

Report: Hybrid vigor in response to Eimeria in the HMHZ

Alice

27 September 2018

Contents

Eimeria detection oocysts flotation	2
Improving Eimeria oocysts detection	2
OPG that we keep	2
Eimeria detection PCR	3
Eimeria detection qPCR	4
General stats on sampling	7
General informations on HMHZ	7
Prevalence of our 3 different methods	8
Prevalence tables	8
OPG-PCR	9
OPG-qPCR	10
OPG-qPCR-PCR	11
BCI	12
BCI vs OPG	12
Testing hybrid vigor along HMHZ	13
PART II for comparison: qPCR detection threshold = -3.75	13
Eimeria detection oocysts flotation	13
Improving Eimeria oocysts detection	13
OPG that we keep	13
Eimeria detection PCR	14
Eimeria detection qPCR	15
General stats on sampling	17
General informations on HMHZ	19
Prevalence of our 3 different methods	19
Prevalence tables	19
OPG-PCR	21
OPG-qPCR	22
OPG-qPCR-PCR	24
BCI	25
BCI vs OPG	25

Testing hybrid vigor along HMHZ

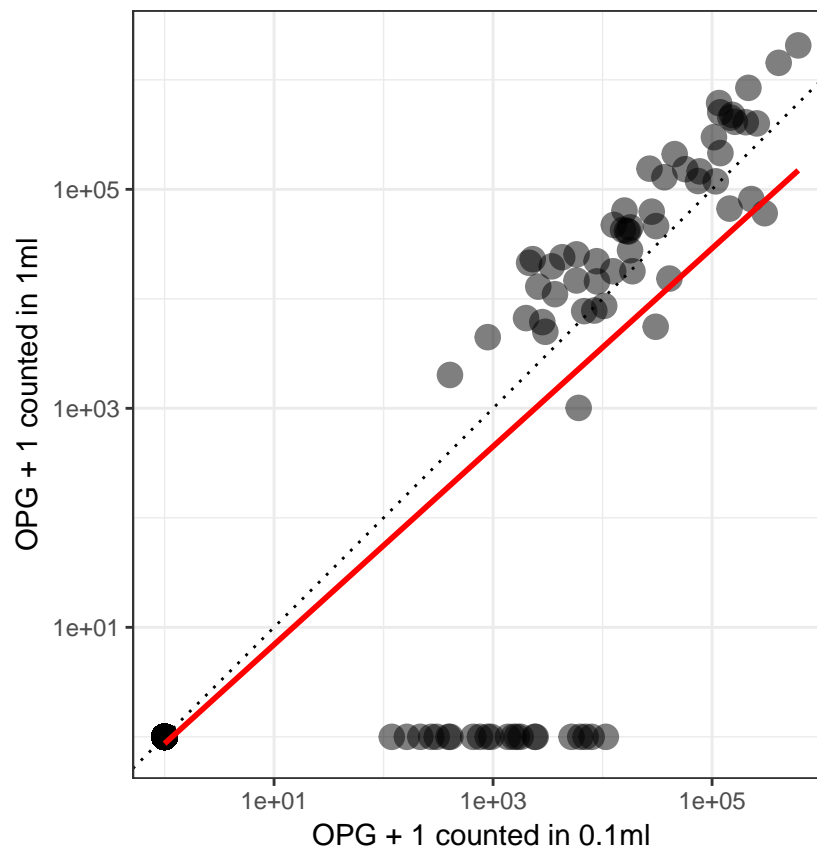
26

Bonus part: genotyping of mice case/control

26

Eimeria detection oocysts flotation

Improving Eimeria oocysts detection



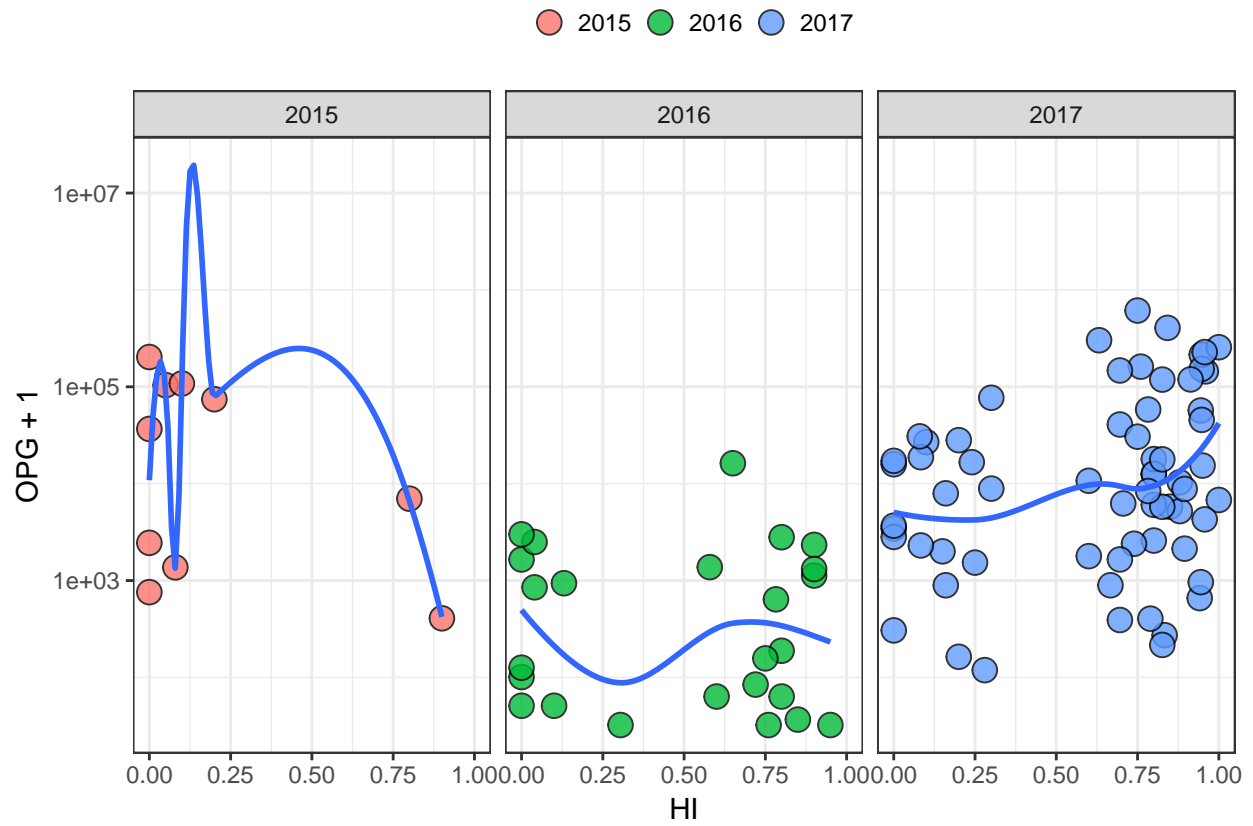
22 new samples were detected while diluting by 0.1mL PBS instead of 1mL before counting in Neubauer chamber.

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

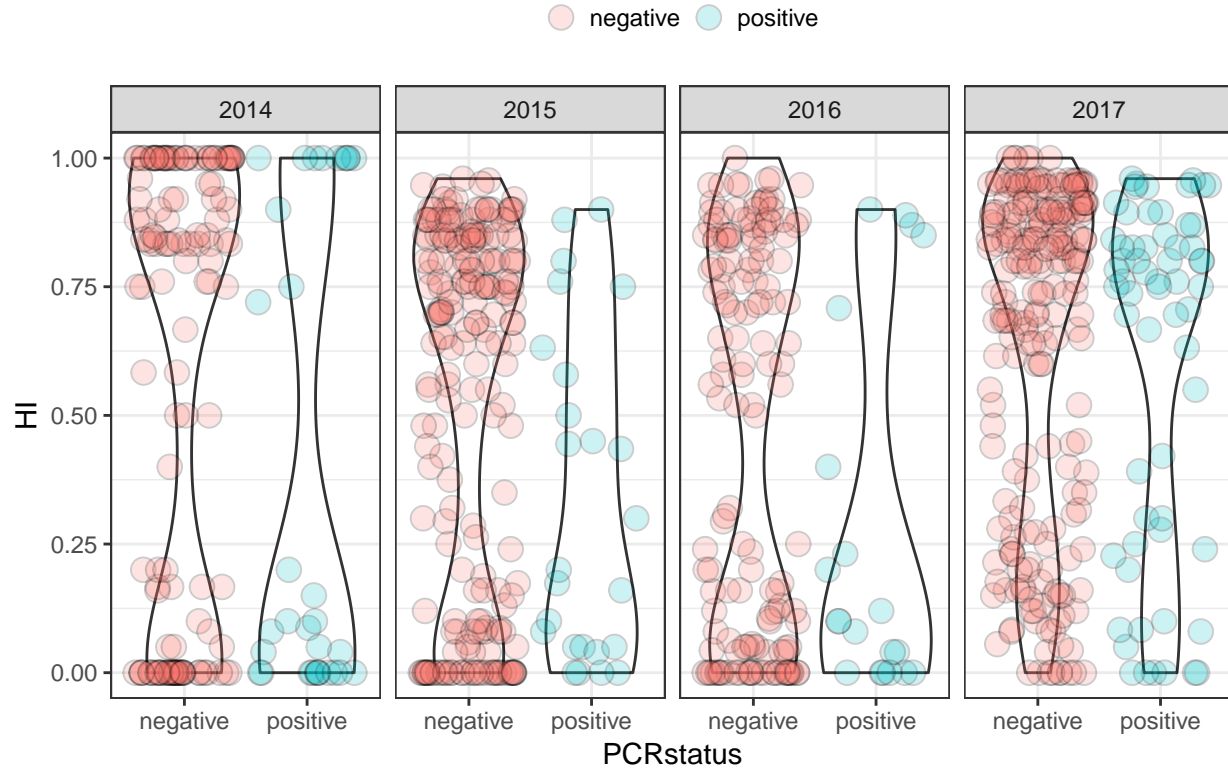
```
## `geom_smooth()` using method = 'loess'
```



