

# Statistical analysis of temperature effect on *Wolbachia*-induced protection to viruses

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## Figure 1

### Survival at different post-infections temperatures

```
surv_2temp<-fread("dataset_s1.txt")

#make a variable describing each unique set of conditions
surv_2temp[,RepFull:=interaction(Temp,Wolb,Dose,Replicate,sep = "_")]
surv_2temp<-surv_2temp[,lapply(.SD,char_asfactor)]

##Diagnostics
```

```

#How many individuals were tested
ftable(xtabs(~Temp+Dose+Wolb,surv_2temp))

##           Wolb Wolb- Wolb+
## Temp Dose
## 18C  E5           50    50
##      E6           50    50
##      E7           50    50
##      E8           50    50
##      E9           50    50
## 25C  E5           50    50
##      E6           50    50
##      E7           50    50
##      E8           50    50
##      E9           50    50

##Data analysis
# Wolbachia * Dose * Temperature comparisons
## Full model

cox_2temp_full<-coxme(Surv(Time,Status)~Wolb*Dose*Temp+(1|RepFull), surv_2temp)

# Anova Table
Anova(cox_2temp_full,test.statistic = "LR")

## Analysis of Deviance Table (Type II tests)
##           LR Chisq Df Pr(>Chisq)
## Wolb           63.810  1  1.370e-15 ***
## Dose          220.370  4  < 2.2e-16 ***
## Temp          98.543  1  < 2.2e-16 ***
## Wolb:Dose        8.001  4   0.09154 .
## Wolb:Temp       19.497  1  1.007e-05 ***
## Dose:Temp       30.601  4  3.693e-06 ***
## Wolb:Dose:Temp   9.123  4   0.05810 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# minimum model

cox_2temp_final<-coxme(Surv(Time,Status)~Temp*(Dose+Wolb)+(1|RepFull), surv_2temp)

Anova(cox_2temp_final,test.statistic = "LR")

## Analysis of Deviance Table (Type II tests)
##           LR Chisq Df Pr(>Chisq)
## Temp          94.853  1  < 2.2e-16 ***
## Dose          220.370  4  < 2.2e-16 ***
## Wolb           63.810  1  1.370e-15 ***
## Temp:Dose      31.862  4  2.041e-06 ***
## Temp:Wolb     17.551  1  2.798e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(cox_2temp_final)

## Cox mixed-effects model fit by maximum likelihood

```

```
## Data: surv_2temp
## events, n = 655, 1000
## Iterations= 17 126
##          NULL Integrated      Fitted
## Log-likelihood -4237.264 -3581.165 -3523.542
##
##          Chisq    df p      AIC      BIC
## Integrated loglik 1312.20 12.00 0 1288.20 1234.38
## Penalized loglik 1427.44 52.22 0 1323.01 1088.85
##
## Model: Surv(Time, Status) ~ Temp * (Dose + Wolb) + (1 | RepFull)
## Fixed coefficients
##          coef exp(coef) se(coef)      z      p
## Temp25C      -0.3599373  0.6977201 0.5636204 -0.64 5.2e-01
## DoseE6         1.6146846  5.0263023 0.4537914  3.56 3.7e-04
## DoseE7         3.3818780 29.4259800 0.4337873  7.80 6.3e-15
## DoseE8         3.8383442 46.4485027 0.4323228  8.88 0.0e+00
## DoseE9         4.1150815 61.2572096 0.4318331  9.53 0.0e+00
## WolbWolb+     -1.8410024  0.1586583 0.1885979 -9.76 0.0e+00
## Temp25C:DoseE6  0.9830450  2.6725819 0.6366543  1.54 1.2e-01
## Temp25C:DoseE7  2.2328572  9.3264756 0.6158195  3.63 2.9e-04
## Temp25C:DoseE8  2.8684844 17.6103079 0.6146590  4.67 3.1e-06
## Temp25C:DoseE9  2.3895115 10.9081641 0.6126688  3.90 9.6e-05
## Temp25C:WolbWolb+ 1.0934571  2.9845742 0.2510025  4.36 1.3e-05
##
## Random effects
## Group Variable Std Dev Variance
## RepFull Intercept 0.4178493 0.1745981
```

Significant interaction between *Wolbachia* and Temperature, and between Dose and Temperature. No interaction between *Wolbachia* and Dose.

```
#Comparisons between hazard ratios
```

```
#Hazard ratios between wolb+ and wolb- at both temp
```

```
contr_2temp_Wolb<-lsmeans::lsmeans(cox_2temp_final,pairwise~Wolb|Temp)
contr_2temp_Wolb
```

```
## $lsmeans
## Temp = 18C:
## Wolb lsmean SE df asymp.LCL asymp.UCL
## Wolb- -0.0203 0.109 Inf -0.234 0.194
## Wolb+ -1.8613 0.137 Inf -2.129 -1.593
##
## Temp = 25C:
## Wolb lsmean SE df asymp.LCL asymp.UCL
## Wolb- 1.3146 0.116 Inf 1.087 1.542
## Wolb+ 0.5670 0.118 Inf 0.335 0.799
##
## Results are averaged over the levels of: Dose
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
```

```
## $contrasts
## Temp = 18C:
## contrast      estimate      SE df z.ratio p.value
## Wolb- - Wolb+    1.841 0.189 Inf 9.762  <.0001
##
## Temp = 25C:
## contrast      estimate      SE df z.ratio p.value
## Wolb- - Wolb+    0.748 0.171 Inf 4.372  <.0001
##
## Results are averaged over the levels of: Dose
## Results are given on the log (not the response) scale.
contrast(contr_2temp_Wolb$contrasts,method="pairwise",by="contrast")

## contrast = Wolb- - Wolb+:
## contrast1 estimate      SE df z.ratio p.value
## 18C - 25C      1.09 0.251 Inf 4.356  <.0001
##
## Results are averaged over the levels of: Dose
## Results are given on the log (not the response) scale.
```

*Wolbachia* has an effect at both temperatures, significantly stronger effect at 18°C post-infection temperature.

```
#Hazards ratios between temperatures, at all doses
mcp_temps_final<-lsmeans::lsmeans(cox_2temp_final,pairwise~Temp|Dose)
mcp_temps_final

## $lsmeans
## Dose = E5:
## Temp lsmean      SE df asymp.LCL asymp.UCL
## 18C  -3.531 0.364 Inf  -4.2434  -2.818
## 25C  -3.344 0.371 Inf  -4.0711  -2.617
##
## Dose = E6:
## Temp lsmean      SE df asymp.LCL asymp.UCL
## 18C  -1.916 0.229 Inf  -2.3655  -1.467
## 25C  -0.746 0.192 Inf  -1.1222  -0.370
##
## Dose = E7:
## Temp lsmean      SE df asymp.LCL asymp.UCL
## 18C  -0.149 0.179 Inf  -0.4990   0.201
## 25C   2.271 0.176 Inf   1.9256   2.616
##
## Dose = E8:
## Temp lsmean      SE df asymp.LCL asymp.UCL
## 18C   0.308 0.172 Inf  -0.0299   0.645
## 25C   3.363 0.185 Inf   3.0010   3.725
##
## Dose = E9:
## Temp lsmean      SE df asymp.LCL asymp.UCL
## 18C   0.584 0.169 Inf   0.2526   0.916
## 25C   3.161 0.181 Inf   2.8050   3.516
##
```

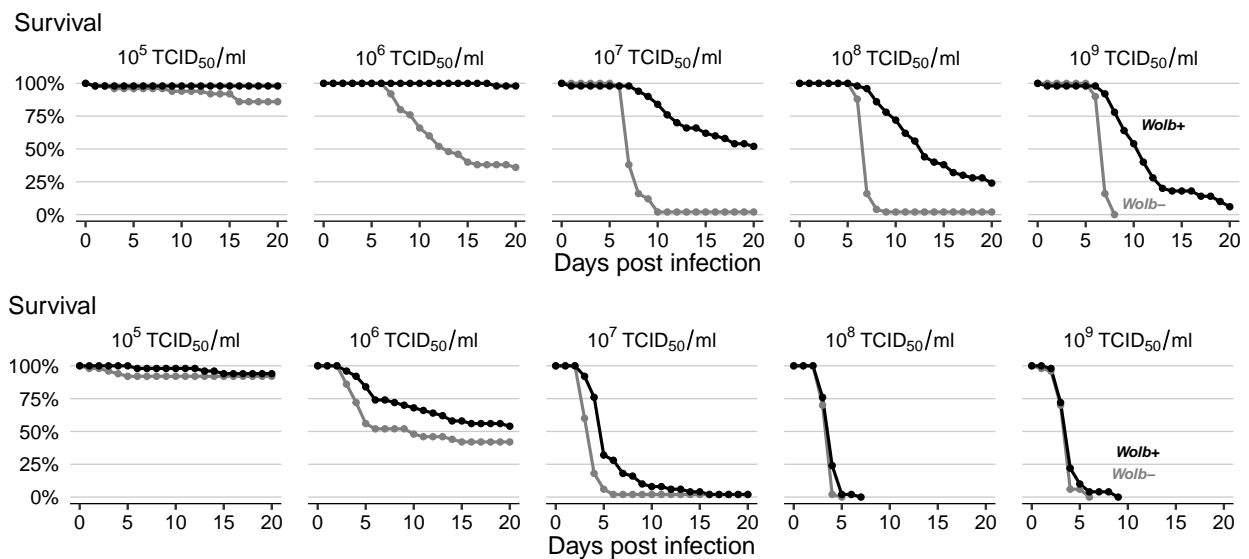
```

## Results are averaged over the levels of: Wolb
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## Dose = E5:
## contrast estimate SE df z.ratio p.value
## 18C - 25C -0.187 0.563 Inf -0.332 0.7402
##
## Dose = E6:
## contrast estimate SE df z.ratio p.value
## 18C - 25C -1.170 0.308 Inf -3.804 0.0001
##
## Dose = E7:
## contrast estimate SE df z.ratio p.value
## 18C - 25C -2.420 0.254 Inf -9.527 <.0001
##
## Dose = E8:
## contrast estimate SE df z.ratio p.value
## 18C - 25C -3.055 0.251 Inf -12.170 <.0001
##
## Dose = E9:
## contrast estimate SE df z.ratio p.value
## 18C - 25C -2.576 0.245 Inf -10.514 <.0001
##
## Results are averaged over the levels of: Wolb
## Results are given on the log (not the response) scale.

```

Temperature has a significant effect in survival, except for lower dose ( $10^5$  TCID<sub>50</sub>/ml), which has very low lethality even without *Wolbachia*.

**Figures 1A and B**



## DCV Titers at different post-infections temperatures

```
#Read the data
DCV_bytemp<-fread("dataset_s2.txt")
DCV_bytemp<-DCV_bytemp[,lapply(.SD,char_asfactor)]
DCV_bytemp[,logRatio:=log10(Ratio)][,inter:=interaction(Wolb,Temp,Dose)]
str(DCV_bytemp)

## Classes 'data.table' and 'data.frame': 100 obs. of 7 variables:
## $ Sample : Factor w/ 2 levels "138","iso": 1 1 1 1 1 1 1 1 1 1 ...
## $ Wolb : Factor w/ 2 levels "Wolb-","Wolb+": 2 2 2 2 2 2 2 2 2 2 ...
## $ Ratio : num 6.06e-04 2.77e-05 3.21e-05 9.63e-06 2.28e-05 ...
## $ Temp : Factor w/ 2 levels "18C","25C": 1 1 1 1 1 1 1 1 1 1 ...
## $ Dose : Factor w/ 5 levels "E5","E6","E7",...: 1 1 1 1 1 2 2 2 2 2 ...
## $ logRatio: num -3.22 -4.56 -4.49 -5.02 -4.64 ...
## $ inter : Factor w/ 20 levels "Wolb-.18C.E5",...: 2 2 2 2 2 6 6 6 6 6 ...
## - attr(*, ".internal.selfref")=<externalptr>

##Diagnostics
#How many individuals were tested
ftable(xtabs(~Wolb+Temp+Dose,data=DCV_bytemp))

##           Dose E5 E6 E7 E8 E9
## Wolb Temp
## Wolb- 18C      5  5  5  5  5
##        25C      5  5  5  5  5
## Wolb+ 18C      5  5  5  5  5
##        25C      5  5  5  5  5

##Data analysis
#linear model
mod.log<-lm(logRatio~Wolb*Temp*Dose,data=DCV_bytemp)
Anova(mod.log)

## Anova Table (Type II tests)
##
## Response: logRatio
##           Sum Sq Df F value    Pr(>F)
## Wolb       122.434  1 164.4990 < 2.2e-16 ***
## Temp       112.567  1 151.2427 < 2.2e-16 ***
## Dose        107.331  4  36.0517 < 2.2e-16 ***
## Wolb:Temp      6.928  1   9.3086  0.003094 **
## Wolb:Dose       3.087  4   1.0369  0.393453
## Temp:Dose       4.650  4   1.5620  0.192507
## Wolb:Temp:Dose 24.034  4   8.0729  1.61e-05 ***
## Residuals      59.543 80
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(mod.log)

##
## Call:
## lm(formula = logRatio ~ Wolb * Temp * Dose, data = DCV_bytemp)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
```

```
## -2.8369 -0.2048 0.0012 0.1655 3.1067
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      -3.7890     0.3858  -9.821 2.16e-15 ***
## WolbWolb+       -0.5964     0.5456  -1.093 0.27762
## Temp25C          3.1187     0.5456   5.716 1.81e-07 ***
## DoseE6           1.2352     0.5456   2.264 0.02629 *
## DoseE7           3.5966     0.5456   6.592 4.27e-09 ***
## DoseE8           4.0454     0.5456   7.414 1.12e-10 ***
## DoseE9           4.3099     0.5456   7.899 1.28e-11 ***
## WolbWolb+:Temp25C -2.2382     0.7716  -2.901 0.00481 **
## WolbWolb+:DoseE6 -1.5190     0.7716  -1.969 0.05247 .
## WolbWolb+:DoseE7 -3.2261     0.7716  -4.181 7.38e-05 ***
## WolbWolb+:DoseE8 -3.4387     0.7716  -4.456 2.68e-05 ***
## WolbWolb+:DoseE9 -2.5312     0.7716  -3.280 0.00154 **
## Temp25C:DoseE6   -0.2890     0.7716  -0.375 0.70899
## Temp25C:DoseE7   -2.0023     0.7716  -2.595 0.01125 *
## Temp25C:DoseE8   -2.4612     0.7716  -3.190 0.00203 **
## Temp25C:DoseE9   -2.8632     0.7716  -3.711 0.00038 ***
## WolbWolb+:Temp25C:DoseE6 2.0791     1.0913   1.905 0.06035 .
## WolbWolb+:Temp25C:DoseE7 4.6631     1.0913   4.273 5.28e-05 ***
## WolbWolb+:Temp25C:DoseE8 5.1238     1.0913   4.695 1.09e-05 ***
## WolbWolb+:Temp25C:DoseE9 4.5895     1.0913   4.206 6.74e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.8627 on 80 degrees of freedom
## Multiple R-squared:  0.8649, Adjusted R-squared:  0.8328
## F-statistic: 26.94 on 19 and 80 DF, p-value: < 2.2e-16
```

