

Transmission-blocking activity of artesunate, chloroquine and methylene blue on Plasmodium vivax gametocytes

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Foreword

This analysis was carried out by Dr. Victor Chaumeau* under the supervision of Dr. James A. Waston, who wrote the Stan code for the Bayesian multi-level model used in this study.

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Session information

```
## R version 4.2.2 Patched (2022-11-10 r83330)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Debian GNU/Linux 12 (bookworm)
##
## Matrix products: default
## BLAS:   /usr/lib/x86_64-linux-gnu/atlas/libblas.so.3.10.3
## LAPACK: /usr/lib/x86_64-linux-gnu/atlas/liblapack.so.3.10.3
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8      LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8  LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8     LC_NAME=C
##  [9] LC_ADDRESS=C             LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods    base
##
## other attached packages:
##  [1] lme4_1.1-35.1      Matrix_1.6-5      forcats_1.0.0      stringr_1.5.1
##  [5] dplyr_1.1.4        purrr_1.0.2       readr_2.1.5        tibble_3.2.1
##  [9] ggplot2_3.4.4      tidyverse_1.3.2   EnvStats_2.8.1     scales_1.3.0
## [13] plotrix_3.8-2      rstan_2.32.5      StanHeaders_2.32.5 tidyr_1.3.1
## [17] extrafont_0.19     RColorBrewer_1.1-3
##
## loaded via a namespace (and not attached):
##  [1] httr_1.4.5          splines_4.2.2      jsonlite_1.8.8
##  [4] QuickJSR_1.1.3      modelr_0.1.11      RcppParallel_5.1.7
##  [7] stats4_4.2.2        googlesheets4_1.0.1 cellranger_1.1.0
## [10] yaml_2.3.8          lattice_0.22-5     Rttf2pt1_1.3.12
## [13] pillar_1.9.0        backports_1.4.1    glue_1.7.0
## [16] extrafontdb_1.0     digest_0.6.34      minqa_1.2.6
## [19] rvest_1.0.3         colorspace_2.1-0   htmltools_0.5.7
## [22] pkgconfig_2.0.3     broom_1.0.5        haven_2.5.4
## [25] tzdb_0.4.0          timechange_0.3.0   googledrive_2.0.0
## [28] generics_0.1.3     withr_3.0.0        cli_3.6.2
## [31] magrittr_2.0.3      crayon_1.5.2       readxl_1.4.3
## [34] evaluate_0.23       fs_1.6.3           fansi_1.0.6
## [37] nlme_3.1-162        MASS_7.3-60.0.1    xml2_1.3.3
## [40] pkgbuild_1.4.3      tools_4.2.2        loo_2.6.0
## [43] hms_1.1.3           gargle_1.3.0       lifecycle_1.0.4
## [46] matrixStats_1.2.0   V8_4.2.2           munsell_0.5.0
## [49] reprex_2.1.0        compiler_4.2.2     rlang_1.1.3
## [52] nloptr_2.0.3        grid_4.2.2         rstudioapi_0.15.0
## [55] rmarkdown_2.25      boot_1.3-28.1      gtable_0.3.4
## [58] codetools_0.2-19    inline_0.3.19      DBI_1.2.1
## [61] curl_5.0.0          R6_2.5.1           gridExtra_2.3
## [64] lubridate_1.9.3     knitr_1.45         fastmap_1.1.1
## [67] utf8_1.2.4          stringi_1.8.3      parallel_4.2.2
## [70] Rcpp_1.0.12         vctrs_0.6.5        dbplyr_2.4.0
## [73] tidyselect_1.2.0    xfun_0.41
```

Summary of assay runs

Table 1: Table 1. Summary of assay runs

					No. dis- sected pairs of salivary glands in baseline batches	No. dis- sected pairs of salivary glands in control batches	No. dis- sected pairs of salivary glands in treated batches
No. of blood sam- ples	No. base- line batches	No. con- trol batches	No. con- trol batches	No. treated baseline batches	No. dis- sected guts in control batch	No. dis- sected guts in treated batch	No. dis- sected guts in treated batch
AS	9	9	36	36	450	1797	1798
CQ	21	21	84	84	1008	4026	4036
MB	8	8	32	32	404	1592	1599

38 vivax malaria patients took part in the study and provided a blood sample before receiving antimalarial drug regimen, 342 mosquito batches were fed on these samples and 20908 specimens were dissected for assessment of either oocyst or sporozoite count.

Baseline characteristics of the samples

Asexual parasitaemia

```
quantile(dta$AsexualParasitaemia, probs = c(0.5, 0.25, 0.75))

##          50%          25%          75%
## 13160.700  6980.831 27798.019

aggregate(AsexualParasitaemia~Drug, FUN = quantile, probs = c(0.5,0.25,0.75), data = dta)

##   Drug AsexualParasitaemia.50% AsexualParasitaemia.25% AsexualParasitaemia.75%
## 1   AS                12108.60                8257.90                16608.38
## 2   CQ                12740.00                3610.45                21652.31
## 3   MB                17717.60                10116.38                28675.35
```

Gametocytæmia

```
quantile(dta$Gametocytæmia, probs = c(0.5,0.25,0.75))

##          50%          25%          75%
## 1091.5500  473.2875 2009.2125

aggregate(Gametocytæmia~Drug, FUN = quantile, probs = c(0.5,0.25,0.75), data = dta)

##   Drug Gametocytæmia.50% Gametocytæmia.25% Gametocytæmia.75%
## 1   AS                766.700                524.475                1350.250
## 2   CQ                897.750                195.000                1849.175
## 3   MB                1821.050                1308.666                4062.525
```

Oocyst index

```
quantile(dta_agg$`NbOocysts > 0`, probs = c(0.5,0.25,0.75))

## 50% 25% 75%
## 0.96 0.84 0.98
```

```
aggregate(`NbOocysts > 0`~Drug, FUN = quantile, probs = c(0.5,0.25,0.75), data = dta_agg)
```

```
## Drug NbOocysts > 0.50% NbOocysts > 0.25% NbOocysts > 0.75%
## 1 AS 0.9800000 0.9400000 0.9800000
## 2 CQ 0.9591837 0.6800000 0.9800000
## 3 MB 0.9600000 0.9158824 0.9850000
```

