Report: Hybrid vigor in response to Eimeria in the HMHZ

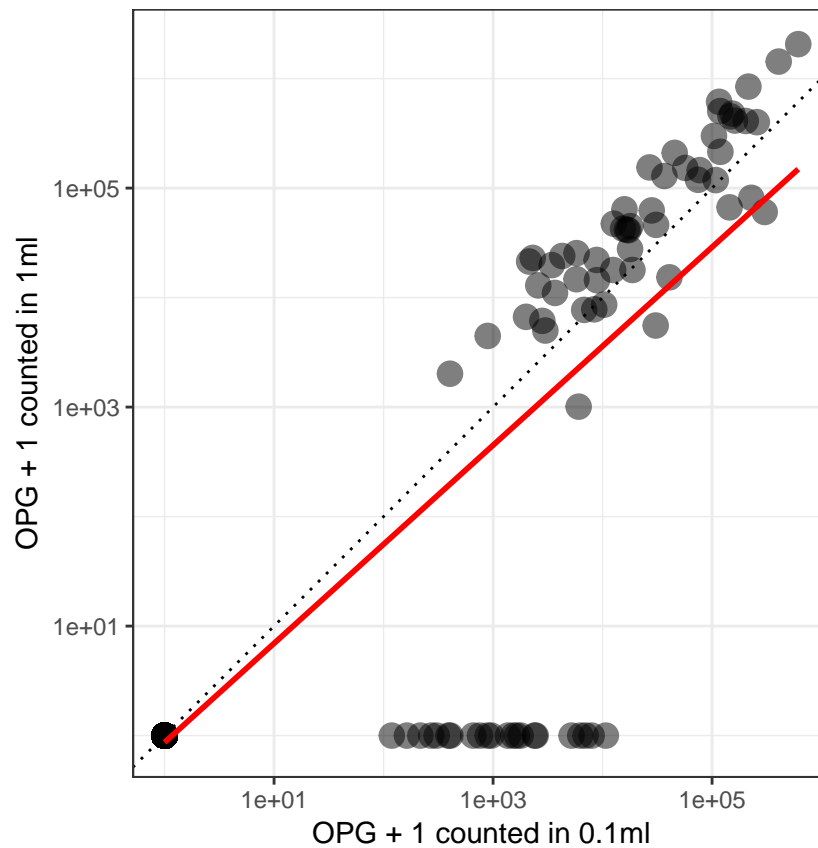*Alice*

19 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

Number of Mus musculus caught with OPG values: 486

```
## `geom_smooth()` using method = 'loess'
```
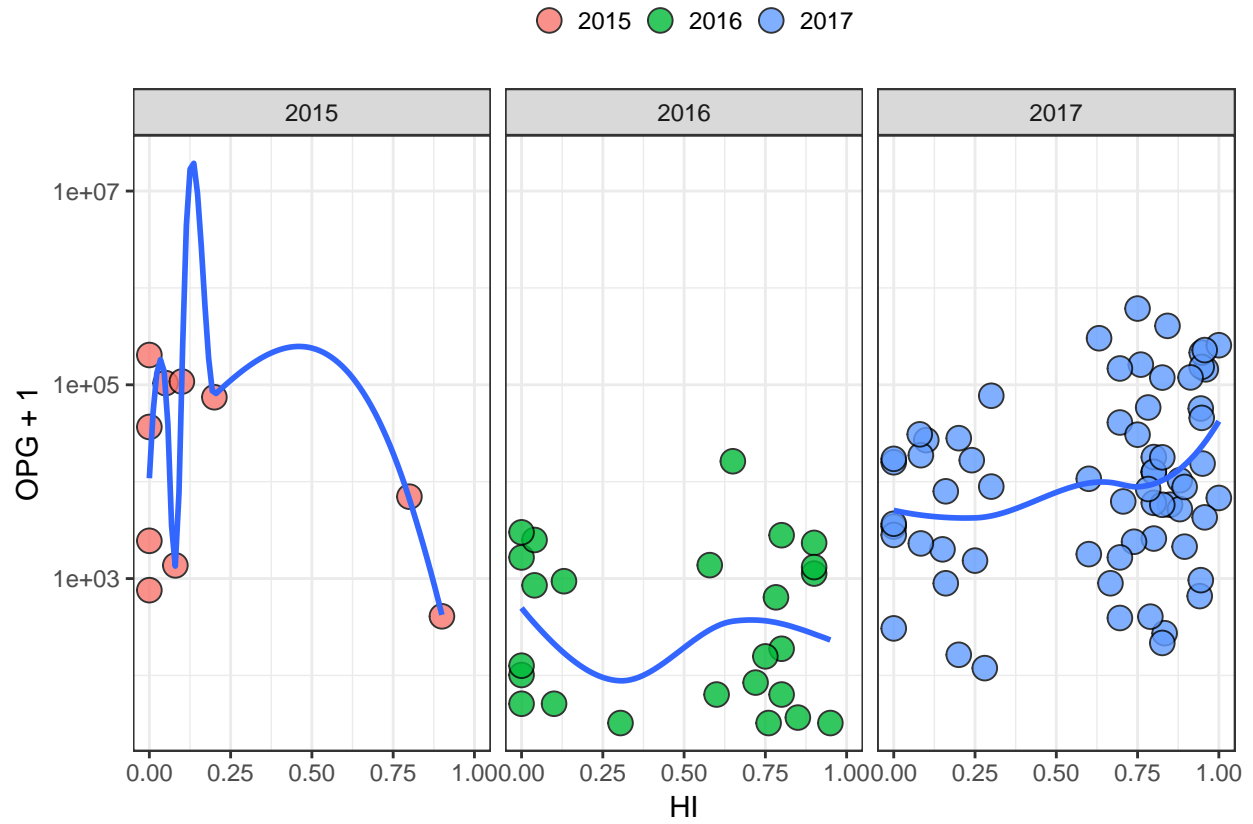
