

## 4. Gene\_expressions\_analysis - Analyzing differences between field and lab gene expression data

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### Aims:

- Compare immune gene expression between lab and field data
- How do eimeria infections affect the expression of various immune genes?
- Does the expression vary between lab and field data?

### load libraries

```
library(ggplot2)
library(tidyverse)

## -- Attaching packages ----- tidyverse 1.3.1 --

## v tibble  3.1.8      v dplyr    1.0.9
## v tidyr   1.2.0      v stringr 1.4.0
## v readr   2.1.2      v forcats 0.5.1
## v purrr   0.3.4

## -- Conflicts ----- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()     masks stats::lag()
```

### Import data:

Here, we have the experimental / field data, including imputed data

```
lab <- read.csv("output_data/gene_expression/data_products/lab_imputed_gene_expression.csv")
field <- read.csv("output_data/gene_expression/data_products/field_imputed_gene_expression.csv")
```

### Selecting genes

```
# vectors for selecting gene columns
Genes_lab <- c("IFNy", "CXCR3", "IL.6", "IL.10", "IL.13", "IL1RN", "CASP1",
              "CXCL9", "IDO1", "IRGM1", "MPO", "MUC2", "MUC5AC",
              "MYD88", "NCR1", "PRF1", "RETNLB", "SOCS1", "TICAM1", "TNF")

Genes_field <- c("IFNy", "CXCR3", "IL.6", #"GBP2", "IL.12", "IRG6",
                "IL.10", "IL.13", "IL1RN",
                "CXCR3", "CASP1", "CXCL9",
                "IDO1", "IRGM1", "MPO", "MUC2", "MUC5AC", "MYD88",
                "NCR1", "PRF1", "RETNLB", "SOCS1", "TICAM1", "TNF")
```

## Adding a variable that signifies if sample is infected or not

detection threshold: delta ct = -5 (Jarquin Diaz et al)

```
field <- field %>%
  mutate(
    infected_eimeria =
      case_when(
        delta_ct_cewe_MminusE > -5 ~ "infected",
        delta_ct_cewe_MminusE < -5 ~ "uninfected"
      )
  )

lab <- lab %>%
  mutate(
    infected_eimeria =
      case_when(
        delta > -5 ~ "infected",
        delta < -5 ~ "uninfected"
      )
  )
```

## Compare the differences in uninfected and infected data and field / lab

Write a function which does a t test to quantify the differences of gene expression between uninfected and infected mice in the lab and field infections and also compares the uninfected lab and field and the infected lab and field

```
eimeria_t.test <- function(x, y) {
  # select the uninfected (delta ct under -5 mice)
  lab_uni <- lab %>%
    dplyr::filter(infected_eimeria == "uninfected")
  # select the infected lab mice
  lab_inf <- lab %>%
    dplyr::filter(infected_eimeria == "infected")
  # select the uninfected field mice (delta ct under -5 mice)
  field_uni <- field %>%
    dplyr::filter(infected_eimeria == "uninfected")
  # select the infected lab mice
  field_inf <- field %>%
    dplyr::filter(infected_eimeria == "infected")

  print(c("Testing differences in expression in uninfected and infected lab mice. Gene:", toString(x)))
  print(t.test(lab_uni %>% dplyr::select(all_of(x)), lab_inf %>% dplyr::select(all_of(x))))

  print(c("Testing differences in expression in uninfected and infected field mice. Gene:", toString(x)))
  print(t.test(field_uni %>% dplyr::select(all_of(x)), field_inf %>% dplyr::select(all_of(x))))

  print(c("Testing differences in expression in uninfected lab vs field mice. Gene:", toString(x)))
  print(t.test(lab_uni %>% dplyr::select(all_of(x)), field_uni %>% dplyr::select(all_of(x))))

  print(c("Testing differences in expression in infected lab vs field mice. Gene:", toString(x)))
  print(t.test(lab_inf %>% dplyr::select(all_of(x)), field_inf %>% dplyr::select(all_of(x))))
}
```

### 1. IFNy

```
ggplot(lab, aes(x = delta, y = IFNy, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
```

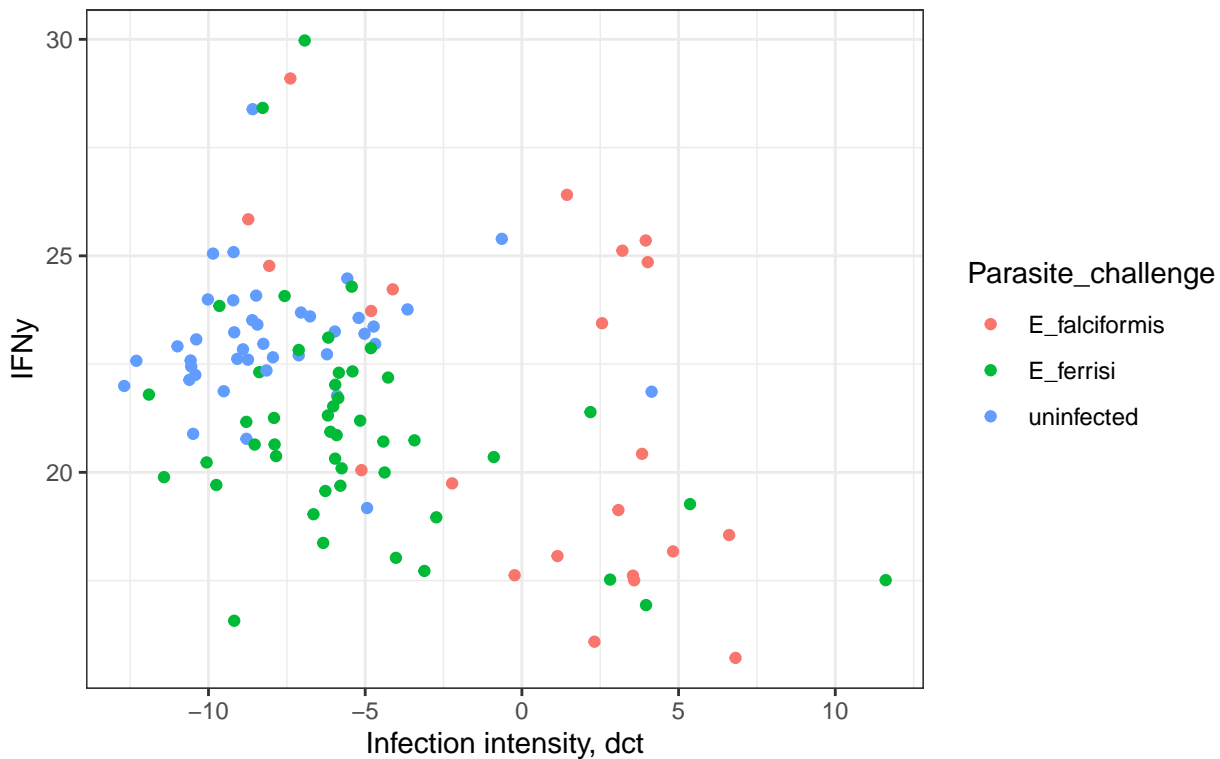
```

    title ="Immune gene expression against infection intensity in lab
infections") +
theme_bw()

```

## Warning: Removed 6 rows containing missing values (geom\_point).

### Immune gene expression against infection intensity in lab infections



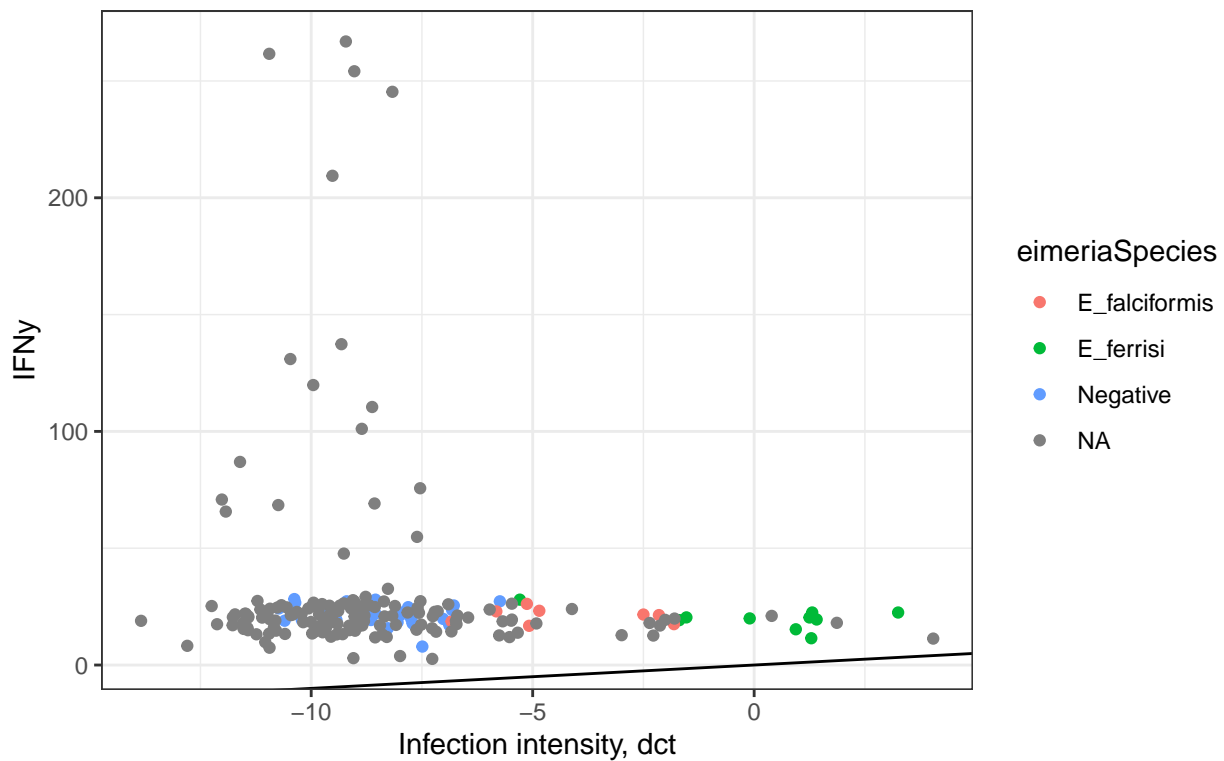
```

ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IFNγ, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
    title ="Immune gene expression against infection intensity in field
samples") +
  theme_bw()