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



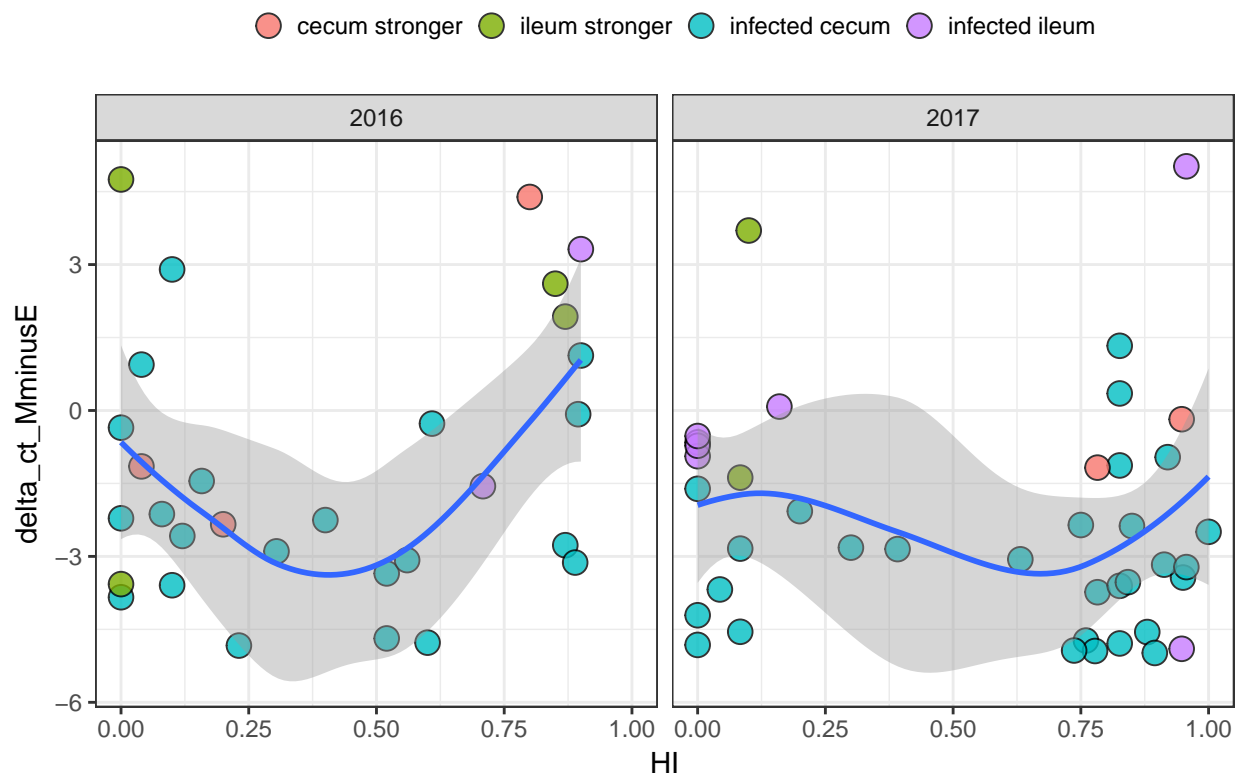
PCR positive = one of the 3 markers 18S, COI or ORF470) gave a sequence. Number of Mus musculus caught with PCR performed: 962

Eimeria detection qPCR

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

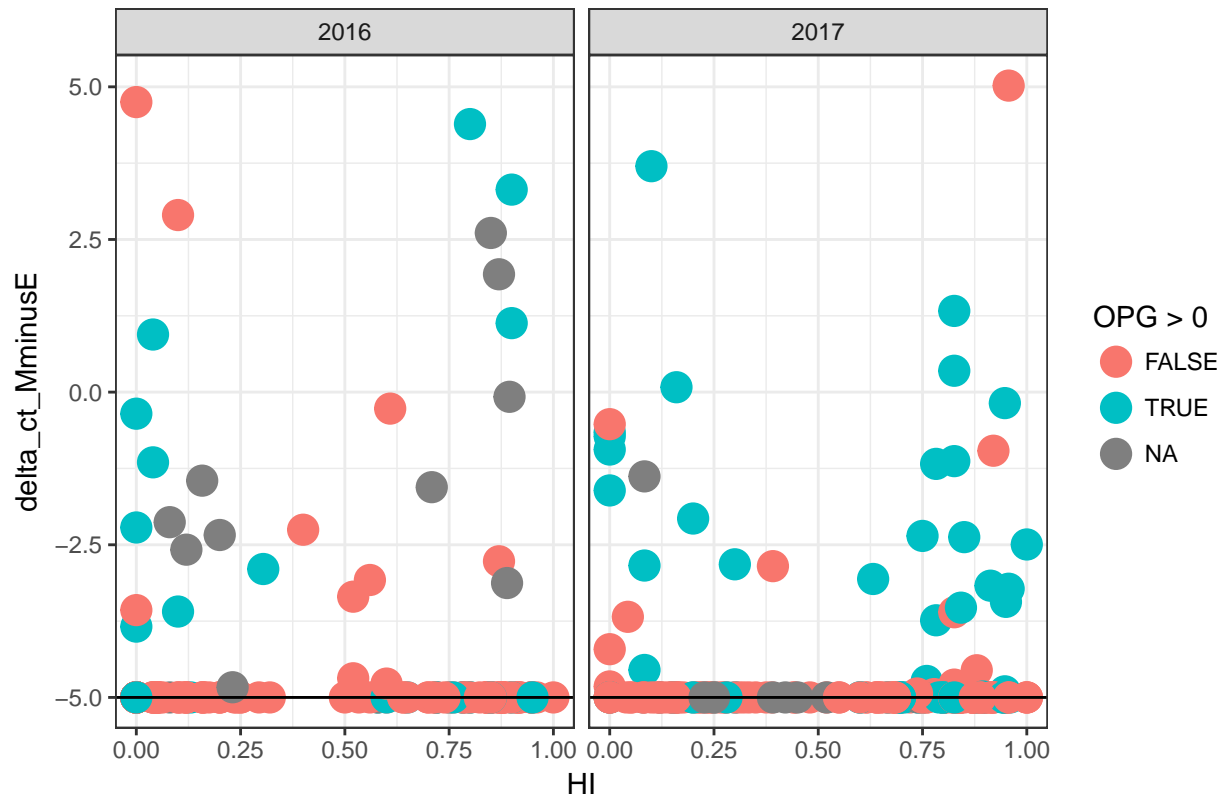
```
## `geom_smooth()` using method = 'loess'
```

Smooth on qPCR data (positive only (> -5))



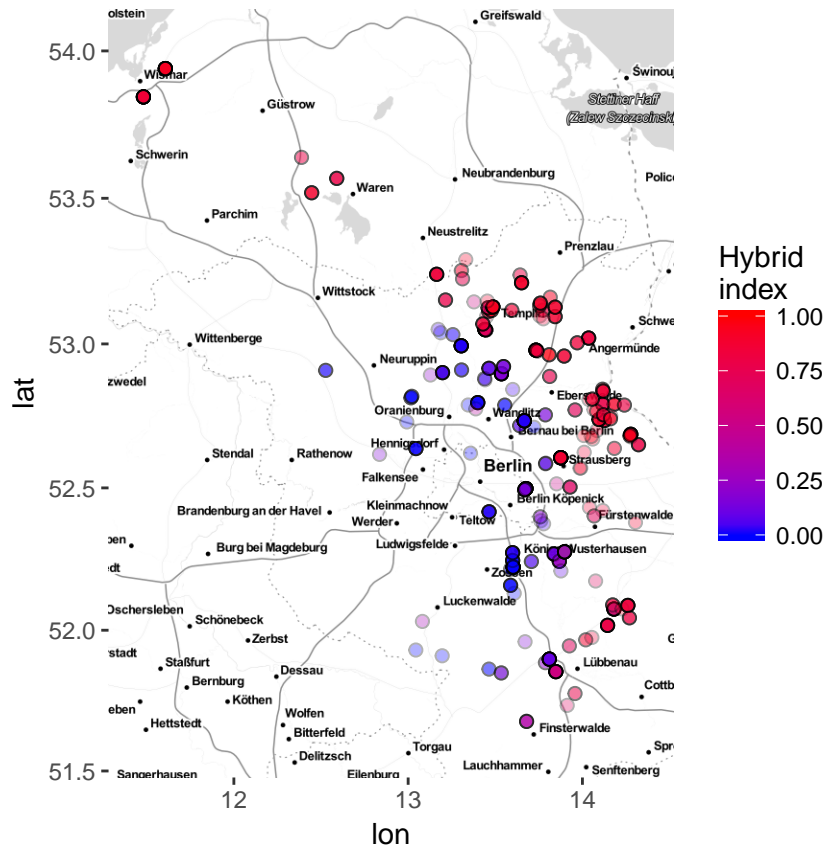
Warning: Removed 168 rows containing missing values (geom_point).

