# Report: Hybrid vigor in response to Eimeria in the HMHZ

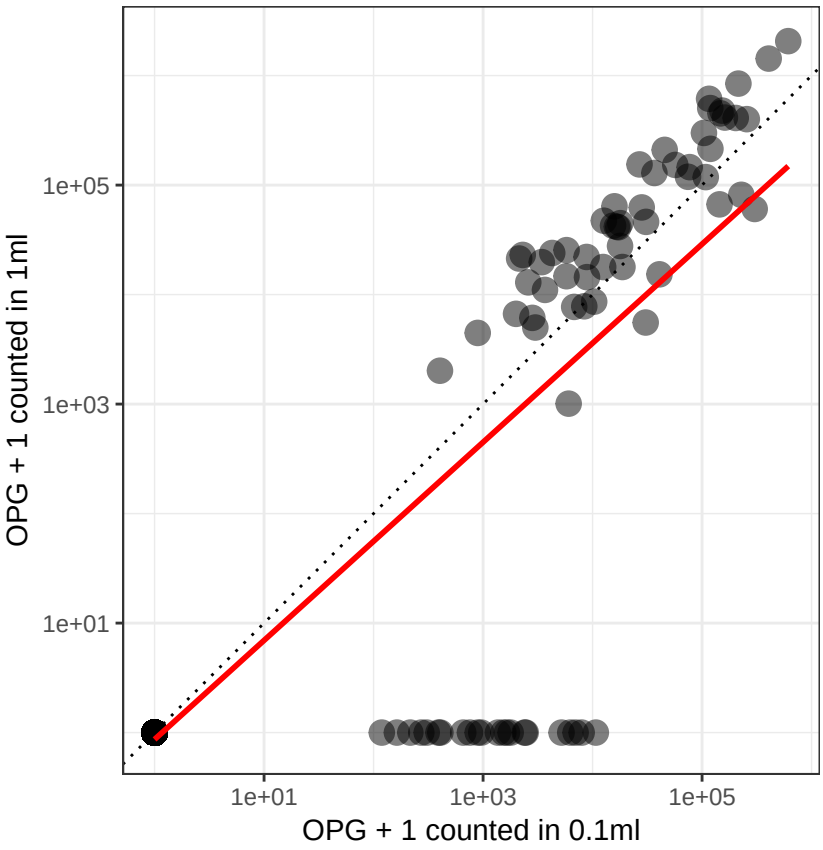
*Alice*

*05 September 2018*

## Contents

# Eimeria detection oocysts flotation

## Improving Eimeria oocysts detection

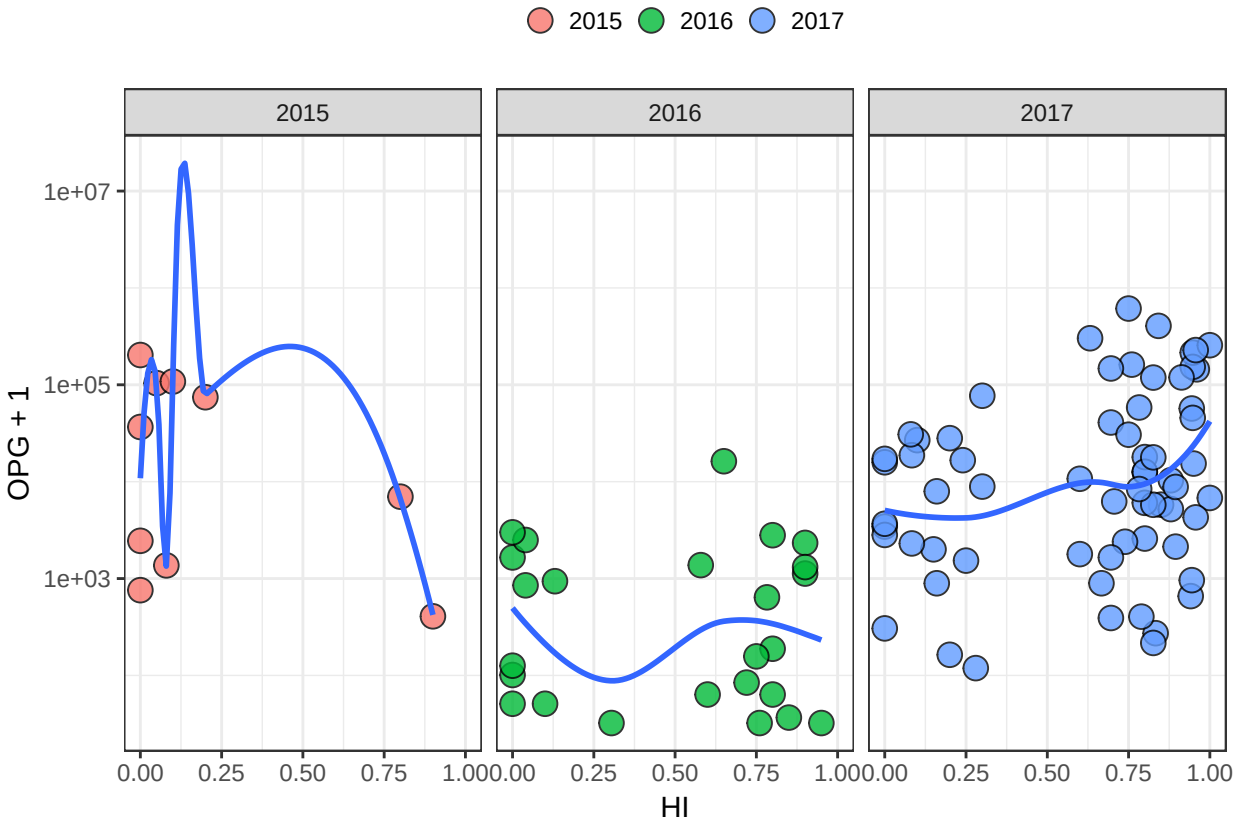



22 new samples were detected while diluting by 0.1mL PBS instead of 1mL before counting in Neubauer chamber.