Interaction between *Wolbachia*, Dose, and Temperature

```
#Least square means
```

```
#effect of Wolbachia at different doses, at different temperatures
```

```
lsm_bywolb.log<-lsmeans::lsmeans(mod.log,pairwise~Wolb|Dose:Temp,adj="none")
summary(lsm_bywolb.log,by=NULL,adj="holm")
```

```
## $lsmeans
##   Wolb Dose Temp lsmean    SE df lower.CL upper.CL
## Wolb- E5  18C  -3.789 0.386 80  -4.994  -2.584
## Wolb+ E5  18C  -4.385 0.386 80  -5.590  -3.181
## Wolb- E6  18C  -2.554 0.386 80  -3.758  -1.349
## Wolb+ E6  18C  -4.669 0.386 80  -5.874  -3.465
## Wolb- E7  18C  -0.192 0.386 80  -1.397   1.012
## Wolb+ E7  18C  -4.015 0.386 80  -5.219  -2.810
## Wolb- E8  18C   0.256 0.386 80  -0.948   1.461
## Wolb+ E8  18C  -3.779 0.386 80  -4.983  -2.574
## Wolb- E9  18C   0.521 0.386 80  -0.684   1.725
## Wolb+ E9  18C  -2.607 0.386 80  -3.811  -1.402
## Wolb- E5  25C  -0.670 0.386 80  -1.875   0.534
## Wolb+ E5  25C  -3.505 0.386 80  -4.710  -2.300
```

```
## Wolb- E6 25C 0.276 0.386 80 -0.929 1.480
## Wolb+ E6 25C -1.999 0.386 80 -3.203 -0.794
## Wolb- E7 25C 0.924 0.386 80 -0.281 2.128
## Wolb+ E7 25C -0.474 0.386 80 -1.678 0.731
## Wolb- E8 25C 0.914 0.386 80 -0.291 2.118
## Wolb+ E8 25C -0.236 0.386 80 -1.440 0.969
## Wolb- E9 25C 0.776 0.386 80 -0.428 1.981
## Wolb+ E9 25C 0.000 0.386 80 -1.205 1.205
##
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 20 estimates
##
## $contrasts
## contrast Dose Temp estimate SE df t.ratio p.value
## Wolb- - Wolb+ E5 18C 0.596 0.546 80 1.093 0.3173
## Wolb- - Wolb+ E6 18C 2.115 0.546 80 3.877 0.0011
## Wolb- - Wolb+ E7 18C 3.822 0.546 80 7.006 <.0001
## Wolb- - Wolb+ E8 18C 4.035 0.546 80 7.395 <.0001
## Wolb- - Wolb+ E9 18C 3.128 0.546 80 5.732 <.0001
## Wolb- - Wolb+ E5 25C 2.835 0.546 80 5.195 <.0001
## Wolb- - Wolb+ E6 25C 2.275 0.546 80 4.169 0.0005
## Wolb- - Wolb+ E7 25C 1.398 0.546 80 2.561 0.0492
## Wolb- - Wolb+ E8 25C 1.150 0.546 80 2.107 0.1148
## Wolb- - Wolb+ E9 25C 0.776 0.546 80 1.423 0.3173
##
## P value adjustment: holm method for 10 tests
```

*Wolbachia* has an effect at most doses and temperatures combinations. At 18°C *Wolbachia* effect is significant at all doses except lowest one. At 25°C *Wolbachia* effect is significant at the three lowest doses (out of 5) doses.

#### #effect of Wolbachia at different temperatures

```
lsm_bywolbtemp.log<-lsmeans::lsmeans(mod.log,pairwise~Wolb|Temp,adj="none")
```

## NOTE: Results may be misleading due to involvement in interactions

```
summary(lsm_bywolbtemp.log,by=NULL,adj="holm")
```

```
## $lsmeans
## Wolb Temp lsmean SE df lower.CL upper.CL
## Wolb- 18C -1.152 0.173 80 -1.59250 -0.711
## Wolb+ 18C -3.891 0.173 80 -4.33193 -3.450
## Wolb- 25C 0.444 0.173 80 0.00302 0.885
## Wolb+ 25C -1.243 0.173 80 -1.68354 -0.802
##
## Results are averaged over the levels of: Dose
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 4 estimates
##
## $contrasts
## contrast Temp estimate SE df t.ratio p.value
## Wolb- - Wolb+ 18C 2.74 0.244 80 11.227 <.0001
## Wolb- - Wolb+ 25C 1.69 0.244 80 6.912 <.0001
##
```



```
## Results are averaged over the levels of: Dose
## P value adjustment: holm method for 2 tests
#fold difference 18C
log_bywoltbtemp_18C <- summary(lsm_bywoltbtemp.log,by=NULL,adj="holm")$contrasts[1,3]
`(`(10,log_bywoltbtemp_18C)

## [1] 548.8167
#fold difference 25C
log_bywoltbtemp_25C <- summary(lsm_bywoltbtemp.log,by=NULL,adj="holm")$contrasts[2,3]
`(`(10,log_bywoltbtemp_25C)

## [1] 48.5923
#Directly test the differences
contrast(lsm_bywoltbtemp.log$contrasts,"pairwise",by="contrast")

## contrast = Wolb- - Wolb+:
## contrast1 estimate SE df t.ratio p.value
## 18C - 25C 1.05 0.345 80 3.051 0.0031
##
## Results are averaged over the levels of: Dose
```

On average *Wolbachia* gives more resistance at 18°C

```
#effect of temp at different doses and Wolb presence
lsm_byTemp.log<-lsmeans::lsmeans(mod.log,pairwise~Temp|Dose:Wolb,adj="none")
summary(lsm_byTemp.log,by=NULL,adj="holm")

## $lsmeans
## Temp Dose Wolb lsmean SE df lower.CL upper.CL
## 18C E5 Wolb- -3.789 0.386 80 -4.994 -2.584
## 25C E5 Wolb- -0.670 0.386 80 -1.875 0.534
## 18C E6 Wolb- -2.554 0.386 80 -3.758 -1.349
## 25C E6 Wolb- 0.276 0.386 80 -0.929 1.480
## 18C E7 Wolb- -0.192 0.386 80 -1.397 1.012
## 25C E7 Wolb- 0.924 0.386 80 -0.281 2.128
## 18C E8 Wolb- 0.256 0.386 80 -0.948 1.461
## 25C E8 Wolb- 0.914 0.386 80 -0.291 2.118
## 18C E9 Wolb- 0.521 0.386 80 -0.684 1.725
## 25C E9 Wolb- 0.776 0.386 80 -0.428 1.981
## 18C E5 Wolb+ -4.385 0.386 80 -5.590 -3.181
## 25C E5 Wolb+ -3.505 0.386 80 -4.710 -2.300
## 18C E6 Wolb+ -4.669 0.386 80 -5.874 -3.465
## 25C E6 Wolb+ -1.999 0.386 80 -3.203 -0.794
## 18C E7 Wolb+ -4.015 0.386 80 -5.219 -2.810
## 25C E7 Wolb+ -0.474 0.386 80 -1.678 0.731
## 18C E8 Wolb+ -3.779 0.386 80 -4.983 -2.574
## 25C E8 Wolb+ -0.236 0.386 80 -1.440 0.969
## 18C E9 Wolb+ -2.607 0.386 80 -3.811 -1.402
## 25C E9 Wolb+ 0.000 0.386 80 -1.205 1.205
##
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 20 estimates
```

```
##
## $contrasts
## contrast Dose Wolb estimate SE df t.ratio p.value
## 18C - 25C E5 Wolb- -3.119 0.546 80 -5.716 <.0001
## 18C - 25C E6 Wolb- -2.830 0.546 80 -5.186 <.0001
## 18C - 25C E7 Wolb- -1.116 0.546 80 -2.046 0.1762
## 18C - 25C E8 Wolb- -0.657 0.546 80 -1.205 0.4636
## 18C - 25C E9 Wolb- -0.255 0.546 80 -0.468 0.6409
## 18C - 25C E5 Wolb+ -0.880 0.546 80 -1.614 0.3317
## 18C - 25C E6 Wolb+ -2.671 0.546 80 -4.894 <.0001
## 18C - 25C E7 Wolb+ -3.541 0.546 80 -6.490 <.0001
## 18C - 25C E8 Wolb+ -3.543 0.546 80 -6.493 <.0001
## 18C - 25C E9 Wolb+ -2.607 0.546 80 -4.777 <.0001
##
## P value adjustment: holm method for 10 tests
#average effect of temperature
lsm_byTemponly.log<-lsmeans::lsmeans(mod.log,pairwise~Temp,adj="none")

## NOTE: Results may be misleading due to involvement in interactions
summary(lsm_byTemponly.log,by=NULL,adj="holm")

## $lsmeans
## Temp lsmean SE df lower.CL upper.CL
## 18C -2.521 0.122 80 -2.800 -2.243
## 25C -0.399 0.122 80 -0.678 -0.121
##
## Results are averaged over the levels of: Wolb, Dose
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 2 estimates
##
## $contrasts
## contrast estimate SE df t.ratio p.value
## 18C - 25C -2.12 0.173 80 -12.298 <.0001
##
## Results are averaged over the levels of: Wolb, Dose
```

On average DCV titres are lower at 18°C than 25°C

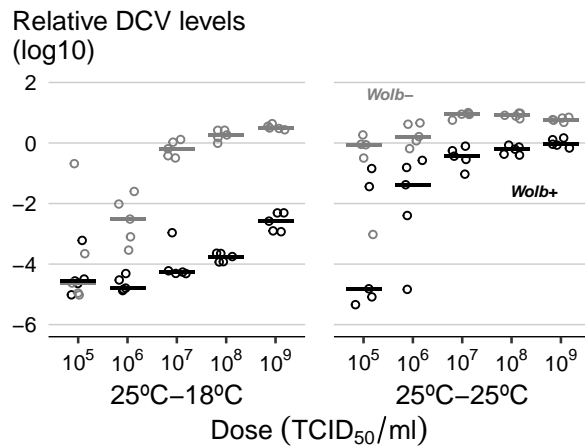
```
#average effect of Wolbachia
lsm_byWolbonly.log<-lsmeans::lsmeans(mod.log,pairwise~Wolb,adj="none")

## NOTE: Results may be misleading due to involvement in interactions
summary(lsm_byWolbonly.log,by=NULL,adj="holm")

## $lsmeans
## Wolb lsmean SE df lower.CL upper.CL
## Wolb- -0.354 0.122 80 -0.633 -0.0751
## Wolb+ -2.567 0.122 80 -2.846 -2.2881
##
## Results are averaged over the levels of: Temp, Dose
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 2 estimates
```

```
##
## $contrasts
## contrast      estimate      SE df t.ratio p.value
## Wolb- - Wolb+      2.21 0.173 80 12.826 <.0001
##
## Results are averaged over the levels of: Temp, Dose
```

Figure 1C



### *Wolbachia* levels post-infection at different temperatures

```
wolb_DCV<-fread("dataset_s3.txt")
unique(wolb_DCV$Treatment)

## [1] "buffer" "CTRL" "DCV"

wolb_DCV[,pre_post:=interaction(Pre_Temp,Post_Temp,sep="_")] [,inter:=droplevels(interaction(Treatment,D
wolb_DCV[,logWolb:=log10(Wolb)] [,inter:=relevel(inter,"CTRL.day3.25C_18C")]
wolb_DCV<-wolb_DCV[,lapply(.SD,char_asfactor)]
#remove day 0 from analysis
wolb_DCV_day3 <- filter(wolb_DCV, Day != "day0")
head(wolb_DCV)
```

```
##           Sample Treatment Day Pre_Temp Post_Temp      Wolb pre_post
## 1: buffer_day_3_18C_a    buffer day3      25C      18C 1.4373219 25C_18C
## 2: buffer_day_3_18C_b    buffer day3      25C      18C 1.5426671 25C_18C
## 3: buffer_day_3_18C_c    buffer day3      25C      18C 1.5763952 25C_18C
## 4: buffer_day_3_18C_d    buffer day3      25C      18C 1.3177769 25C_18C
## 5: buffer_day_3_18C_e    buffer day3      25C      18C 1.0030623 25C_18C
## 6: buffer_day_3_25C_a    buffer day3      25C      25C 0.9432642 25C_25C
##           inter      logWolb
## 1: buffer.day3.25C_18C 0.157554047
## 2: buffer.day3.25C_18C 0.188272227
## 3: buffer.day3.25C_18C 0.197665103
## 4: buffer.day3.25C_18C 0.119841880
## 5: buffer.day3.25C_18C 0.001327906
## 6: buffer.day3.25C_25C -0.025366649
```

```
#lm
wolb_model<-lm(logWolb~Treatment*Post_Temp,data=wolb_DCV_day3)
Anova(wolb_model)
```

```
## Anova Table (Type II tests)
##
## Response: logWolb
##
##           Sum Sq Df F value Pr(>F)
## Treatment      0.016584  2  0.8847  0.4259
## Post_Temp       0.000217  1  0.0232  0.8803
## Treatment:Post_Temp 0.013996  2  0.7466  0.4846
## Residuals      0.224945 24
```

No effect of DCV infection or post-infection temperature on *Wolbachia* levels

Figure 1D

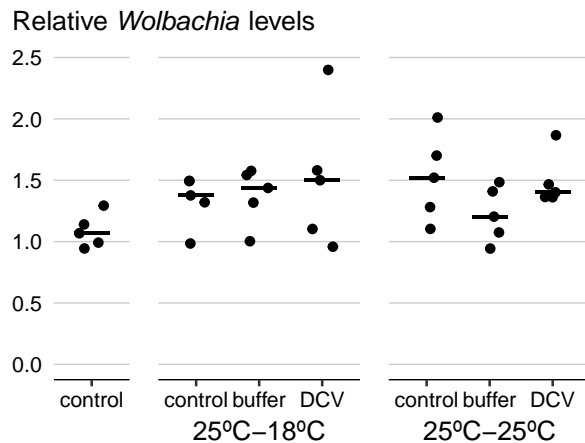


Figure 2 and S1

Effect of different pre and post-infection temperatures on survival

```
##Data import and create column with replicate information
surv_pre_post_ini<-fread("dataset_s4.txt")

surv_pre_post_ini<-surv_pre_post_ini[, "]="(Pre_Temp=as.factor(Pre_Temp),
      Post_Temp=as.factor(Post_Temp),
      RepFull=interaction(Wolb,Pre_Temp,Post_Temp,Replicate,sep = "_"))
][,lapply(.SD,char_asfactor)]

surv_cyc<-droplevels(surv_pre_post_ini[Pre_Temp=="cycling"])
surv_pre_post<-droplevels(surv_pre_post_ini[Pre_Temp!="cycling"])
surv_pre_post[,Wolb:=relevel(Wolb,"Wolb+")]

##Diagnostics
```

```

#How many individuals were tested
ftable(xtabs(~Pre_Temp+Post_Temp+Wolb,surv_pre_post))

##              Wolb Wolb+ Wolb-
## Pre_Temp Post_Temp
## 18          18          100  100
##          25          100  100
## 25          18          100  100
##          25          100  100

ftable(xtabs(~Pre_Temp+Post_Temp+Wolb,surv_cyc))

##              Wolb Wolb- Wolb+
## Pre_Temp Post_Temp
## cycling cycling          100  100

##Data analysis
# Full model
cox_pre_post_full<-coxme(Surv(Time,Status)~Wolb*Pre_Temp*Post_Temp+(1|RepFull),
                        surv_pre_post)
Anova(cox_pre_post_full)

## Analysis of Deviance Table (Type II tests)
##
## Response: Surv(Time, Status)
##              Df    Chisq Pr(>Chisq)
## Wolb           1 18.4874 1.710e-05 ***
## Pre_Temp        1 16.3617 5.233e-05 ***
## Post_Temp        1 30.5079 3.325e-08 ***
## Wolb:Pre_Temp    1  7.0238  0.008043 **
## Wolb:Post_Temp   1  0.0049  0.944290
## Pre_Temp:Post_Temp 1  0.0596  0.807088
## Wolb:Pre_Temp:Post_Temp 1  2.3646  0.124117
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#simpler model
cox_pre_post_final<-coxme(Surv(Time,Status)~Wolb*Pre_Temp+Post_Temp+(1|RepFull),
                        surv_pre_post)
Anova(cox_pre_post_final)

## Analysis of Deviance Table (Type II tests)
##
## Response: Surv(Time, Status)
##              Df    Chisq Pr(>Chisq)
## Wolb           1 18.2611 1.926e-05 ***
## Pre_Temp        1 16.1596 5.822e-05 ***
## Post_Temp        1 29.8053 4.777e-08 ***
## Wolb:Pre_Temp    1  6.7722  0.009259 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(cox_pre_post_final)

## Cox mixed-effects model fit by maximum likelihood
##   Data: surv_pre_post
##   events, n = 500, 800