```

## Warning: Removed 154 rows containing missing values (geom\_point).

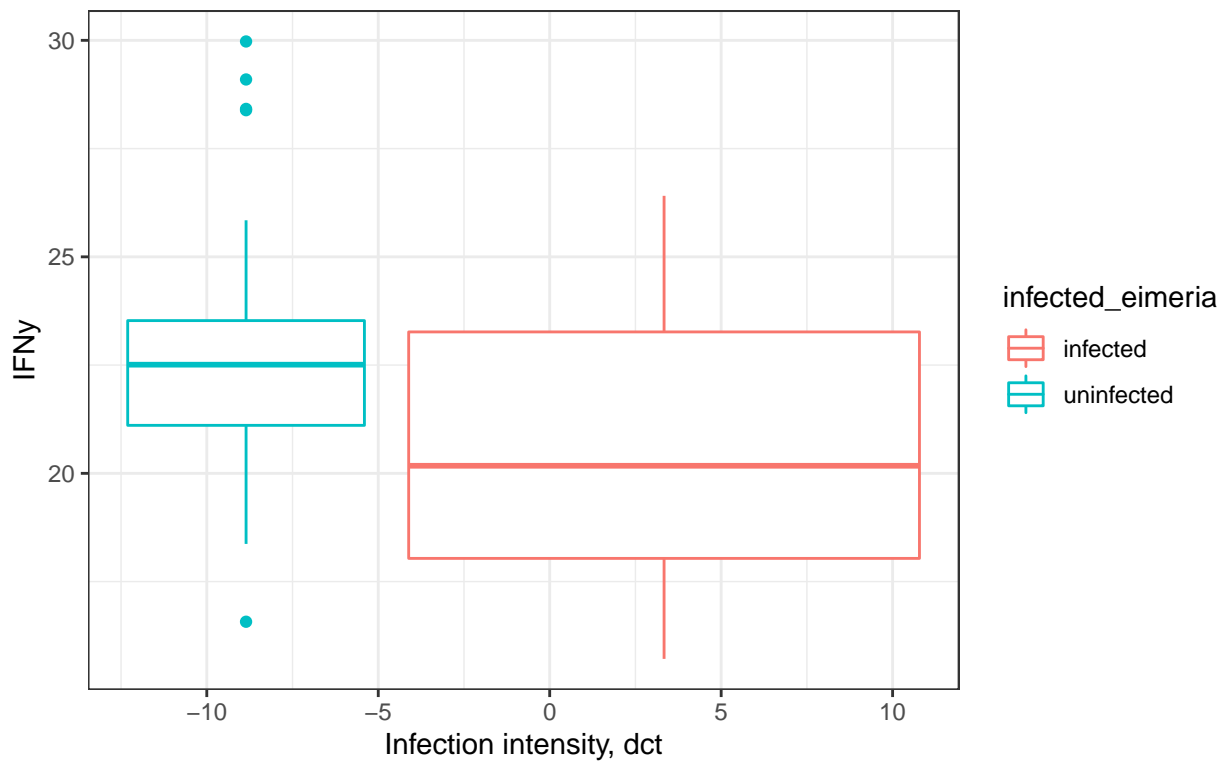
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = IFN $\gamma$ , color = infected_eimeria)) +  
  geom_boxplot() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity in lab  
infections") +  
  theme_bw()
```

```
## Warning: Removed 6 rows containing missing values (stat_boxplot).
```

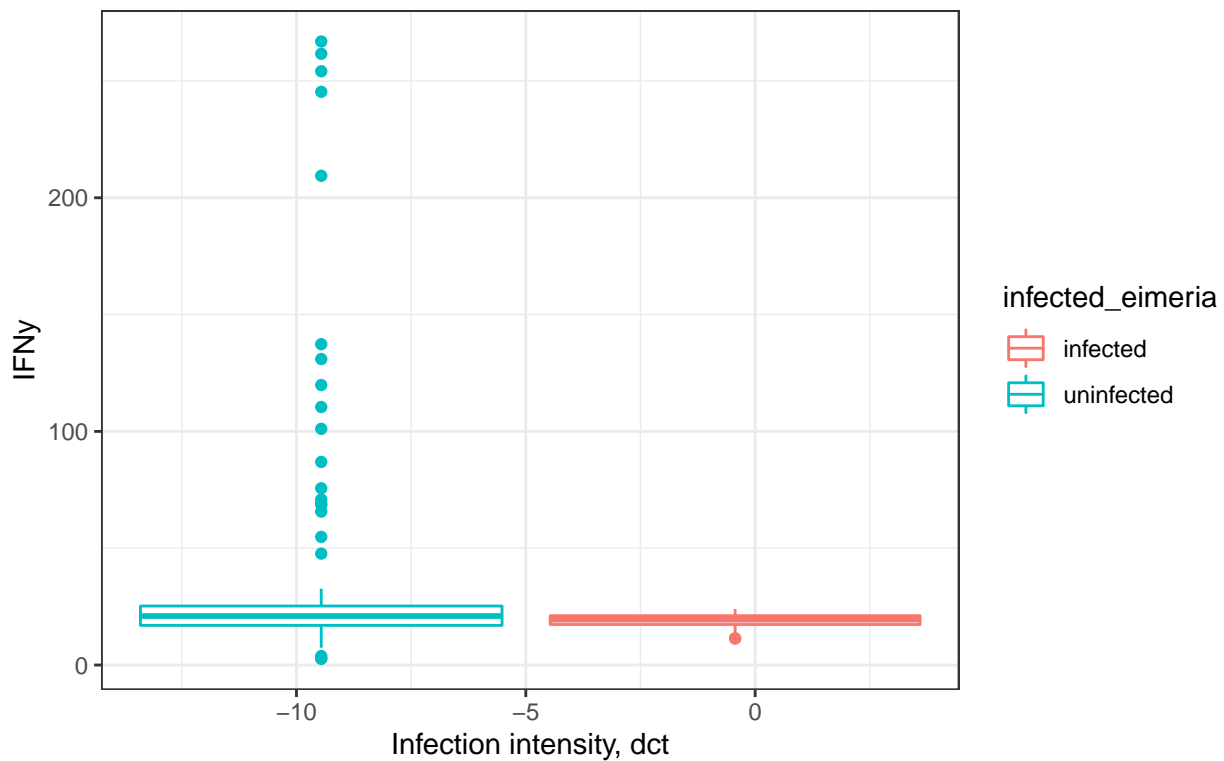
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IFNγ,  
                  color = infected_eimeria)) +  
  geom_boxplot() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity in field  
samples") +  
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples

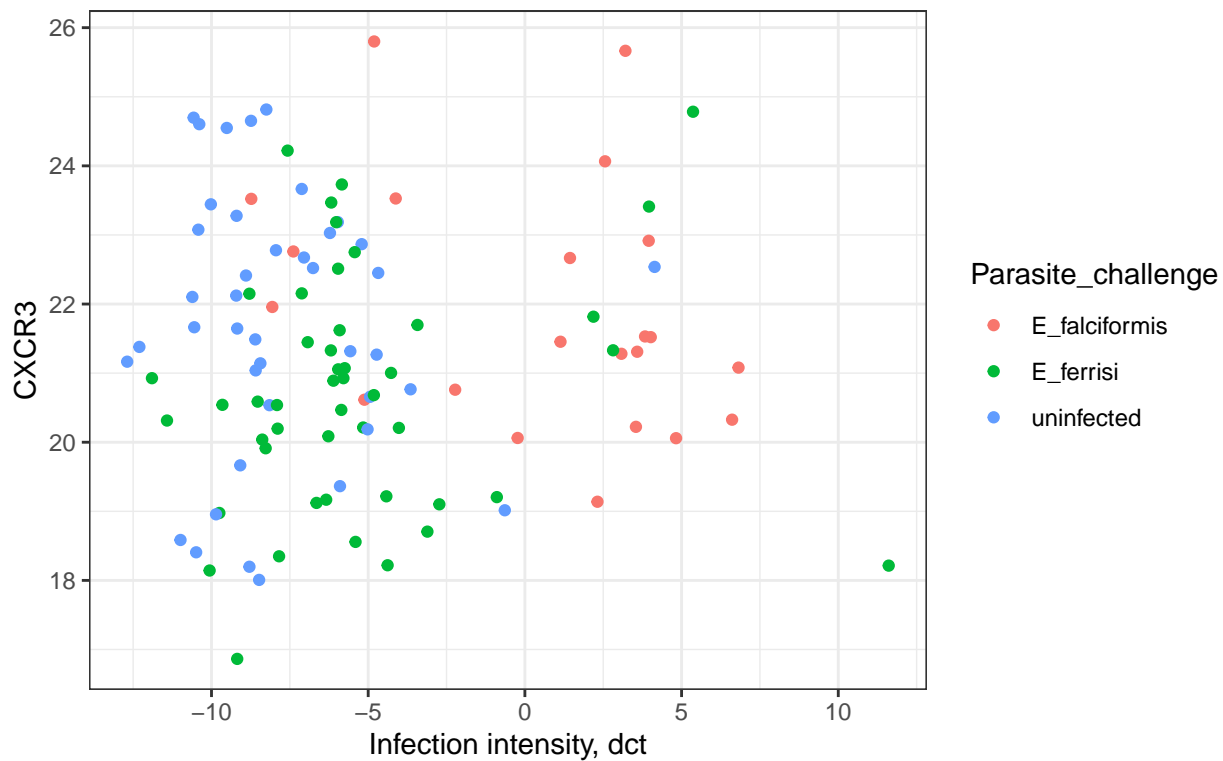


## 2. CXCR3

```
ggplot(lab, aes(x = delta, y = CXCR3_bio, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct", y = "CXCR3",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).

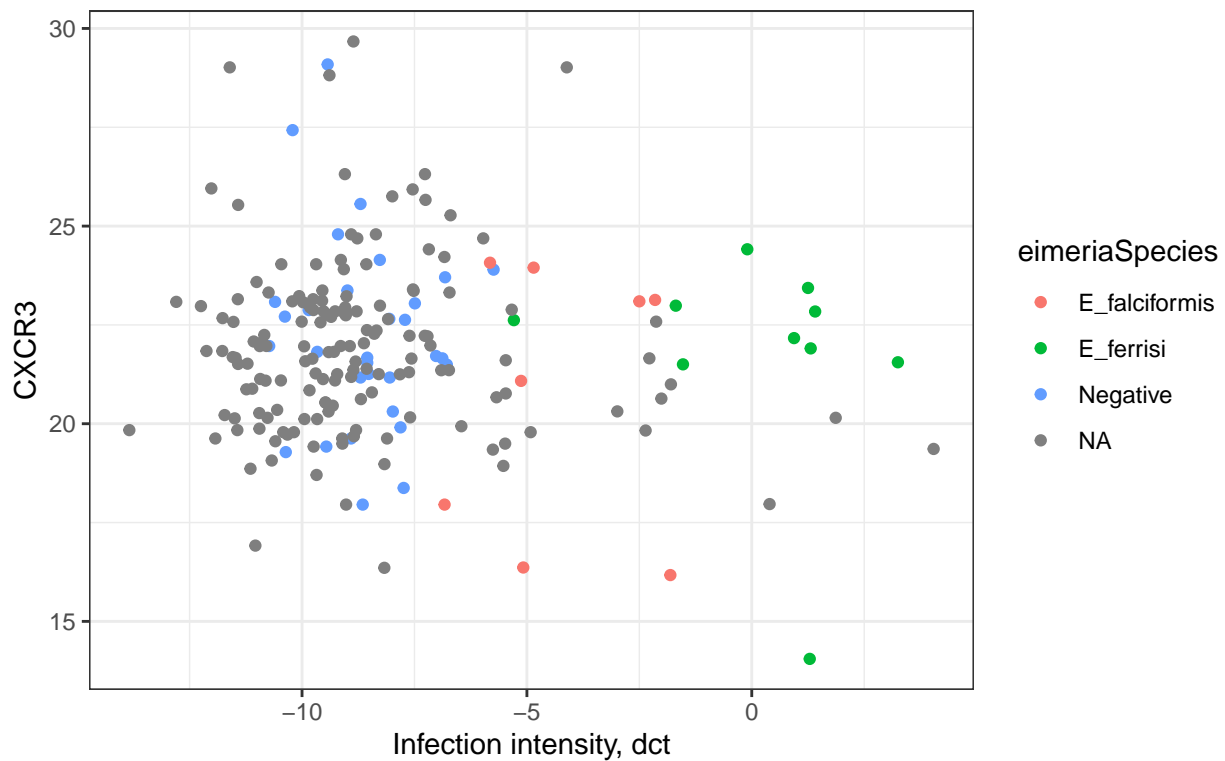
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = CXCR3, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in
       field samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