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



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 HMMZ

- 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)

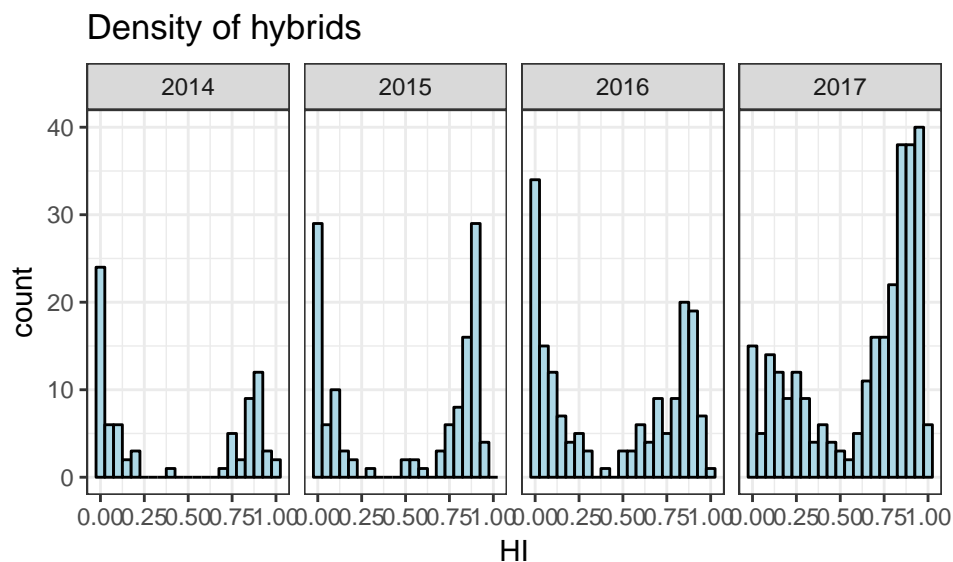


Figure 1: Number of animals caught along the hybrid index

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 if one 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

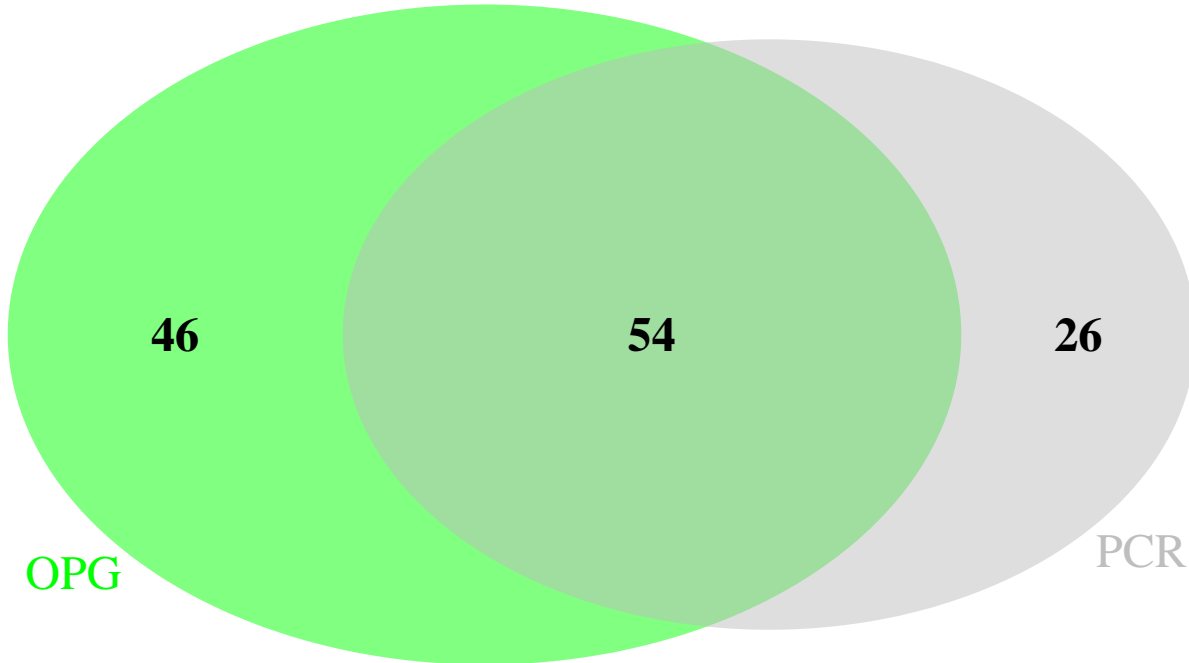
Table 3: Prevalence of Eimeria per year, based on qPCR

	2014	2015	2016	2017
FALSE	0	0	134.00	167.00
TRUE	0	0	30.00	40.00
total	0	0	164.00	207.00
prevalence(%)	NaN	NaN	18.29	19.32

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

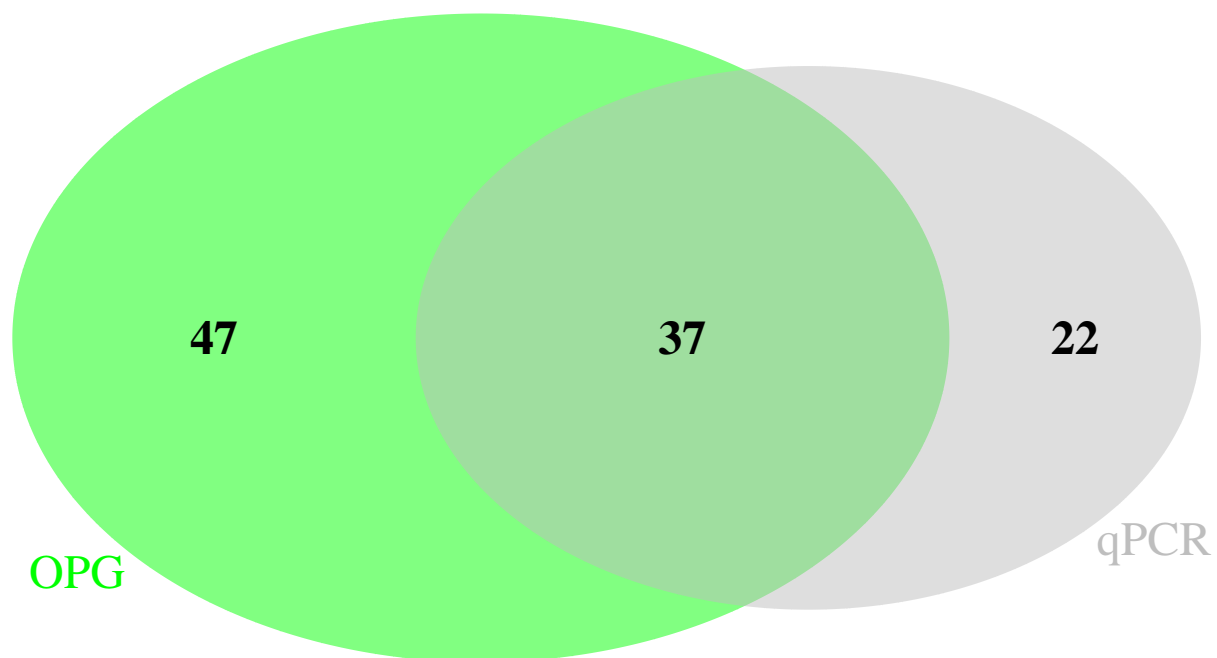
	2014	2015	2016	2017
negative	53.00	105.00	122.00	195.00
positive	23.00	17.00	45.00	95.00
total	76.00	122.00	167.00	290.00
prevalence(%)	30.26	13.93	26.95	32.76

OPG-PCR



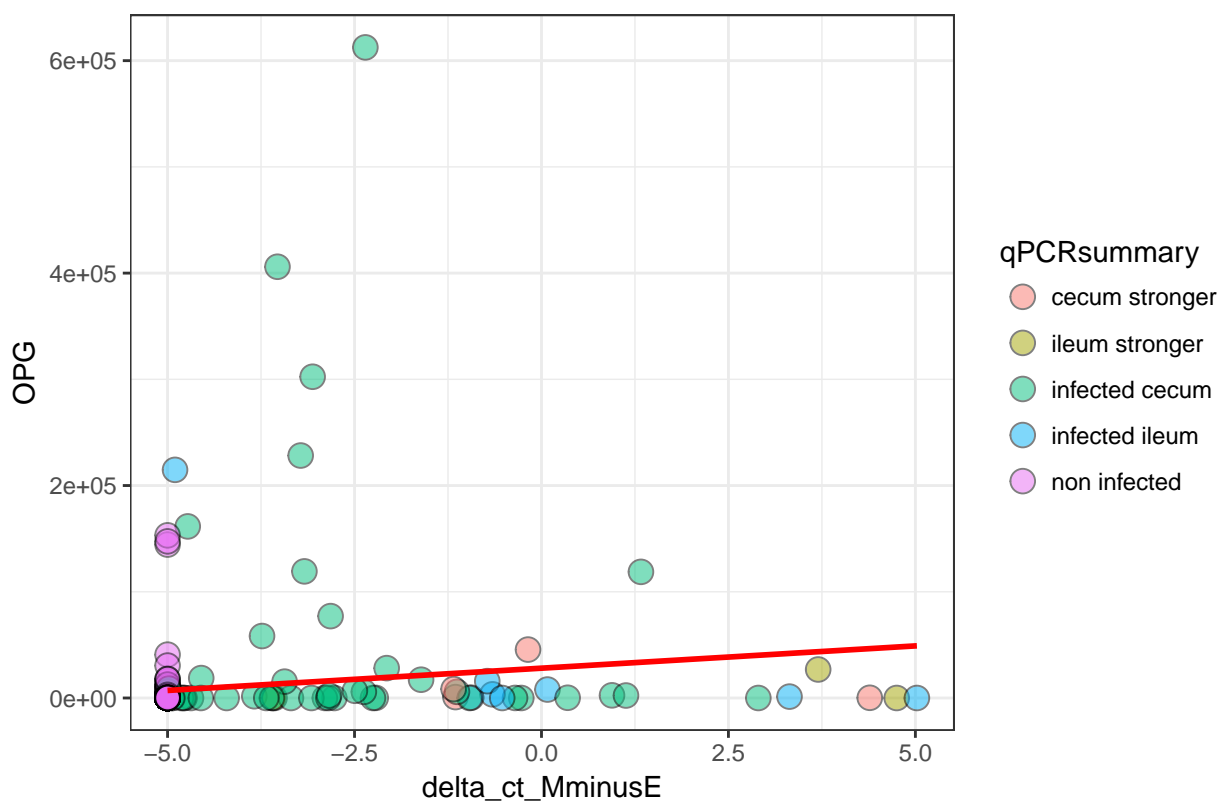
(polygon[GRID.polygon.805], polygon[GRID.polygon.806], polygon[GRID.polygon.807], polygon[GRID.polygon.808])