```

```
## Iterations= 14 77
##          NULL Integrated      Fitted
## Log-likelihood -3137.045 -2996.654 -2909.238
##
##          Chisq      df p      AIC      BIC
## Integrated loglik 280.78  5.00 0 270.78 249.71
## Penalized loglik 455.61 60.98 0 333.65  76.64
##
## Model:  Surv(Time, Status) ~ Wolb * Pre_Temp + Post_Temp + (1 | RepFull)
## Fixed coefficients
##          coef exp(coef)  se(coef)      z      p
## WolbWolb-      0.3450515 1.4120627 0.2736339  1.26 2.1e-01
## Pre_Temp25     -1.3396287 0.2619429 0.2876196 -4.66 3.2e-06
## Post_Temp25      1.0147959 2.7588002 0.1858797  5.46 4.8e-08
## WolbWolb-:Pre_Temp25 1.0392922 2.8272153 0.3993688  2.60 9.3e-03
##
## Random effects
## Group  Variable Std Dev  Variance
## RepFull Intercept 0.7535451 0.5678303
```

Significant interaction between *Wolbachia* and pre-infection temperature

```
#Hazarads ratios between wolb+ and wolb- at both temp
pre_temp_Wolb<-lsmeans::lsmeans(cox_pre_post_final,pairwise~Wolb|Pre_Temp)
summary(pre_temp_Wolb,by=NULL,adj="holm")
```

```
## $lsmeans
## Wolb Pre_Temp lsmean SE df asymp.LCL asymp.UCL
## Wolb+ 18      0.237 0.170 Inf -0.187 0.661
## Wolb- 18      0.583 0.168 Inf 0.162 1.003
## Wolb+ 25     -1.102 0.179 Inf -1.548 -0.656
## Wolb- 25      0.282 0.170 Inf -0.143 0.708
##
## Results are averaged over the levels of: Post_Temp
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 4 estimates
##
## $contrasts
## contrast      Pre_Temp estimate SE df z.ratio p.value
## Wolb+ - Wolb- 18      -0.345 0.274 Inf -1.261 0.2073
## Wolb+ - Wolb- 25      -1.384 0.287 Inf -4.823 <.0001
##
## Results are averaged over the levels of: Post_Temp
## Results are given on the log (not the response) scale.
## P value adjustment: holm method for 2 tests
```

*Wolbachia* only makes a difference if pre-infection temperature is 25°C

```
#direct comparison of Wolb effect at different temp
contrast(pre_temp_Wolb$contrasts,"pairwise",by="contrast")
```

```
## contrast = Wolb+ - Wolb-:
## contrast1 estimate SE df z.ratio p.value
## 18 - 25 1.04 0.399 Inf 2.602 0.0093
##
## Results are averaged over the levels of: Post_Temp
## Results are given on the log (not the response) scale.
```

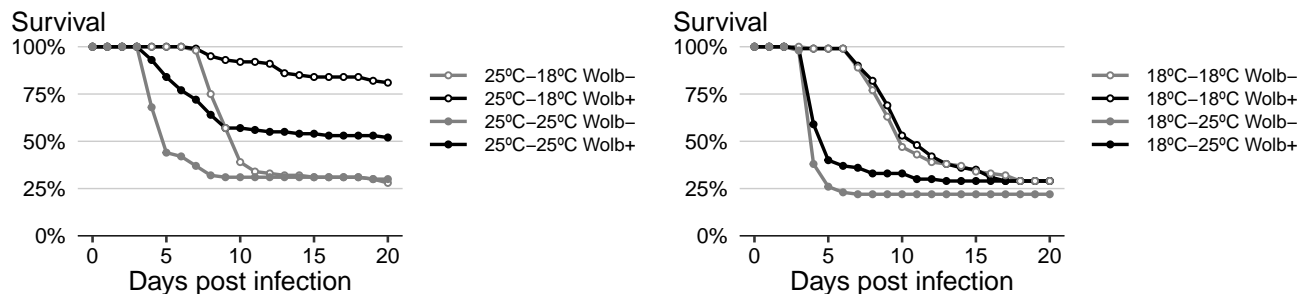
*Wolbachia* effect is significantly different between pre-infection temperatures

```
#Hazarads ratios between pre-temps when wolb is either present or not
Wolb_pre_temp<-lsmeans::lsmeans(cox_pre_post_final,pairwise~Pre_Temp|Wolb)
summary(Wolb_pre_temp,by=NULL,adj="holm")
```

```
## $lsmeans
## Pre_Temp Wolb lsmean SE df asymp.LCL asymp.UCL
## 18 Wolb+ 0.237 0.170 Inf -0.187 0.661
## 25 Wolb+ -1.102 0.179 Inf -1.548 -0.656
## 18 Wolb- 0.583 0.168 Inf 0.162 1.003
## 25 Wolb- 0.282 0.170 Inf -0.143 0.708
##
## Results are averaged over the levels of: Post_Temp
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 4 estimates
##
## $contrasts
## contrast Wolb estimate SE df z.ratio p.value
## 18 - 25 Wolb+ 1.34 0.288 Inf 4.658 <.0001
## 18 - 25 Wolb- 0.30 0.276 Inf 1.090 0.2759
##
## Results are averaged over the levels of: Post_Temp
## Results are given on the log (not the response) scale.
## P value adjustment: holm method for 2 tests
```

Pre-infection temperature only makes a difference if *Wolbachia* is present

Figures 2A and 2B



## DCV levels and different pre-infection temperatures

```
#load data
pre_DCV<-fread("dataset_s5.txt")
pre_DCV<-pre_DCV[,pre_wolb:=paste(Pre_inf,Wolb,sep="_")] [,lapply(.SD,char_asfactor)]
pre_DCV<-pre_DCV[,logRatio:=log10(Ratio)]
pre_DCV$Pre_inf<-as.factor(as.character(pre_DCV$Pre_inf))

head(pre_DCV)

##      Pre_inf Wolb Sample_name      Ratio Rep pre_wolb logRatio
## 1:      18 Wolb+      CS 18 10  662.4316   a 18_Wolb+ 2.821141
## 2:      18 Wolb+      CS 18 5  1045.3680   a 18_Wolb+ 3.019269
## 3:      18 Wolb+      CS 18 6   713.7923   a 18_Wolb+ 2.853572
## 4:      18 Wolb+      CS 18 7  1820.3450   a 18_Wolb+ 3.260154
## 5:      18 Wolb+      CS 18 8  1001.5730   a 18_Wolb+ 3.000683
## 6:      18 Wolb+      CS 18 9   539.2514   a 18_Wolb+ 2.731791

#lmer

mod_DCV<-lmer(logRatio~Pre_inf*Wolb + (1|Rep),data=pre_DCV)
Anova(mod_DCV)

## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: logRatio
##              Chisq Df Pr(>Chisq)
## Pre_inf        45.093  1  1.879e-11 ***
## Wolb          145.361  1  < 2.2e-16 ***
## Pre_inf:Wolb   33.996  1  5.522e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(mod_DCV)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: logRatio ~ Pre_inf * Wolb + (1 | Rep)
## Data: pre_DCV
##
## REML criterion at convergence: 148.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -4.3523 -0.2458  0.1647  0.4542  2.2721
##
## Random effects:
## Groups Name Variance Std.Dev.
## Rep (Intercept) 1.3033  1.142
## Residual      0.5374  0.733
## Number of obs: 64, groups: Rep, 2
##
## Fixed effects:
##              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)      3.1264      0.8281  1.0776   3.775   0.151
## Pre_inf25        -0.1621      0.2592 59.0000  -0.625   0.534
```



```
## WolbWolb+          -1.1410      0.2592 59.0000 -4.402 4.56e-05 ***
## Pre_inf25:WolbWolb+ -2.1371      0.3665 59.0000 -5.831 2.47e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) Pr_n25 WlbWl+
## Pre_inf25    -0.156
## WolbWolb+    -0.156  0.500
## Pr_nf25:WW+  0.111 -0.707 -0.707
```

There is interaction between pre-infection temperature and *Wolbachia*

```
lsm_pre_DCV_wolb<-lsmeans::lsmeans(mod_DCV,pairwise~Wolb|Pre_inf,adj="none")
summary(lsm_pre_DCV_wolb,by=NULL,adj="holm")
```

```
## $lsmeans
##   Wolb Pre_inf lsmean    SE  df lower.CL upper.CL
##   Wolb- 18      3.126 0.828 1.08   -29.1    35.4
##   Wolb+ 18      1.985 0.828 1.08   -30.3    34.3
##   Wolb- 25      2.964 0.828 1.08   -29.3    35.2
##   Wolb+ 25     -0.314 0.828 1.08   -32.6    32.0
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 4 estimates
##
## $contrasts
##   contrast      Pre_inf estimate    SE df t.ratio p.value
##   Wolb- - Wolb+ 18          1.14 0.259 59  4.402  <.0001
##   Wolb- - Wolb+ 25          3.28 0.259 59 12.648  <.0001
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: holm method for 2 tests
```

```
#fold changes
'^(10,summary(lsm_pre_DCV_wolb,by=NULL,adj="holm")$contrasts$estimate)
```

```
## [1] 13.83501 1896.84848
```

```
#direct comparison of Wolb effect at different temp
contrast(lsm_pre_DCV_wolb$contrasts,"pairwise",by="contrast")
```

```
## contrast = Wolb- - Wolb+:
##   contrast1 estimate    SE df t.ratio p.value
##   18 - 25      -2.14 0.367 59 -5.831  <.0001
##
## Degrees-of-freedom method: kenward-roger
```

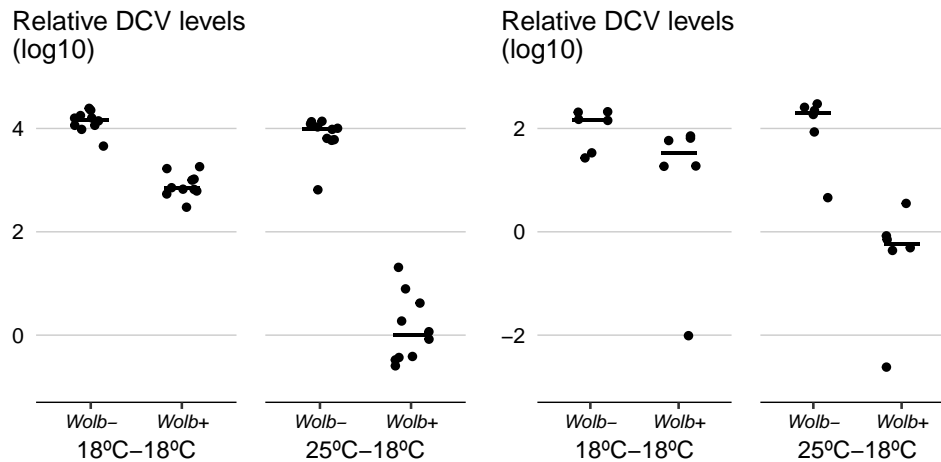
*Wolbachia* increases resistance at both pre-infection temperatures. But *Wolbachia* induces more resistance at pre-infection temperature of 25°C

```
#differences between pre-temp in absence or presence of Wolb
lsm_pre_DCV_temp<-lsmeans::lsmeans(mod_DCV, pairwise~Pre_inf|Wolb, adj="none")
summary(lsm_pre_DCV_temp, by=NULL, adj="holm")
```

```
## $lsmeans
## Pre_inf Wolb lsmean SE df lower.CL upper.CL
## 18 Wolb- 3.126 0.828 1.08 -29.1 35.4
## 25 Wolb- 2.964 0.828 1.08 -29.3 35.2
## 18 Wolb+ 1.985 0.828 1.08 -30.3 34.3
## 25 Wolb+ -0.314 0.828 1.08 -32.6 32.0
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 4 estimates
##
## $contrasts
## contrast Wolb estimate SE df t.ratio p.value
## 18 - 25 Wolb- 0.162 0.259 59 0.625 0.5341
## 18 - 25 Wolb+ 2.299 0.259 59 8.871 <.0001
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: holm method for 2 tests
```

Pre-infection temperature only changes resistance when *Wolbachia* is present

Figures 2C and S1



### *Wolbachia* levels and different pre-infection temperatures

```
##Fig 2D
##Wolbachia levels

pre_Wolb<-fread("dataset_s6.txt")[,lapply(.SD, char_asfactor)]
pre_Wolb[,Wolb:=rel_wolb][,logWolb:=log10(Wolb)]
```

```

##Data Analysis
##Linear model

pre_wolb_lm<-lm(logWolb~Temp, data=pre_Wolb)
summary(pre_wolb_lm)

##
## Call:
## lm(formula = logWolb ~ Temp, data = pre_Wolb)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.103011 -0.047381  0.002781  0.047650  0.094834
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) -0.002111   0.018891  -0.112   0.912
## Temp25C      0.155470   0.026716   5.819 1.63e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.05974 on 18 degrees of freedom
## Multiple R-squared:  0.653, Adjusted R-squared:  0.6337
## F-statistic: 33.87 on 1 and 18 DF, p-value: 1.635e-05

Anova(pre_wolb_lm)

## Anova Table (Type II tests)
##
## Response: logWolb
##           Sum Sq Df F value    Pr(>F)
## Temp       0.120854  1  33.866 1.635e-05 ***
## Residuals  0.064235 18
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#estimated fold differences in Wolbachia levels between pre-temp
'^'(10,pre_wolb_lm$coefficients)

## (Intercept)      Temp25C
##   0.9951519    1.4304396

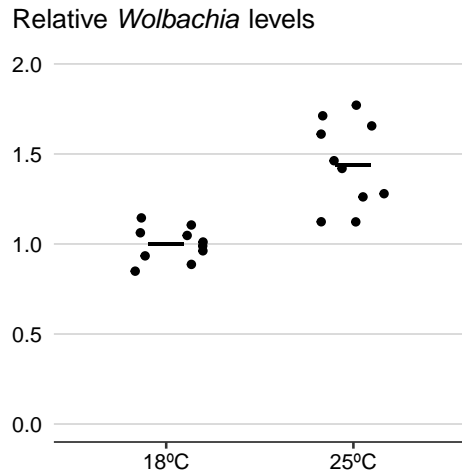
1 / '^'(10,pre_wolb_lm$coefficients)

## (Intercept)      Temp25C
##   1.0048717    0.6990858

```

*Wolbachia* levels differ between development temperatures, are higher at higher temperature

## Figures 2D



## Figure 3 and S2-4

Effect of pre-infection temperature on survival to DCV infection in different host and *Wolbachia* genetic backgrounds

*#Figure 3 A and B*

```
genotypes <- fread("dataset_s7.txt")
```

```
genotypes[,Gen:=gsub("c.*", "", Gen)][,Gen:=tolower(Gen)][,RepFull:=paste(Gen,Dose,Preinf,Wolbachia,Test,1)]
```

```
genotypes[,PlotSeries:=paste(Gen,Test,sep="_")][,Condition:=paste(Wolbachia,Preinf)]
```

```
genotypes<-genotypes[,lapply(.SD,char_asfactor)][,Preinf:=as.factor(Preinf)]
```

```
genotypes_toplot<-data.table(fitsurv(.(Gen,Wolbachia,Preinf,Dose),genotypes))
```

