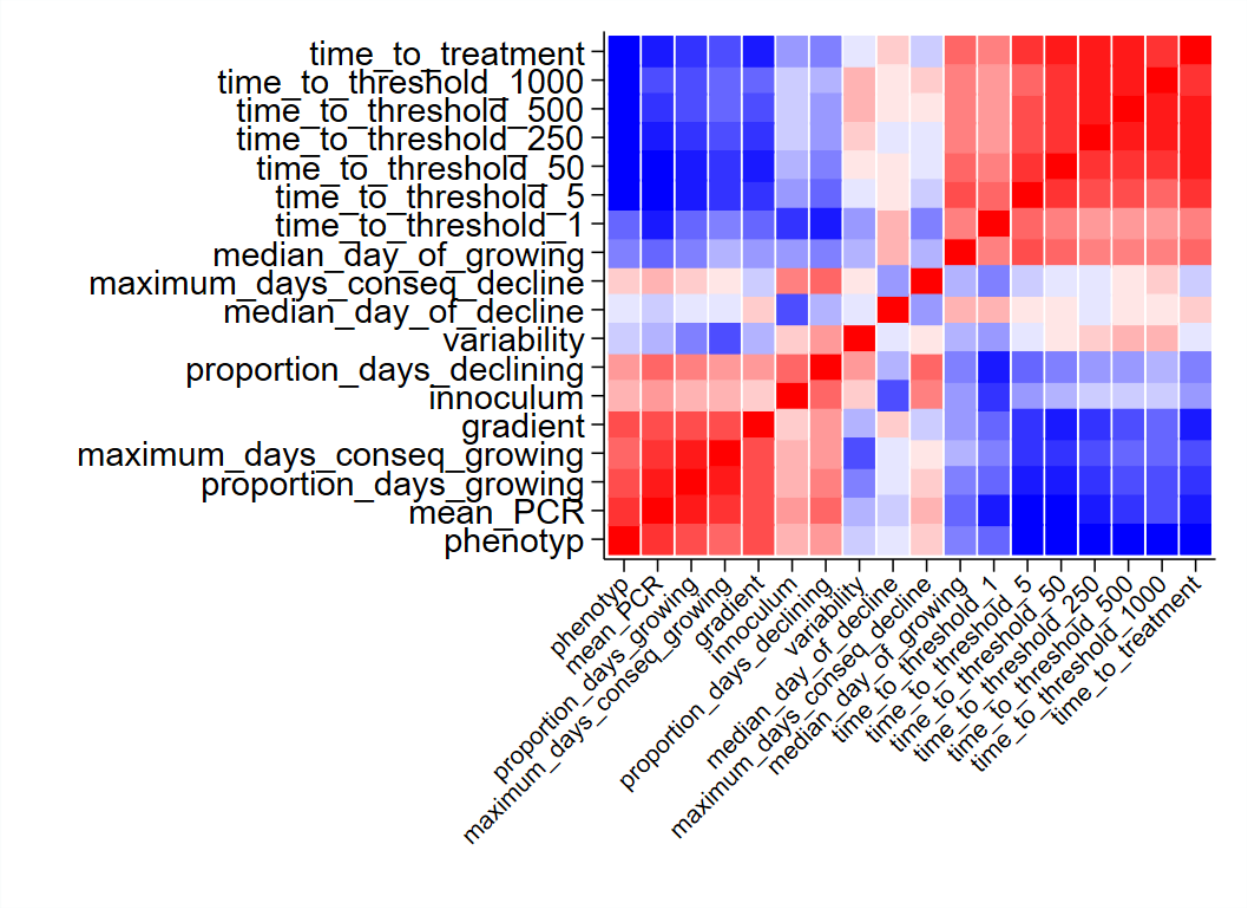
Supplementary Fig. 1 Schizont antibody responses by volunteer location. Violin plots of the anti-schizont antibody units (AU) measured from each of the 142 volunteers at screening by ELISA. Plasma samples obtained at screening from each individual volunteer was assessed for IgG specific responses to schizont extract. Indicated within each violin plat are boxplots with the median (dark solid line), minimum and maximum. Each individual is represented by each individual black closed circle. NS not significant.



Supplementary Fig. 2 Parasite growth based on qPCR outcome and location. Blood samples from C+8 onwards after inoculation were assessed by qPCR to determine parasitaemia from individuals from low transmission (left panel, Kilifi North – N=34) and high transmission (right panel, Kilifi South – N=93 and Ahero – N=15). Parasitaemia was determined by asexual 18S ribosomal RNA gene qPCR done in Kilifi. Blue lines represent individuals who required treatment and reached diagnosis threshold (Treated); green lines represent individuals who did not meet the diagnosis threshold but were qPCR positive (Untreated PCR +ve); orange lines represent individuals who were qPCR negative throughout monitoring (PCR -ve); and red dot denotes individuals who were febrile and who required treatment and reached diagnosis threshold (Febrile).