## Immune gene expression against infection intensity in field samples



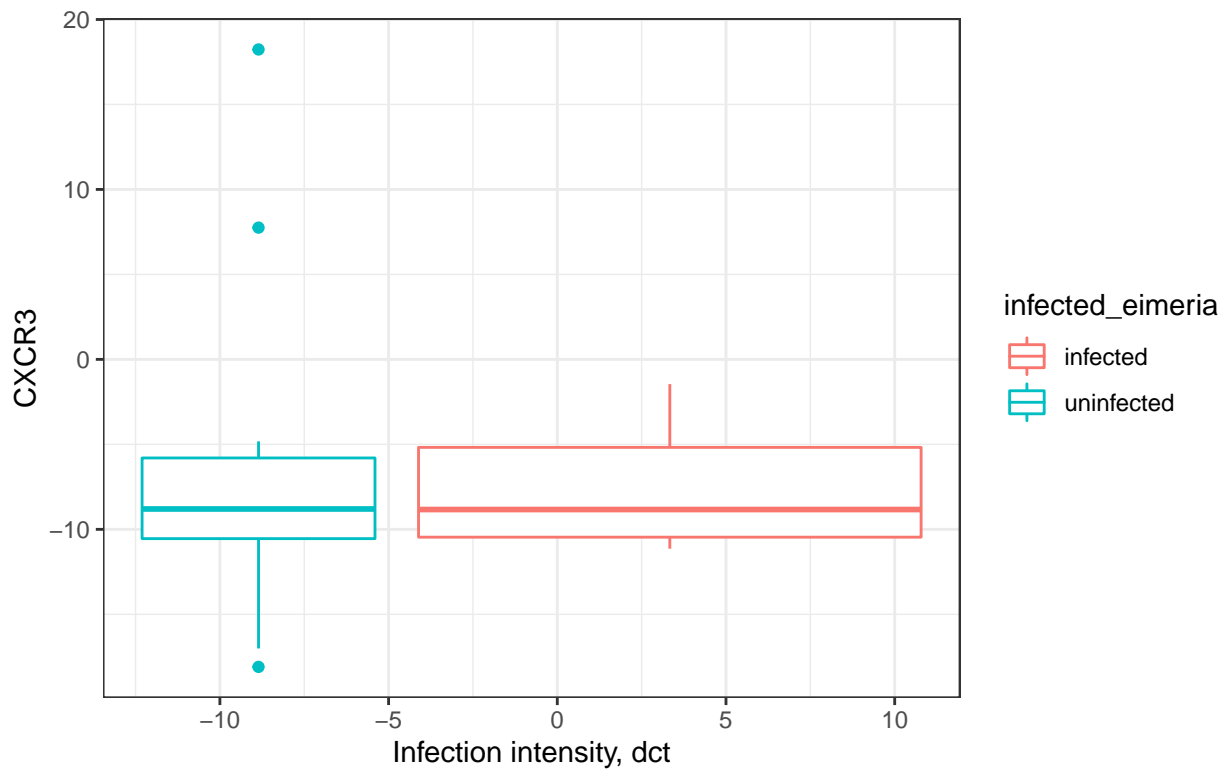
```
ggplot(lab, aes(x = delta, y = CXCR3, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

## Warning: Removed 49 rows containing non-finite values (stat\_boxplot).



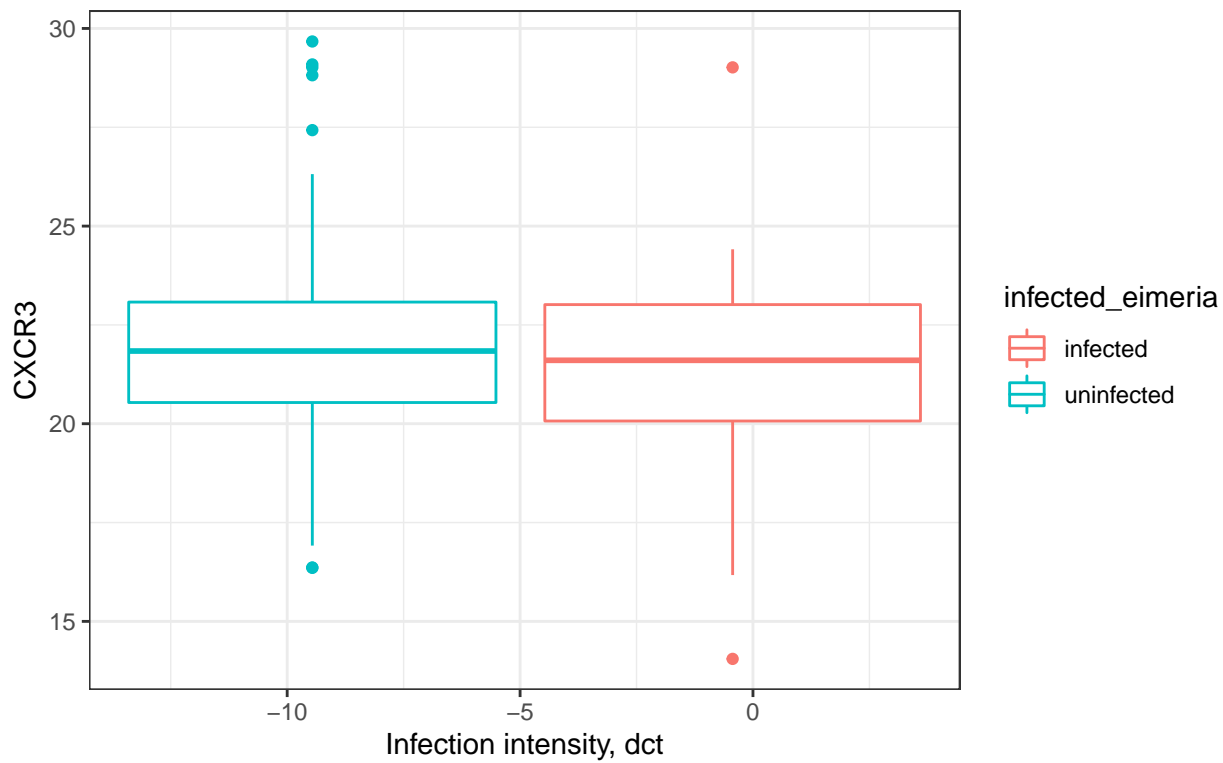
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = CXCR3,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
               samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

### Immune gene expression against infection intensity in field samples

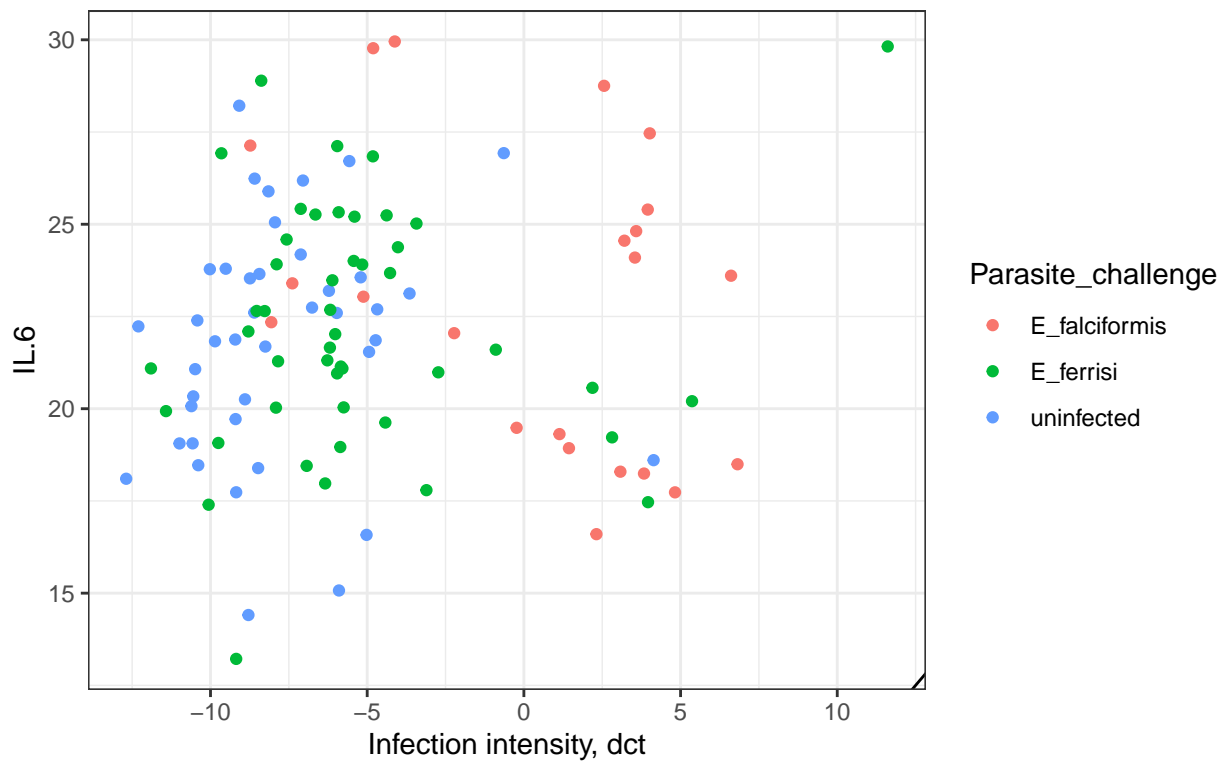


### 3. IL.6

```
ggplot(lab, aes(x = delta, y = IL.6, color = Parasite_challenge)) +  
  geom_jitter() +  
  geom_abline() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity in lab  
infections") +  
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).