*##Diagnostics*

*#How many individuals were tested*

```
fable(xtabs(~Dose+Wolbachia+Gen+Test+Preinf,genotypes))
```

```
##
## Dose Wolbachia Gen Test Preinf 18 25
## E7 Wol- alj1 01_12_2014 40 40
## 27_11_2014 40 40
## w20 01_12_2014 40 40
## 27_11_2014 40 40
## Wol+ alj1 01_12_2014 40 40
## 27_11_2014 40 40
## w20 01_12_2014 40 40
## 27_11_2014 40 40
## E8 Wol- alj1 01_12_2014 40 40
## 27_11_2014 40 40
## w20 01_12_2014 40 40
## 27_11_2014 40 40
## Wol+ alj1 01_12_2014 40 40
## 27_11_2014 40 40
## w20 01_12_2014 40 40
```

```
##          27_11_2014          44 40
## E9   Wol-   alj1 01_12_2014      40 40
##          27_11_2014          40 40
##          w20 01_12_2014          40 40
##          27_11_2014          40 40
##          Wol+   alj1 01_12_2014      40 40
##          27_11_2014          40 40
##          w20 01_12_2014          40 40
##          27_11_2014          40 40
```

```
##Models
```

```
#Analyse one genotype at a time
```

```
#Aljezur flies, Cox model
```

```
#Full model
```

```
alj_all<-genotypes[Gen=="alj1"]
```

```
alj_cox_full<-coxme(Surv(Time,Status)~Wolbachia*Dose*Preinf+(1|Test/RepFull),data=alj_all)
Anova(alj_cox_full,test.statistic = "LR")
```

```
## Analysis of Deviance Table (Type II tests)
```

```
##          LR Chisq Df Pr(>Chisq)
## Wolbachia      22.498  1  2.104e-06 ***
## Dose           50.914  2  8.793e-12 ***
## Preinf         5.258  1   0.02184 *
## Wolbachia:Dose   3.009  2   0.22211
## Wolbachia:Preinf 27.037  1  1.996e-07 ***
## Dose:Preinf      0.729  2   0.69451
## Wolbachia:Dose:Preinf 1.407  2   0.49485
```

```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#simplified model
```

```
alj_cox_model2<-coxme(Surv(Time,Status)~Wolbachia*Preinf+Dose+(1|Test/RepFull),data=alj_all)
Anova(alj_cox_model2,test.statistic = "LR")
```

```
## Analysis of Deviance Table (Type II tests)
```

```
##          LR Chisq Df Pr(>Chisq)
## Wolbachia      22.376  1  2.242e-06 ***
## Preinf         5.223  1   0.0223 *
## Dose           50.914  2  8.793e-12 ***
## Wolbachia:Preinf 26.183  1  3.105e-07 ***
```

```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(alj_cox_model2)
```

```
## Cox mixed-effects model fit by maximum likelihood
```

```
##   Data: alj_all
```

```
##   events, n = 842, 960
```

```
##   Iterations= 20 105
```

```
##          NULL Integrated      Fitted
```

```
## Log-likelihood -5188.363   -5049.27 -4982.198
```

```
##
```

```
##          Chisq    df p      AIC      BIC
```

```
## Integrated loglik 278.19 7.00 0 264.19 231.03
## Penalized loglik 412.33 55.92 0 300.48 35.64
##
## Model: Surv(Time, Status) ~ Wolbachia * Preinf + Dose + (1 | Test/RepFull)
## Fixed coefficients
##               coef exp(coef) se(coef)      z      p
## WolbachiaWol+ -0.04456881 0.9564098 0.1475228 -0.30 7.6e-01
## Preinf25      0.29156245 1.3385172 0.1483443 1.97 4.9e-02
## DoseE8        0.57182227 1.7714923 0.1307041 4.37 1.2e-05
## DoseE9        1.05424879 2.8698185 0.1299828 8.11 5.6e-16
## WolbachiaWol+:Preinf25 -1.14555241 0.3180482 0.2109993 -5.43 5.7e-08
##
## Random effects
## Group      Variable      Std Dev      Variance
## Test/RepFull (Intercept) 0.38367135 0.14720370
## Test        (Intercept) 0.17308333 0.02995784
```

Interaction between *Wolbachia* and temperature in Aljezur1 flies

```
#Test the effect of wolbachia at different pre-infection temperature
alj_cox_contr=lsmeans::lsmeans(alj_cox_model2,pairwise~Wolbachia|Preinf)
summary(alj_cox_contr$contrasts,by=NULL,adj="holm")
```

```
## contrast      Preinf estimate      SE  df z.ratio p.value
## Wol- - Wol+ 18      0.0446 0.148 Inf 0.302 0.7626
## Wol- - Wol+ 25      1.1901 0.151 Inf 7.898 <.0001
##
## Results are averaged over the levels of: Dose
## Results are given on the log (not the response) scale.
## P value adjustment: holm method for 2 tests
```

```
#direct comparison of Wolb effect at different temp
contrast(alj_cox_contr$contrasts,"pairwise",by="contrast")
```

```
## contrast = Wol- - Wol+:
## contrast1 estimate      SE  df z.ratio p.value
## 18 - 25      -1.15 0.211 Inf -5.429 <.0001
##
## Results are averaged over the levels of: Dose
## Results are given on the log (not the response) scale.
```

*Wolbachia* only has an effect if flies raised at 25°C

```
#W20 flies, Cox model
#Full model
w20_all<-genotypes[Gen=="w20"]

w20_cox_full<-coxme(Surv(Time,Status)~Wolbachia*Dose*Preinf+(1|Test/RepFull),data=w20_all)
Anova(w20_cox_full,test.statistic = "LR")

## Analysis of Deviance Table (Type II tests)
##               LR Chisq Df Pr(>Chisq)
```

```

## Wolbachia          80.541  1  < 2.2e-16 ***
## Dose               53.989  2  1.889e-12 ***
## Preinf            19.556  1  9.771e-06 ***
## Wolbachia:Dose      1.083  2    0.5819
## Wolbachia:Preinf    29.042  1  7.081e-08 ***
## Dose:Preinf         0.777  2    0.6779
## Wolbachia:Dose:Preinf 0.836  2    0.6584
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Simplified model
w20_cox_model2<-coxme(Surv(Time,Status)~Wolbachia*Preinf+Dose+(1|Test/RepFull),data=w20_all)
Anova(w20_cox_model2,test.statistic = "LR")

## Analysis of Deviance Table (Type II tests)
##              LR Chisq Df Pr(>Chisq)
## Wolbachia      80.263  1  < 2.2e-16 ***
## Preinf         19.451  1  1.032e-05 ***
## Dose           53.989  2  1.889e-12 ***
## Wolbachia:Preinf 28.363  1  1.006e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(w20_cox_model2)

## Cox mixed-effects model fit by maximum likelihood
##   Data: w20_all
##   events, n = 734, 964
##   Iterations= 7 51
##              NULL Integrated   Fitted
## Log-likelihood -4639.69 -4246.389 -4142.787
##
##              Chisq    df p    AIC    BIC
## Integrated loglik 786.60  7.00 0 772.60 740.41
## Penalized loglik 993.81 72.38 0 849.04 516.18
##
## Model:  Surv(Time, Status) ~ Wolbachia * Preinf + Dose + (1 | Test/RepFull)
## Fixed coefficients
##              coef exp(coef) se(coef)      z      p
## WolbachiaWol+ -1.21292804 0.2973254 0.2127377 -5.70 1.2e-08
## Preinf25      0.01231417 1.0123903 0.2240180  0.05 9.6e-01
## DoseE8        1.18444422 3.2688696 0.2018180  5.87 4.4e-09
## DoseE9        1.60143882 4.9601641 0.1982039  8.08 6.7e-16
## WolbachiaWol+:Preinf25 -1.80380488 0.1646711 0.3303489 -5.46 4.8e-08
##
## Random effects
## Group      Variable      Std Dev      Variance
## Test/RepFull (Intercept) 0.6762892716 0.4573671789
## Test        (Intercept) 0.0201313478 0.0004052712

```

Interaction between *Wolbachia* and temperature in W20 flies

```

#Test the effect of wolbachia at different pre-infection temperature (minimal model)**
w20_cox_contr=lsmeans::lsmeans(w20_cox_model2,pairwise~Wolbachia|Preinf)

```

```
summary(w20_cox_contr$contrasts,by=NULL,adj="holm")
```

```
## contrast      Preinf estimate      SE  df z.ratio p.value
## Wol- - Wol+ 18          1.21 0.213 Inf   5.702 <.0001
## Wol- - Wol+ 25          3.02 0.235 Inf  12.821 <.0001
##
```

```
## Results are averaged over the levels of: Dose
## Results are given on the log (not the response) scale.
## P value adjustment: holm method for 2 tests
```

```
#direct comparison of Wolb effect at different temp
```

```
contrast(w20_cox_contr$contrasts,"pairwise",by="contrast")
```

```
## contrast = Wol- - Wol+:
```

```
## contrast1 estimate      SE  df z.ratio p.value
## 18 - 25          -1.8 0.33 Inf  -5.460 <.0001
##
```

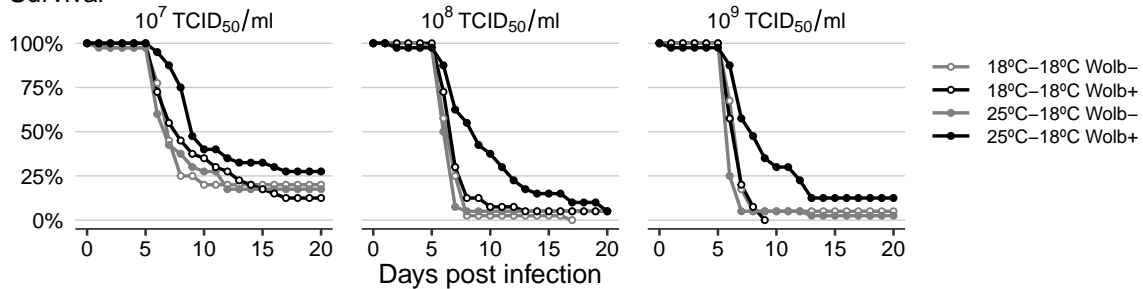
```
## Results are averaged over the levels of: Dose
## Results are given on the log (not the response) scale.
```

*Wolbachia* has an effect at both temperatures, effect is stronger in flies raised at 25°C

Figures 3A, 3B, S2A, S2B

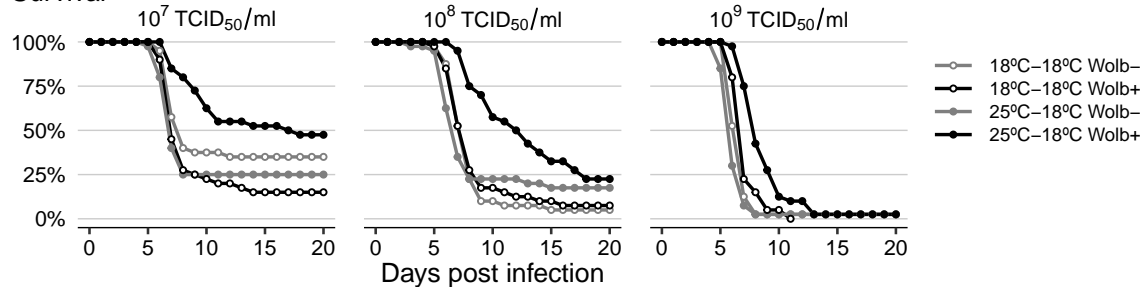
#### A Aljezur1, DCV

Survival



#### A Aljezur1, DCV

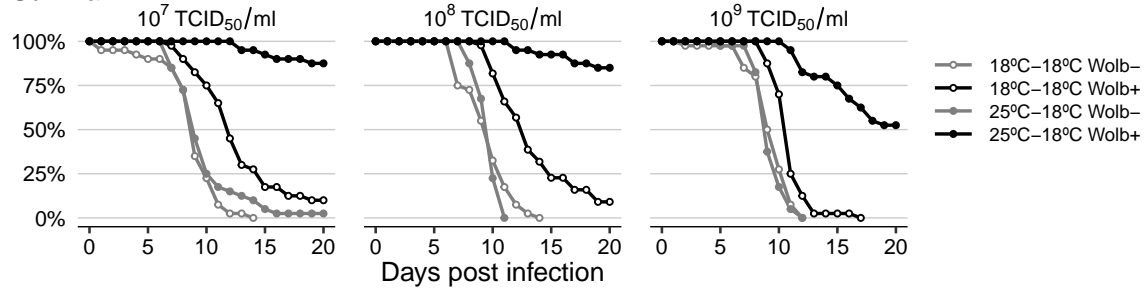
Survival





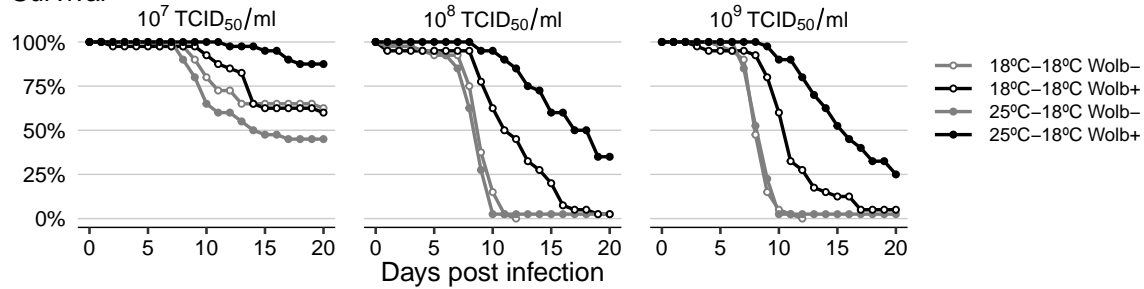
## B Oregon-R (W-20), DCV

### Survival



## B Oregon-R (W-20), DCV

### Survival



## Effect of pre-infection temperature on survival to FHV infection

```
###Effect of Pre-temp on FHV
##Data import
surv_FHV<-fread("dataset_s8.txt")

surv_FHV<-surv_FHV[,Preinf:=as.factor(Preinf)]
surv_FHV[,RepFull:=interaction(Preinf,Wolbachia,Dose,Replicate,sep = "_")] [,Condition:=paste(Wolbachia,Preinf)]
surv_FHV<-surv_FHV[,lapply(.SD,char_asfactor)]

##Diagnostics
#How many individuals were tested
ftable(xtabs(~Test+Preinf+Dose+Wolbachia,surv_FHV))
```

##	## Test	Preinf	Dose	Wolbachia Wol-	Wol+
##	09_02_2015	18	E6	50	51
##			E7	50	50
##			E8	49	50
##			E9	0	0
##		25	E6	49	48
##			E7	50	50
##			E8	50	50
##			E9	0	0
##	21_11_2014	18	E6	0	0
##			E7	50	50
##			E8	50	50
##			E9	50	50
##		25	E6	0	0

```
##          E7          50  50
##          E8          50  50
##          E9          50  50

#Kill one fly of wolb+ 25-18 E6 sample, in the last day, to allow model to converge
surv_FHVa <- surv_FHV
surv_FHVa$Status[which(surv_FHVa$Genotype == "CS 25-18 E6")[1]]<-1

#Data analysis
#Cox models
#Full model
surv_FHV_cox_full<-coxme(Surv(Time,Status)~(Wolbachia*Dose*Preinf)+(1|Test/RepFull),
                        data=surv_FHVa)
Anova(surv_FHV_cox_full,test.statistic = "LR")
```

```
## Analysis of Deviance Table (Type II tests)
##          LR Chisq Df Pr(>Chisq)
## Wolbachia      102.003  1 < 2.2e-16 ***
## Dose           194.917  3 < 2.2e-16 ***
## Preinf         48.565  1 3.195e-12 ***
## Wolbachia:Dose    4.409  3   0.22056
## Wolbachia:Preinf 143.421  1 < 2.2e-16 ***
## Dose:Preinf       0.958  3   0.81143
## Wolbachia:Dose:Preinf 10.825  3   0.01271 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

There is a significant interaction between pre-infection temperature, *Wolbachia*, and dose of infection

```
#effect of Wolb at different temp and doses
surv_FHV_temp_contr=lsmeans::lsmeans(surv_FHV_cox_full,pairwise~Wolbachia|Preinf:Dose)
summary(surv_FHV_temp_contr,by=NULL,adj="holm")
```