OPG-qPCR



```
## (polygon[GRID.polygon.814], polygon[GRID.polygon.815], polygon[GRID.polygon.816], polygon[GRID.polygon.817])
```

Compare qPCR results and OPG



```
##
```

```
## Call:
```

```
## lm(formula = data1$OPG ~ data1$delta_ct_MminusE)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -49104  -7092  -7092  -7092  594318
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      28056       7882   3.559 0.000425 ***
## data1$delta_ct_MminusE    4193       1662   2.522 0.012119 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 49140 on 341 degrees of freedom
## Multiple R-squared:  0.01831,    Adjusted R-squared:  0.01543
## F-statistic: 6.361 on 1 and 341 DF,  p-value: 0.01212
```

OPG-qPCR-PCR

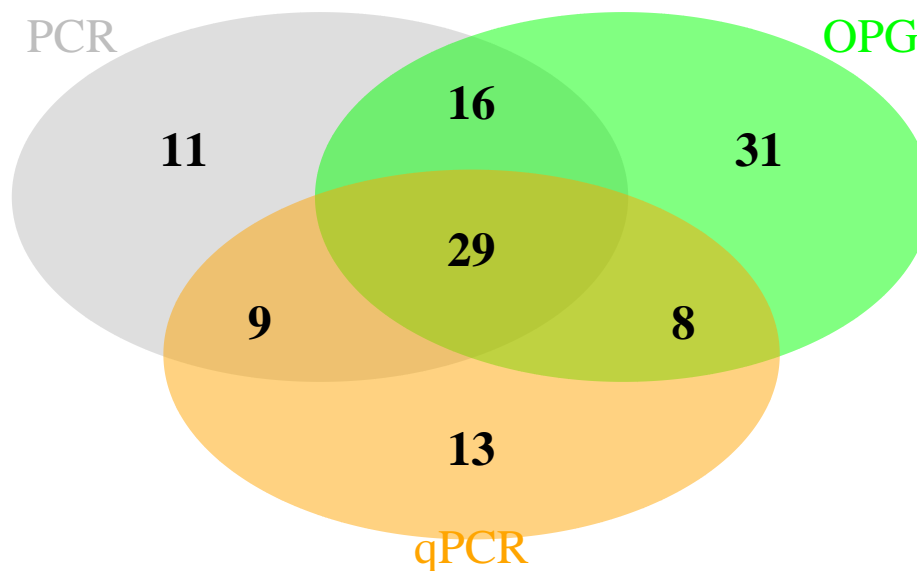
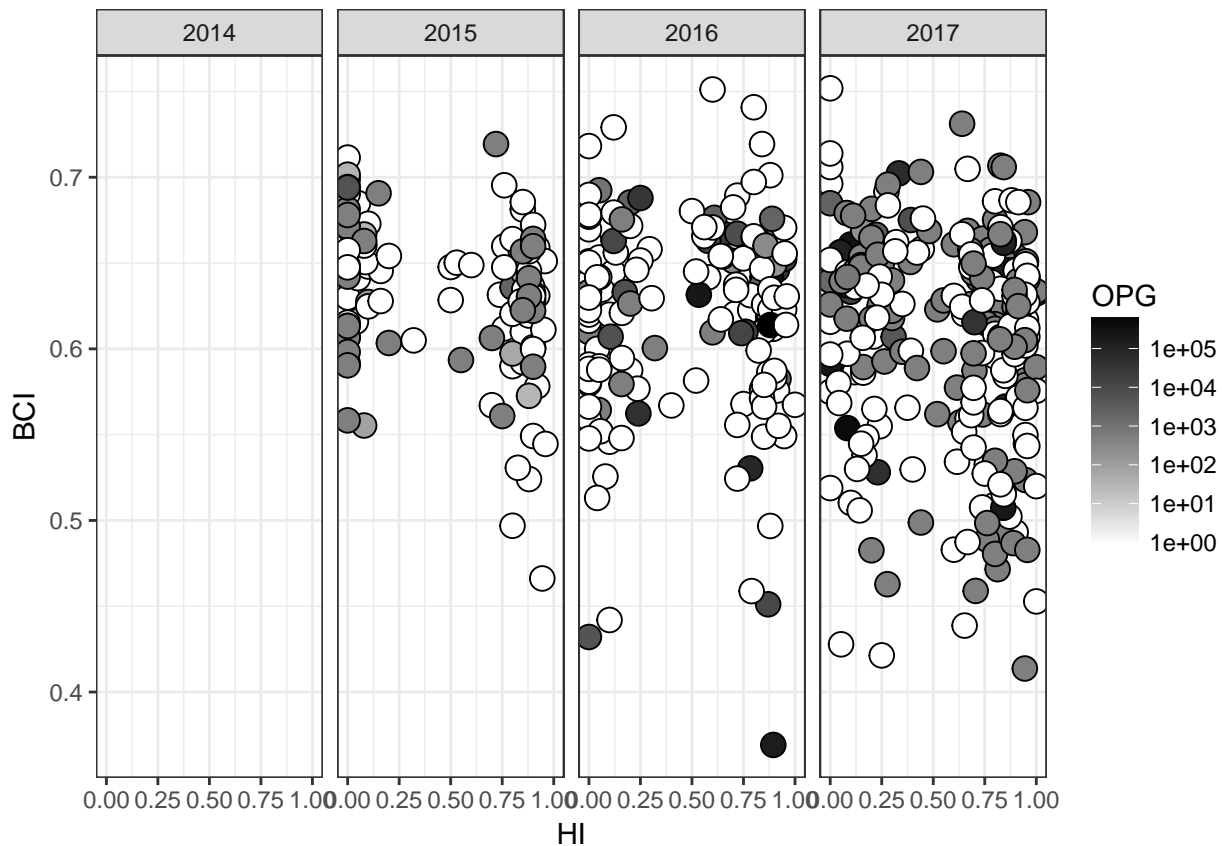


Figure 2: Comparison of detection: PCR vs flotation vs qPCR

```
## (polygon[GRID.polygon.900], polygon[GRID.polygon.901], polygon[GRID.polygon.902], polygon[GRID.polygon.903])
```

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
```

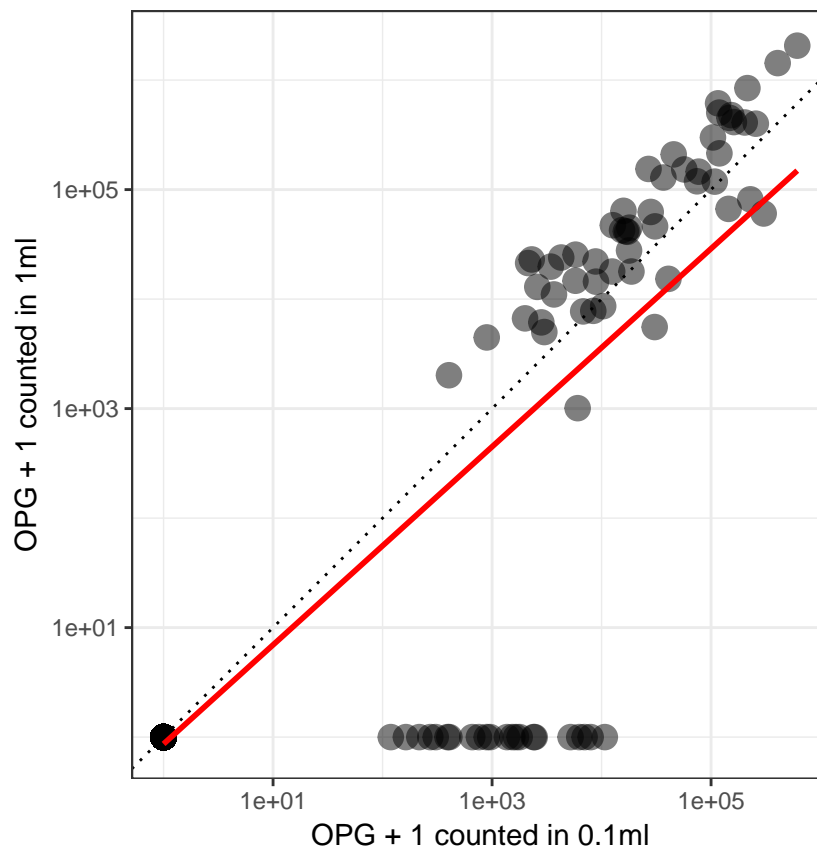
Testing hybrid vigor along HMMZ

PART II for comparison: qPCR detection threshold = -3.75