Oocyst count

```
## 50% 25% 75%
## 63.750 4.125 124.875

## Drug NbOocysts.50% NbOocysts.25% NbOocysts.75%
## 1 AS 95.000 48.500 127.000
## 2 CQ 18.500 2.000 92.000
## 3 MB 114.500 54.625 183.875
```

Sporozoite index

```
quantile(dta_agg$`NbSporozoites2 > 0`, probs = c(0.5,0.25,0.75))
```

```
## 50% 25% 75%
## 0.70 0.35 0.90
```

```
aggregate(`NbSporozoites2 > 0`~Drug, FUN = quantile, probs = c(0.5,0.25,0.75), data = dta_agg)
```

```
## Drug NbSporozoites2 > 0.50% NbSporozoites2 > 0.25% NbSporozoites2 > 0.75%
## 1 AS 0.60000 0.10000 0.70000
## 2 CQ 0.65000 0.27500 0.85625
## 3 MB 0.95000 0.87500 1.00000
```

Sporozoite count

```
quantile(dta_agg$NbSporozoites2, probs = c(0.5,0.25,0.75))
```

```
## 50% 25% 75%
## 211 5 4321
```

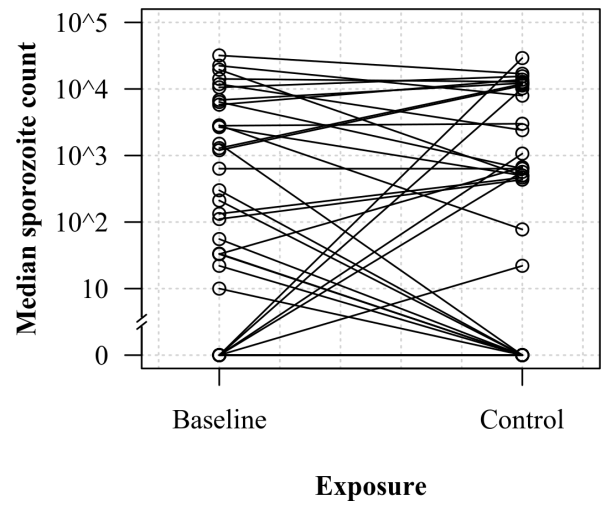
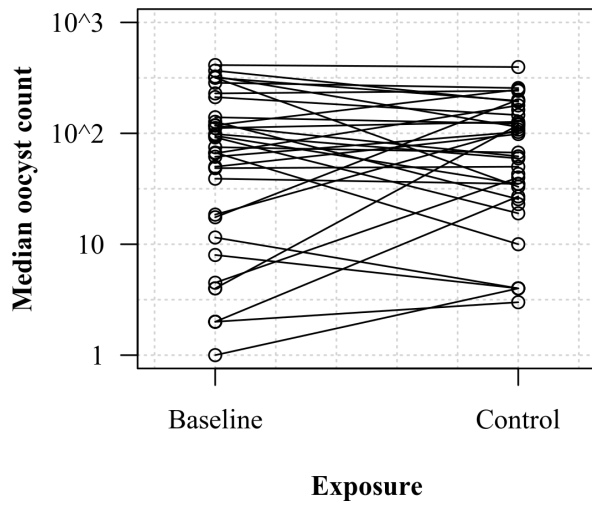
```
aggregate(NbSporozoites2~Drug, FUN = quantile, probs = c(0.5,0.25,0.75), data = dta_agg)
```

```
## Drug NbSporozoites2.50% NbSporozoites2.25% NbSporozoites2.75%
## 1 AS 33.0 0.0 211.0
## 2 CQ 122.0 2.5 2377.5
## 3 MB 4604.5 2402.5 10876.0
```

Overall, the median asexual parasitaemia was 13161 parasites /uL (inter-quartile range, IQR: 6981 to 27798) and the median gametocytaemia was 1092 gametocytes /uL (IQR: 473 to 2009). All blood samples but one were infectious to mosquitoes (97.4%, the sample that was not infectious during baseline became infectious after 24 hours of incubation). The median oocyst index (i.e., the proportion of mosquitoes harboring malaria oocysts) and oocyst count in mosquito samples were 0.96 (IQR: 0.84 to 0.98) and 63.8 oocysts per mosquito (IQR: 4.1 to 124.9). The corresponding figures for the sporozoite stage were 0.7 (IQR: 0.35 to 0.9) and 211 sporozoites per mosquito (IQR: 5 to 4321).

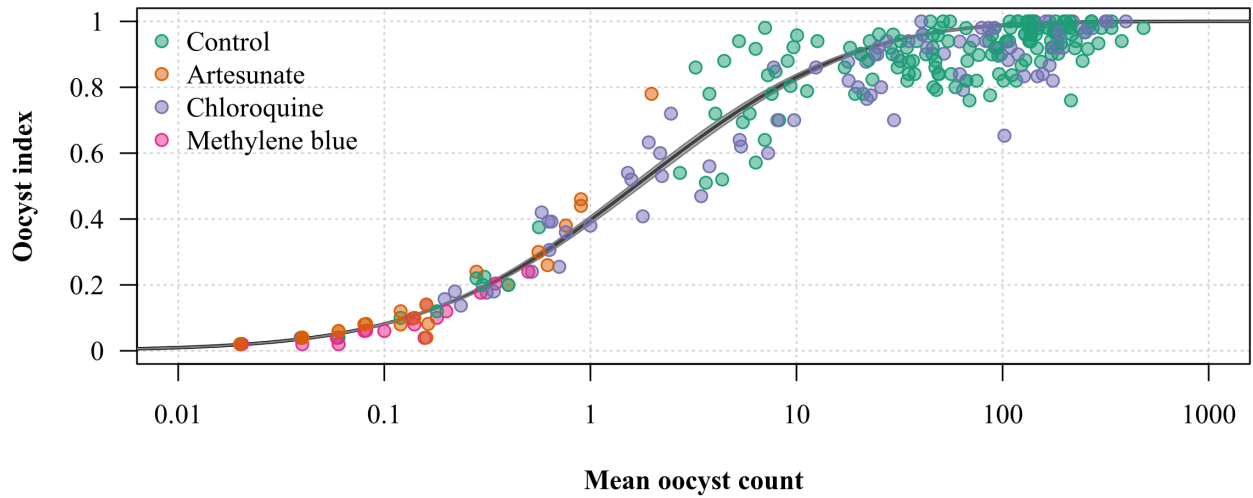
Evolution of parasite count during incubation ex vivo

The median ratio of the median parasite count in the controls to the median parasite count at baseline was 0.93 (IQR: 0.51 to 3.68) and 0.9 (IQR: 0.01 to 6.56) for the oocyst and sporozoite stage, respectively.