```
## $lsmeans
## Wolbachia Preinf Dose lsmean SE df asymp.LCL asymp.UCL
## Wol-      18     E6  -1.772 0.313 Inf  -2.698 -0.846
## Wol+      18     E6  -3.193 0.565 Inf  -4.864 -1.522
## Wol-      25     E6  -1.847 0.326 Inf  -2.812 -0.882
## Wol+      25     E6  -4.268 0.967 Inf  -7.125 -1.411
## Wol-      18     E7   1.195 0.139 Inf   0.785  1.604
## Wol+      18     E7   0.188 0.153 Inf  -0.264  0.641
## Wol-      25     E7   1.134 0.138 Inf   0.725  1.542
## Wol+      25     E7  -2.717 0.387 Inf  -3.862 -1.572
## Wol-      18     E8   2.370 0.140 Inf   1.957  2.782
## Wol+      18     E8   1.598 0.136 Inf   1.197  1.999
## Wol-      25     E8   2.406 0.140 Inf   1.992  2.820
## Wol+      25     E8  -3.132 0.469 Inf  -4.517 -1.747
## Wol-      18     E9   2.441 0.188 Inf   1.887  2.995
## Wol+      18     E9   2.032 0.187 Inf   1.479  2.585
## Wol-      25     E9   2.210 0.187 Inf   1.657  2.762
## Wol+      25     E9  -1.780 0.567 Inf  -3.455 -0.106
##
## Results are given on the log (not the response) scale.
```

```
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 16 estimates
##
## $contrasts
## contrast      Preinf Dose estimate      SE df z.ratio p.value
## Wol- - Wol+ 18      E6        1.420 0.660 Inf  2.153  0.0648
## Wol- - Wol+ 25      E6        2.421 1.054 Inf  2.297  0.0648
## Wol- - Wol+ 18      E7        1.006 0.186 Inf  5.405 <.0001
## Wol- - Wol+ 25      E7        3.851 0.432 Inf  8.920 <.0001
## Wol- - Wol+ 18      E8        0.772 0.167 Inf  4.632 <.0001
## Wol- - Wol+ 25      E8        5.538 0.523 Inf 10.596 <.0001
## Wol- - Wol+ 18      E9        0.409 0.229 Inf  1.786  0.0742
## Wol- - Wol+ 25      E9        3.990 0.606 Inf  6.581 <.0001
##
## Results are given on the log (not the response) scale.
## P value adjustment: holm method for 8 tests
#interaction of Wolb and temp at different doses
surv_FHV_temp_contr_inter=contrast(surv_FHV_temp_contr$contrasts,by=c("contrast","Dose"),method="pairwi
summary(surv_FHV_temp_contr_inter,by=NULL,adj="holm")

## contrast1 contrast      Dose estimate      SE df z.ratio p.value
## 18 - 25      Wol- - Wol+ E6        -1.00 1.244 Inf -0.805  0.4208
## 18 - 25      Wol- - Wol+ E7        -2.84 0.469 Inf -6.069 <.0001
## 18 - 25      Wol- - Wol+ E8        -4.77 0.544 Inf -8.757 <.0001
## 18 - 25      Wol- - Wol+ E9        -3.58 0.647 Inf -5.536 <.0001
##
## Results are given on the log (not the response) scale.
## P value adjustment: holm method for 4 tests
```

*Wolbachia* effect is stronger at pre-infection temperature of 25°C at all doses except 10<sup>6</sup> TCID<sub>50</sub>/ml

```
#effect of pre-infection temp with and without Wolb
summary(lsmeans::lsmeans(surv_FHV_cox_full,pairwise~Preinf|Dose:Wolbachia),by=NULL,adj = "holm")

## $lsmeans
## Preinf Dose Wolbachia lsmean      SE df asymp.LCL asymp.UCL
## 18      E6      Wol-      -1.772 0.313 Inf   -2.698   -0.846
## 25      E6      Wol-      -1.847 0.326 Inf   -2.812   -0.882
## 18      E7      Wol-       1.195 0.139 Inf    0.785    1.604
## 25      E7      Wol-       1.134 0.138 Inf    0.725    1.542
## 18      E8      Wol-       2.370 0.140 Inf    1.957    2.782
## 25      E8      Wol-       2.406 0.140 Inf    1.992    2.820
## 18      E9      Wol-       2.441 0.188 Inf    1.887    2.995
## 25      E9      Wol-       2.210 0.187 Inf    1.657    2.762
## 18      E6      Wol+      -3.193 0.565 Inf   -4.864   -1.522
## 25      E6      Wol+      -4.268 0.967 Inf   -7.125   -1.411
## 18      E7      Wol+       0.188 0.153 Inf   -0.264    0.641
## 25      E7      Wol+      -2.717 0.387 Inf   -3.862   -1.572
## 18      E8      Wol+       1.598 0.136 Inf    1.197    1.999
## 25      E8      Wol+      -3.132 0.469 Inf   -4.517   -1.747
## 18      E9      Wol+       2.032 0.187 Inf    1.479    2.585
## 25      E9      Wol+      -1.780 0.567 Inf   -3.455   -0.106
##
```

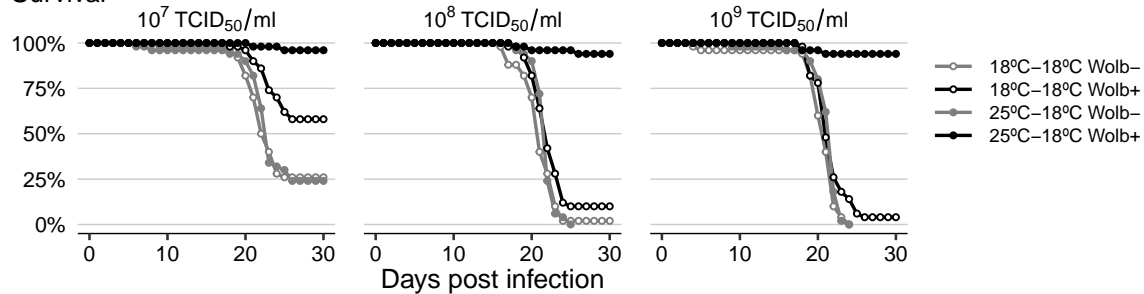
```
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 16 estimates
##
## $contrasts
## contrast Dose Wolbachia estimate SE df z.ratio p.value
## 18 - 25 E6 Wol- 0.0746 0.449 Inf 0.166 1.0000
## 18 - 25 E7 Wol- 0.0613 0.172 Inf 0.357 1.0000
## 18 - 25 E8 Wol- -0.0364 0.162 Inf -0.224 1.0000
## 18 - 25 E9 Wol- 0.2314 0.227 Inf 1.021 1.0000
## 18 - 25 E6 Wol+ 1.0756 1.159 Inf 0.928 1.0000
## 18 - 25 E7 Wol+ 2.9055 0.436 Inf 6.660 <.0001
## 18 - 25 E8 Wol+ 4.7299 0.519 Inf 9.109 <.0001
## 18 - 25 E9 Wol+ 3.8121 0.606 Inf 6.292 <.0001
##
## Results are given on the log (not the response) scale.
## P value adjustment: holm method for 8 tests
```

Pre-infection temperature is not significant in the absence of *Wolbachia*. It is significant in the presence of *Wolbachia* at all doses except  $10^6$  TCID<sub>50</sub>/ml.

Figures 3C, S2C

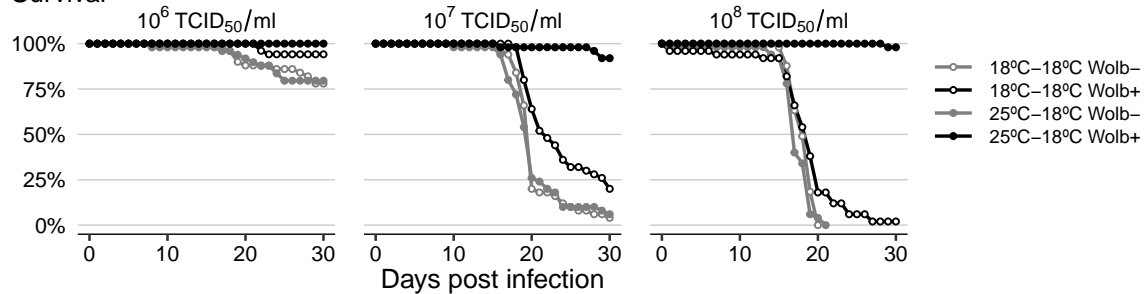
### C FHV

#### Survival



### C FHV

#### Survival



## Survival with temperature cycling

```
#Cox model
cox_cycling<-coxme(Surv(Time,Status)~Wolb+(1|RepFull),surv_cyc)

Anova(cox_cycling, test.statistic = "LR")

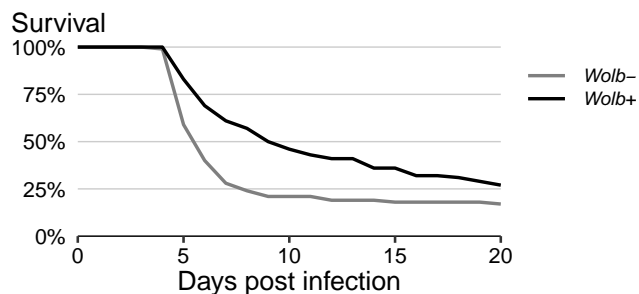
## Analysis of Deviance Table
##  Cox model: response is Surv(Time, Status)
## Terms added sequentially (first to last)
##
##      loglik  Chisq Df Pr(>|Chi|)
## NULL -737.91
## Wolb -725.48 24.867  1  6.142e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(cox_cycling)

## Cox mixed-effects model fit by maximum likelihood
##  Data: surv_cyc
##  events, n = 156, 200
##  Iterations= 7 32
##
##              NULL Integrated      Fitted
## Log-likelihood -737.9147  -725.4812 -709.5113
##
##              Chisq    df          p    AIC    BIC
## Integrated loglik 24.87   2.00 3.9828e-06 20.87 14.77
## Penalized loglik 56.81 12.61 1.4132e-07 31.60 -6.85
##
## Model:  Surv(Time, Status) ~ Wolb + (1 | RepFull)
## Fixed coefficients
##              coef exp(coef) se(coef)      z      p
## WolbWolb+ -0.6671881 0.5131495 0.2719185 -2.45 0.014
##
## Random effects
## Group  Variable Std Dev  Variance
## RepFull Intercept 0.4861021 0.2362953
```

*Wolbachia* increases survival in cycling temperatures

Figure S3



## Effect of development and aging temperature before infection on survival

```
Surv_aging <- fread("dataset_s9.txt")[,lapply(.SD,char_asfactor)]
Surv_aging<-Surv_aging[,Rep:=paste(Test,Wolbachia, Development_temperature, Aging_temperature,Replicate)]
Surv_aging$Development_temperature<-as.factor(as.character(Surv_aging$Development_temperature))
Surv_aging$Aging_temperature<-as.factor(as.character(Surv_aging$Aging_temperature))

#Tested individuals
xtabs(~Wolbachia+RepTemp+Test,Surv_aging)

## , , Test = A
##
##      RepTemp
## Wolbachia 18_18 18_25 25_18 25_25
##      Wol-    50    50    50    50
##      Wol+    50    50    50    49
##
## , , Test = B
##
##      RepTemp
## Wolbachia 18_18 18_25 25_18 25_25
##      Wol-    50    50    50    50
##      Wol+    50    50    50    50
##
## , , Test = C
##
##      RepTemp
## Wolbachia 18_18 18_25 25_18 25_25
##      Wol-    50    50    50    50
##      Wol+    50    50    50    50
##
#full model
cox_Surv_aging_full<-coxme(Surv(Time,Status)~Wolbachia*Development_temperature*Aging_temperature+(1|Test))
Anova(cox_Surv_aging_full,test.statistic = "LR")

## Analysis of Deviance Table (Type II tests)
##
##              LR Chisq Df Pr(>Chisq)
## Wolbachia          75.774  1 < 2.2e-16 ***
## Development_temperature 29.275  1 6.279e-08 ***
## Aging_temperature        0.035  1  0.85061
## Wolbachia:Development_temperature 40.163  1 2.336e-10 ***
## Wolbachia:Aging_temperature    6.521  1  0.01066 *
## Development_temperature:Aging_temperature 0.633  1  0.42637
## Wolbachia:Development_temperature:Aging_temperature 0.513  1  0.47375
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#simpler model
cox_Surv_aging_simple <-coxme(Surv(Time,Status)~Wolbachia*Aging_temperature+Wolbachia*Development_temper
Anova(cox_Surv_aging_simple,test.statistic = "LR")

## Analysis of Deviance Table (Type II tests)
##
##              LR Chisq Df Pr(>Chisq)
## Wolbachia          75.576  1 < 2.2e-16 ***
```

```
## Aging_temperature          0.035  1    0.85061
## Development_temperature    29.275  1  6.279e-08 ***
## Wolbachia:Aging_temperature    6.348  1    0.01175 *
## Wolbachia:Development_temperature 39.902  1  2.671e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(cox_Surv_aging_simple)

## Cox mixed-effects model fit by maximum likelihood
##   Data: Surv_aging
##   events, n = 1064, 1199
##   Iterations= 12 64
##               NULL Integrated    Fitted
## Log-likelihood -6774.882 -6532.167 -6463.197
##
##               Chisq    df p    AIC    BIC
## Integrated loglik 485.43  7.00 0 471.43 436.64
## Penalized loglik 623.37 58.64 0 506.08 214.63
##
## Model: Surv(Time, Status) ~ Wolbachia * Aging_temperature + Wolbachia * Development_temperature
## Fixed coefficients
##
##               coef exp(coef) se(coef)      z
## WolbachiaWol+    -0.237051814 0.7889504 0.1447667 -1.64
## Aging_temperature25    0.222724245 1.2494760 0.1175657  1.89
## Development_temperature25 -0.001842135 0.9981596 0.1174962 -0.02
## WolbachiaWol+:Aging_temperature25 -0.431474262 0.6495508 0.1699900 -2.54
## WolbachiaWol+:Development_temperature25 -1.158294456 0.3140213 0.1713643 -6.76
##
##               P
## WolbachiaWol+    1.0e-01
## Aging_temperature25    5.8e-02
## Development_temperature25    9.9e-01
## WolbachiaWol+:Aging_temperature25    1.1e-02
## WolbachiaWol+:Development_temperature25 1.4e-11
##
## Random effects
##   Group   Variable   Std Dev   Variance
## Test/Rep (Intercept) 0.31406601 0.09863746
## Test      (Intercept) 0.48075177 0.23112226
```

There is a significant interaction between *Wolbachia* and aging temperature, and between *Wolbachia* and development temperature.