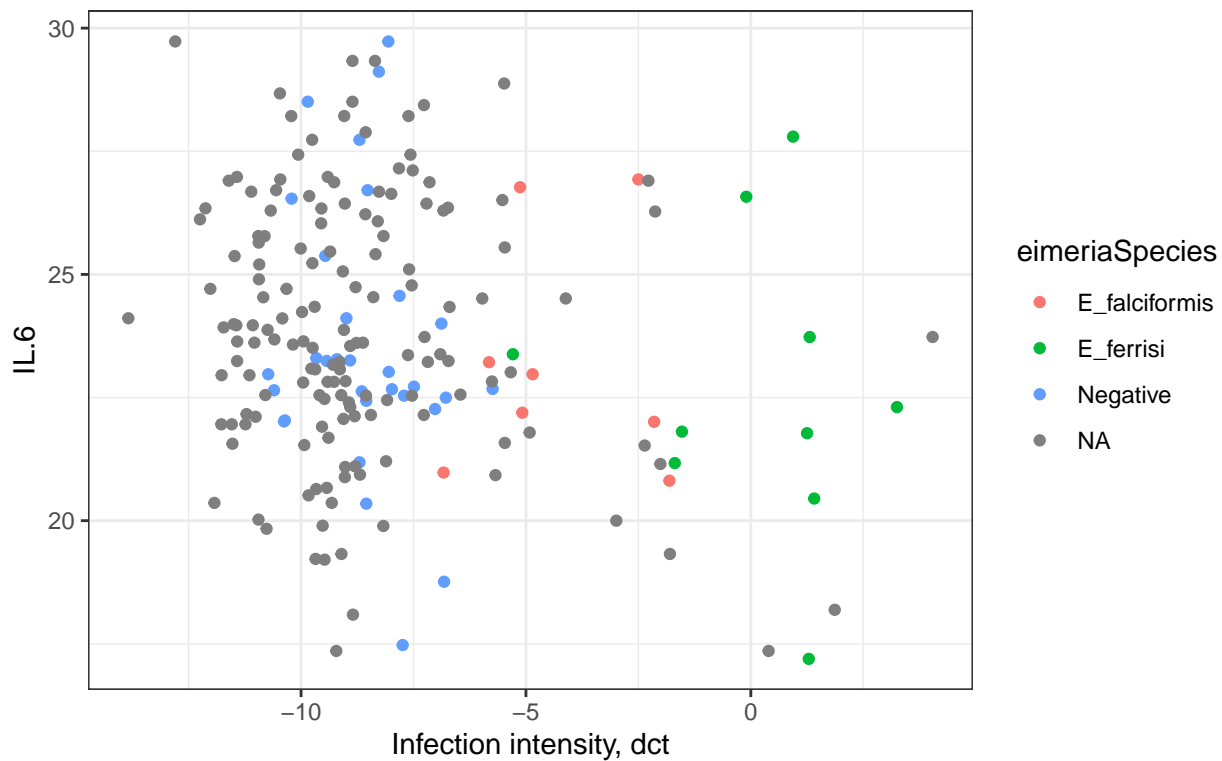
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IL.6, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
               samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