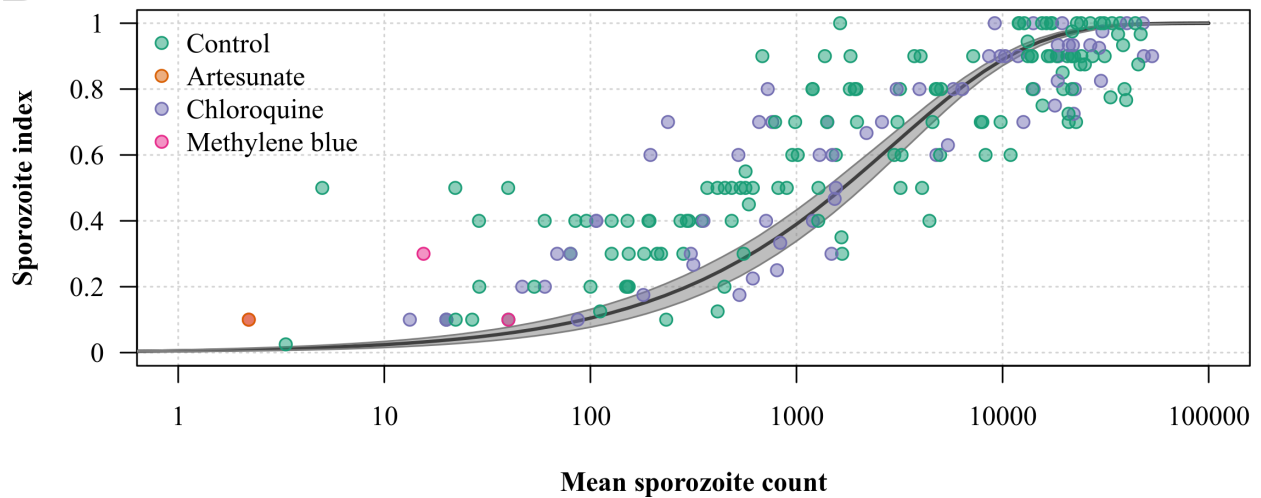


Prevalence estimates

A



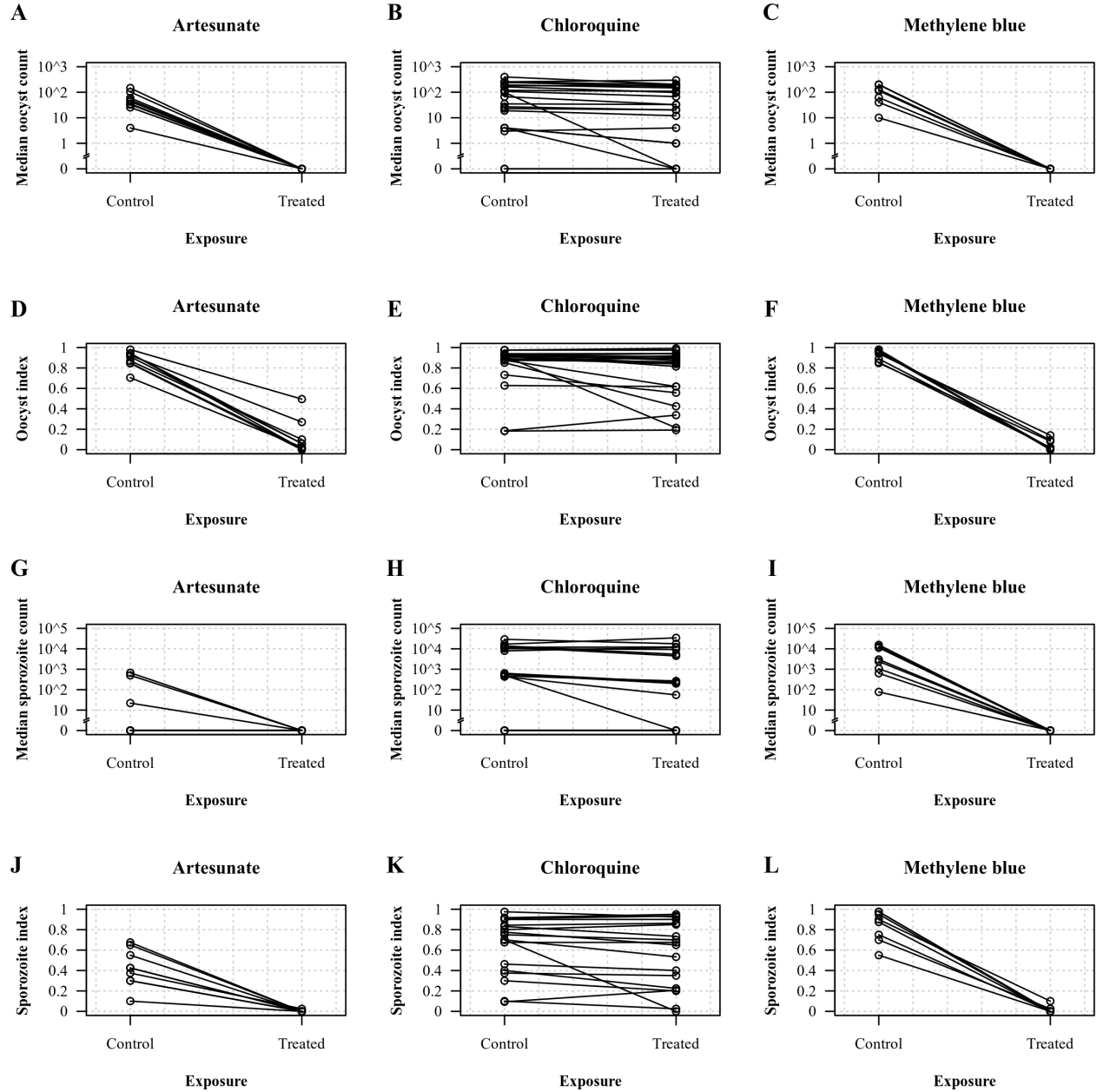
B



Drug effects on parasite count and parasite index

Chloroquine exhibited little transmission-blocking activity on *P. vivax* gametocyte transmission, despite the high concentrations used (Figure 1). Of all dissected mosquitoes in the chloroquine spiked samples, 2974/4036 (73.69%) carried oocysts in the treated replicates compared with 3299/4026 (81.94%) in the controls (relative risk, RR: 0.85 [95%CI 0.7 to 0.98], $\log_{10}[\text{p-value}] = -24.73$), and 701/1177 (59.56%) carried sporozoites in the treated replicates compared with 785/1228 (63.93%) in the controls (RR: 0.89 [95%CI 0.86 to 0.92], $\log_{10}[\text{p-value}] = -2.3$). In contrast, artesunate and methylene blue almost completely interrupted gametocyte transmission. For artesunate, only 207/1798 (11.51%) of the mosquitoes carried oocysts in the treated replicates compared with 1591/1797 (88.54%) in the controls (RR: 0.04 [95%CI 4e-04 to 0.94], $\log_{10}[\text{p-value}] = -258.52$) and 1/360 (0.28%) dissected mosquitoes carried sporozoites in the treated replicates versus 152/360 (42.22%) in the controls (RR: 0.0078 [95%CI 2e-04 to 0.31], $\log_{10}[\text{p-value}] = -8.14$); for methylene blue, only 76/1599 (4.75%) carried oocysts in the treated replicates versus 1470/1592 (92.34%) in the controls (RR:

0.017 [95%CI 1e-04 to 0.93], $\log_{10}[\text{p-value}] = -255.89$) and only 5/320 (1.56%) carried sporozoites in the treated replicates versus 267/320 (83.44%) in the controls (RR: 0.0045 [95%CI 2e-05 to 0.6], $\log_{10}[\text{p-value}] = -32.12$).



Look at model coefficient estimates

As observed previously, there was considerable heterogeneity in the count data across mosquitos and a considerable variability in the count data across blood samples and experimental batches. To account for this heterogeneity and variability, we estimated the drug effects under a Bayesian hierarchical model (mixed effects) whereby the count data are modelled as negative binomial with the dispersion parameter as a parametric function of the mean count (Table 2; see Methods) (20). In contrast to the previous data describing proportions of mosquitos with parasites, the model parameterizes the drug effect as a reduction in the mean number of parasites per mosquito, accounting for variability across blood samples and mosquito