```
#Contrasts

#Contrast between with and without Wolbachia at different development temperatures
contr_Wolb_Dev<-lsmeans::lsmeans(cox_Surv_aging_simple,pairwise~Wolbachia|Development_temperature)
summary(contr_Wolb_Dev)

## $lsmeans
## Development_temperature = 18:
## Wolbachia lsmean SE df asymp.LCL asymp.UCL
## Wol-      0.5159 0.0728 Inf 0.3732 0.659
```

```
## Wol+      0.0631 0.0727 Inf   -0.0795    0.206
##
## Development_temperature = 25:
## Wolbachia lsmean      SE  df asymp.LCL asymp.UCL
## Wol-      0.5140 0.0730 Inf    0.3710    0.657
## Wol+      -1.0970 0.0785 Inf   -1.2510   -0.943
##
## Results are averaged over the levels of: Aging_temperature
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## Development_temperature = 18:
## contrast      estimate      SE  df z.ratio p.value
## Wol- - Wol+    0.453 0.118 Inf   3.838 0.0001
##
## Development_temperature = 25:
## contrast      estimate      SE  df z.ratio p.value
## Wol- - Wol+    1.611 0.125 Inf  12.858 <.0001
##
## Results are averaged over the levels of: Aging_temperature
## Results are given on the log (not the response) scale.
```

```
contrast(contr_Wolb_Dev$contrasts,"pairwise",by="contrast")
```

```
## contrast = Wol- - Wol+:
## contrast1 estimate      SE  df z.ratio p.value
## 18 - 25      -1.16 0.171 Inf -6.759 <.0001
##
## Results are averaged over the levels of: Aging_temperature
## Results are given on the log (not the response) scale.
```

*Wolbachia* has an effect at both development temperatures but effect is higher at 25°C

```
#Contrast between with and without Wolbachia at different aging temperatures
contr_Wolb_Aging<-lsmeans::lsmeans(cox_Surv_aging_simple,pairwise~Wolbachia|Aging_temperature)
summary(contr_Wolb_Aging)
```

```
## $lsmeans
## Aging_temperature = 18:
## Wolbachia lsmean      SE  df asymp.LCL asymp.UCL
## Wol-      0.404 0.0728 Inf    0.261    0.546
## Wol+      -0.413 0.0741 Inf   -0.558   -0.267
##
## Aging_temperature = 25:
## Wolbachia lsmean      SE  df asymp.LCL asymp.UCL
## Wol-      0.626 0.0731 Inf    0.483    0.770
## Wol+      -0.621 0.0760 Inf   -0.770   -0.472
##
## Results are averaged over the levels of: Development_temperature
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
```



```
## $contrasts
## Aging_temperature = 18:
## contrast estimate SE df z.ratio p.value
## Wol- - Wol+ 0.816 0.120 Inf 6.806 <.0001
##
## Aging_temperature = 25:
## contrast estimate SE df z.ratio p.value
## Wol- - Wol+ 1.248 0.122 Inf 10.186 <.0001
##
## Results are averaged over the levels of: Development_temperature
## Results are given on the log (not the response) scale.
```

```
contrast(contr_Wolb_Aging$contrasts,"pairwise",by="contrast")
```

```
## contrast = Wol- - Wol+:
## contrast1 estimate SE df z.ratio p.value
## 18 - 25 -0.431 0.17 Inf -2.538 0.0111
##
## Results are averaged over the levels of: Development_temperature
## Results are given on the log (not the response) scale.
```

*Wolbachia* has an effect at both aging temperatures but effect is higher at 25°C

```
#Contrast between with and without Wolbachia at different development temperatures and aging temperatures
contr_Wolb_dev_temp_Aging<-lsmeans::lsmeans(cox_Surv_aging_simple,pairwise~Wolbachia|Development_temperature)
summary(contr_Wolb_dev_temp_Aging,adj="holm",by=NULL)
```

```
## $lsmeans
## Wolbachia Development_temperature Aging_temperature lsmean SE df
## Wol- 18 18 0.4045 0.0931 Inf
## Wol+ 18 18 0.1675 0.0951 Inf
## Wol- 25 18 0.4027 0.0940 Inf
## Wol+ 25 18 -0.9927 0.0982 Inf
## Wol- 18 25 0.6273 0.0941 Inf
## Wol+ 18 25 -0.0413 0.0951 Inf
## Wol- 25 25 0.6254 0.0934 Inf
## Wol+ 25 25 -1.2014 0.1010 Inf
## asymp.LCL asymp.UCL
## 0.1500 0.659
## -0.0927 0.428
## 0.1457 0.660
## -1.2612 -0.724
## 0.3700 0.884
## -0.3013 0.219
## 0.3699 0.881
## -1.4777 -0.925
##
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 8 estimates
##
## $contrasts
## contrast Development_temperature Aging_temperature estimate SE df
```

```
## Wol- - Wol+ 18      18      0.237 0.145 Inf
## Wol- - Wol+ 25      18      1.395 0.150 Inf
## Wol- - Wol+ 18      25      0.669 0.146 Inf
## Wol- - Wol+ 25      25      1.827 0.153 Inf
## z.ratio p.value
## 1.637 0.1015
## 9.305 <.0001
## 4.578 <.0001
## 11.953 <.0001
##
## Results are given on the log (not the response) scale.
## P value adjustment: holm method for 4 tests
```

```
contrast(contr_Wolb_dev_temp_Aging$contrasts,"pairwise",by="contrast")
```

```
## contrast = Wol- - Wol+:
## contrast1      estimate      SE df z.ratio p.value
## 18,18 - 25,18   -1.158 0.171 Inf -6.759 <.0001
## 18,18 - 18,25   -0.431 0.170 Inf -2.538 0.0542
## 18,18 - 25,25   -1.590 0.242 Inf -6.558 <.0001
## 25,18 - 18,25    0.727 0.240 Inf  3.024 0.0133
## 25,18 - 25,25   -0.431 0.170 Inf -2.538 0.0542
## 18,25 - 25,25   -1.158 0.171 Inf -6.759 <.0001
##
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 4 estimates
```

```
#Contrast between development temperatures with and without Wolbachia
```

```
contr_Dev_Wolb<-lsmeans::lsmeans(cox_Surv_aging_simple,pairwise~Development_temperature|Wolbachia)
summary(contr_Dev_Wolb)
```

```
## $lsmeans
## Wolbachia = Wol-:
## Development_temperature  lsmean      SE df asymp.LCL asymp.UCL
## 18                      0.5159 0.0728 Inf    0.3732    0.659
## 25                      0.5140 0.0730 Inf    0.3710    0.657
##
## Wolbachia = Wol+:
## Development_temperature  lsmean      SE df asymp.LCL asymp.UCL
## 18                      0.0631 0.0727 Inf    -0.0795    0.206
## 25                      -1.0970 0.0785 Inf    -1.2510   -0.943
##
## Results are averaged over the levels of: Aging_temperature
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## Wolbachia = Wol-:
## contrast estimate      SE df z.ratio p.value
## 18 - 25    0.00184 0.117 Inf 0.016 0.9875
##
## Wolbachia = Wol+:
## contrast estimate      SE df z.ratio p.value
## 18 - 25    1.16014 0.124 Inf 9.338 <.0001
##
```

```
## Results are averaged over the levels of: Aging_temperature
## Results are given on the log (not the response) scale.
```

Development temperature only has an effect in the presence of *Wolbachia*

```
#Contrast between aging temperatures with and without Wolbachia
contr_Aging_Wolb <-lsmeans::lsmeans(cox_Surv_aging_simple,pairwise~Aging_temperature|Wolbachia)
summary(contr_Aging_Wolb)

## $lsmeans
## Wolbachia = Wol-:
##   Aging_temperature lsmean      SE  df asymp.LCL asymp.UCL
##   18                0.404 0.0728 Inf    0.261    0.546
##   25                0.626 0.0731 Inf    0.483    0.770
##
## Wolbachia = Wol+:
##   Aging_temperature lsmean      SE  df asymp.LCL asymp.UCL
##   18               -0.413 0.0741 Inf   -0.558   -0.267
##   25               -0.621 0.0760 Inf   -0.770   -0.472
##
## Results are averaged over the levels of: Development_temperature
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
##
## $contrasts
## Wolbachia = Wol-:
##   contrast estimate      SE  df z.ratio p.value
##   18 - 25      -0.223 0.118 Inf -1.894  0.0582
##
## Wolbachia = Wol+:
##   contrast estimate      SE  df z.ratio p.value
##   18 - 25       0.209 0.123 Inf  1.703  0.0886
##
## Results are averaged over the levels of: Development_temperature
## Results are given on the log (not the response) scale.
```

Aging temperature does not have an effect in direct contrasts. The interaction significance comes from slightly deleterious effect of higher temperature in the absence of *Wolbachia* and slightly beneficial effect in its presence.

Figure S4

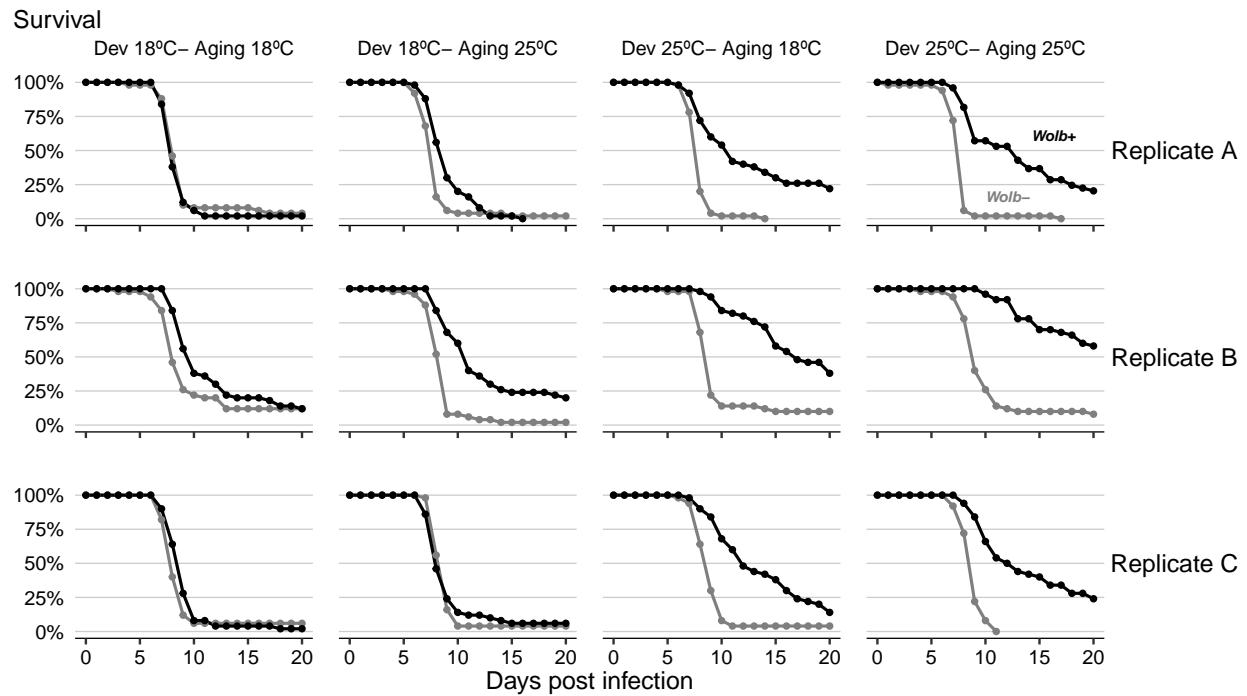


Figure 4 and S5

### DCV levels at early infection

```
DCV_early <- fread("dataset_s10.txt")[,lapply(.SD,char_asfactor)]
DCV_early <- filter(DCV_early, Treatment == "DCV")
DCV_early$logDCV <- ifelse(is.na(DCV_early$rel_DCV),log10(min(DCV_early$rel_DCV,na.rm = T)/10),log10(DCV_early$rel_DCV))
DCV_early$Time <- as.factor(as.character(DCV_early$Time))

#linear model
lm_DCV_early <- lm(logDCV~Wolb*Time, data=DCV_early)
Anova(lm_DCV_early)

## Anova Table (Type II tests)
##
## Response: logDCV
##          Sum Sq Df  F value    Pr(>F)
## Wolb         26.662  1  63.4876 2.462e-10 ***
## Time        288.807  5 137.5440 < 2.2e-16 ***
## Wolb:Time    15.928  5   7.5856 2.571e-05 ***
## Residuals    20.158 48
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(lm_DCV_early)

##
## Call:
## lm(formula = logDCV ~ Wolb * Time, data = DCV_early)
```

```
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -2.06961 -0.29621 -0.01134  0.20753  1.55543
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -1.66618    0.28981  -5.749 6.04e-07 ***
## WolbWolb+      0.29534    0.40985   0.721 0.474652
## Time0.25     -0.09621    0.40985  -0.235 0.815412
## Time0.5       2.17278    0.40985   5.301 2.87e-06 ***
## Time1        3.19419    0.40985   7.793 4.52e-10 ***
## Time2        5.49313    0.40985  13.403 < 2e-16 ***
## Time3        6.99756    0.40985  17.073 < 2e-16 ***
## WolbWolb+:Time0.25 -0.49534    0.57962  -0.855 0.397022
## WolbWolb+:Time0.5 -2.37547    0.57962  -4.098 0.000159 ***
## WolbWolb+:Time1   -1.79437    0.57962  -3.096 0.003273 **
## WolbWolb+:Time2   -2.28847    0.57962  -3.948 0.000257 ***
## WolbWolb+:Time3   -2.81762    0.57962  -4.861 1.29e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.648 on 48 degrees of freedom
## Multiple R-squared:  0.9427, Adjusted R-squared:  0.9295
## F-statistic: 71.74 on 11 and 48 DF, p-value: < 2.2e-16
```

There is an interaction between *Wolbachia* and time of infection

```
#compare effect of Wolb at the several time points
lsm_lm_DCV_early <- lsmeans::lsmeans(lm_DCV_early, pairwise~Wolb|Time,adj="none")
summary(lsm_lm_DCV_early,by=NULL,adj="holm")
```

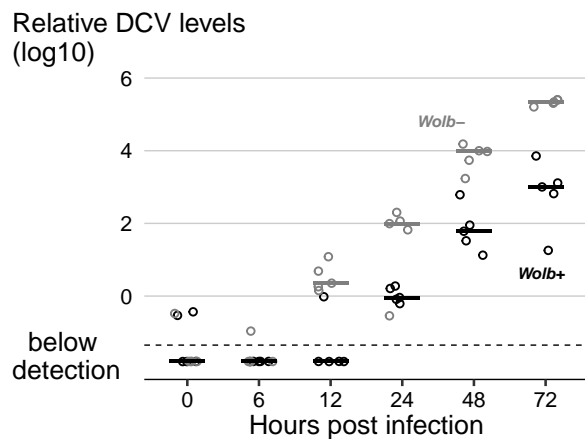
```
## $lsmeans
##   Wolb Time lsmean   SE df lower.CL upper.CL
##   Wolb- 0    -1.666 0.29 48   -2.538   -0.794
##   Wolb+ 0    -1.371 0.29 48   -2.243   -0.499
##   Wolb- 0.25 -1.762 0.29 48   -2.634   -0.890
##   Wolb+ 0.25 -1.962 0.29 48   -2.834   -1.090
##   Wolb- 0.5   0.507 0.29 48   -0.365    1.379
##   Wolb+ 0.5  -1.574 0.29 48   -2.446   -0.701
##   Wolb- 1     1.528 0.29 48    0.656    2.400
##   Wolb+ 1     0.029 0.29 48   -0.843    0.901
##   Wolb- 2     3.827 0.29 48    2.955    4.699
##   Wolb+ 2     1.834 0.29 48    0.962    2.706
##   Wolb- 3     5.331 0.29 48    4.459    6.203
##   Wolb+ 3     2.809 0.29 48    1.937    3.681
##
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 12 estimates
##
## $contrasts
##   contrast      Time estimate   SE df t.ratio p.value
##   Wolb- - Wolb+ 0      -0.295 0.41 48  -0.721  0.9493
```

```
## Wolb- - Wolb+ 0.25      0.200 0.41 48  0.488  0.9493
## Wolb- - Wolb+ 0.5      2.080 0.41 48  5.075  <.0001
## Wolb- - Wolb+ 1        1.499 0.41 48  3.657  0.0019
## Wolb- - Wolb+ 2        1.993 0.41 48  4.863  0.0001
## Wolb- - Wolb+ 3        2.522 0.41 48  6.154  <.0001
##
## P value adjustment: holm method for 6 tests
log_DCV_early <- summary(lsm_lm_DCV_early,by=NULL,adj="holm")$contrasts[,3]
print(10,log_DCV_early)

## [1] 0.5065949 1.5848932 120.2627078 31.5522757 98.4306741 332.8744891
```

*Wolbachia* has a significant effect from 12h on.

Figure 4A



*#another data set of early time points of DCV infection*

```
DCV_early_doses <- fread("dataset_s11.txt")[,lapply(.SD,char_asfactor)]
DCV_early_doses$Time <- as.factor(as.character(DCV_early_doses$Time))
DCV_early_doses$logDCV <- ifelse(is.na(DCV_early_doses$Ratio),log10(min(DCV_early_doses$Ratio,na.rm = T)),log(DCV_early_doses$Ratio))

#lm
lm_DCV_early_doses <- lm(logDCV~Genotype*Time*Dose, data=DCV_early_doses)
Anova(lm_DCV_early_doses)
```