Eimeria detection PCR

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

Violin plots on PCR data
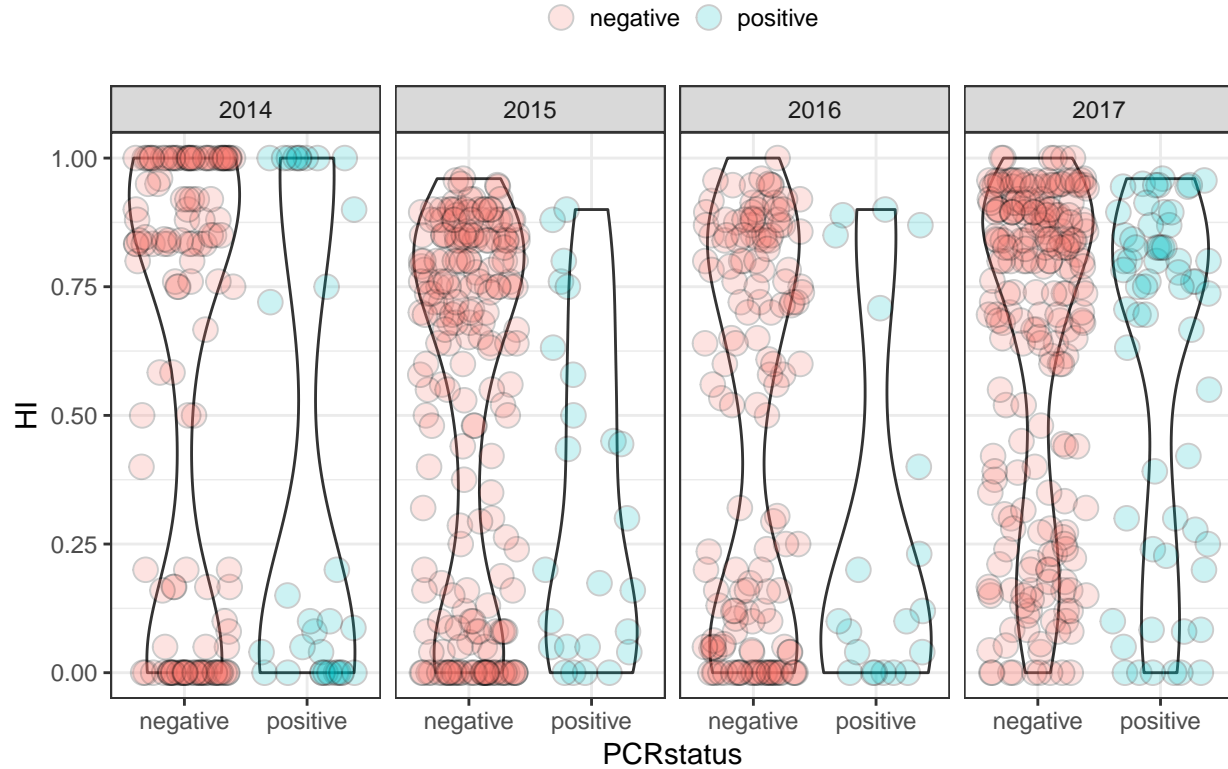
PCR positive = one of the 3 markers 18S, COI or ORF470) gave a sequence. Number of Mus musculus caught with PCR performed: 962