Adjusted R-squared = 0.81 represents the amount of variation in y explained by x.

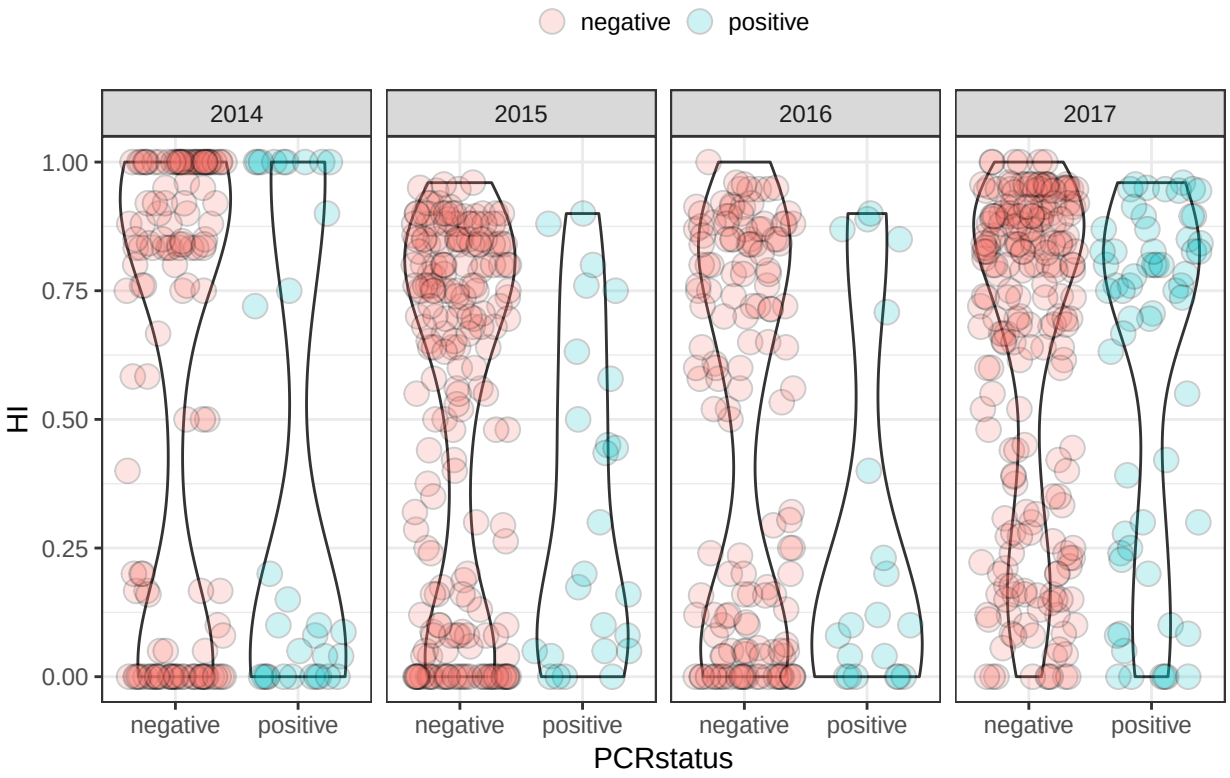
## OPG that we keep

```
## `geom_smooth()` using method = 'loess'
```