```
## Warning in makeMiceTable("../Data_important/"): NAs introduced by coercion
## Warning in makeMiceTable("../Data_important/"): NAs introduced by coercion
```

Eimeria detection oocysts flotation

Improving Eimeria oocysts detection



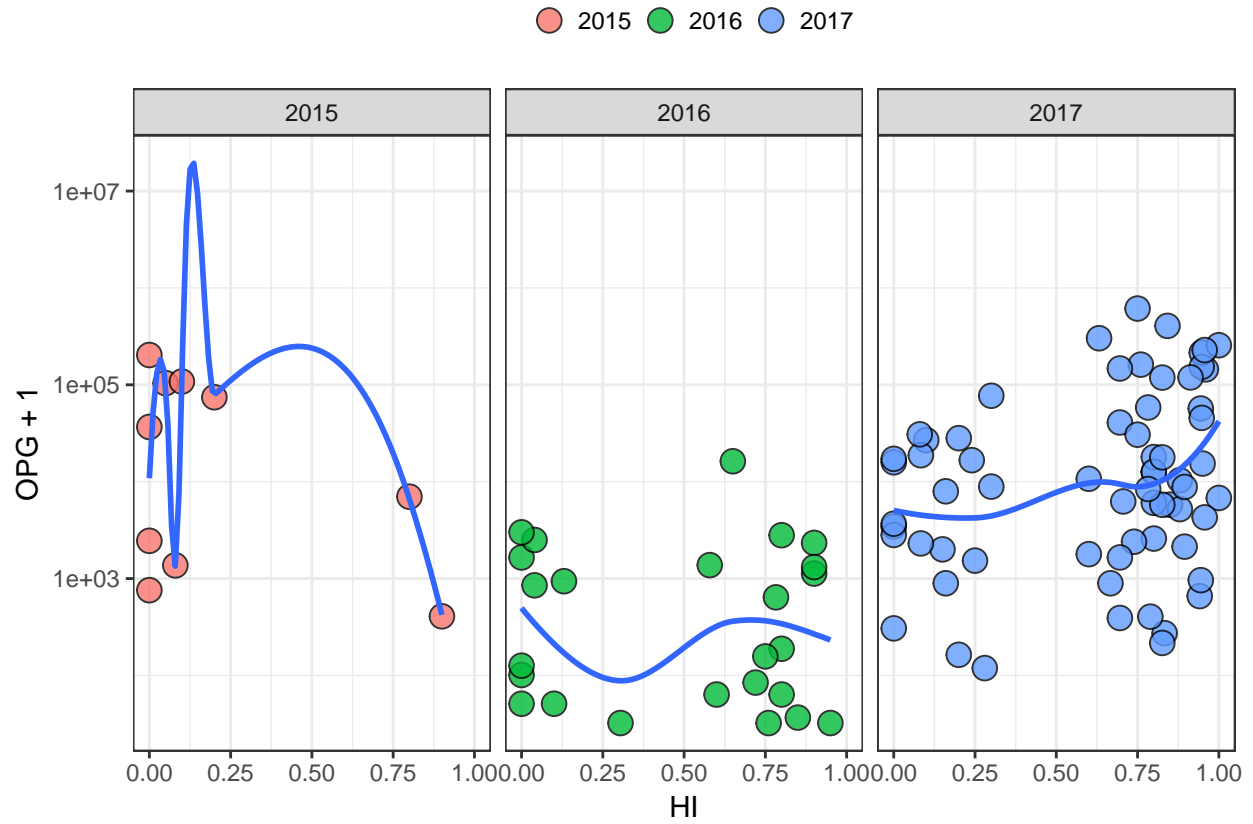
22 new samples were detected while diluting by 0.1mL PBS instead of 1mL before counting in Neubauer chamber.

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

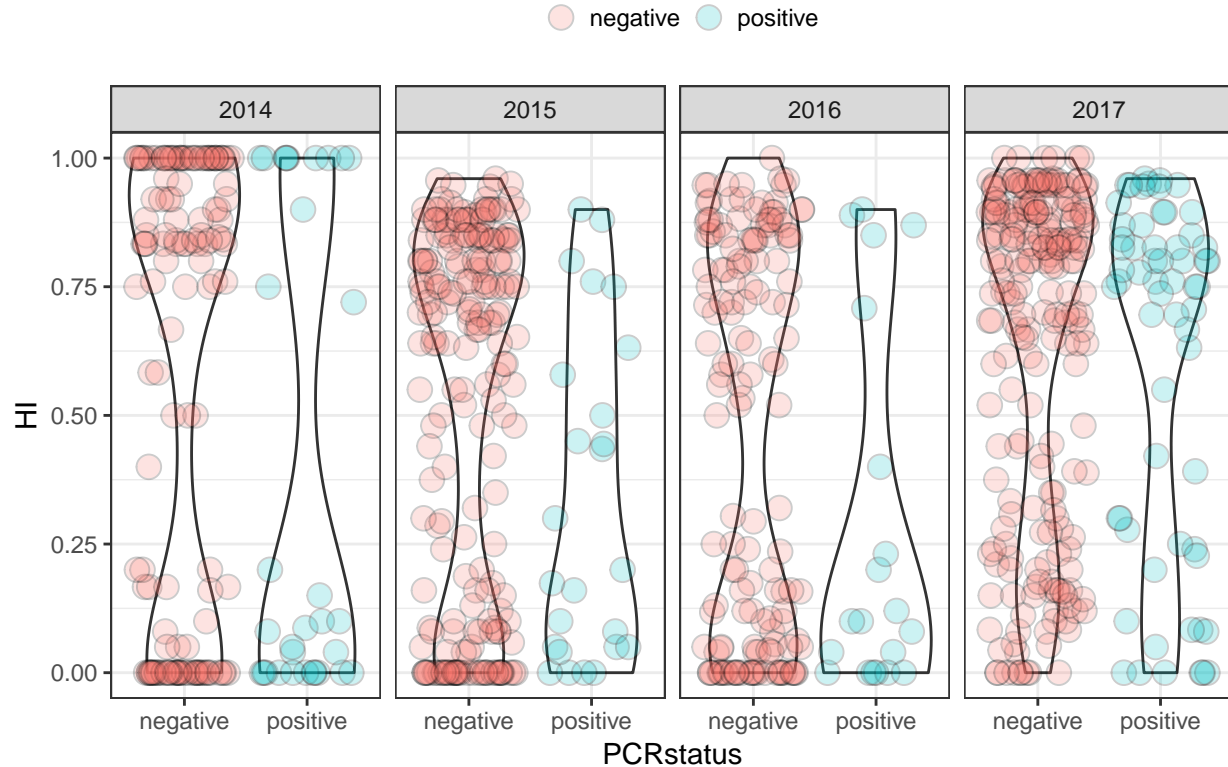
```
## `geom_smooth()` using method = 'loess'
```



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



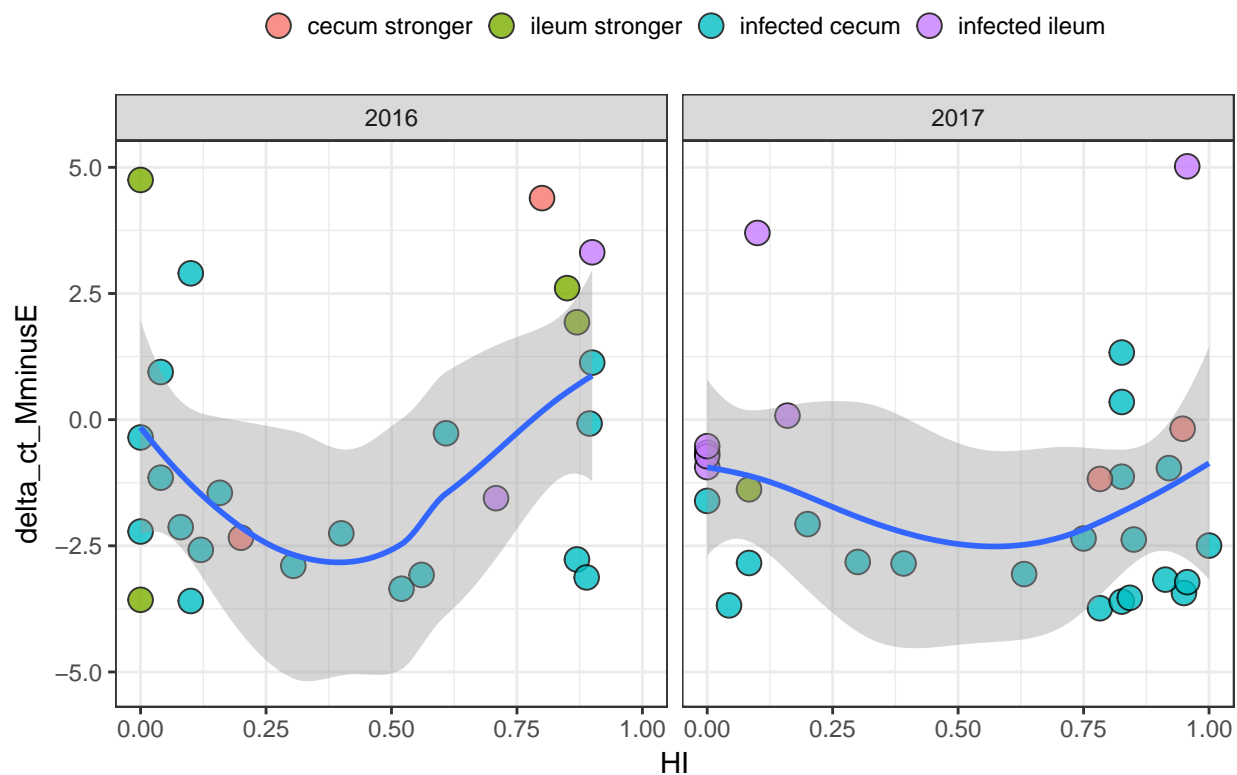
PCR positive = one of the 3 markers 18S, COI or ORF470) gave a sequence. Number of *Mus musculus* caught with PCR performed: 962

Eimeria detection qPCR

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

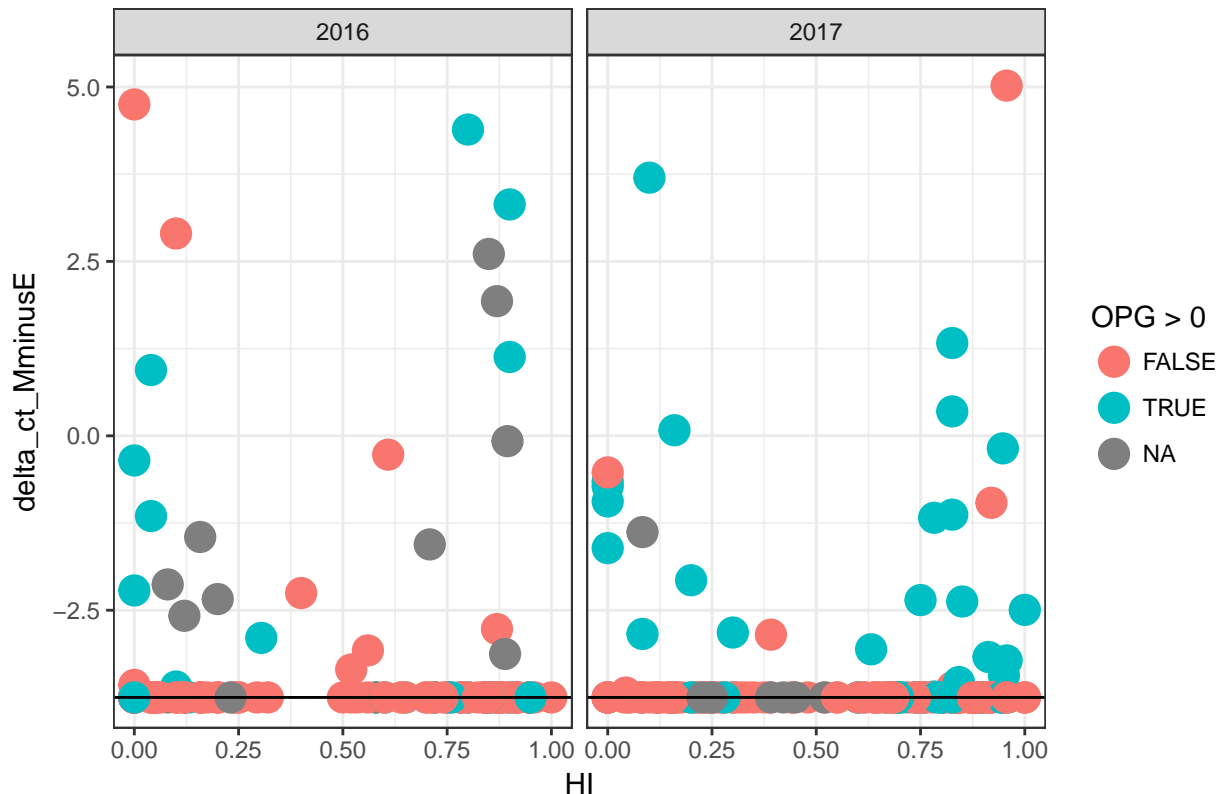
```
## `geom_smooth()` using method = 'loess'
```

Smooth on qPCR data (positive only (> -3.75))



Warning: Removed 168 rows containing missing values (geom_point).

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



Number of Mus musculus caught with qPCR performed: 371

General stats on sampling