Eimeria detection qPCR

We keep only the values for mice having been tested for BOTH ileum and cecum!

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

Smooth on qPCR data (positive only (> −6)
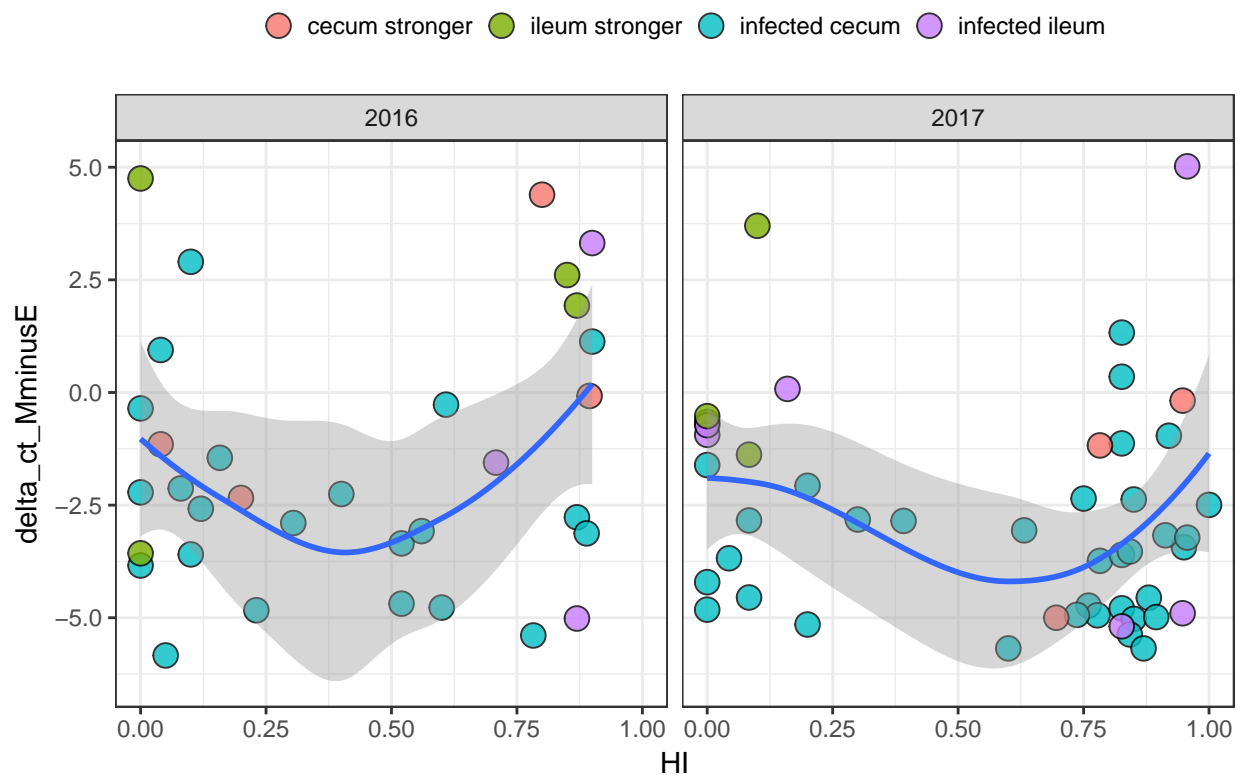
Warning: Removed 168 rows containing missing values (geom_point).