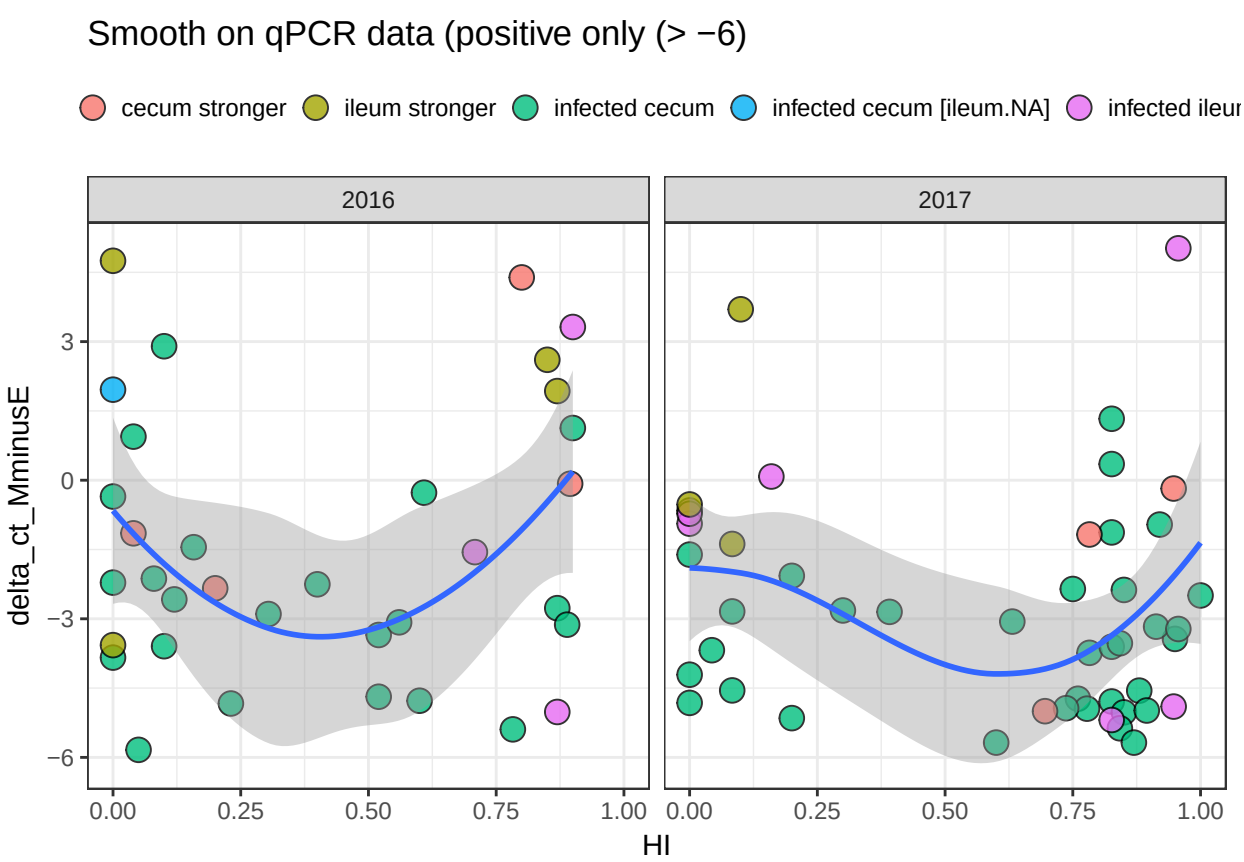

## Eimeria detection PCR

PCR positive = one of the 3 other markers than AP5 sequenced (Ap5 was used for detection only, the other markers for confirmation)