batches.

Table 2: Parameter estimates given by the output of transmission-blocking activity model fitted to oocyst and sporozoite data

	Oocyst_Median	Oocyst_CI	Sporozoite_Median	Sporozoite_CI
mu_population	44.6000	31.3 to 63.7	4906.6000	2875 to 8158.8
sigma_patient	0.9200	0.74 to 1.15	1.2200	0.98 to 1.54
sigma_batch	0.4600	0.41 to 0.53	0.3100	0.23 to 0.41
a0	0.4091	0.3719 to 0.4469	0.0007	4e-04 to 0.0012
a1	0.1865	0.1637 to 0.2099	0.6103	0.5556 to 0.6826
artesunate_effect	0.0021	0.0015 to 0.0029	0.0068	0.0021 to 0.0165
chloroquine_effect	0.7142	0.606 to 0.8359	0.7444	0.6032 to 0.8951
methylene_blue_effect	0.0007	5e-04 to 0.001	0.0019	8e-04 to 0.0041
baseline_oocyst_count	1.8000	1.2 to 2.5	1.2000	0.7 to 2
baseline_parasitemia	1.1000	0.6 to 2.1	1.2000	0.5 to 3
baseline_gametocytemia	6.5000	3 to 13.4	3.6000	1.2 to 9.2
wash	0.8000	0.4 to 1.7	0.3000	0.1 to 0.6

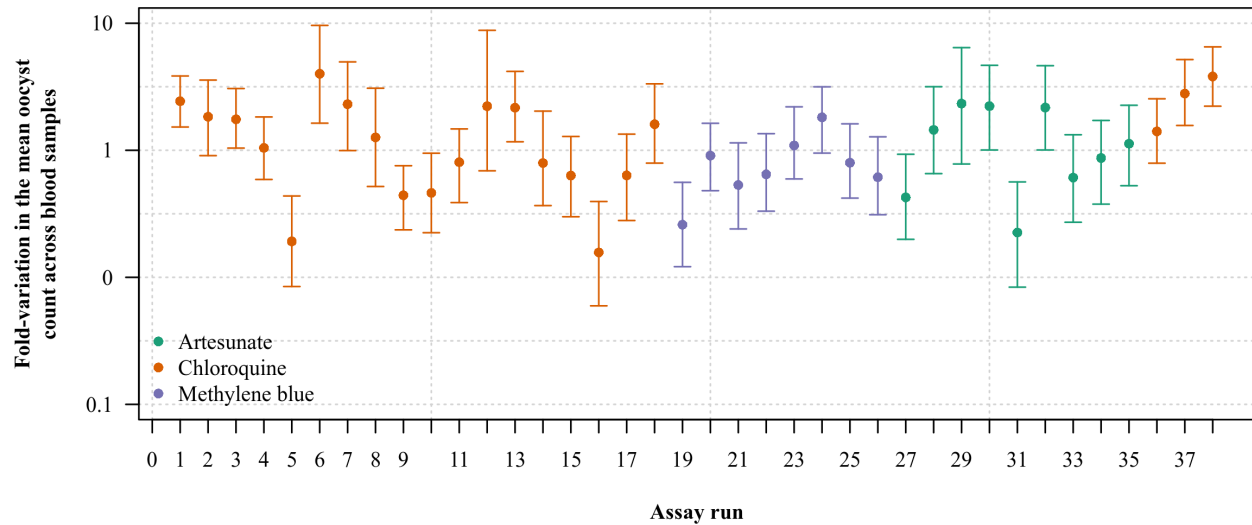
Under the model, gametocyte exposure to chloroquine decreased the mean oocyst count 1.4-fold [95% credible interval [CrI]: 1.65 to 1.2-fold] (from 100.33 to 69.44 oocysts per mosquito in the controls and treated replicates, respectively) and the mean sporozoite count 1.34-fold [95%CrI 1.66 to 1.12-fold] (from 14414.5 to 11131.9 sporozoites per mosquito in the controls and treated replicates, respectively). The corresponding figures for artesunate and methylene blue were a 469.42-fold reduction [95%CrI 650.45 to 344.73-fold] (from 60.56 to 0.22 oocysts per mosquito in the controls and treated replicates, respectively) and a 1437.88-fold reduction [95%CI 2063.86 to 969.55-fold] (from 107.25 to 0.08 oocysts per mosquito in the controls and treated replicates, respectively) in oocyst count respectively and a 147.77-fold [95%CrI 469.56 to 60.64-fold] (from 1303.3 to 0.1 sporozoites per mosquito in the controls and treated replicates, respectively) and a 536.1-fold [95%CrI 1310.51 to 245.99-fold] (from 13914.5 to 1.8 sporozoites per mosquito in the controls and treated replicates, respectively) reduction in sporozoite count, respectively.