Remark of J. Wolinska: some individuals here HAVE qPCR value, but no oc

Number of Mus musculus caught with qPCR performed: 371

General stats on sampling

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

- Number of Mus musculus caught with OPG counted: 485

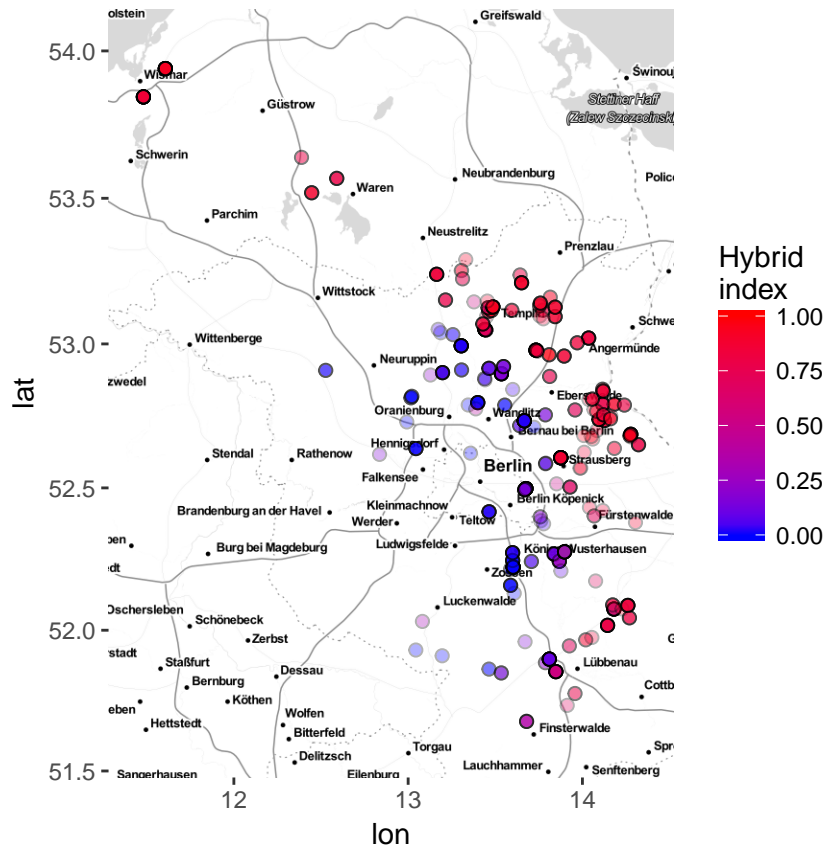- Number of Mus musculus caught with qPCR performed: 371

- Number of Mus musculus caught with either OPG counted or qPCR performed: 513

- Number of Mus musculus caught with OPG counted AND qPCR performed: 343

- 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

- 655 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),

- 371 mice had Eimeria detected by qPCR on intestinal tissues

- On average, 4.04 mice were caught per farm (95% CI 0.34)

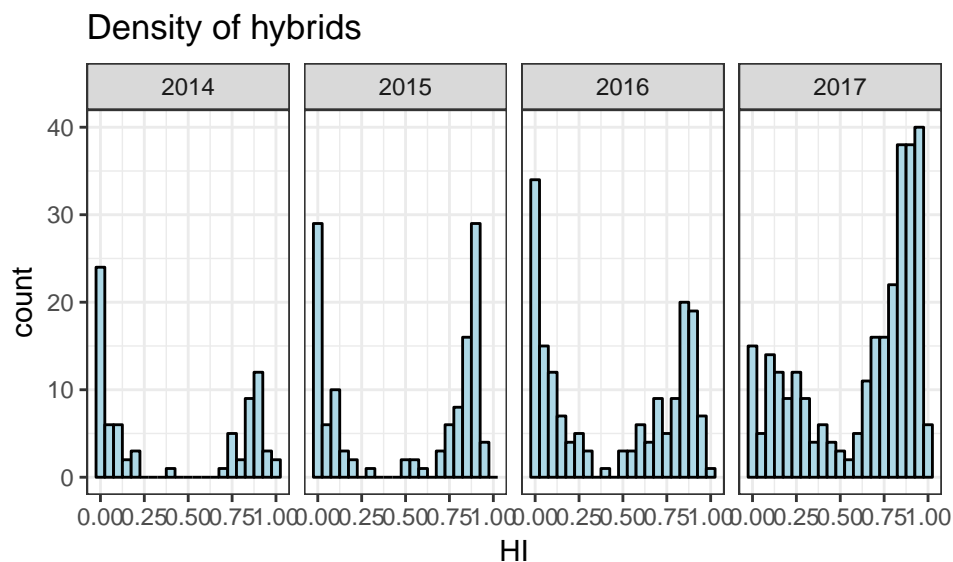- **Hybrid indexes** were calculated as ratio of M.m.d/M.m.m alleles (between 4 and 14, on average 13 loci)