## Immune gene expression against infection intensity in field samples

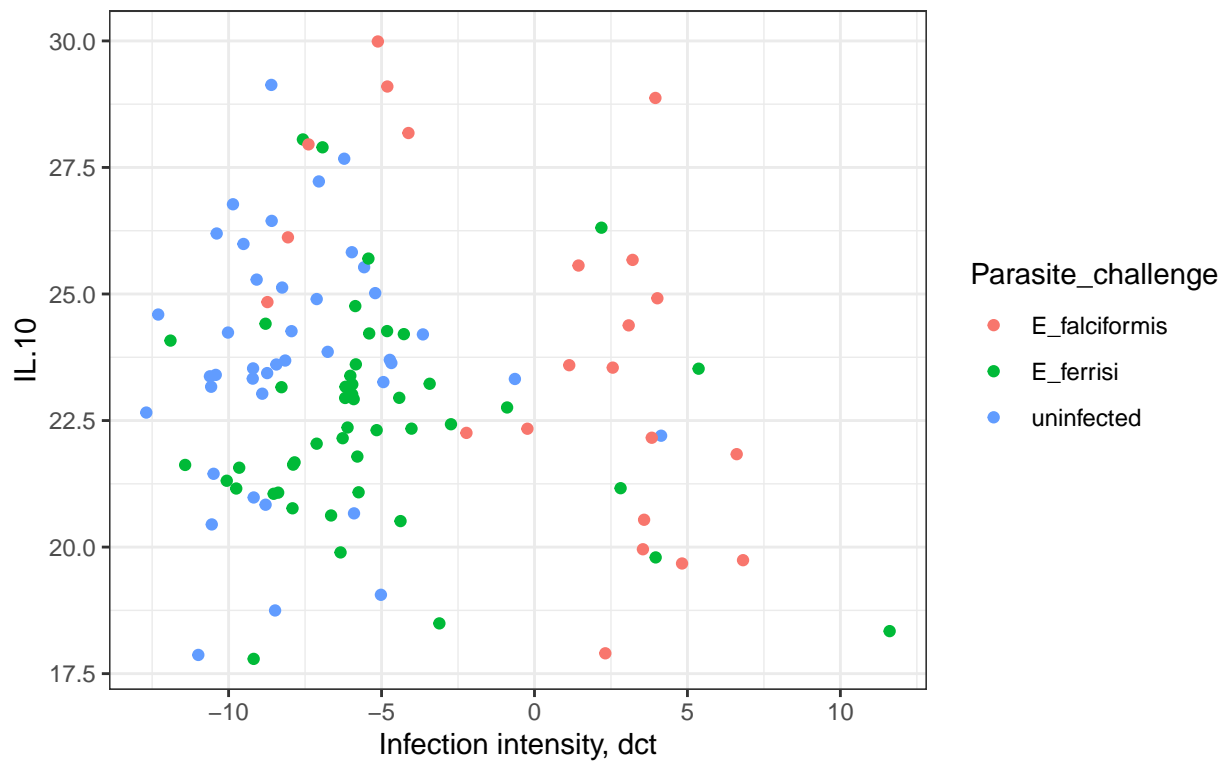


### 4. IL.10

```
ggplot(lab, aes(x = delta, y = IL.10, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).

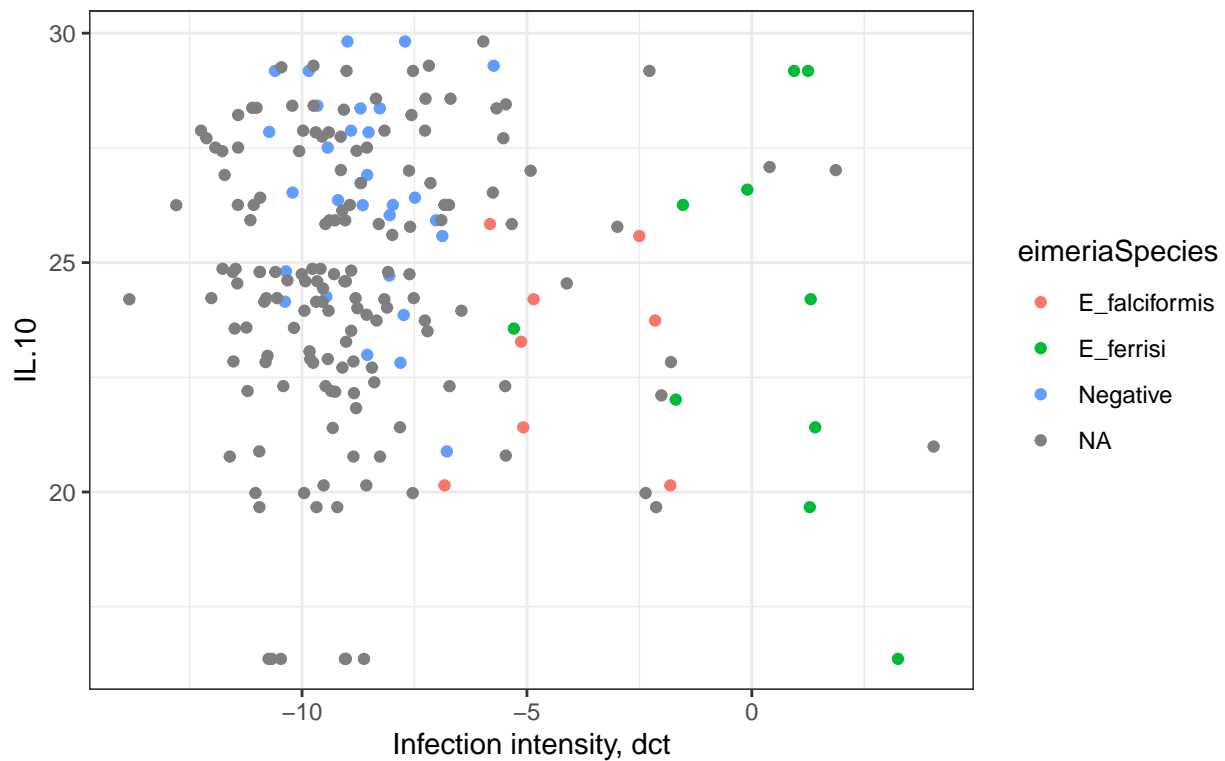
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IL.10, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

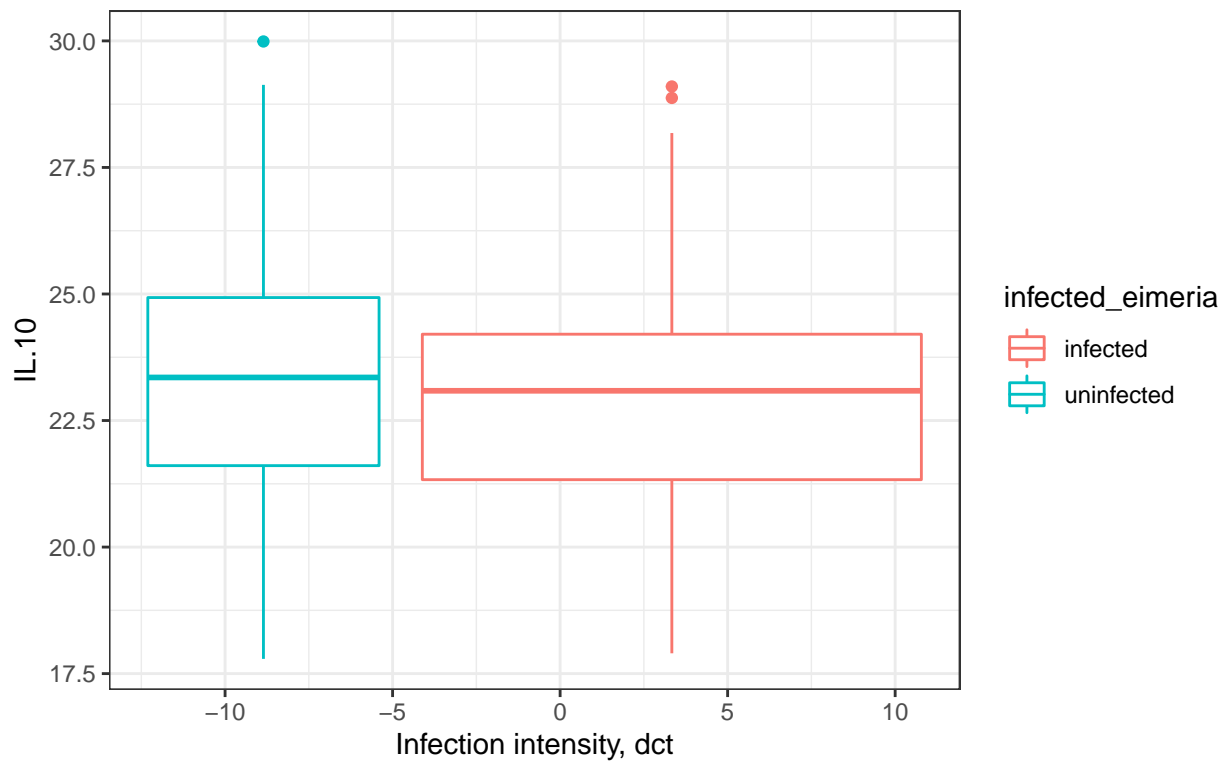
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = IL.10, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

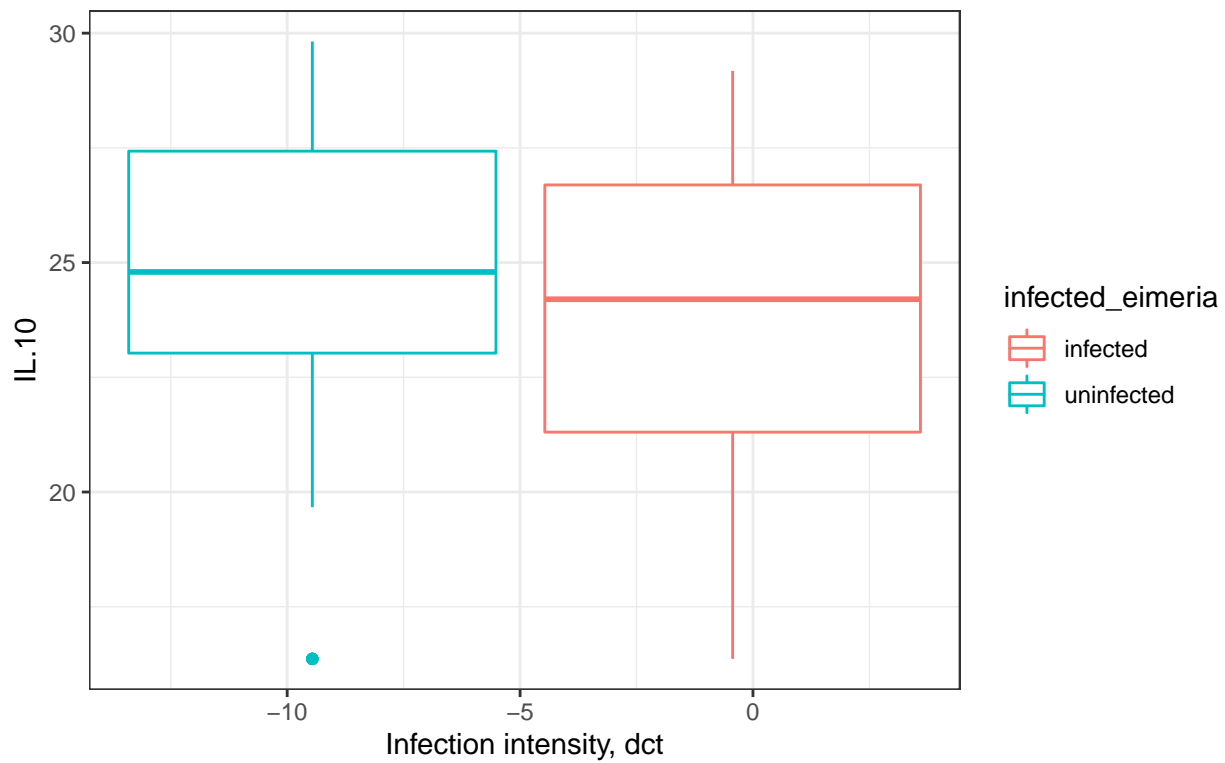
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IL.10,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples



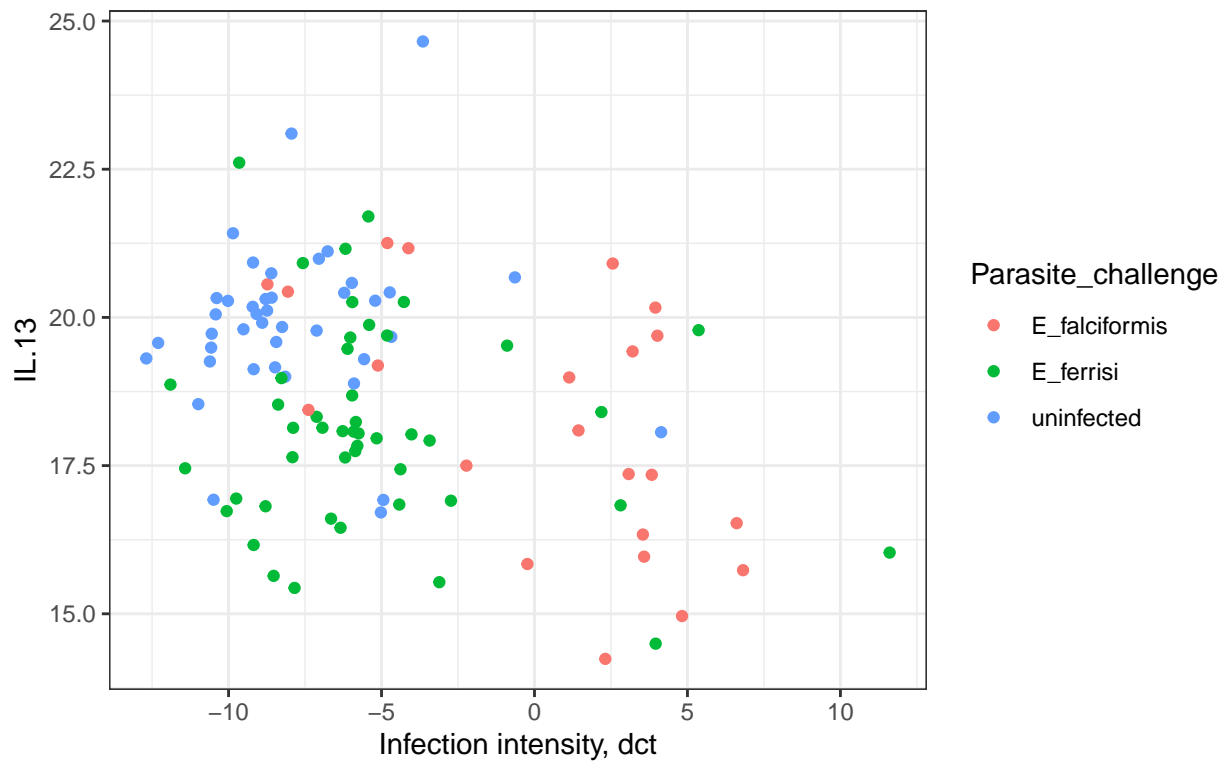
### 5. IL.13

```
ggplot(lab, aes(x = delta, y = IL.13, color = Parasite_challenge)) +  
  geom_jitter() +  
  geom_abline() +  
  labs(x = "Infection intensity, dct",  
        title = "Immune gene expression against infection intensity  
in lab infections") +  
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).



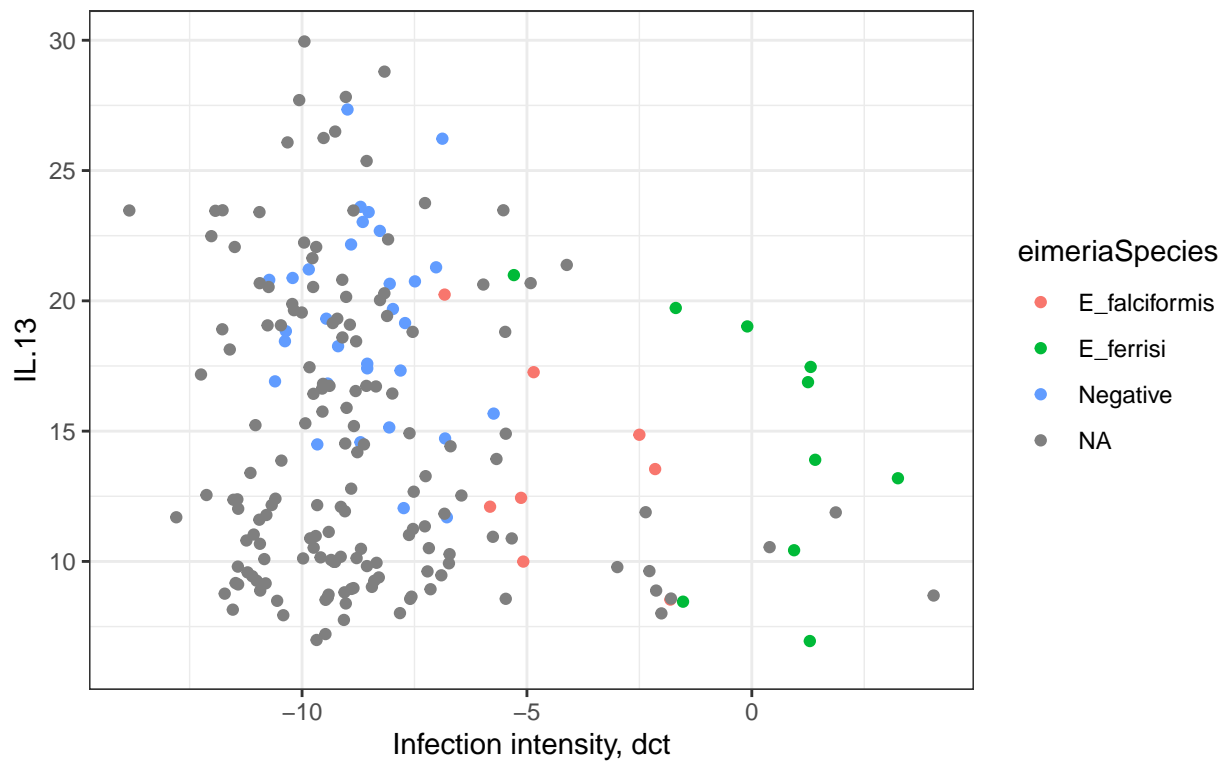
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IL.13, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