To consider baseline variations in blood meal infectiousness to mosquitoes across blood samples, the log10oocyst count, log10asexual parasitaemia and log10gametocytaemia assessed on admission (i.e., on the collection day before the 24-hour incubation time with or without drug) were introduced as linear predictors of the mean parasite count in mosquito samples of the experimental replicates (i.e. after 24 hours of incubation with or without drug). A 10-fold increase in the mean oocyst count and gametocytaemia at baseline were respectively associated with a 1.76 [95%CI: 1.2 to 2.48] and a 6.47-fold increase [95%CI: 3.04 to 13.37] in the mean oocyst count in the experimental replicates; there was no significant association between the mean oocyst count in experimental replicate and baseline asexual parasitaemia (model coefficient: 1.08 [95%CI 0.56 to 2.11]) or artesunate wash off (model coefficient: 0.84 [95%CI 0.41 to 1.71]). A 10-fold increase in baseline gametocytaemia and artesunate wash off were respectively associated with a 3.56-fold increase [95%CI 1.21 to 9.2] and a 0.26-fold decrease [95%CI 0.1 to 0.61] in the mean sporozoite count in the experimental replicates; there was no significant association between the mean sporozoite count in the experimental replicate and mean oocyst count (model coefficient: 1.21 [95%CI 0.72 to 1.96]) or asexual parasitaemia (model coefficient: 1.22 [95%CI 0.48 to 2.96]) at baseline.

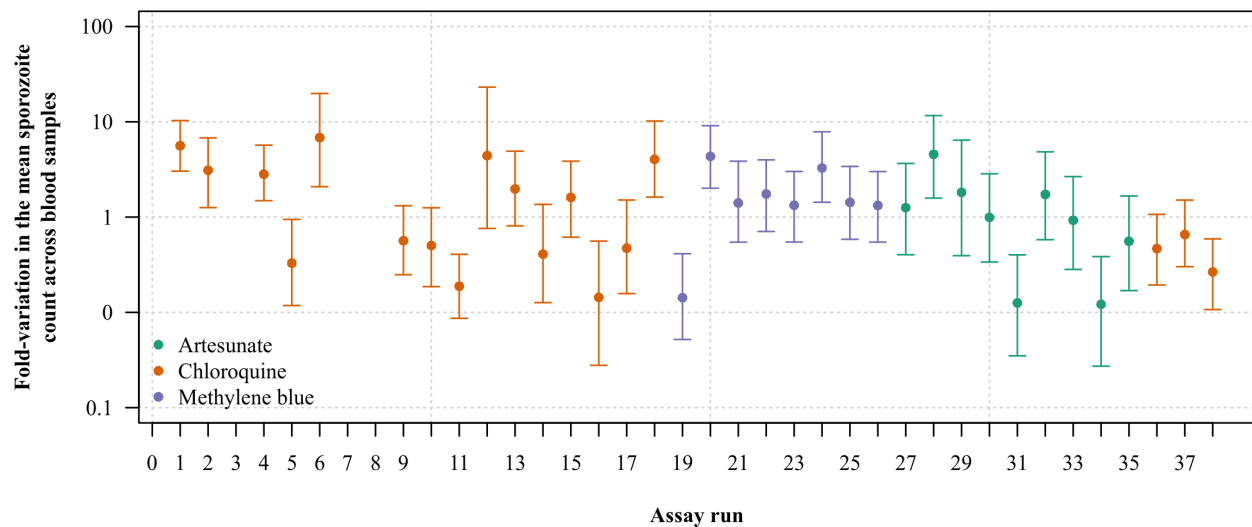
Assay variability

Inter-experiment variability

A



B



These are the figures for inter-experiment variability (in fold-variation in the mean parasite count across blood samples):

```
quantile(my_table$median.oocyst)
```

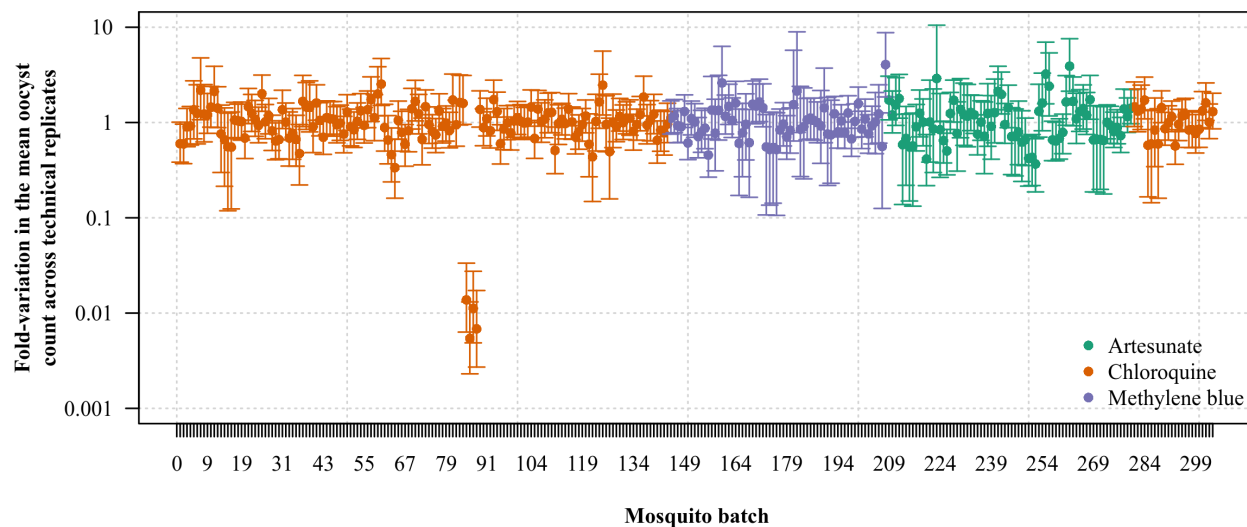
```
##          0%          25%          50%          75%         100%
## 0.1569302 0.6182925 1.0669434 2.0812507 4.0001822
```

```
quantile(my_table$median.sporozoite, na.rm = T)
```