Density of hybrids

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

	2014	2015	2016	2017
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	33.00	47.00
total	0	0	164.00	207.00
prevalence(%)	NaN	NaN	20.12	22.71

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
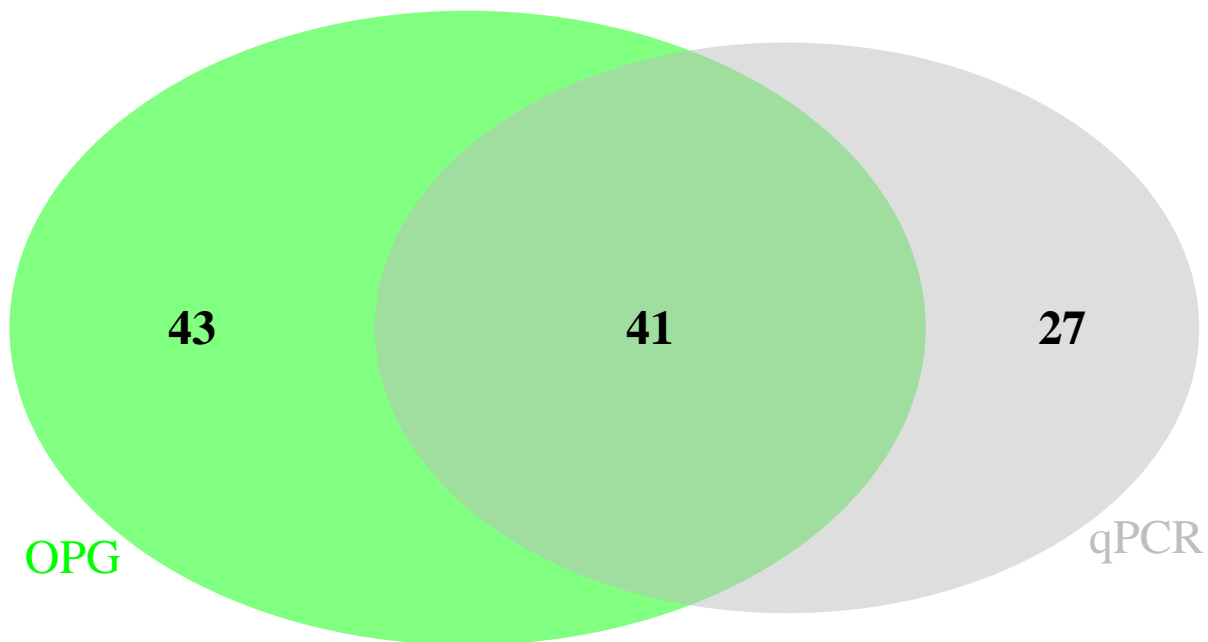
	2014	2015	2016	2017
negative	53.00	105.00	119.00	193.00
positive	23.00	17.00	48.00	97.00
total	76.00	122.00	167.00	290.00
prevalence(%)	30.26	13.93	28.74	33.45