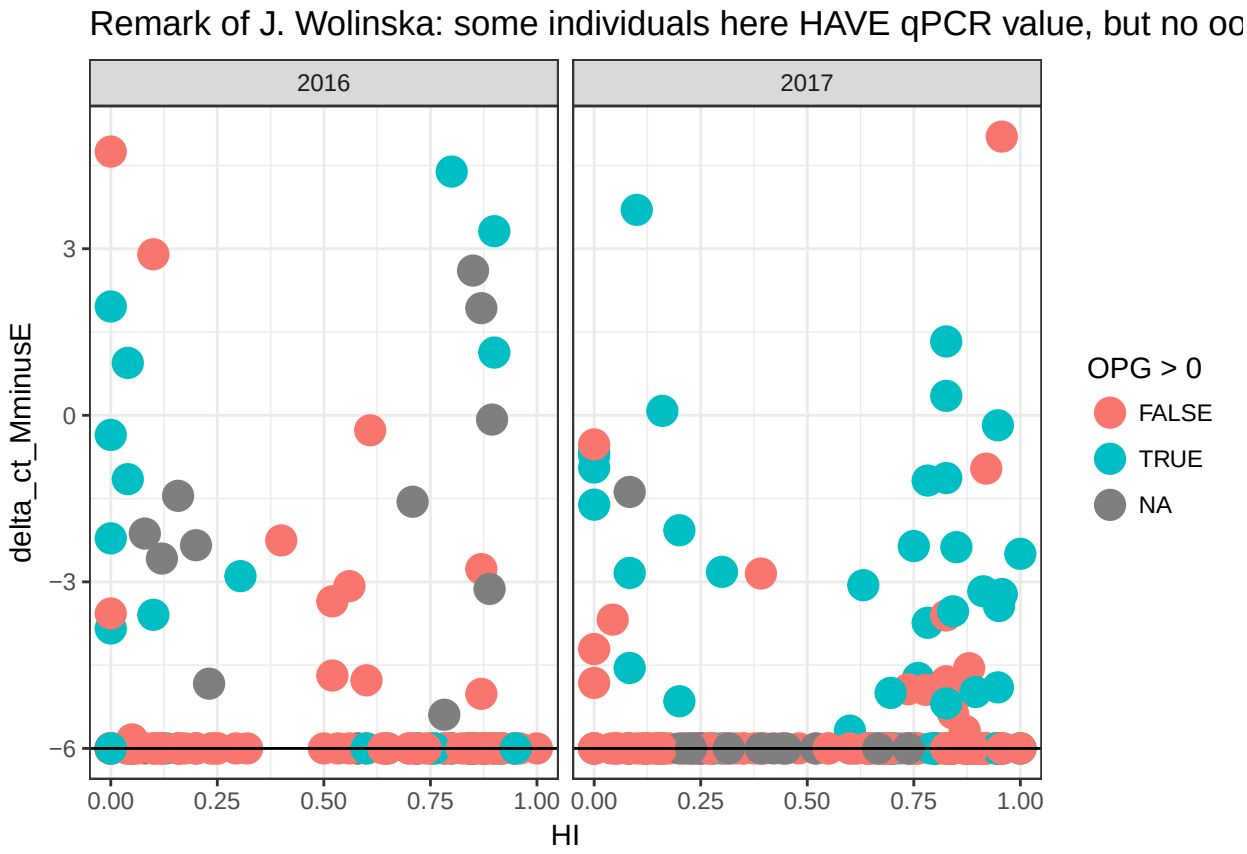



## Eimeria detection qPCR

```
## `geom_smooth()` using method = 'loess'
```

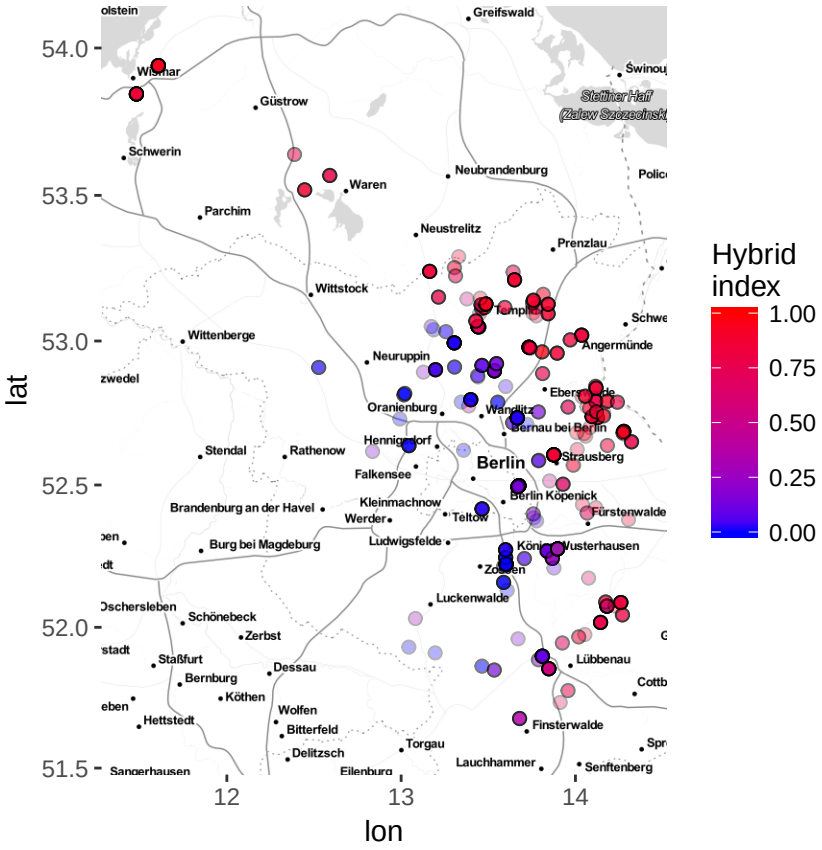



```
## Warning: Removed 142 rows containing missing values (geom_point).
```




## General stats on sampling

We keep mice with OPG, PCR or qPCR status, in North Germany.




- Some information regarding latitude and longitude are missing for the following mice:

SK_3174

- We still miss info (HI) on the following mice (ask Jarda):

AA_0411, AA_0412, AA_0420, SK_2668, SK_2669, SK_2671, SK_2674, SK_2675, SK_2676, SK_2677, SK_2678, SK_2681, SK_2682, SK_2684, SK_2685, SK_2687, SK_2688, SK_2690, SK_2692, SK_2693, SK_2695, SK_2696, SK_2699, SK_2700, SK_2701, SK_2702, SK_2703, SK_2704, SK_2705, SK_2710, SK_2713, SK_2715, SK_2724, SK_2727, SK_2729, SK_2733, SK_2734, SK_2736, SK_2737, SK_2738, SK_2739, SK_2745, SK_2750, SK_2751, SK_2752, SK_2754, SK_2755, SK_2756, SK_2758, SK_2759, SK_2760, SK_2761, SK_2775, SK_2778, SK_2780, SK_2782, SK_2789, SK_2792, SK_2793, SK_2794, SK_2795, SK_2798, SK_2799, SK_2800, SK_2801, SK_2802, SK_2803, SK_2804, SK_2805, SK_3174

# General informations on HMHZ

- 656 mice were captured over three years, from 157 farms
- From these mice:
- 485 mice had Eimeria detected by feces flotation,
- 652 mice had Eimeria detected by colon content PCR (cf paper Victor),
- 397 mice had Eimeria detected by qPCR on intestinal tissues
- On average, 4.04 mice were caught per farm (95% CI 0.34)
- **Hybrid indexes** were calculated as ratio of M.m.d/M.m.m alleles (between 4 and 14, on average 13 loci)