Supplementary Fig. 3 Anti-schizont antibody titres in relation to CHMI qPCR category outcomes. Violin plots of the anti-schizont antibody units (AU) measured from each of the 142 volunteers at screening by ELISA. Plasma samples obtained at screening from each individual volunteer was assessed for IgG specific responses to schizont extract. Indicated within each violin plat are boxplots with the median (dark solid line), minimum and maximum. Each individual is represented by each individual closed circle based on either low transmission (blue) or high transmission (peach).



Supplementary Fig. 4 Spearman correlations of qPCR metrics. Correlation matrix of qPCR metrics showed collinearity for phenotype (treated vs untreated), time to thresholds (1, 000 parasites/µl; 500 parasites/µl; 250 parasites/µl; 50 parasites/µl; 5 parasites/µl; and 1 parasite/µl) or to diagnosis, and mean qPCR/gradient/days of growing (maximum days consecutively growing; median days of growth; proportion of days growing) or decline (maximum days consecutively decline; median days of decline; proportion of days declining), and other parasite growth metrics. Inoculum represents peak at days from days 8.5 to 10 post-infection; proportion of days with parasite growth represents analysis of smoothed data; proportion of days with parasite growth represents analysis of raw data; and variability represents the summed/average day to day increase or decrease.

Supplementary Table 1 Sub-group analysis of qPCR outcome in relation to anti-schizont antibody responses

		Anti-schizont antibody concentration (AU)	Proportion resident at high transmission
Outcome	N	Odds Ratio	
Treated non-febrile	30	0.52 (0.20 to 0.85)	0.56 (0.38 to 0.75)
Treated febrile	26	-0.1 (-0.3 to 0.09)	0.31 (0.12 to 0.49)
Untreated PCR (+) – early/late	19	0.85 (0.50 to 1.1)	1 (1 to 1)
Untreated PCR (+) – early	18	1.2 (1.0 to 1.4)	1 (1 to 1)
Untreated PCR (+) – late	16	0.76 (0.50 to 1.0)	0.93 (0.81 to 1.0)
PCR (-)	33	1.2 (0.95 to 1.5)	0.93 (0.86 to 1.0)

p=0.15 for the outcome categories on log likelihood ratio testing compared with the outcome categories for Table 1 in predicting schizont antibody concentrations. AU, antibody units. [a]Median antibody responses with 95%CI in parenthesis. [b]Proportion with 95%CI in parenthesis. N number of volunteers in the analysis.

Supplementary Table 2 Parametric analysis of qPCR parameters with anti-schizont antibody responses and location

Parameter	Anti-schizont antibody concentration (AU)	*p* value anti-schizont antibody	Location	*p* value Location
Inoculum[a]	955.0 (776.3 to 1,202.3)	0.74	0.53 (0.07 to 1)	**0.03**
Mean parasite density	955.0 (645.7 to 1,445.4)	0.86	0.7 (-0.08 to 1.47)	0.08
Proportion of days with parasite growth[b]	354.8 (794.3 to 1,258.9)	0.11	-0.42 (-2.7 to 1.93)	0.73
Proportion of days with parasite growth[c]	446.7 (63.1 to 3,235.9)	0.42	1.66 (-2.2 to 5.57)	0.4
Proportion of days with declining parasite numbers	1,348.9 (120.2 to 14,791.1)	0.81	2.86 (-1.5 to 7.29)	0.21
Days of longest consecutive parasite decline	933.3 (691.8 to 1,258.9)	0.64	0.48 (-0.2 to 1.15)	0.17
Median point of days with parasite growth	831.8 (707.9 to 955.0)	**0.02**	-0.23 (-0.61 to 0.15)	0.24
Median point of days with declining parasites	1,000.0 (912.0 to 1,071.5)	0.83	-0.1 (-0.28 to 0.07)	0.25
Maximum days of consecutive decline	933.3 (851.1 to 1,000.0)	**0.03**	-0.06 (-0.17 to 0.05)	0.31
Gradient	1,288.3 (912.0 to 1,819.7)	0.14	0.16 (-0.27 to 0.6)	0.46
Variability[d]	1,122.0 (1,023.3 to 1,230.3)	**0.01**	0.13 (-0.04 to 0.29)	0.13

[a]Peak at days considered are from days 8.5 to 10 post-infection; [b]analysis of smoothed data; [c]analysis of raw data; [d]represents the summed/average day to day increase or decrease. AU, antibody units.