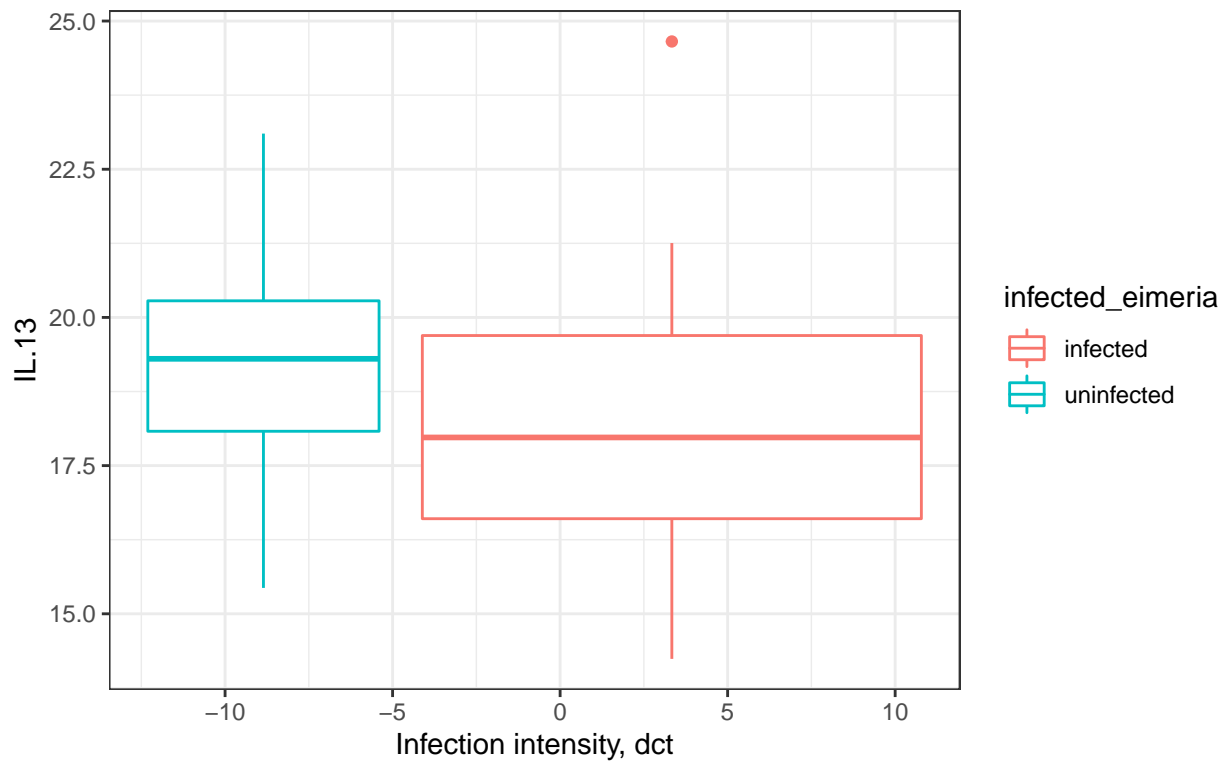
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = IL.13, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

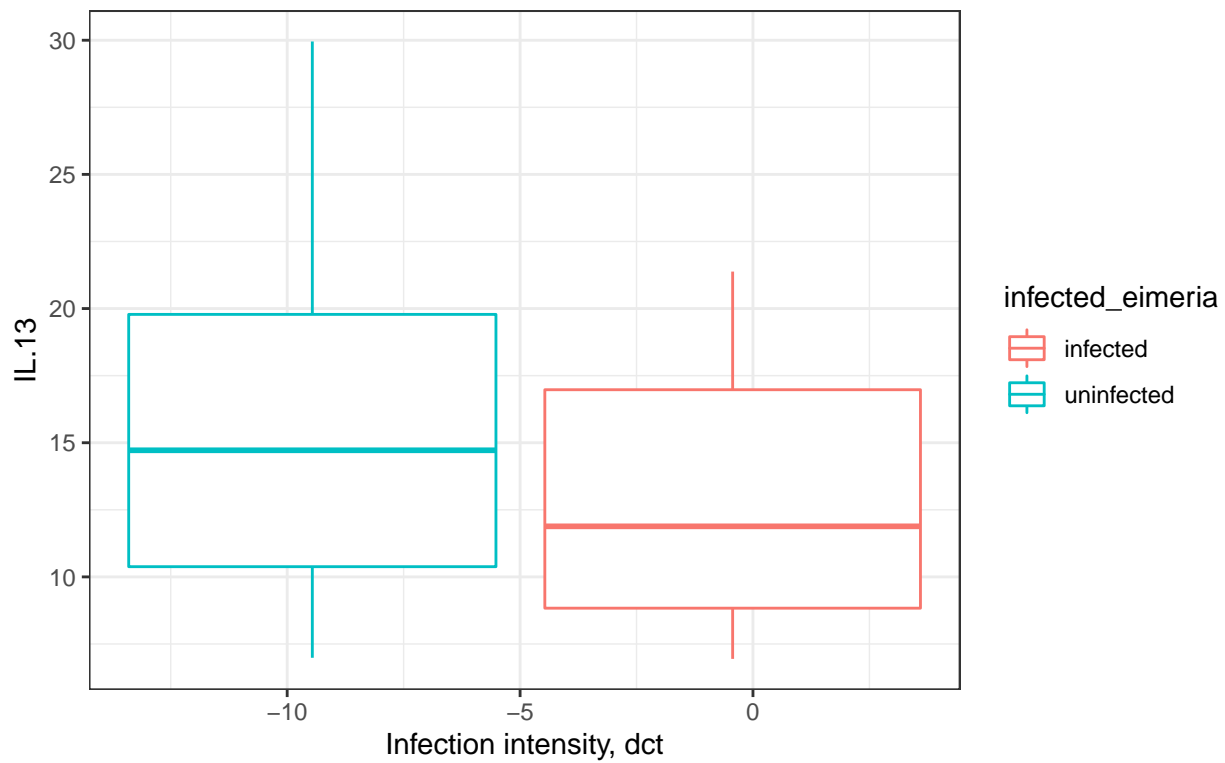
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IL.13,  
                  color = infected_eimeria)) +  
  geom_boxplot() +  
  labs(x = "Infection intensity, dct",  
        title = "Immune gene expression against infection intensity in field  
samples") +  
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples