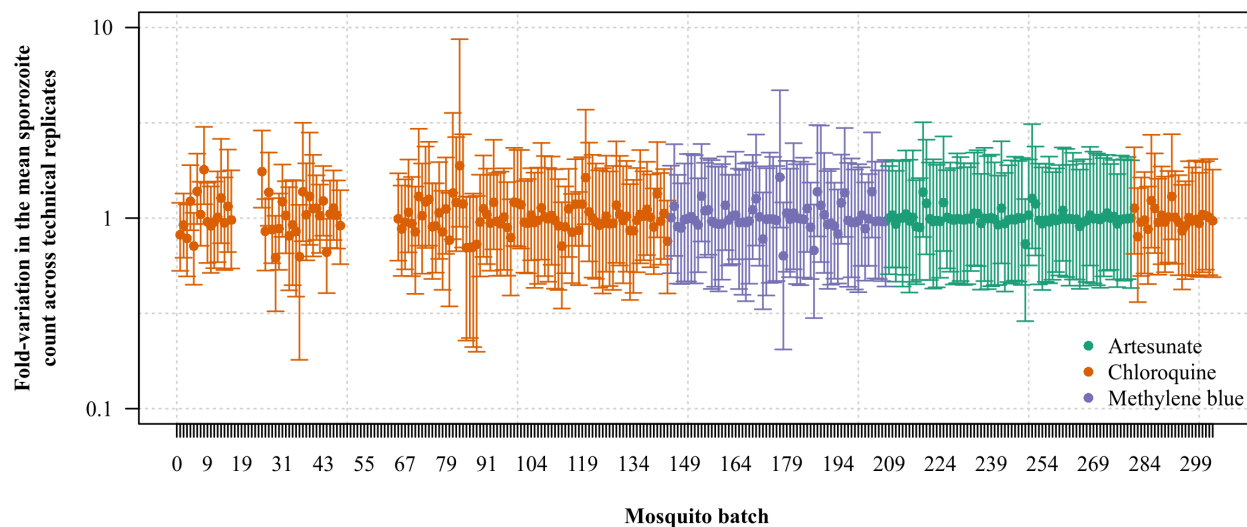
```
##          0%          25%          50%          75%         100%
## 0.1215025 0.4695308 1.3234454 2.3978970 6.8286956
```

Intra-experiment variability

A



B



These are the figures for intra-experiment variability (in fold-variation in the mean parasite count across technical replicates):

```
quantile(my_table$median.oocyst)
```

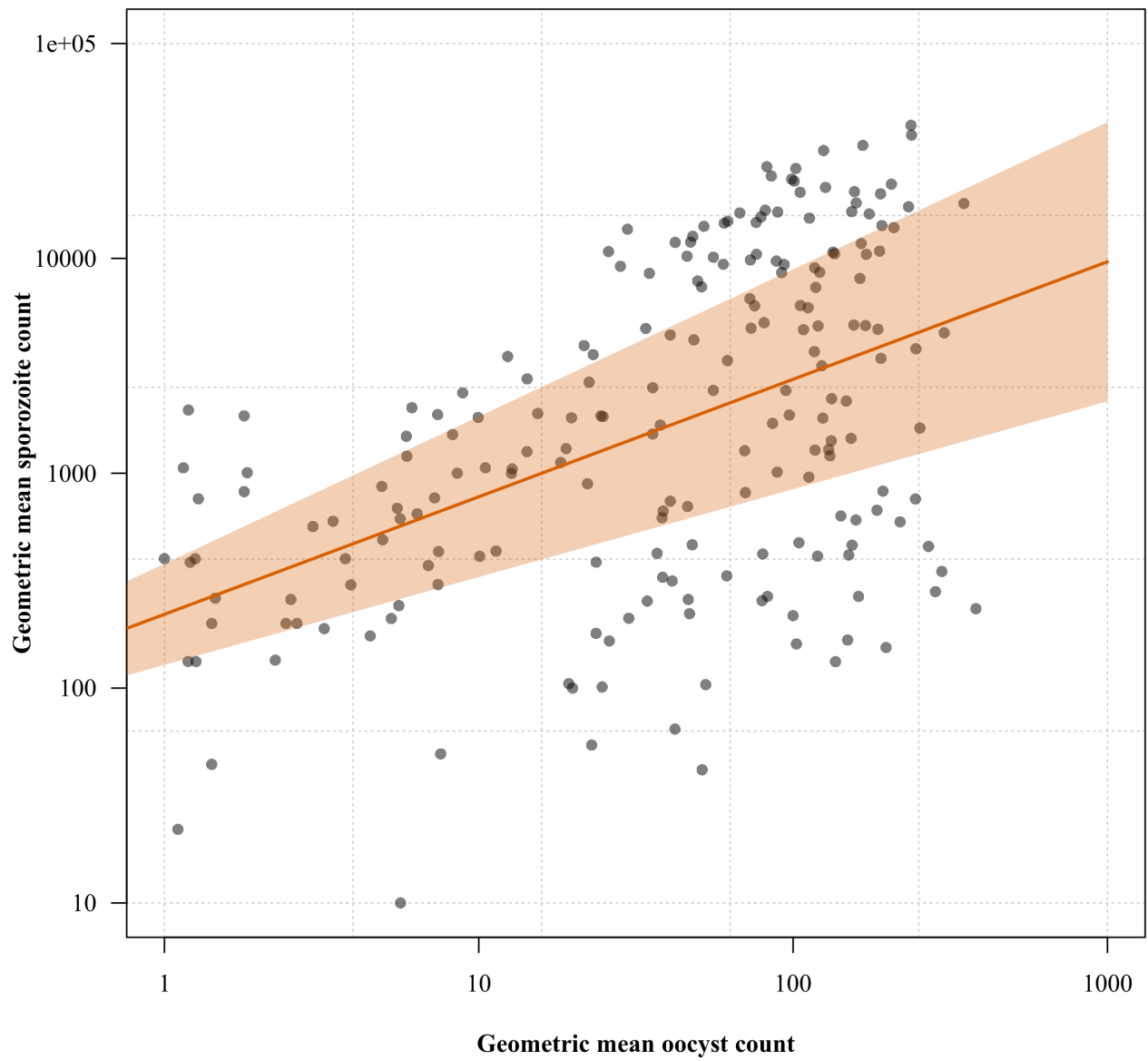
```
##           0%          25%          50%          75%          100%
## 0.005421552 0.768113629 1.001994206 1.298951808 4.045166536
```

```
quantile(my_table$median.sporozoite, na.rm = T)
```

```
##           0%          25%          50%          75%          100%
## 0.6208217 0.9386475 0.9875390 1.0514436 1.8824427
```