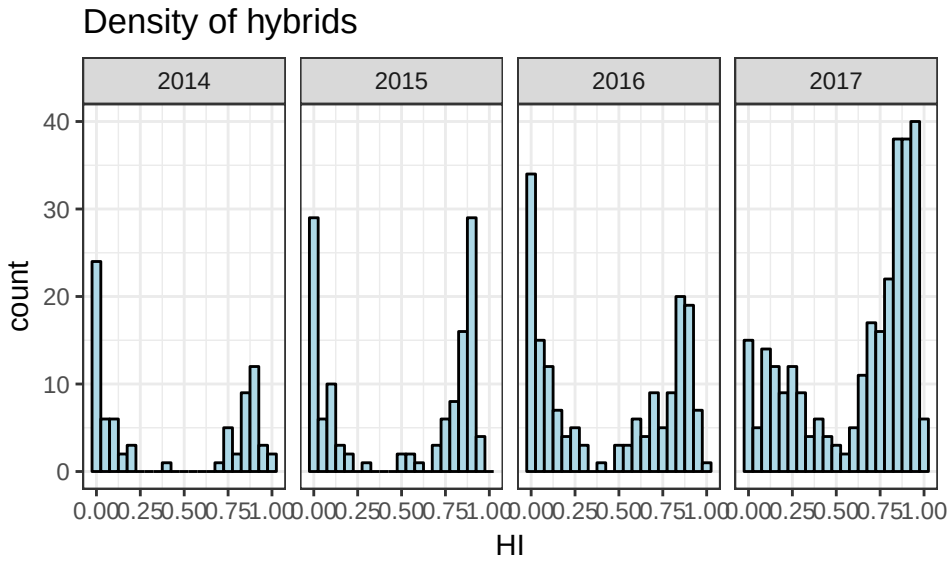



Figure 1: Number of animals caught along the hybrid index

# Prevalence of our 3 different methods

## Prevalence tables

Table 1: Prevalence of Eimeria per year, based on oocyst flotation

| | 2014 | 2015 | 2016 | 2017 |
|---|---|---|---|---|
| FALSE | 0 | 92.0 | 126.00 | 167.00 |
| TRUE | 0 | 10.0 | 25.00 | 65.00 |
| total | 0 | 102.0 | 151.00 | 232.00 |
| prevalence(%) | NaN | 9.8 | 16.56 | 28.02 |

Table 2: Prevalence of Eimeria per year, based on PCR detection. A mouse was considered infected by Eimeria ifone of the 3 markers (COI, 18S or ORF470) gave a sequence

| | 2014 | 2015 | 2016 | 2017 |
|---|---|---|---|---|
| negative | 53.00 | 110.00 | 146.00 | 226.00 |
| positive | 23.00 | 12.00 | 20.00 | 62.00 |
| total | 76.00 | 122.00 | 166.00 | 288.00 |
| prevalence(%) | 30.26 | 9.84 | 12.05 | 21.53 |

Table 3: Prevalence of Eimeria per year, based on qPCR

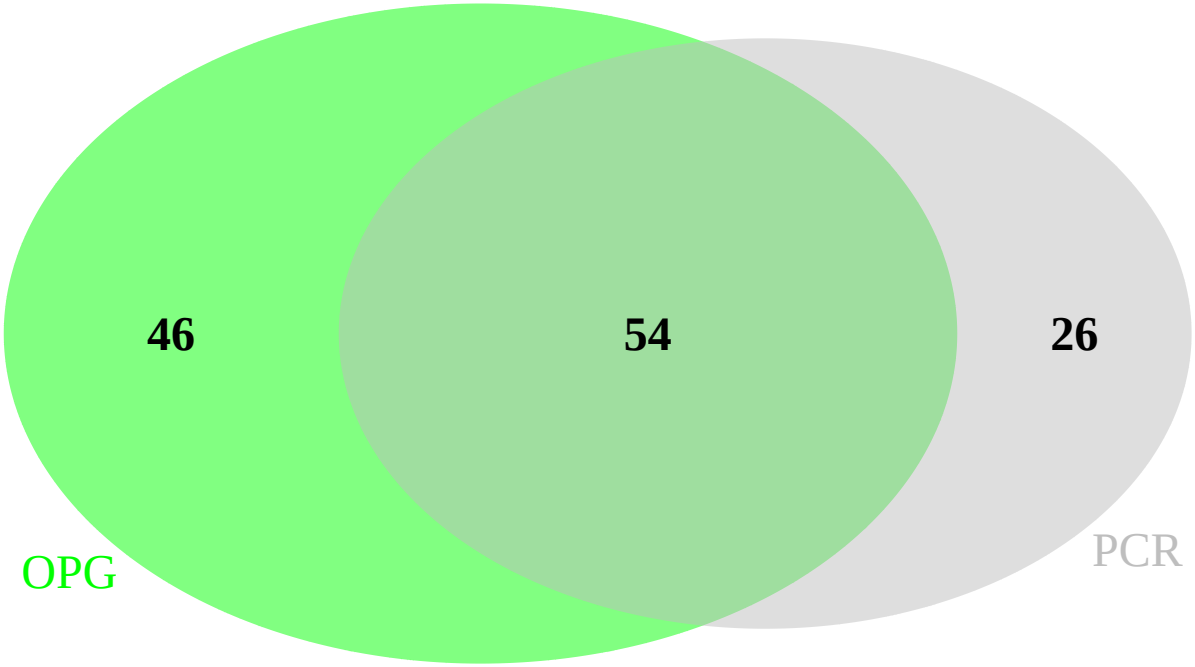
| | 2014 | 2015 | 2016 | 2017 |
|---|---|---|---|---|
| negative | 0 | 0 | 131.00 | 160.00 |
| positive | 0 | 0 | 34.00 | 72.00 |
| total | 0 | 0 | 165.00 | 232.00 |

| | 2014 | 2015 | 2016 | 2017 |
|---|---|---|---|---|
| prevalence(%) | NaN | NaN | 20.61 | 31.03 |

Table 4: Prevalence of Eimeria per year, based on all detections methods. A mouse was considered infected by Eimeria if one of the 3 markers (COI, 18S or ORF470) gave a sequence, OR if it had a positive count of oocysts in its feces, OR if it was qPCR positive