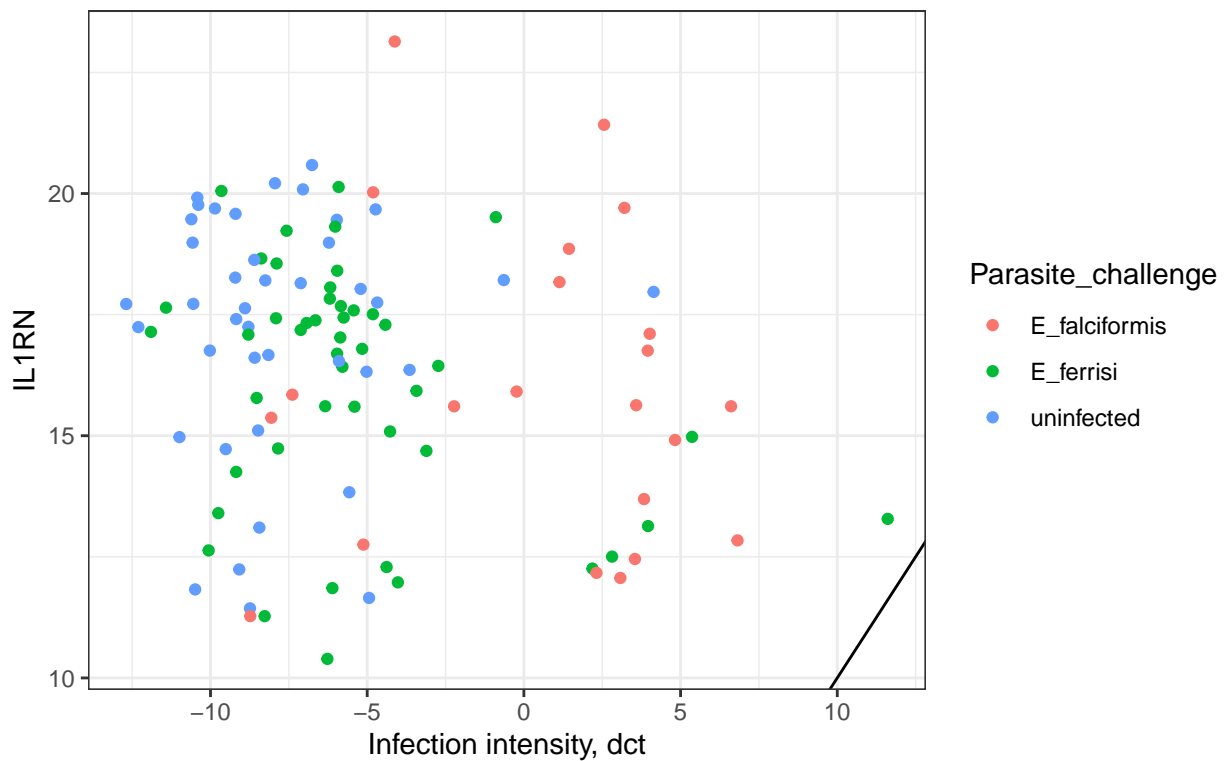


### 6. IL1RN

```
ggplot(lab, aes(x = delta, y = IL1RN, color = Parasite_challenge)) +  
  geom_jitter() +  
  geom_abline() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity  
in lab infections") +  
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).

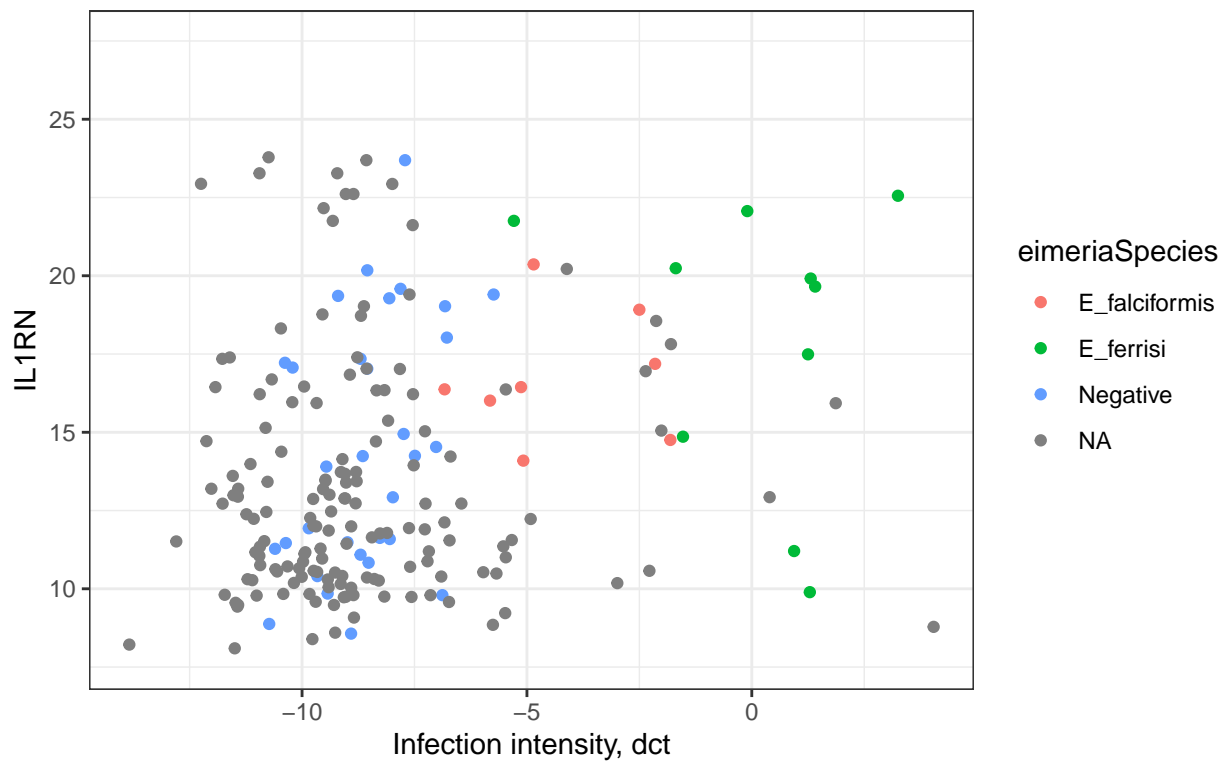
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IL1RN, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
       samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

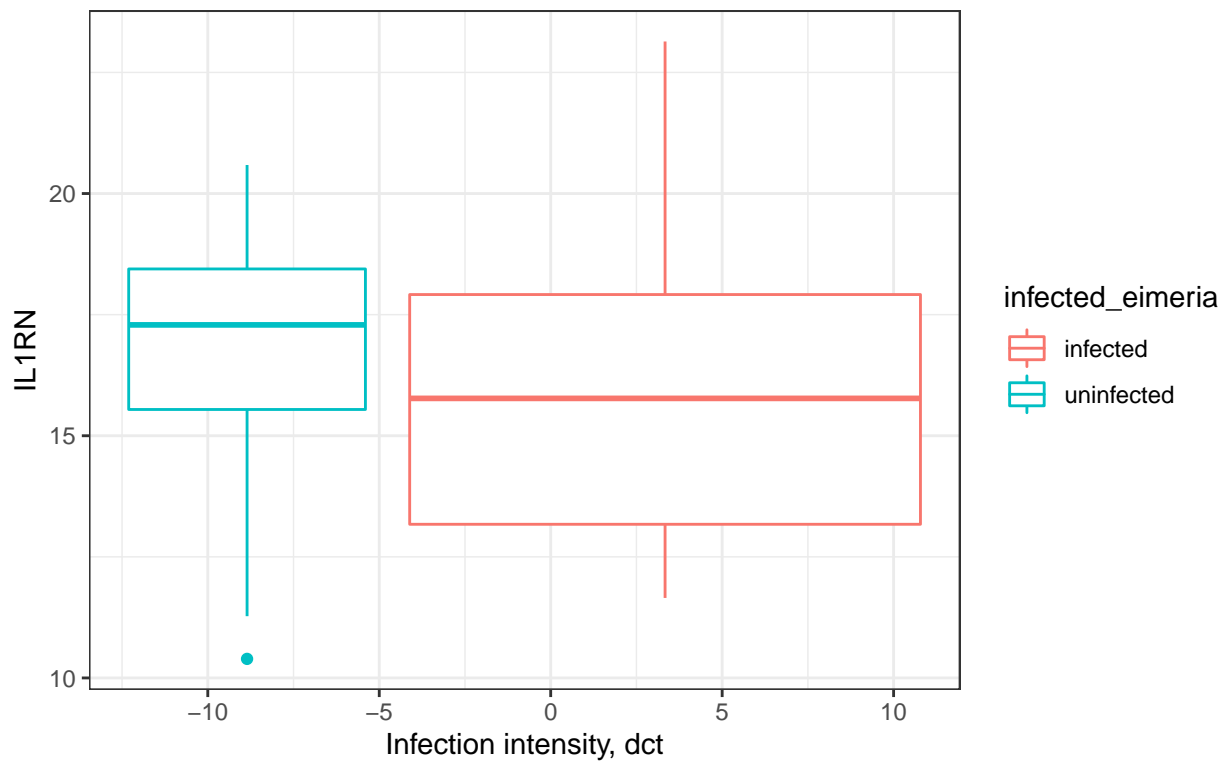
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = IL1RN, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

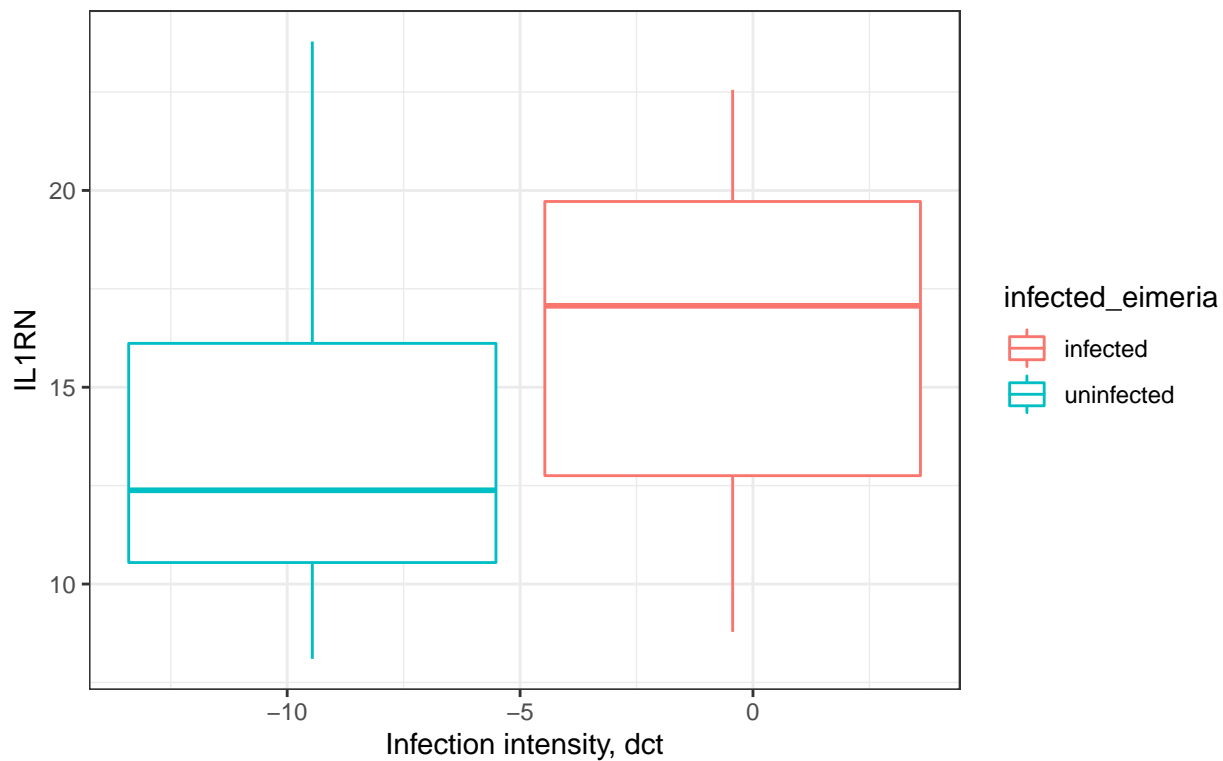
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IL1RN,  
                  color = infected_eimeria)) +  
  geom_boxplot() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity in field  
samples") +  
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples



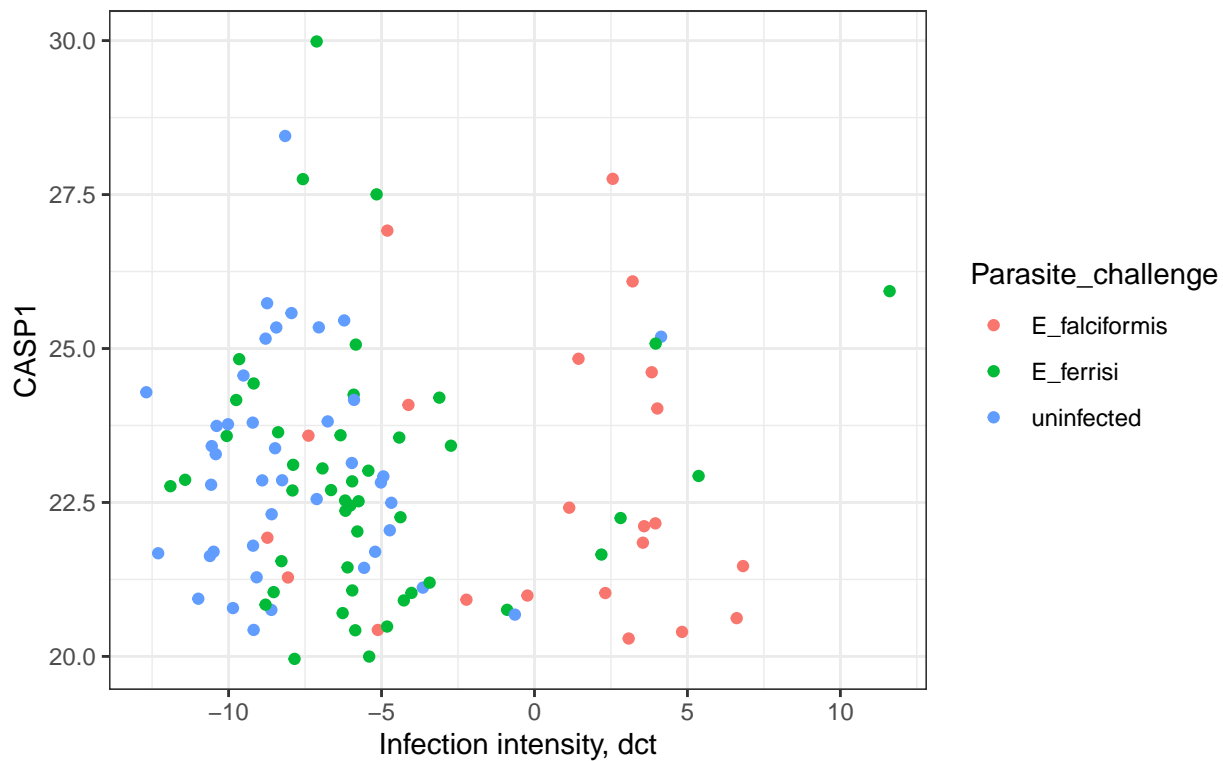
## 7. CASP1

```
ggplot(lab, aes(x = delta, y = CASP1, color = Parasite_challenge)) +  
  geom_jitter() +  
  geom_abline() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity  
in lab infections") +  
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).