```
## Warning in file.remove(index[[url]]): cannot remove file
## '470a3353e013428057316f09bebc2634.rds', reason 'No such file or directory'

## Warning in file.remove(index[[url]]): cannot remove file
## '38fbfc523793116165689a0313c890a2.rds', reason 'No such file or directory'

## Warning in file.remove(index[[url]]): cannot remove file
## '77d569e91de72d296943f667074aa269.rds', reason 'No such file or directory'

## Warning in file.remove(index[[url]]): cannot remove file
## '022b99a7810e5f121831862b7ae5585c.rds', reason 'No such file or directory'

## Warning in file.remove(index[[url]]): cannot remove file
## '304d0f0a73e271eaf822f46811766f2c.rds', reason 'No such file or directory'

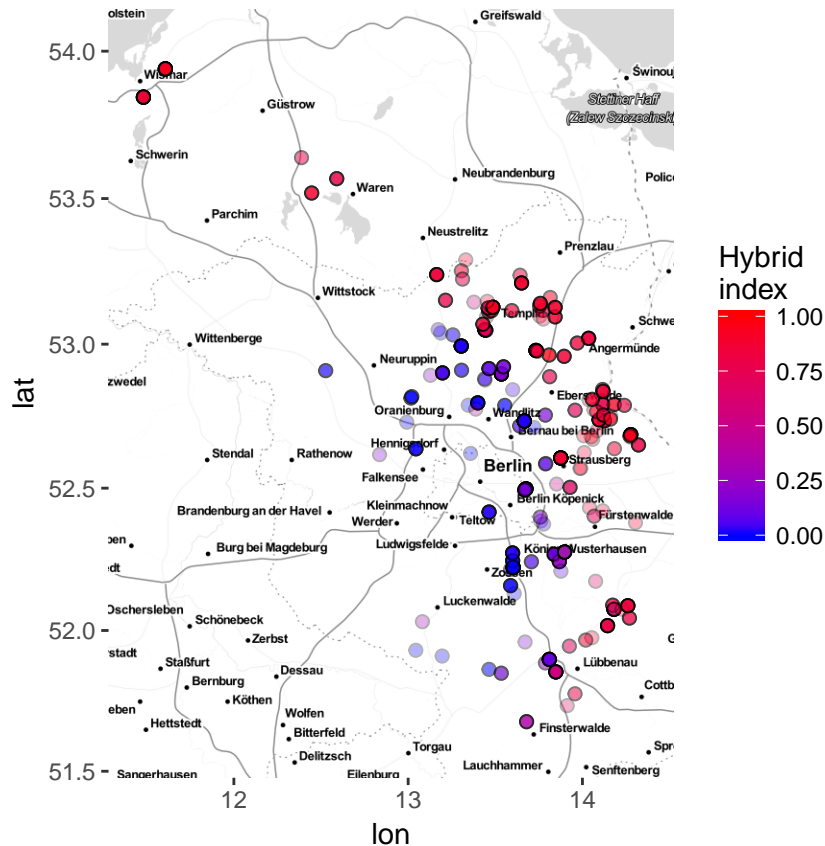
## Warning in file.remove(index[[url]]): cannot remove file
## '0334f529ea54c1d0ccf1cd725091cad0.rds', reason 'No such file or directory'

## Warning in file.remove(index[[url]]): cannot remove file
## '64129c1d3ac5deae5e8ed253206b774d.rds', reason 'No such file or directory'

## Warning in file.remove(index[[url]]): cannot remove file
## '72f10416ae8d9fb0622179a5c939c1ff.rds', reason 'No such file or directory'