Correlation between oocyst and sporozoite count

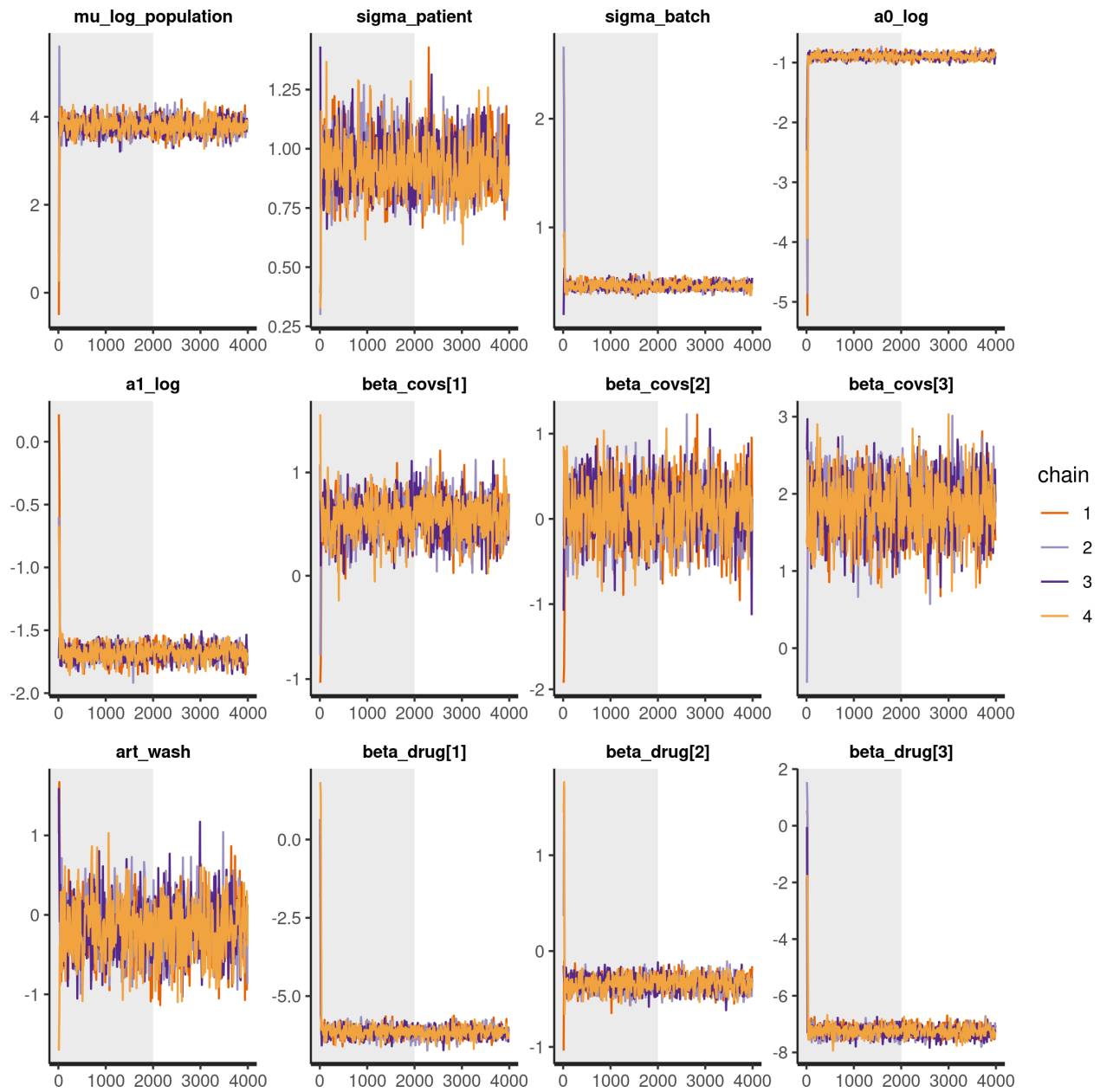
```
##
## Call:
## lm(formula = yval ~ xval)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.75436 -0.41638  0.08999  0.52068  1.03513
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  2.34347    0.11931  19.642 < 2e-16 ***
## xval         0.54698    0.07005   7.809 2.98e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6681 on 204 degrees of freedom
## Multiple R-squared:  0.2301, Adjusted R-squared:  0.2263
## F-statistic: 60.98 on 1 and 204 DF,  p-value: 2.979e-13
```