```
## Anova Table (Type II tests)
##
## Response: logDCV
##          Sum Sq Df F value    Pr(>F)
## Genotype    18.178  1 23.2119 2.473e-05 ***
## Time        55.014  1 70.2489 4.473e-10 ***
## Dose        16.738  1 21.3727 4.491e-05 ***
## Genotype:Time  0.790  1  1.0092  0.321608
## Genotype:Dose  0.775  1  0.9895  0.326331
## Time:Dose     7.165  1  9.1498  0.004505 **
## Genotype:Time:Dose 0.037  1  0.0469  0.829772
```

```
## Residuals          28.976 37
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

lm_DCV_early_doses_simple <- lm(logDCV~Genotype+Time*Dose, data=DCV_early_doses)
Anova(lm_DCV_early_doses_simple)

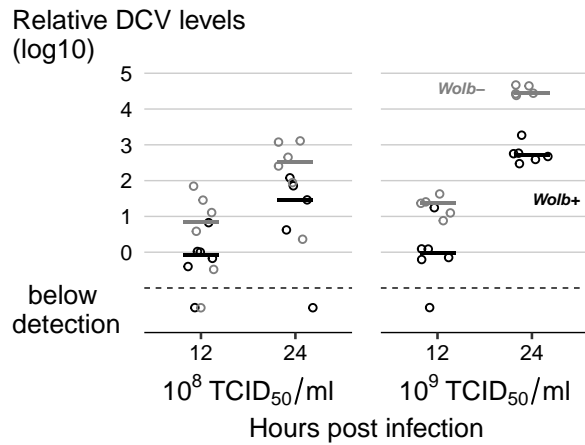
## Anova Table (Type II tests)
##
## Response: logDCV
##           Sum Sq Df F value    Pr(>F)
## Genotype  18.178  1 23.7496 1.771e-05 ***
## Time      54.720  1 71.4915 1.929e-10 ***
## Dose       16.611  1 21.7026 3.490e-05 ***
## Time:Dose   6.981  1  9.1213 0.004387 **
## Residuals 30.616 40
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(lm_DCV_early_doses_simple)

##
## Call:
## lm(formula = logDCV ~ Genotype + Time * Dose, data = DCV_early_doses)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -2.4281 -0.2828  0.1308  0.4261  1.3118
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.7896     0.2844   2.776 0.008330 **
## GenotypewMelCS -1.2750     0.2616  -4.873 1.77e-05 ***
## Time24         1.4349     0.3654   3.927 0.000331 ***
## DoseE9         0.4480     0.3654   1.226 0.227349
## Time24:DoseE9  1.5771     0.5222   3.020 0.004387 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.8749 on 40 degrees of freedom
## Multiple R-squared:  0.7593, Adjusted R-squared:  0.7352
## F-statistic: 31.54 on 4 and 40 DF,  p-value: 6.901e-12
```

*Wolbachia* confers significant resistance at these early time points

Figure S5A



## FHV levels at early infection

```
FHV_early <- fread("dataset_s12.txt")[,lapply(.SD,char_asfactor)]
FHV_early <- filter(FHV_early, Treatment == "FHV")
FHV_early$logFHV<- ifelse(is.na(FHV_early$rel_FHV),log10(min(FHV_early$rel_FHV,na.rm = T)/10),log10(FHV_early$rel_FHV))
FHV_early$Time <- as.factor(as.character(FHV_early$Time))
```

```
#linear model
lm_FHV_early <- lm(logFHV~Wolb*Time, data=FHV_early)
Anova(lm_FHV_early)
```

```
## Anova Table (Type II tests)
##
## Response: logFHV
##          Sum Sq Df F value    Pr(>F)
## Wolb      1.123  1  42.3198 2.428e-06 ***
## Time     95.652  4 901.1750 < 2.2e-16 ***
## Wolb:Time  0.421  4   3.9703  0.01571 *
## Residuals  0.531 20
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(lm_FHV_early)
```

```
##
## Call:
## lm(formula = logFHV ~ Wolb * Time, data = FHV_early)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.27644 -0.05261  0.00000  0.02928  0.41231
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  -1.247e+00  9.405e-02 -13.257 2.29e-11 ***
## WolbWolb+      3.430e-16  1.330e-01   0.000 1.000000
## Time1          1.673e+00  1.330e-01  12.578 5.90e-11 ***
```



```
## Time2          2.757e+00  1.330e-01  20.731 5.44e-15 ***
## Time3          3.912e+00  1.330e-01  29.412 < 2e-16 ***
## Time6          5.352e+00  1.330e-01  40.240 < 2e-16 ***
## WolbWolb+:Time1 -4.261e-01  1.881e-01  -2.265 0.034751 *
## WolbWolb+:Time2 -7.455e-01  1.881e-01  -3.963 0.000766 ***
## WolbWolb+:Time3 -4.046e-01  1.881e-01  -2.151 0.043894 *
## WolbWolb+:Time6 -3.586e-01  1.881e-01  -1.906 0.071085 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1629 on 20 degrees of freedom
## Multiple R-squared:  0.9946, Adjusted R-squared:  0.9921
## F-statistic: 407 on 9 and 20 DF, p-value: < 2.2e-16
```

There is a significant interaction between *Wolbachia* and time

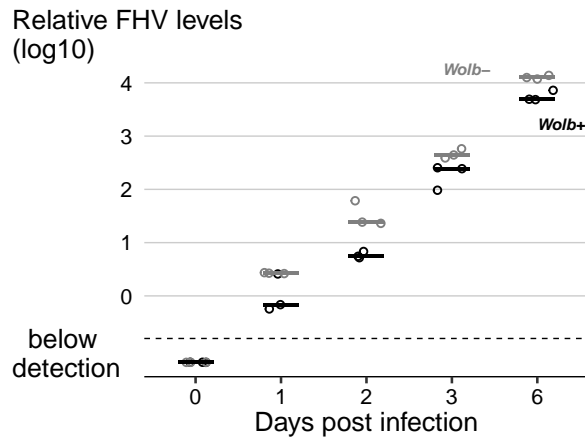
```
#compare effect of Wolb at the several timepoints
lsm_lm_FHV_early <- lsmeans::lsmeans(lm_FHV_early, pairwise~Wolb|Time,adj="none")
summary(lsm_lm_FHV_early,by=NULL,adj="holm")
```

```
## $lsmeans
## Wolb Time lsmean SE df lower.CL upper.CL
## Wolb- 0 -1.247 0.094 20 -1.543 -0.950
## Wolb+ 0 -1.247 0.094 20 -1.543 -0.950
## Wolb- 1 0.426 0.094 20 0.130 0.723
## Wolb+ 1 0.000 0.094 20 -0.297 0.297
## Wolb- 2 1.510 0.094 20 1.214 1.807
## Wolb+ 2 0.765 0.094 20 0.468 1.062
## Wolb- 3 2.665 0.094 20 2.369 2.962
## Wolb+ 3 2.261 0.094 20 1.964 2.557
## Wolb- 6 4.105 0.094 20 3.809 4.402
## Wolb+ 6 3.747 0.094 20 3.450 4.043
##
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 10 estimates
##
## $contrasts
## contrast Time estimate SE df t.ratio p.value
## Wolb- - Wolb+ 0 0.000 0.133 20 0.000 1.0000
## Wolb- - Wolb+ 1 0.426 0.133 20 3.204 0.0178
## Wolb- - Wolb+ 2 0.746 0.133 20 5.605 0.0001
## Wolb- - Wolb+ 3 0.405 0.133 20 3.042 0.0193
## Wolb- - Wolb+ 6 0.359 0.133 20 2.696 0.0278
##
## P value adjustment: holm method for 5 tests
log_FHV_early <- summary(lsm_lm_FHV_early,by=NULL,adj="holm")$contrasts[,3]
p.adjust(10,log_FHV_early)

## [1] 1.000000 2.667471 5.565594 2.538451 2.283328
```

*Wolbachia* has a small significant effect from 1 day on (2 to 6 fold)

Figure S5B



### *Wolbachia* levels after antibiotics treatment

```
Antibiotics_levels <- fread("dataset_s13.txt")[,lapply(.SD,char_asfactor)]
Antibiotics_levels$logratio <- log10(Antibiotics_levels$ratio)
Antibiotics_levels$timepoint <- as.factor(as.character(Antibiotics_levels$timepoint))
Antibiotics_levels[,treatment:=relevel(treatment,"water")]
Antibiotics_treat <- filter(Antibiotics_levels, timepoint != "0")

#linear model
lmer_ant <- lmer(logratio~treatment*timepoint + (1|replicate), data=Antibiotics_treat)
Anova(lmer_ant)

## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: logratio
##               Chisq Df Pr(>Chisq)
## treatment      1869.3483  5 < 2.2e-16 ***
## timepoint         0.1531  1   0.6956
## treatment:timepoint  29.3365  5  1.992e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(lmer_ant)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: logratio ~ treatment * timepoint + (1 | replicate)
##   Data: Antibiotics_treat
##
## REML criterion at convergence: -116
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.5493 -0.6249 -0.1255  0.5181  3.7513
##
```

```

## Random effects:
##   Groups      Name      Variance Std.Dev.
## replicate (Intercept) 0.0008164 0.02857
## Residual              0.0207875 0.14418
## Number of obs: 155, groups: replicate, 2
##
## Fixed effects:
##
##              Estimate Std. Error      df t value
## (Intercept)      1.01457    0.04481 12.97522  22.642
## treatmentampicillin -0.07155    0.05772 142.02299  -1.239
## treatmentethanol     0.05314    0.05655 142.00000   0.940
## treatmentrifampicin -0.93840    0.05655 142.00000 -16.594
## treatmentstreptomycin -0.01454    0.05655 142.00000  -0.257
## treatmenttetracycline -0.95028    0.05655 142.00000 -16.804
## timepoint30         0.11133    0.05655 142.00000   1.969
## treatmentampicillin:timepoint30 -0.03037    0.08011 142.04098  -0.379
## treatmentethanol:timepoint30 -0.01597    0.07998 142.00000  -0.200
## treatmentrifampicin:timepoint30 -0.35046    0.07999 142.03508  -4.381
## treatmentstreptomycin:timepoint30 -0.11843    0.08081 142.01176  -1.466
## treatmenttetracycline:timepoint30 -0.20667    0.07998 142.00000  -2.584
##
##              Pr(>|t|)
## (Intercept)      8.17e-12 ***
## treatmentampicillin  0.2172
## treatmentethanol    0.3490
## treatmentrifampicin < 2e-16 ***
## treatmentstreptomycin  0.7975
## treatmenttetracycline < 2e-16 ***
## timepoint30        0.0509 .
## treatmentampicillin:timepoint30  0.7052
## treatmentethanol:timepoint30    0.8420
## treatmentrifampicin:timepoint30 2.28e-05 ***
## treatmentstreptomycin:timepoint30 0.1450
## treatmenttetracycline:timepoint30 0.0108 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) trtmntm trtmntth trtmntr trtmnts trtmnttt tmpn30 trtmntm:30
## trtmntmpc11 -0.618
## tretmntthn1 -0.631  0.490
## trtmntrfmpc -0.631  0.490  0.500
## trtmntstrpt -0.631  0.490  0.500  0.500
## trtmnttttrcy -0.631  0.490  0.500  0.500  0.500
## timepoint30 -0.631  0.490  0.500  0.500  0.500  0.500
## trtmntmp:30  0.445 -0.721 -0.353 -0.353 -0.353 -0.353 -0.706
## trtmntth:30  0.446 -0.346 -0.707 -0.354 -0.354 -0.354 -0.707  0.499
## trtmntrf:30  0.446 -0.347 -0.353 -0.707 -0.353 -0.353 -0.707  0.499
## trtmntst:30  0.442 -0.343 -0.350 -0.350 -0.700 -0.350 -0.700  0.494
## trtmnttt:30  0.446 -0.346 -0.354 -0.354 -0.354 -0.707 -0.707  0.499
##
##              trtmntth:30 trtmntr:30 trtmnts:30
## trtmntmpc11
## tretmntthn1
## trtmntrfmpc
## trtmntstrpt

```

```
## trtmntttcry
## timepoint30
## trtmntmp:30
## trtmntth:30
## trtmntrf:30 0.500
## trtmntst:30 0.495 0.495
## trtmnttt:30 0.500 0.500 0.495
```

There is an interaction between treatment and timepoint

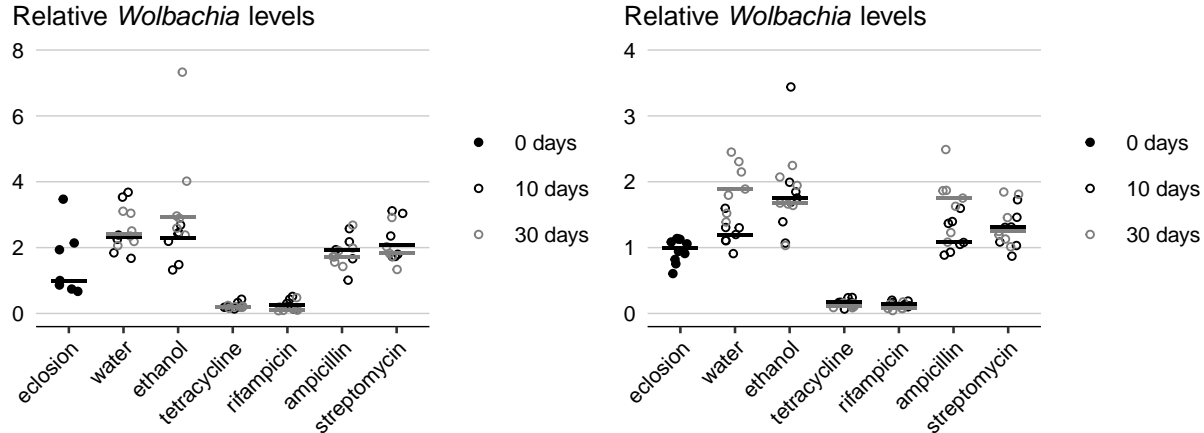
```
#pairwise comparison of treatments at each time point
lsm_ant <- lsmeans::lsmeans(lmer_ant, pairwise~treatment|timepoint,adj="none")
summary(lsm_ant,by=NULL,adj="holm")
```

```
## $lsmeans
## treatment timepoint lsmean SE df lower.CL upper.CL
## water 10 1.0146 0.0448 13.0 0.8591 1.1700
## ampicillin 10 0.9430 0.0463 14.6 0.7859 1.1001
## ethanol 10 1.0677 0.0448 13.0 0.9122 1.2232
## rifampicin 10 0.0762 0.0448 13.0 -0.0793 0.2316
## streptomycin 10 1.0000 0.0448 13.0 0.8446 1.1555
## tetracycline 10 0.0643 0.0448 13.0 -0.0912 0.2198
## water 30 1.1259 0.0448 13.0 0.9704 1.2814
## ampicillin 30 1.0240 0.0435 11.6 0.8694 1.1786
## ethanol 30 1.1631 0.0448 13.0 1.0076 1.3185
## rifampicin 30 -0.1630 0.0448 13.0 -0.3184 -0.0075
## streptomycin 30 0.9929 0.0463 14.6 0.8358 1.1500
## tetracycline 30 -0.0311 0.0448 13.0 -0.1865 0.1244
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 12 estimates
##
## $contrasts
## contrast timepoint estimate SE df t.ratio p.value
## water - ampicillin 10 0.0715 0.0577 142 1.239 1.0000
## water - ethanol 10 -0.0531 0.0566 142 -0.940 1.0000
## water - rifampicin 10 0.9384 0.0566 142 16.594 <.0001
## water - streptomycin 10 0.0145 0.0566 142 0.257 1.0000
## water - tetracycline 10 0.9503 0.0566 142 16.804 <.0001
## ampicillin - ethanol 10 -0.1247 0.0577 142 -2.160 0.3248
## ampicillin - rifampicin 10 0.8668 0.0577 142 15.014 <.0001
## ampicillin - streptomycin 10 -0.0570 0.0577 142 -0.987 1.0000
## ampicillin - tetracycline 10 0.8787 0.0577 142 15.220 <.0001
## ethanol - rifampicin 10 0.9915 0.0566 142 17.533 <.0001
## ethanol - streptomycin 10 0.0677 0.0566 142 1.197 1.0000
## ethanol - tetracycline 10 1.0034 0.0566 142 17.743 <.0001
## rifampicin - streptomycin 10 -0.9239 0.0566 142 -16.337 <.0001
## rifampicin - tetracycline 10 0.0119 0.0566 142 0.210 1.0000
## streptomycin - tetracycline 10 0.9357 0.0566 142 16.547 <.0001
## water - ampicillin 30 0.1019 0.0555 142 1.835 0.6175
## water - ethanol 30 -0.0372 0.0566 142 -0.657 1.0000
## water - rifampicin 30 1.2889 0.0566 142 22.771 <.0001
```