## Warning in file.remove(index[[url]]): cannot remove file
```

```
## '226174bf385d58f14a113ec142144b62.rds', reason 'No such file or directory'
## Warning in file.remove(index[[url]]): cannot remove file
## '3b753e162cfb58b4a26adc1d5b6851ec.rds', reason 'No such file or directory'
## Warning in file.remove(index[[url]]): cannot remove file
## '22009ff43e754b87b1de5c26c172be48.rds', reason 'No such file or directory'
## Warning in file.remove(index[[url]]): cannot remove file
## 'b7c4544b894e579df026e737678920d9.rds', reason 'No such file or directory'
```



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)

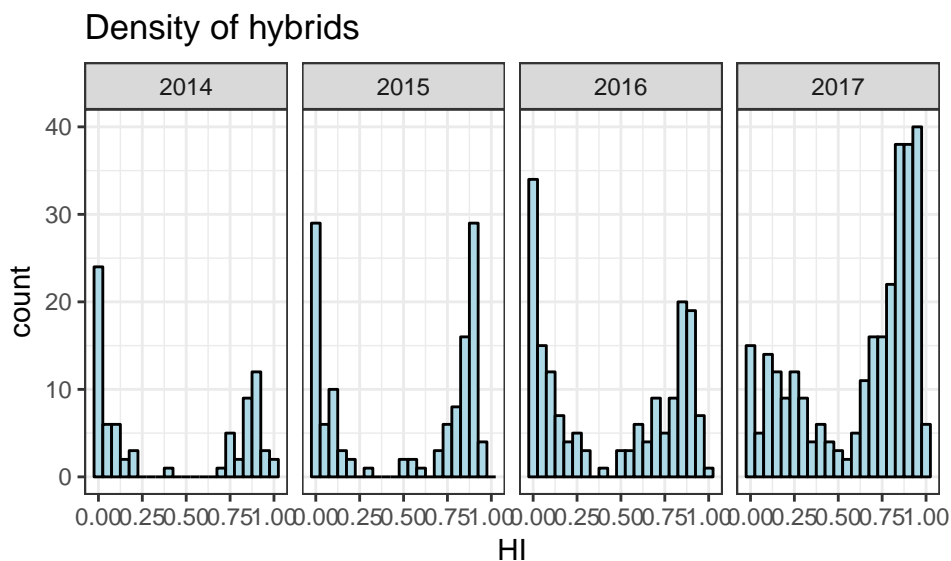


Figure 3: Number of animals caught along the hybrid index

Prevalence of our 3 different methods

Prevalence tables

Table 5: 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 6: Prevalence of Eimeria per year, based on PCR detection. A mouse was considered infected by Eimeria if one 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

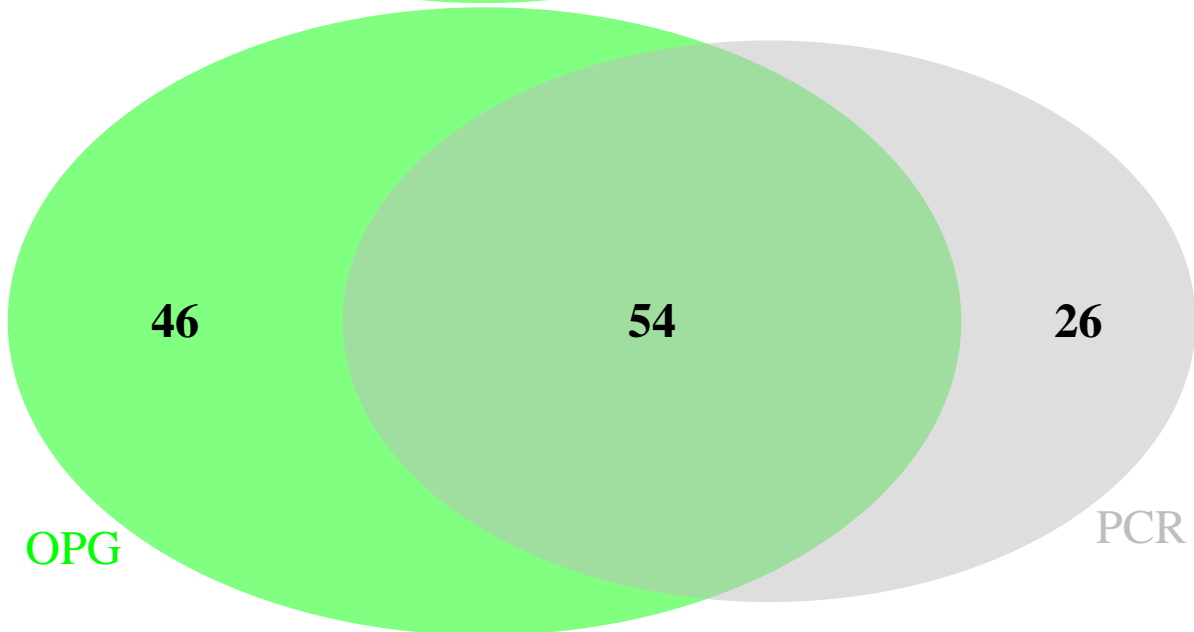
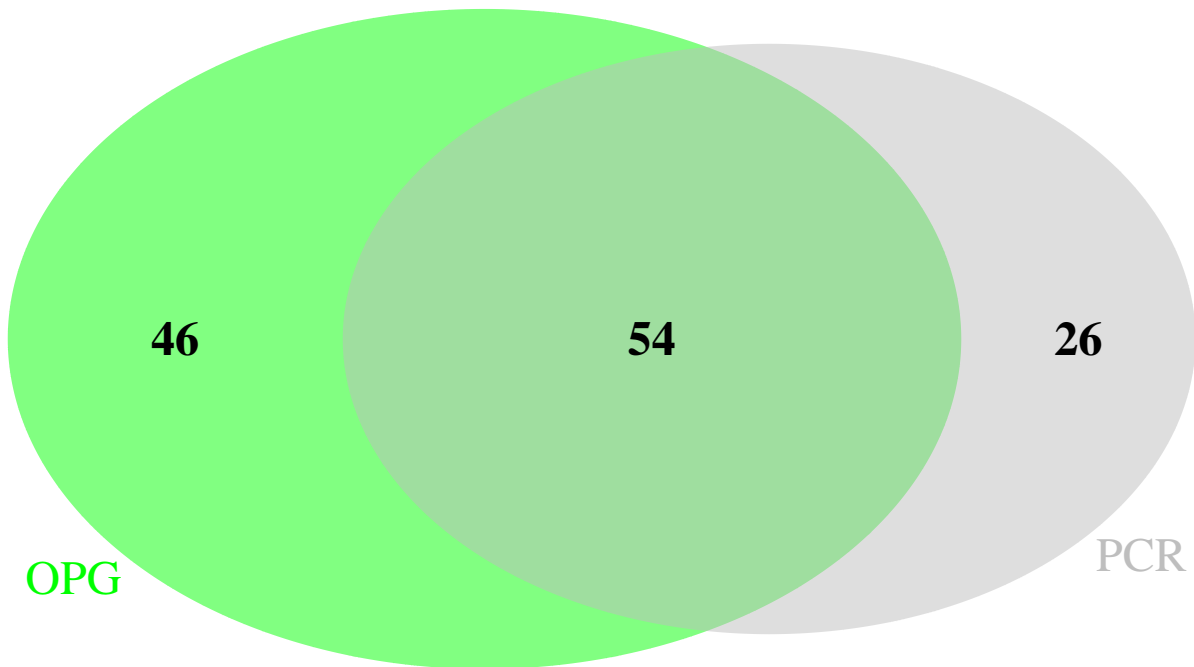
Table 7: Prevalence of Eimeria per year, based on qPCR

	2014	2015	2016	2017
FALSE	0	0	138.00	177.00
TRUE	0	0	26.00	30.00
total	0	0	164.00	207.00
prevalence(%)	NaN	NaN	15.85	14.49

Table 8: 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

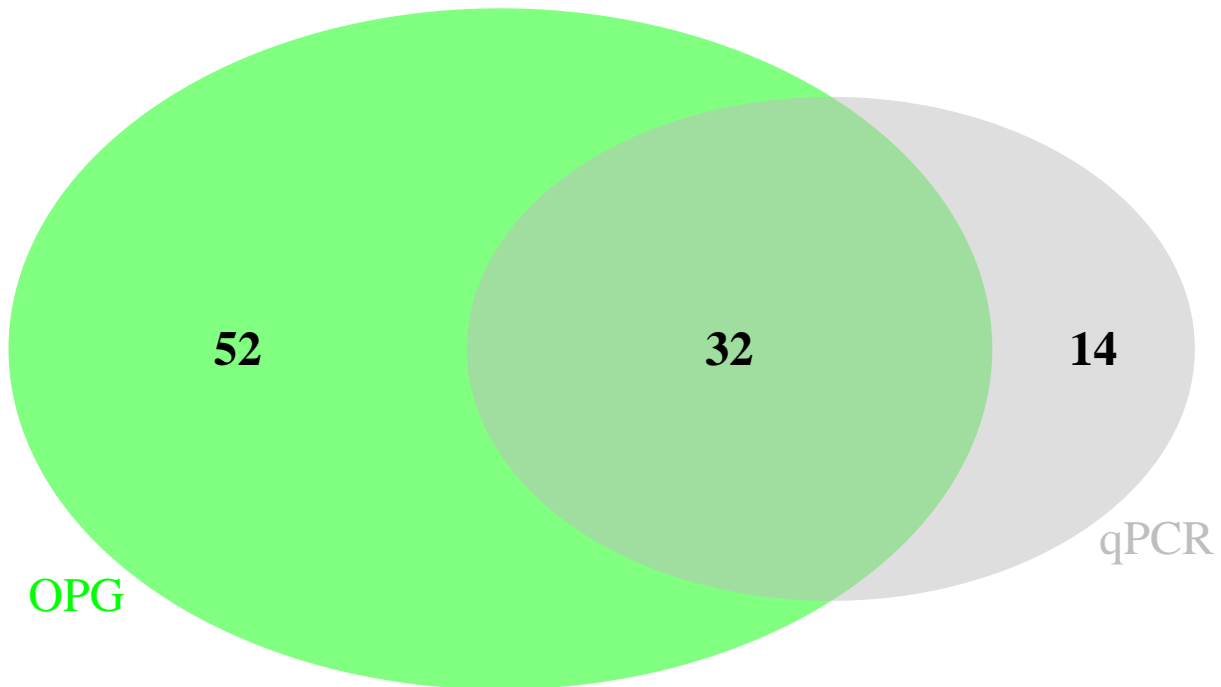
	2014	2015	2016	2017
negative	53.00	105.00	124.00	198.00
positive	23.00	17.00	43.00	92.00
total	76.00	122.00	167.00	290.00
prevalence(%)	30.26	13.93	25.75	31.72

OPG-PCR



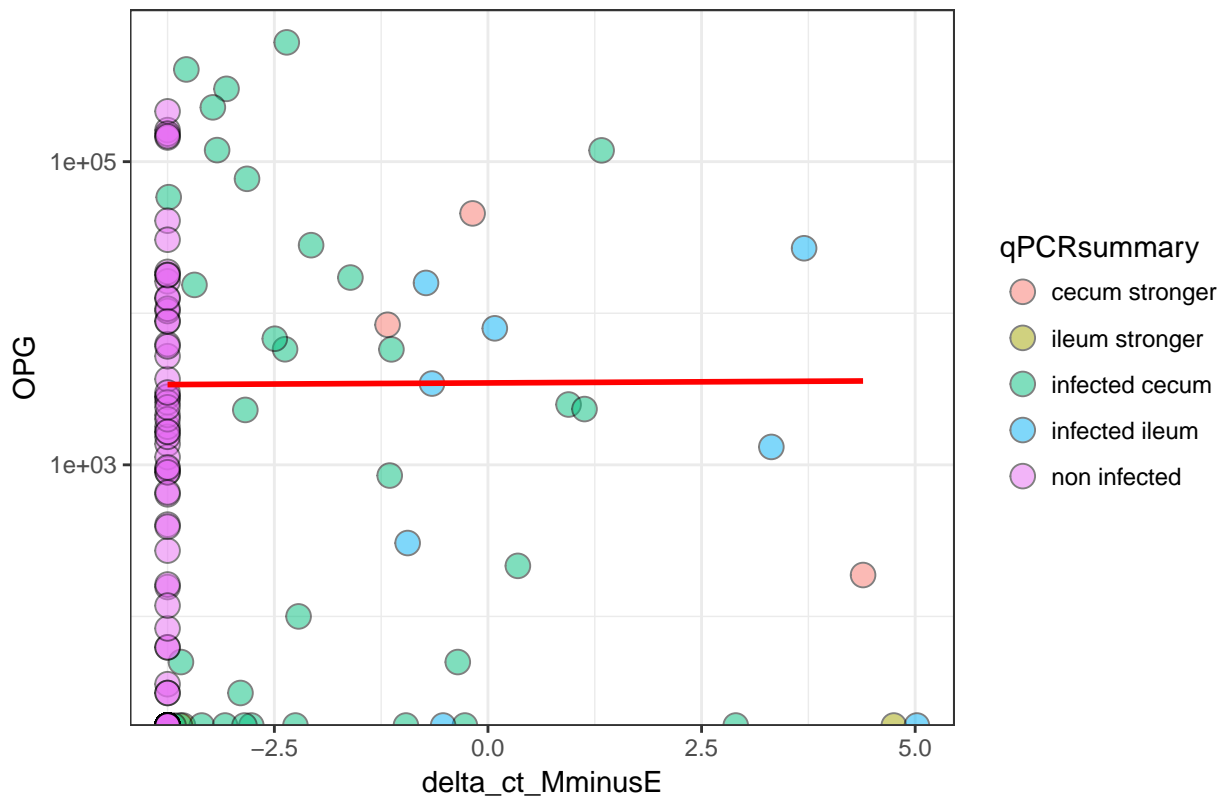
3 methods, threshold for qPCR = - 5

OPG-qPCR



(polygon[GRID.polygon.1869], polygon[GRID.polygon.1870], polygon[GRID.polygon.1871], polygon[GRID.polygon.1872])

Compare qPCR results and OPG



##

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

	Min	1Q	Median	3Q	Max
	-32526	-8357	-8357	-8357	600298