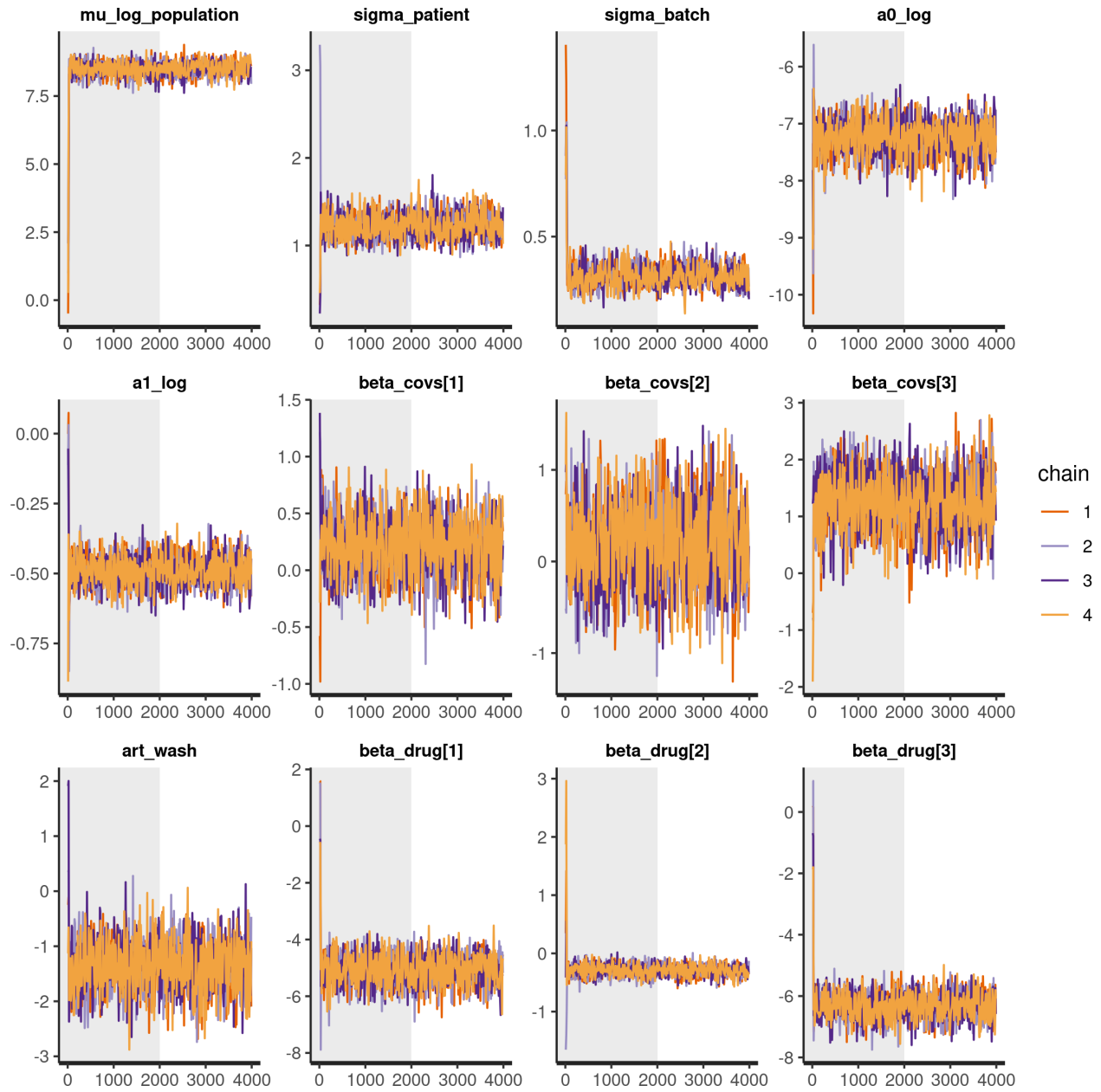
Model assesment

Traceplots

Oocyst stage

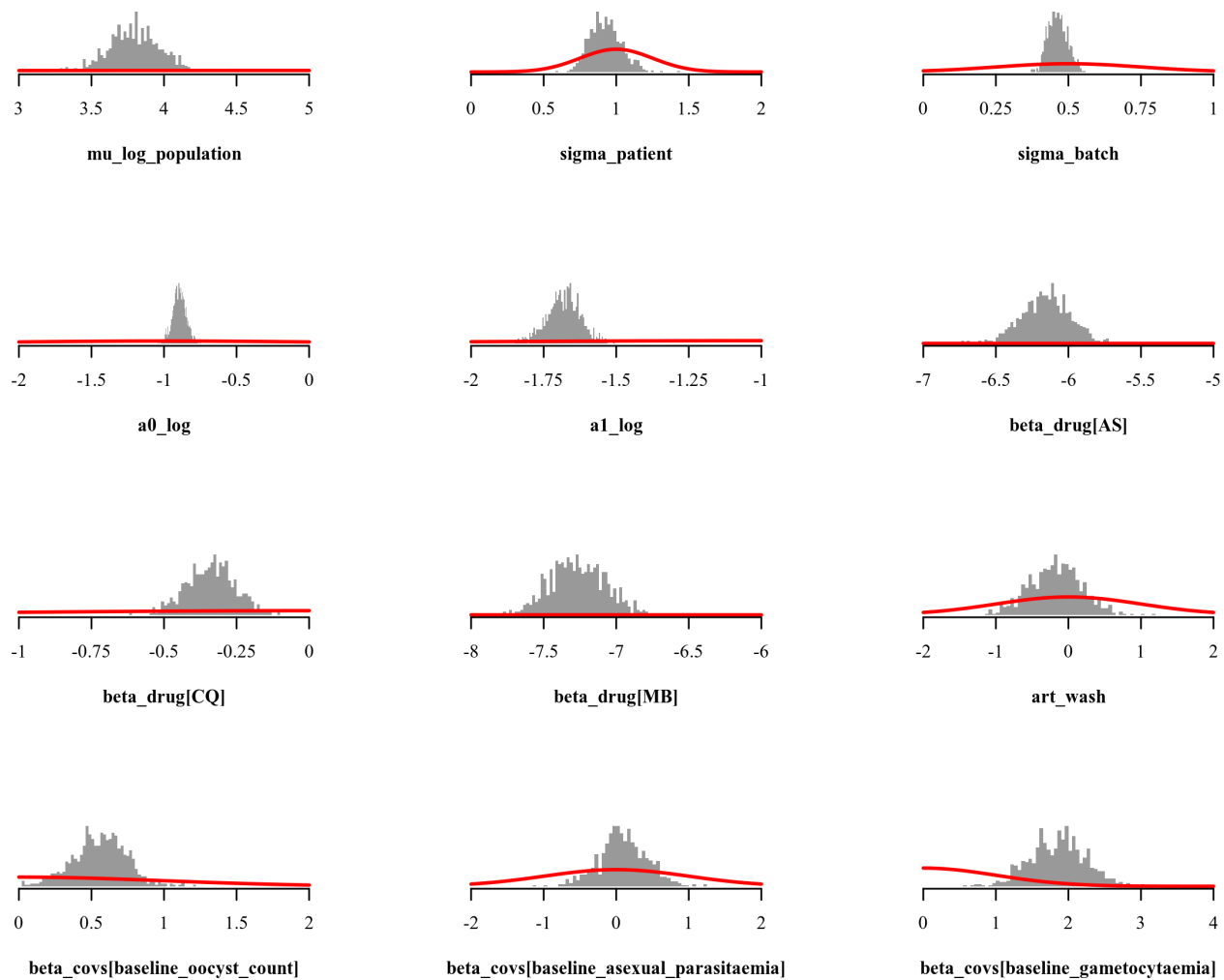


Sporozoite stage



Comparison of priors and posteriors

Oocyst stage



Sporozoite stage