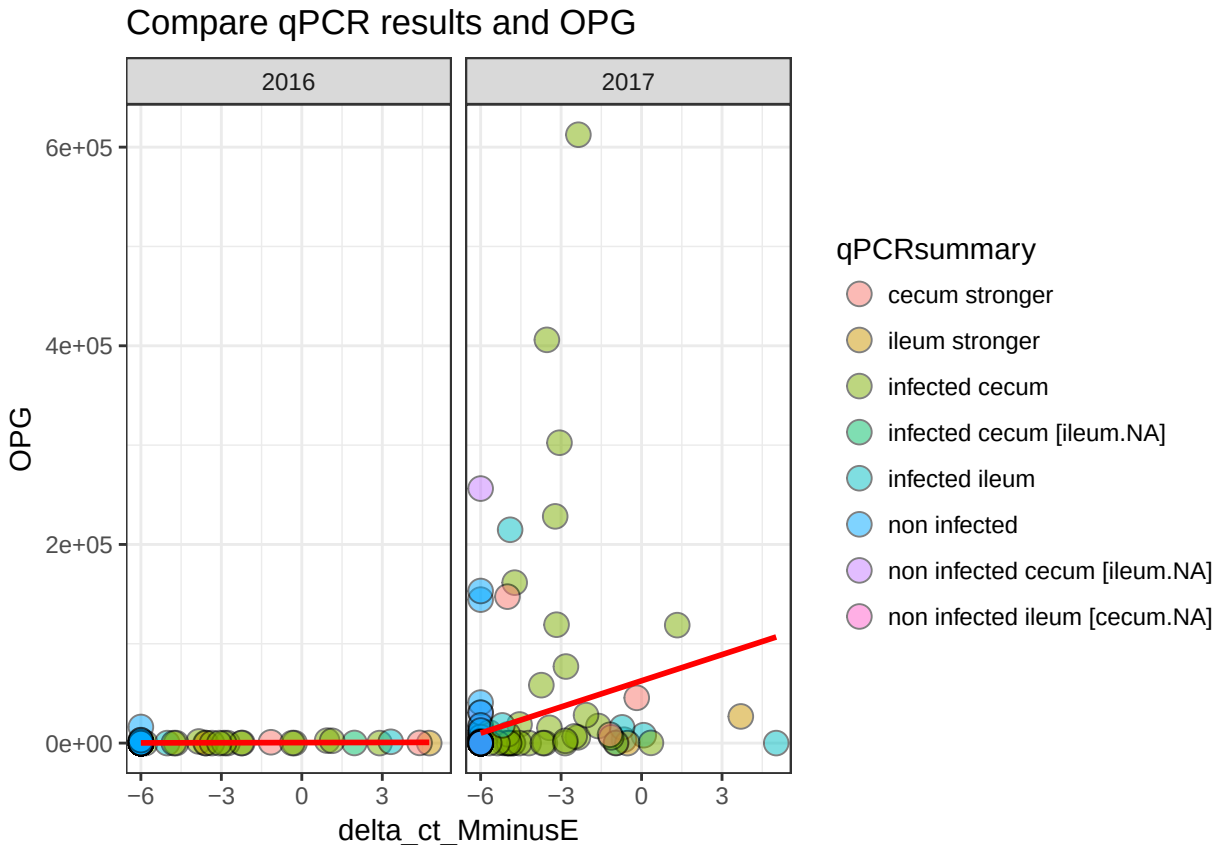
| | 2014 | 2015 | 2016 | 2017 |
|---|---|---|---|---|
| negative | 53.00 | 105.00 | 119.00 | 174.00 |
| positive | 23.00 | 17.00 | 48.00 | 117.00 |
| total | 76.00 | 122.00 | 167.00 | 291.00 |
| prevalence(%) | 30.26 | 13.93 | 28.74 | 40.21 |