```
##
## Coefficients:
```

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	18692	7645	2.445	0.015 *
data1\$delta_ct_MminusE	2756	2110	1.306	0.192

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 49480 on 341 degrees of freedom
## Multiple R-squared:  0.004979,    Adjusted R-squared:  0.002061
## F-statistic: 1.706 on 1 and 341 DF,  p-value: 0.1924

##
## Call:
## lm(formula = data2$OPG ~ data2$delta_ct_MminusE)
##
## Residuals:
```

	Min	1Q	Median	3Q	Max
	-114277	-62907	-46511	15482	522445

```
##
## Coefficients:
```

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	43894	26591	1.651	0.1092
data2\$delta_ct_MminusE	-19601	10955	-1.789	0.0837 .

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 132800 on 30 degrees of freedom
## Multiple R-squared:  0.09642,    Adjusted R-squared:  0.0663
## F-statistic: 3.201 on 1 and 30 DF,  p-value: 0.08368
```

OPG-qPCR-PCR

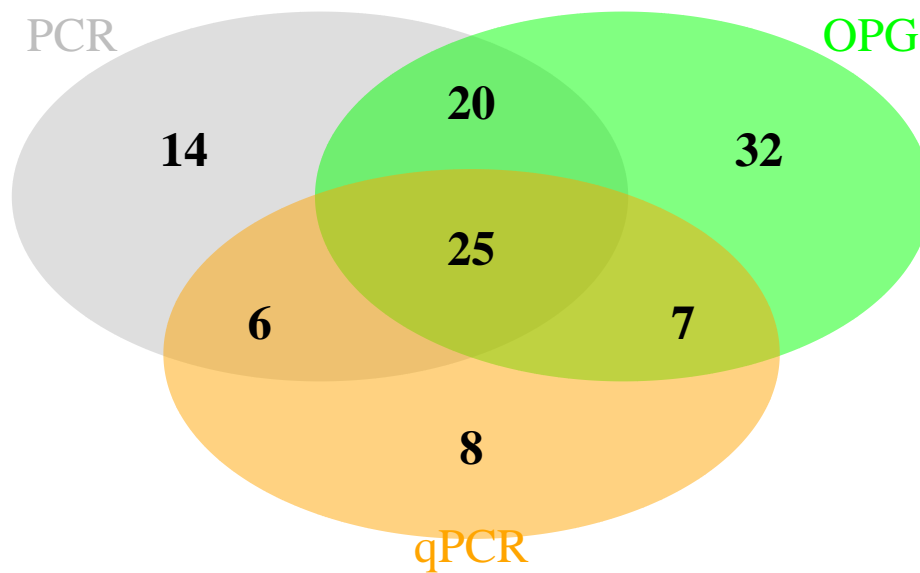
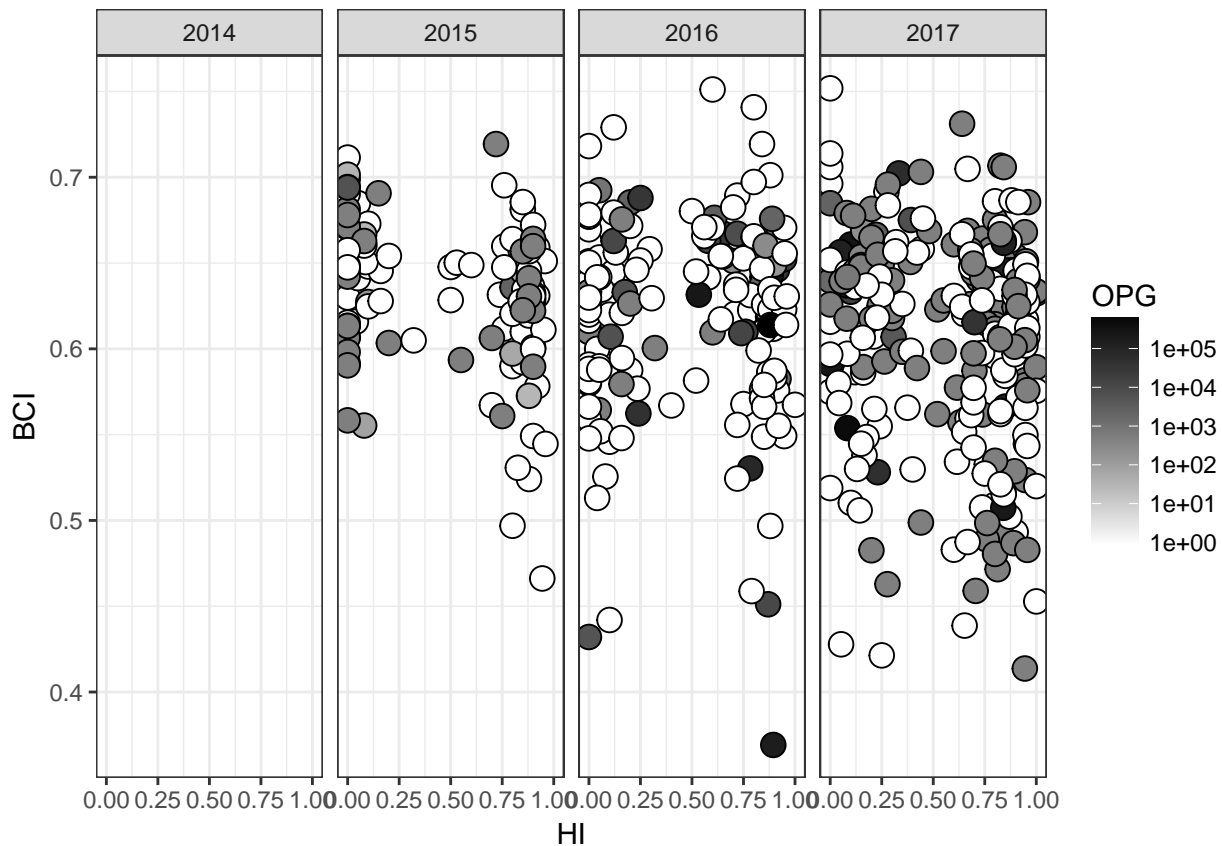


Figure 4: Comparison of detection: PCR vs flotation vs qPCR

```
## (polygon[GRID.polygon.1955], polygon[GRID.polygon.1956], polygon[GRID.polygon.1957], polygon[GRID.po
```


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 HMMZ

Bonus part: genotyping of mice case/control

- 100 out of 483 are positive for flotation and have an hybrid index.
- 56 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 $\sim 400 \pm 70$ infectés SNPchip.
- H_0 : no differences are observed
- Separate <0.5 and >0.5 to see the species effect
- timing : WHEN (for my thesis?)