OPG-PCR
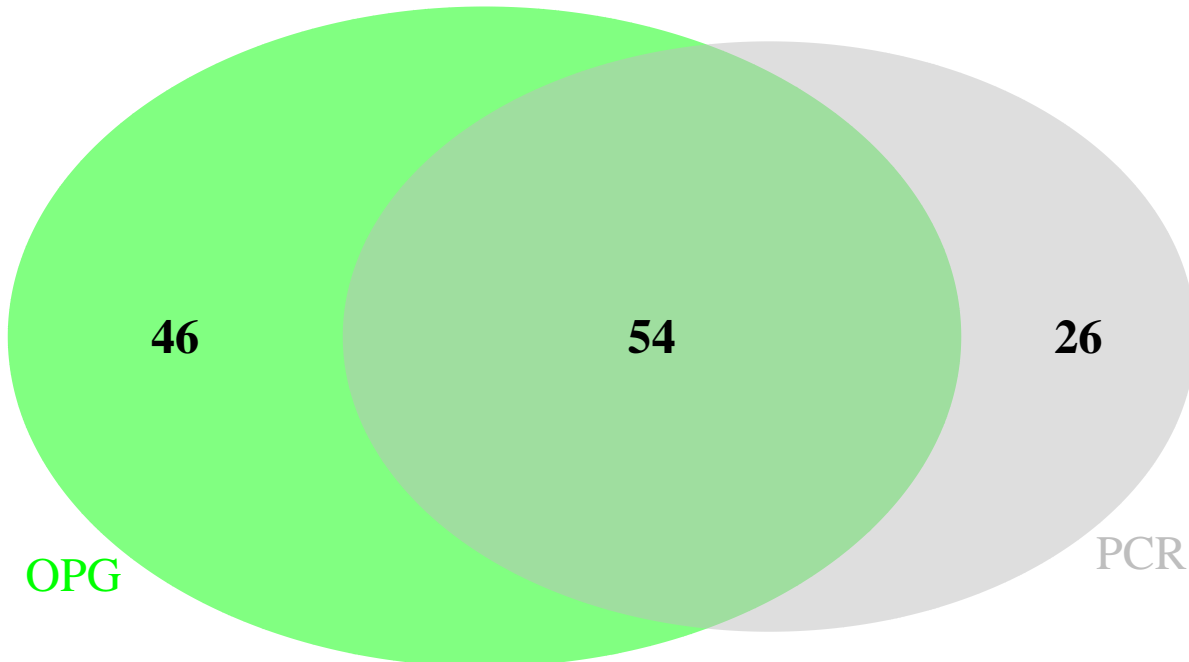
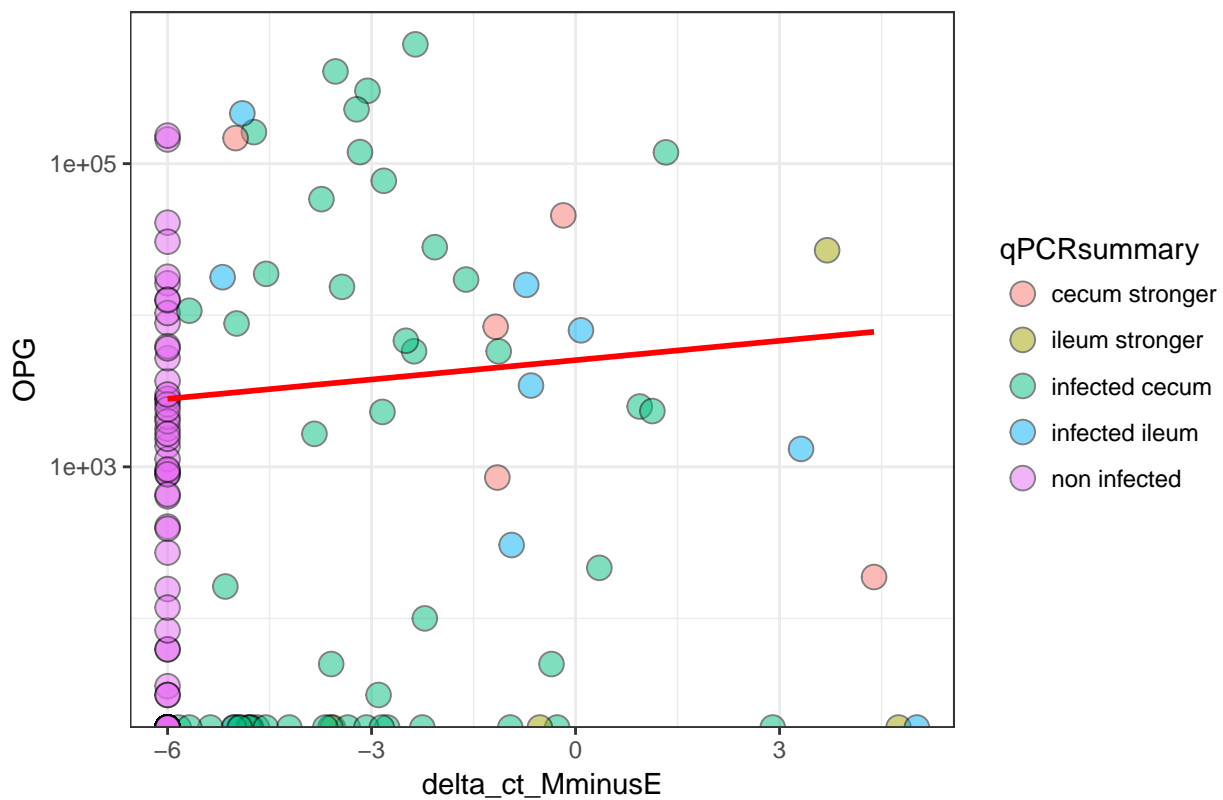## (polygon[GRID.polygon.805], polygon[GRID.polygon.806], polygon[GRID.polygon.807], polygon[GRID.polyg