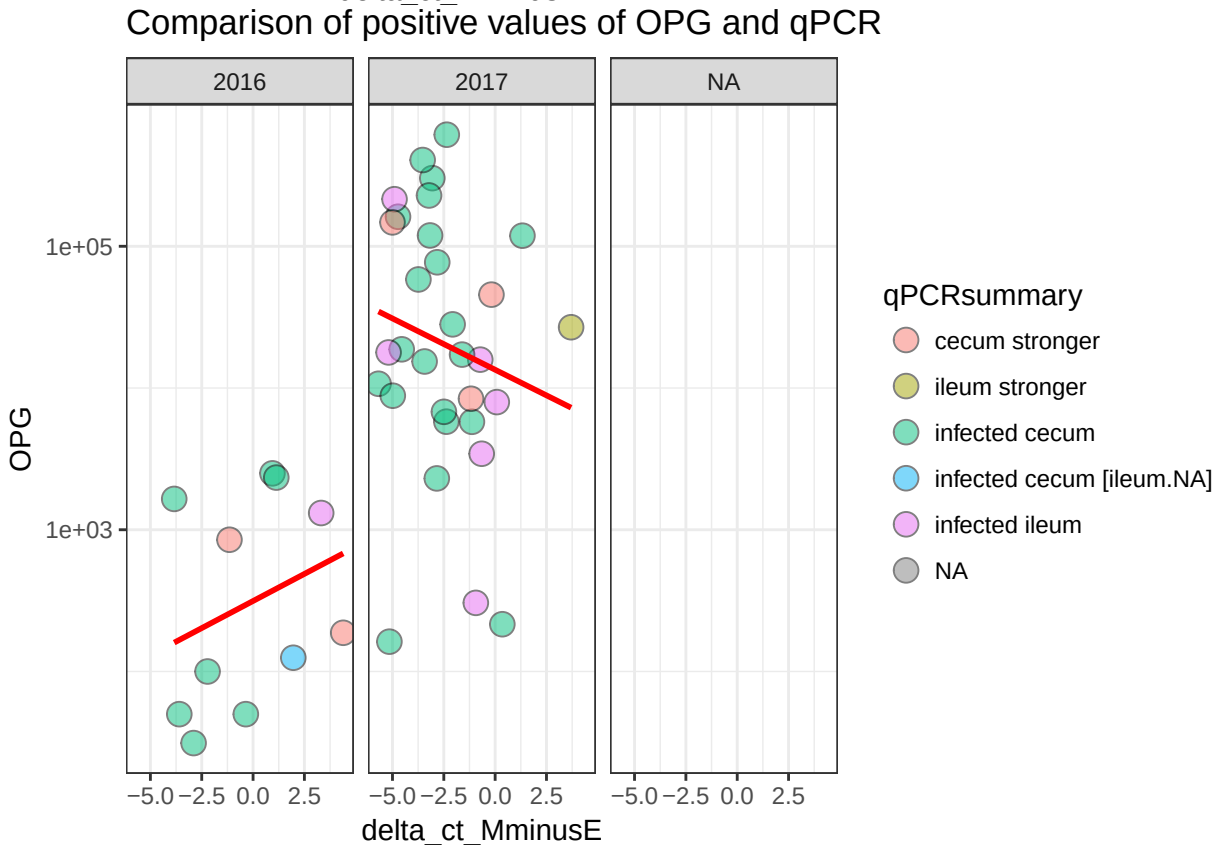
**OPG-PCR**




```
## (polygon[GRID.polygon.810], polygon[GRID.polygon.811], polygon[GRID.polygon.812], polygon[GRID.polyg
```

## OPG-qPCR


Compare qPCR results and OPG
2016
2017
6e+05
4e+05
2e+05
0e+00
OPG
−6
−3
0
3
delta_ct_MminusE
qPCRsummary
cecum stronger
ileum stronger
infected cecum
infected cecum [ileum.NA]
infected ileum
non infected
non infected cecum [ileum.NA]
non infected ileum [cecum.NA]


Comparison of positive values of OPG and qPCR
2016
2017
NA
1e+05
1e+03
OPG
−5.0
−2.5
0.0
2.5
delta_ct_MminusE
qPCRsummary
cecum stronger
ileum stronger
infected cecum
infected cecum [ileum.NA]
infected ileum
NA

```
##
## Call:
## lm(formula = data1$OPG ~ data1$delta_ct_MminusE)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -54567  -6542  -6542  -6542 590073
##
## Coefficients:
##                         Estimate Std. Error t value Pr(>|t|)
## (Intercept)                32690       7705   4.243 2.81e-05 ***
## data1$delta_ct_MminusE      4358       1371   3.179  0.00161 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 49170 on 363 degrees of freedom
## Multiple R-squared:  0.02708,    Adjusted R-squared:  0.0244
## F-statistic:  10.1 on 1 and 363 DF,  p-value: 0.001607
```

```
##
## Call:
## lm(formula = data2$OPG ~ data2$delta_ct_MminusE)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -102684  -66707  -38288    7445  542482
##
## Coefficients:
##                         Estimate Std. Error t value Pr(>|t|)
## (Intercept)                42358      23804   1.779   0.0828 .
## data2$delta_ct_MminusE   -11745       7621  -1.541   0.1311
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 123600 on 40 degrees of freedom
## Multiple R-squared:  0.05605,    Adjusted R-squared:  0.03246
## F-statistic: 2.375 on 1 and 40 DF,  p-value: 0.1311
```