```
## water - streptomycin      30      0.1330 0.0577 142    2.303 0.2542
## water - tetracycline      30      1.1569 0.0566 142   20.458 <.0001
## ampicillin - ethanol      30     -0.1391 0.0555 142   -2.504 0.1744
## ampicillin - rifampicin   30      1.1869 0.0555 142   21.369 <.0001
## ampicillin - streptomycin 30      0.0311 0.0568 142    0.547 1.0000
## ampicillin - tetracycline 30      1.0550 0.0555 142   18.994 <.0001
## ethanol - rifampicin      30      1.3260 0.0566 142   23.427 <.0001
## ethanol - streptomycin    30      0.1701 0.0577 142    2.947 0.0526
## ethanol - tetracycline    30      1.1941 0.0566 142   21.115 <.0001
## rifampicin - streptomycin 30     -1.1559 0.0578 142  -19.984 <.0001
## rifampicin - tetracycline 30     -0.1319 0.0566 142   -2.331 0.2542
## streptomycin - tetracycline 30     1.0240 0.0577 142   17.736 <.0001
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: holm method for 30 tests
```

*Wolbachia* levels in rifampicin and tetracycline flies significantly different from controls and other antibiotics at both time points ( $p < 0.001$  for all). Rifampicin and tetracycline not significantly different at both time points ( $p > 0.25$  for both). Other samples not significantly different from each other at each time point ( $p > 0.05$  for all).

Figures 4B and S5C



## Survival to DCV after antibiotics

```
#load data
Survival_antibiotics <- fread("dataset_s14.txt")[,lapply(.SD,char_asfactor)]

Survival_antibiotics <- tidyr::separate(Survival_antibiotics, Condition, c("Wolbachia", "Treatment"), "
Survival_antibiotics <- tidyr::unite(Survival_antibiotics, RepFull, Treatment,Wolbachia,Test,Replicate,

Survival_antibiotics <- Survival_antibiotics[,lapply(.SD,char_asfactor)]
Survival_antibiotics[,Treatment:=relevel(Treatment,"water")]
```

```

#Diagnostics
#How many individuals were tested**
ftable(xtabs(~Test+Wolbachia*Treatment,Survival_antibiotics))

##           Treatment water ampicillin ethanol rifampicin streptomycin tetracycline
## Test Wolbachia
## A      CS              50           50           50           50           50           50
##      iso              50           50           50           50           50           49
## B      CS              50           50           50           50           50           50
##      iso              50           50           50           50           50           50

##Data analysis
#Full model

Survival_antibiotics_cox<-coxme(Surv(Time,Status)~Wolbachia*Treatment+(1|RepFull)+(1|Test), Survival_an
Anova(Survival_antibiotics_cox,test.statistic = "LR")

## Analysis of Deviance Table (Type II tests)
##           LR Chisq Df Pr(>Chisq)
## Wolbachia          70.955  1 < 2.2e-16 ***
## Treatment          41.817  5 6.416e-08 ***
## Wolbachia:Treatment  67.167  5 3.977e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(Survival_antibiotics_cox)

## Cox mixed-effects model fit by maximum likelihood
##   Data: Survival_antibiotics
##   events, n = 653, 1199
##   Iterations= 13 110
##           NULL Integrated      Fitted
## Log-likelihood -4406.166 -4058.574 -3957.027
##
##           Chisq   df p      AIC      BIC
## Integrated loglik 695.18 13.0 0 669.18 610.92
## Penalized loglik 898.28 76.8 0 744.67 400.48
##
## Model:  Surv(Time, Status) ~ Wolbachia * Treatment + (1 | RepFull) +      (1 | Test)
## Fixed coefficients
##
##           coef      exp(coef)  se(coef)      z
## Wolbachiaiso          2.6484521 14.13214648 0.4222592  6.27
## Treatmentampicillin    -0.5045895  0.60375339 0.5417512 -0.93
## Treatmentethanol        0.4252938  1.53003993 0.4674664  0.91
## Treatmentrifampicin     2.5392613 12.67030856 0.4202059  6.04
## Treatmentstreptomycin   -1.4907877  0.22519521 0.7079285 -2.11
## Treatmenttetracycline    2.5743570 13.12287695 0.4210857  6.11
## Wolbachiaiso:Treatmentampicillin  0.4011888  1.49359923 0.6407198  0.63
## Wolbachiaiso:Treatmentethanol    -0.2192497  0.80312117 0.5777168 -0.38
## Wolbachiaiso:Treatmentrifampicin  -2.4678268  0.08476888 0.5416011 -4.56
## Wolbachiaiso:Treatmentstreptomycin  1.3697108  3.93421287 0.7856511  1.74
## Wolbachiaiso:Treatmenttetracycline -2.5054517  0.08163871 0.5435895 -4.61
##
##           p
## Wolbachiaiso          3.6e-10
## Treatmentampicillin    3.5e-01

```

```
## Treatmentethanol 3.6e-01
## Treatmentrifampicin 1.5e-09
## Treatmentstreptomycin 3.5e-02
## Treatmenttetracycline 9.7e-10
## Wolbachiaiso:Treatmentampicillin 5.3e-01
## Wolbachiaiso:Treatmentethanol 7.0e-01
## Wolbachiaiso:Treatmentrifampicin 5.2e-06
## Wolbachiaiso:Treatmentstreptomycin 8.1e-02
## Wolbachiaiso:Treatmenttetracycline 4.0e-06
##
## Random effects
## Group Variable Std Dev Variance
## RepFull Intercept 0.65522621 0.42932138
## Test Intercept 0.29563700 0.08740124
```

There is an interaction between Treatment and *Wolbachia*

```
# Comparison of hazard ratios of Wolb versus no-Wolb at each treatment
mcp_Survival_antibiotics<-lsmeans::lsmeans(Survival_antibiotics_cox,pairwise~Wolbachia|Treatment)
summary(mcp_Survival_antibiotics,adj="holm",by=NULL)
```

```
## $lsmeans
## Wolbachia Treatment lsmean SE df asymp.LCL asymp.UCL
## CS water -1.629 0.331 Inf -2.576 -0.681
## iso water 1.020 0.239 Inf 0.336 1.703
## CS ampicillin -2.133 0.387 Inf -3.243 -1.024
## iso ampicillin 0.916 0.240 Inf 0.230 1.603
## CS ethanol -1.203 0.297 Inf -2.054 -0.353
## iso ethanol 1.226 0.236 Inf 0.550 1.901
## CS rifampicin 0.910 0.236 Inf 0.234 1.587
## iso rifampicin 1.091 0.239 Inf 0.408 1.774
## CS streptomycin -3.120 0.568 Inf -4.748 -1.491
## iso streptomycin 0.899 0.238 Inf 0.216 1.581
## CS tetracycline 0.946 0.237 Inf 0.266 1.626
## iso tetracycline 1.089 0.242 Inf 0.396 1.781
##
## Results are given on the log (not the response) scale.
## Confidence level used: 0.95
## Conf-level adjustment: bonferroni method for 12 estimates
##
## $contrasts
## contrast Treatment estimate SE df z.ratio p.value
## CS - iso water -2.648 0.422 Inf -6.272 <.0001
## CS - iso ampicillin -3.050 0.477 Inf -6.388 <.0001
## CS - iso ethanol -2.429 0.388 Inf -6.261 <.0001
## CS - iso rifampicin -0.181 0.339 Inf -0.532 1.0000
## CS - iso streptomycin -4.018 0.659 Inf -6.099 <.0001
## CS - iso tetracycline -0.143 0.343 Inf -0.417 1.0000
##
## Results are given on the log (not the response) scale.
## P value adjustment: holm method for 6 tests
```

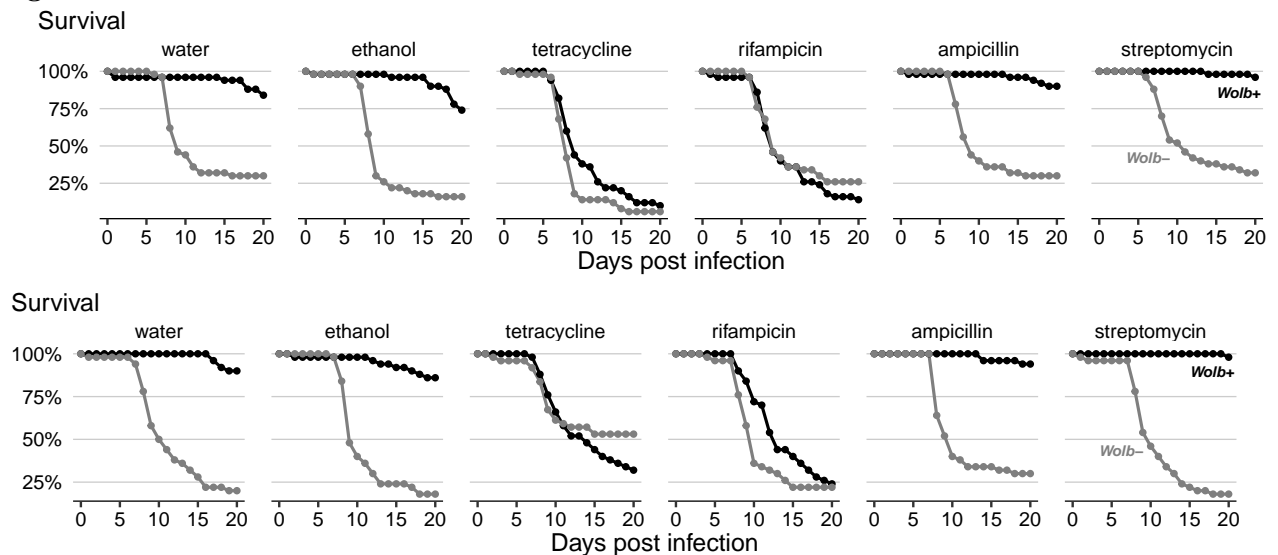
*#Wolb is protective in CTR, amp, and strp. Not in tet, rifa*

```
contrast(mcp_Survival_antibiotics$contrasts,"pairwise",by="contrast")
```

```
## contrast = CS - iso:
## contrast1
## water - ampicillin      estimate    SE  df z.ratio p.value
## water - ethanol         -0.2193  0.578 Inf  -0.380  0.9990
## water - rifampicin      -2.4678  0.542 Inf  -4.557  0.0001
## water - streptomycin    1.3697  0.786 Inf   1.743  0.5029
## water - tetracycline    -2.5055  0.544 Inf  -4.609  0.0001
## ampicillin - ethanol    -0.6204  0.618 Inf  -1.003  0.9170
## ampicillin - rifampicin -2.8690  0.585 Inf  -4.903  <.0001
## ampicillin - streptomycin 0.9685  0.817 Inf   1.186  0.8440
## ampicillin - tetracycline -2.9066  0.587 Inf  -4.952  <.0001
## ethanol - rifampicin    -2.2486  0.516 Inf  -4.355  0.0002
## ethanol - streptomycin  1.5890  0.768 Inf   2.070  0.3029
## ethanol - tetracycline  -2.2862  0.518 Inf  -4.412  0.0001
## rifampicin - streptomycin 3.8375  0.740 Inf   5.184  <.0001
## rifampicin - tetracycline -0.0376  0.484 Inf  -0.078  1.0000
## streptomycin - tetracycline -3.8752  0.742 Inf  -5.226  <.0001
##
## Results are given on the log (not the response) scale.
## P value adjustment: tukey method for comparing a family of 6 estimates
```

*Wolbachia* is protective in all conditions except in flies treated with tetracycline and rifampicin

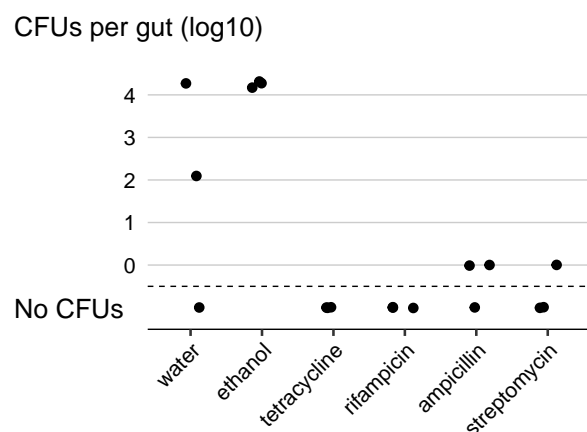
Figures 4C and S5E





## Bacteria levels in the gut after antibiotics

Figure S5D



## Session info

`sessionInfo()`

```
## R version 4.0.0 (2020-04-24)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Catalina 10.15.5
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats graphics grDevices utils datasets methods base
##
## other attached packages:
## [1] broom_0.5.6 lmerTest_3.1-2 lme4_1.1-23 Matrix_1.2-18
## [5] reshape2_1.4.4 car_3.0-7 carData_3.0-3 multcomp_1.4-13
## [9] TH.data_1.0-10 MASS_7.3-51.5 mvtnorm_1.1-0 lsmeans_2.30-0
## [13] emmeans_1.4.6 data.table_1.12.8 coxme_2.2-16 bdsmatrix_1.3-4
## [17] survival_3.1-12 lemon_0.4.4 forcats_0.5.0 stringr_1.4.0
## [21] dplyr_0.8.5 purrr_0.3.4 readr_1.3.1 tidyr_1.0.2
## [25] tibble_3.0.1 ggplot2_3.3.0 tidyverse_1.3.0 plyr_1.8.6
##
## loaded via a namespace (and not attached):
## [1] nlme_3.1-147 pbkrtest_0.4-8.6 fs_1.4.1
## [4] lubridate_1.7.8 httr_1.4.1 numDeriv_2016.8-1.1
## [7] tools_4.0.0 backports_1.1.6 R6_2.4.1
## [10] DBI_1.1.0 colorspace_1.4-1 withr_2.2.0
## [13] tidyselect_1.0.0 gridExtra_2.3 curl_4.3
## [16] compiler_4.0.0 cli_2.0.2 rvest_0.3.5
```

## [19] xml2_1.3.1	sandwich_2.5-1	labeling_0.3
## [22] scales_1.1.0	digest_0.6.25	foreign_0.8-78
## [25] minqa_1.2.4	rmarkdown_2.1	rio_0.5.16
## [28] pkgconfig_2.0.3	htmltools_0.4.0	dbplyr_1.4.3
## [31] rlang_0.4.5	readxl_1.3.1	rstudioapi_0.11
## [34] farver_2.0.3	generics_0.0.2	zoo_1.8-7
## [37] jsonlite_1.6.1	zip_2.0.4	magrittr_1.5
## [40] Rcpp_1.0.4.6	munsell_0.5.0	fansi_0.4.1
## [43] abind_1.4-5	lifecycle_0.2.0	stringi_1.4.6
## [46] yaml_2.2.1	grid_4.0.0	parallel_4.0.0
## [49] crayon_1.3.4	lattice_0.20-41	haven_2.2.0
## [52] splines_4.0.0	hms_0.5.3	knitr_1.28
## [55] pillar_1.4.3	boot_1.3-24	estimability_1.3
## [58] codetools_0.2-16	reprex_0.3.0	glue_1.4.0
## [61] evaluate_0.14	modelr_0.1.7	vctrs_0.2.4
## [64] nloptr_1.2.2.1	cellranger_1.1.0	gtable_0.3.0
## [67] assertthat_0.2.1	xfun_0.13	openxlsx_4.1.4
## [70] xtable_1.8-4	statmod_1.4.34	ellipsis_0.3.0