OPG-qPCR

(polygon[GRID.polygon.814], polygon[GRID.polygon.815], polygon[GRID.polygon.816], polygon[GRID.polyg

Compare qPCR results and OPG

##
Call:
lm(formula = data1$OPG ~ data1$delta_ct_MminusE)

```
##
## Residuals:
##    Min    1Q Median    3Q    Max
## -57721  -5929  -5929  -5929 589440
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 34128       7818   4.365 1.68e-05 ***
## data1$delta_ct_MminusE       4700       1395   3.368 0.000843 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 48800 on 341 degrees of freedom
## Multiple R-squared:  0.0322, Adjusted R-squared:  0.02936
## F-statistic: 11.35 on 1 and 341 DF,  p-value: 0.0008429

##
## Call:
## lm(formula = data2$OPG ~ data2$delta_ct_MminusE)
##
## Residuals:
##     Min     1Q  Median     3Q     Max
## -102183  -67339  -39204    6991  542133
##
## Coefficients:
##                          Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 43423      25002   1.737   0.0903 .
## data2$delta_ct_MminusE     -11441       7946  -1.440   0.1579
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 125100 on 39 degrees of freedom
## Multiple R-squared:  0.05048,    Adjusted R-squared:  0.02613
## F-statistic: 2.073 on 1 and 39 DF,  p-value: 0.1579
```
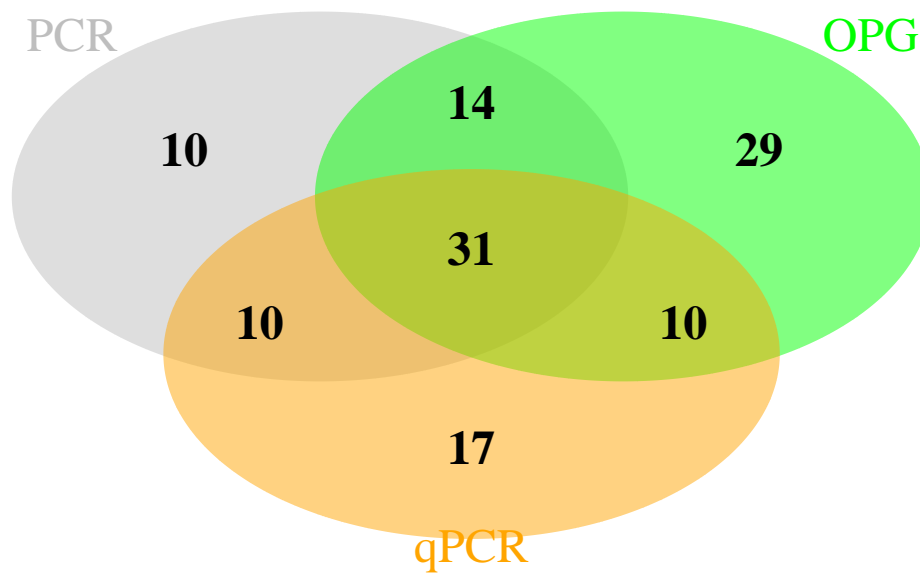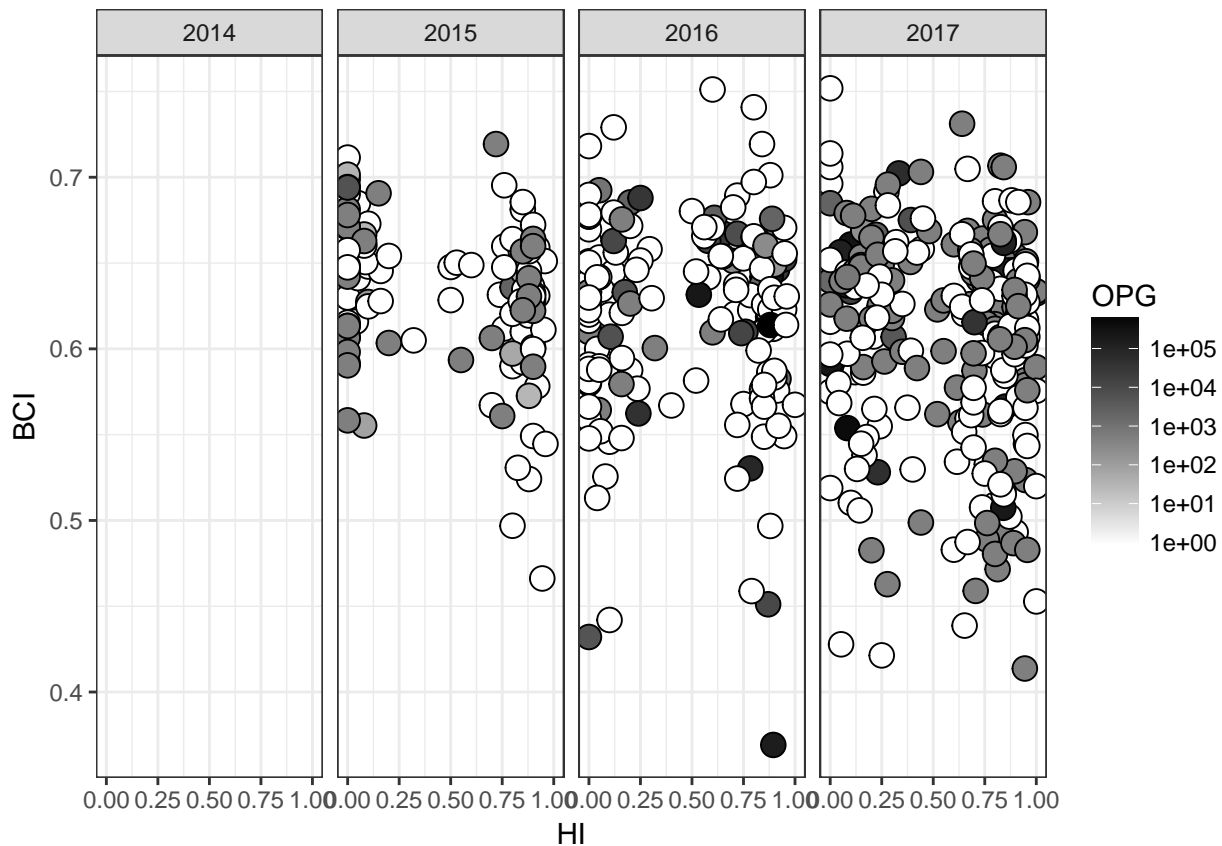
OPG-qPCR-PCR

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

```
## (polygon[GRID.polygon.900], polygon[GRID.polygon.901], polygon[GRID.polygon.902], polygon[GRID.polyg
```

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
##   (176 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.

- 80 out of 369 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?)