**OPG-qPCR-PCR**

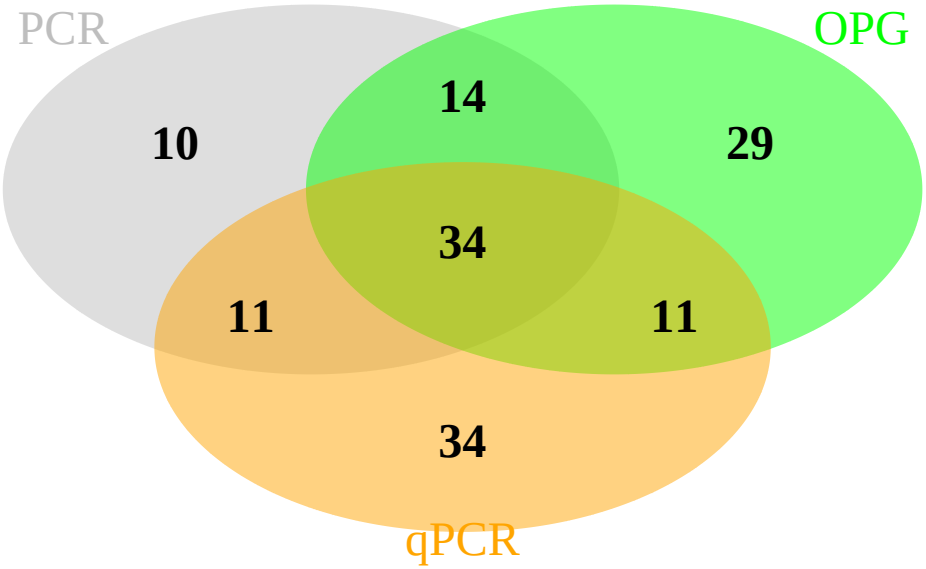
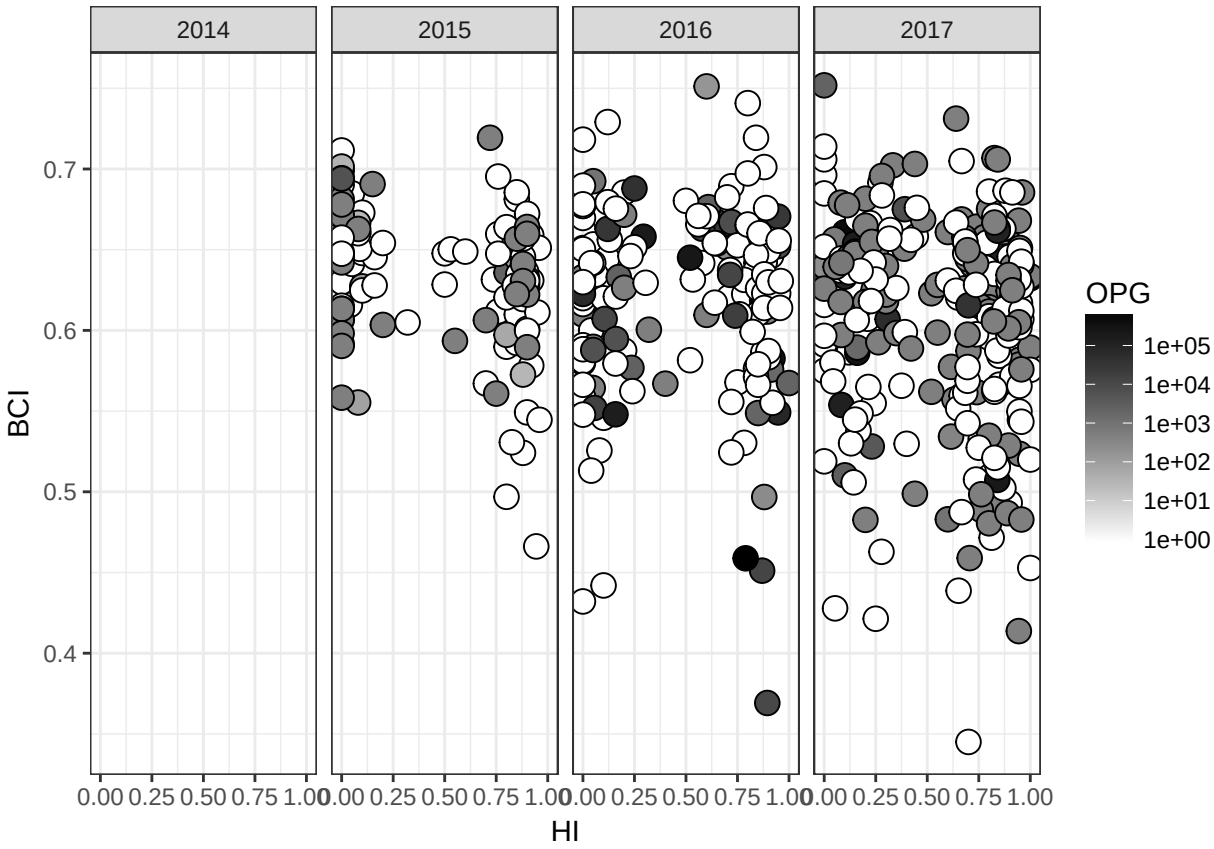



Figure 2: Comparison of detection: PCR vs flotation vs qPCŔ

```
## (polygon[GRID.polygon.1102], polygon[GRID.polygon.1103], polygon[GRID.polygon.1104], polygon[GRID.pol
```

# BCI

## BCI vs OPG




```
##
## Call:
## lm(formula = myData$BCI ~ myData$OPG + myData$HI)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.20165 -0.02542  0.01057  0.03494  0.13137
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  6.304e-01  4.424e-03 142.490  < 2e-16 ***
## myData$OPG  -2.061e-07  5.449e-08  -3.782 0.000176 ***
## myData$HI   -1.760e-02  6.779e-03  -2.596 0.009710 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.05359 on 476 degrees of freedom
##   (177 observations deleted due to missingness)
## Multiple R-squared:  0.04528,    Adjusted R-squared:  0.04127
## F-statistic: 11.29 on 2 and 476 DF,  p-value: 1.625e-05
```

## Testing hybrid vigor along HMHZ

### Oocyst shedding

Statistical model (dvp. . . )

### qPCR proxy

tbc

### BCI proxy

tbc

## Bonus part: genotyping of mice case/control

- 100 out of 483 are positive for flotation and have an hybrid index.
- 106 out of 395 are positive for qPCR and have an hybrid index.

Discussed with Stuart:

- Test distributions 0 or counts. Test all vs only infected ("intensity") distribution. We should be able to fit the distribution of infected on all. Zeros are data. Stochastic move.
- Separation of the zero class. balanced design case/control ~ 400 +/-70infectés SNPchip.
- H0: no differences are observed
- Separate <0.5 and >0.5 to see the species effect
- timing : WHEN (for my thesis?)