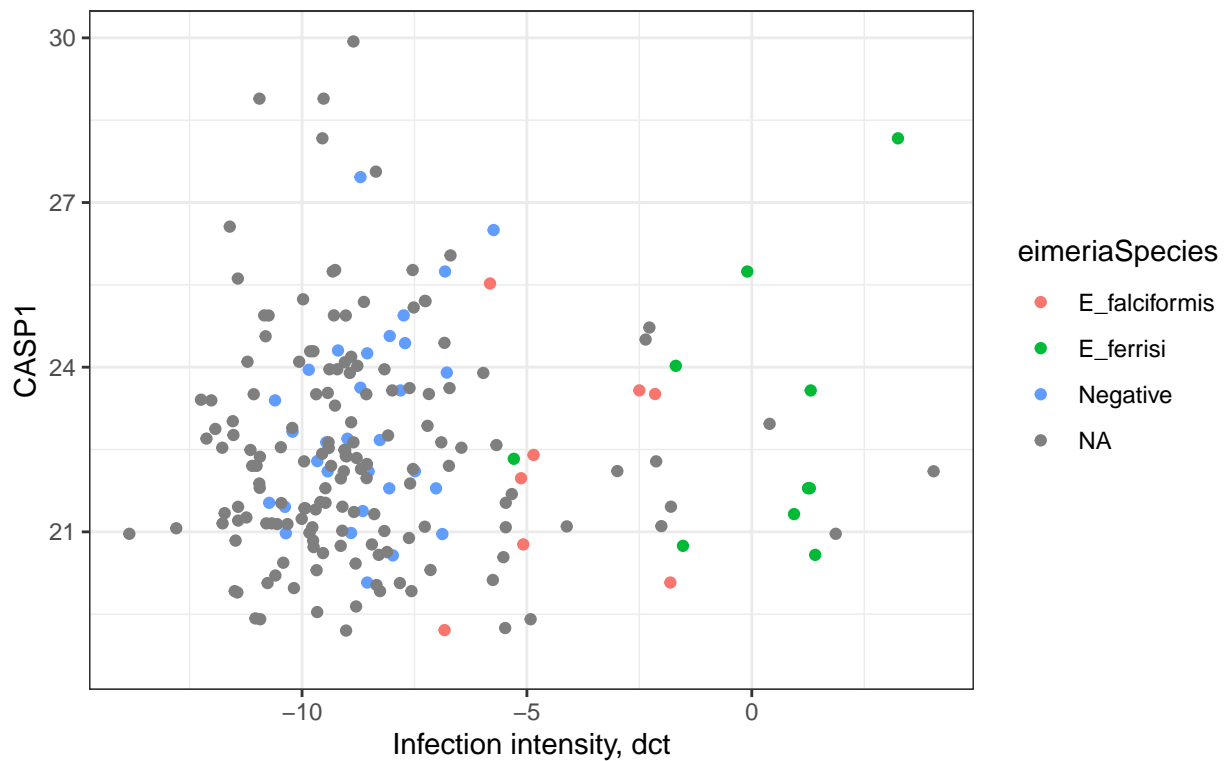
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = CASP1, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

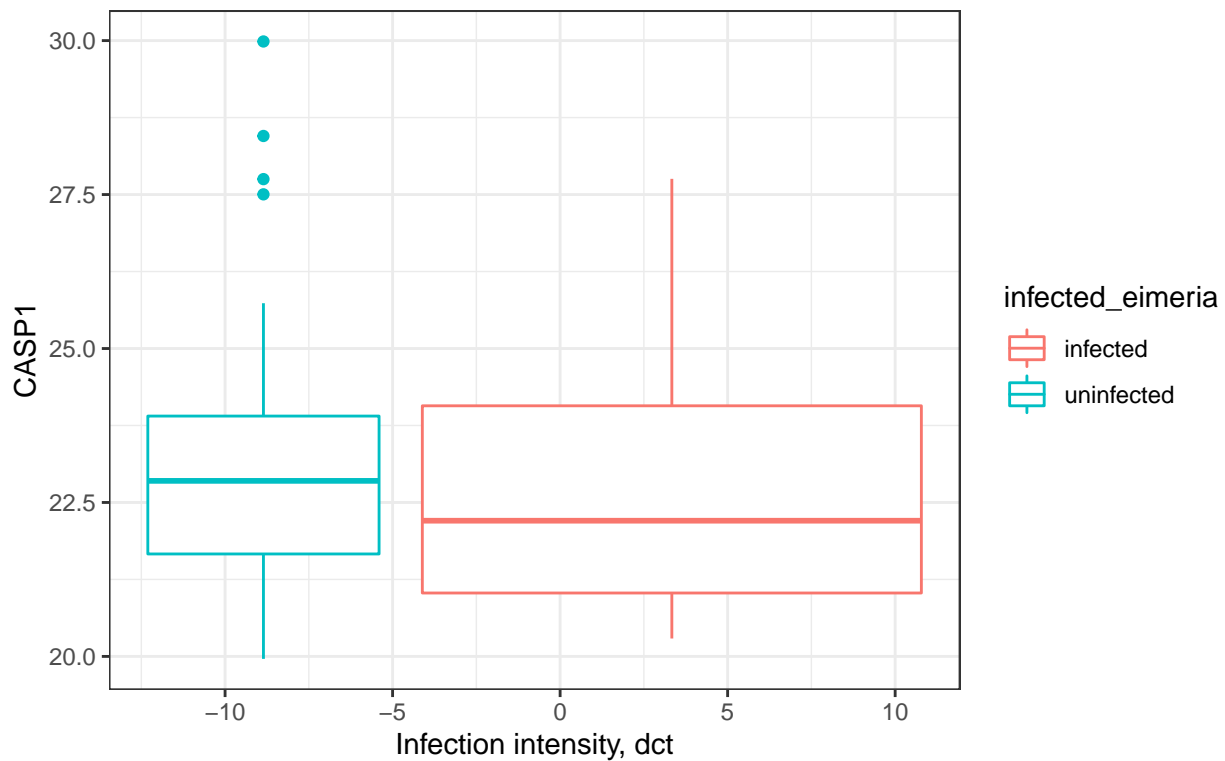
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = CASP1, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

```
## Warning: Removed 6 rows containing missing values (stat_boxplot).
```

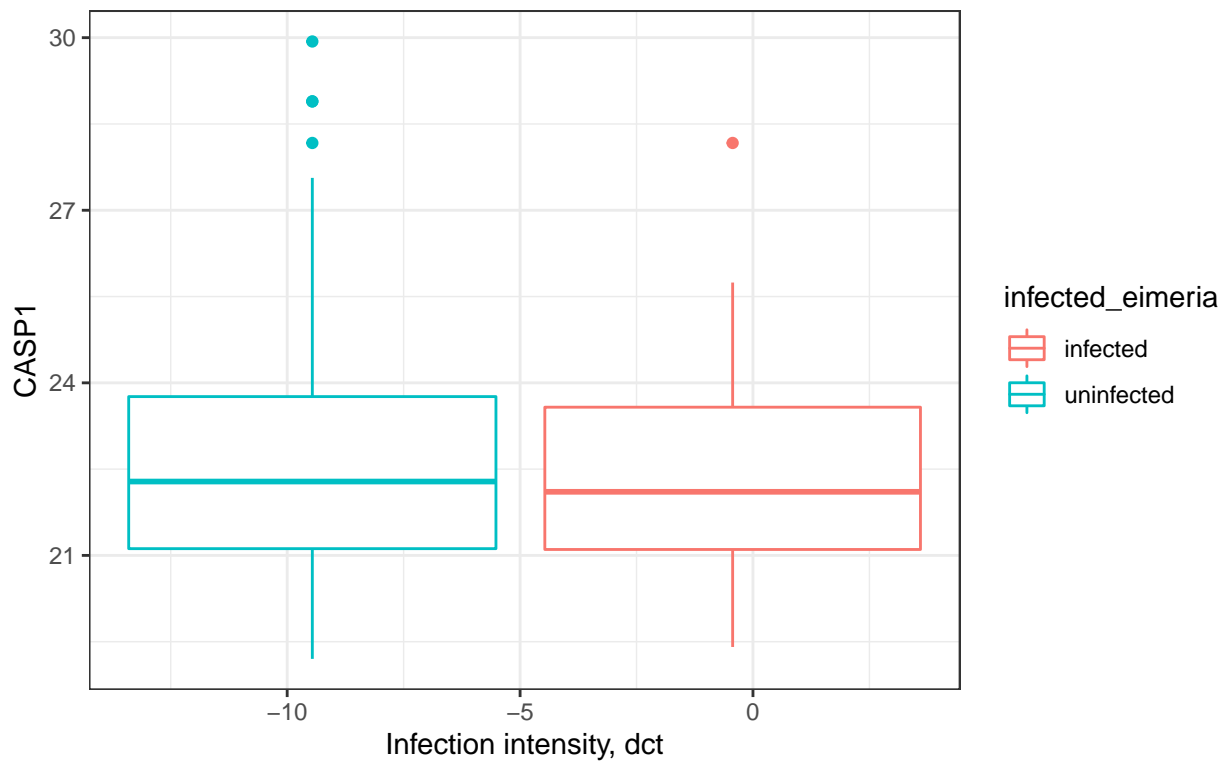
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = CASP1,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples

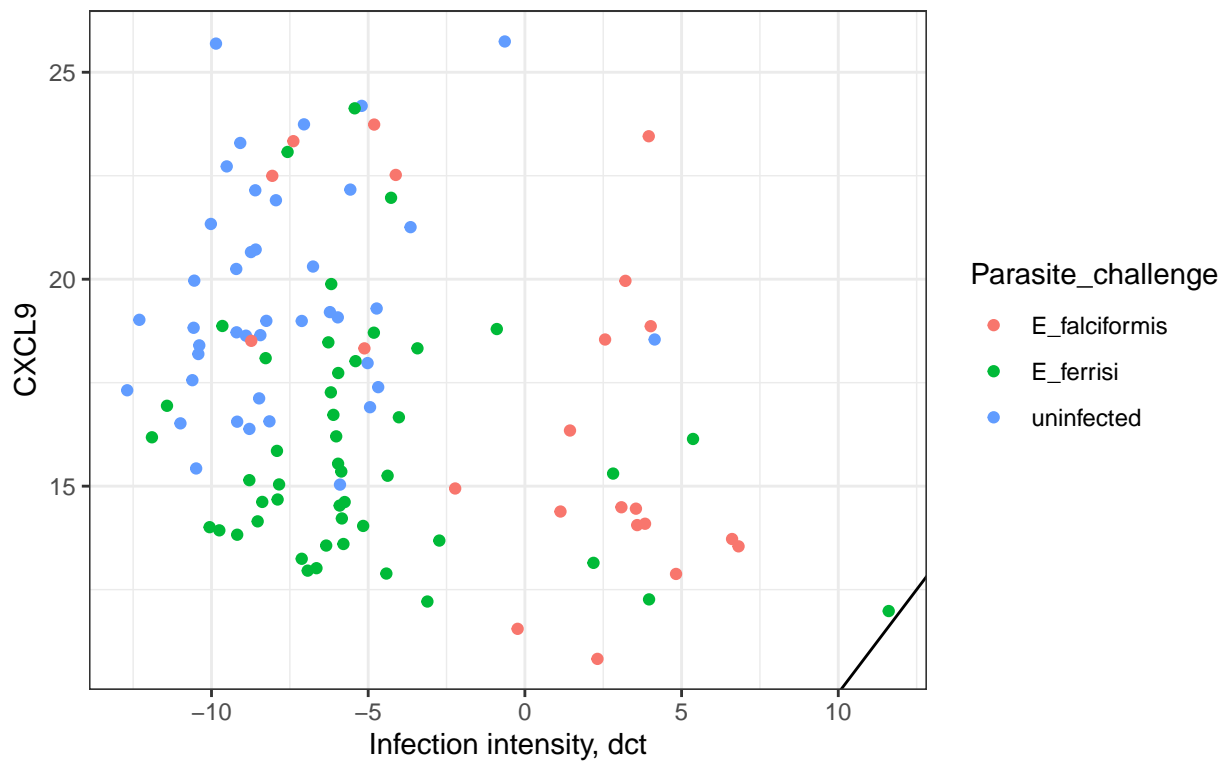


## 8. CXCL9

```
ggplot(lab, aes(x = delta, y = CXCL9, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).

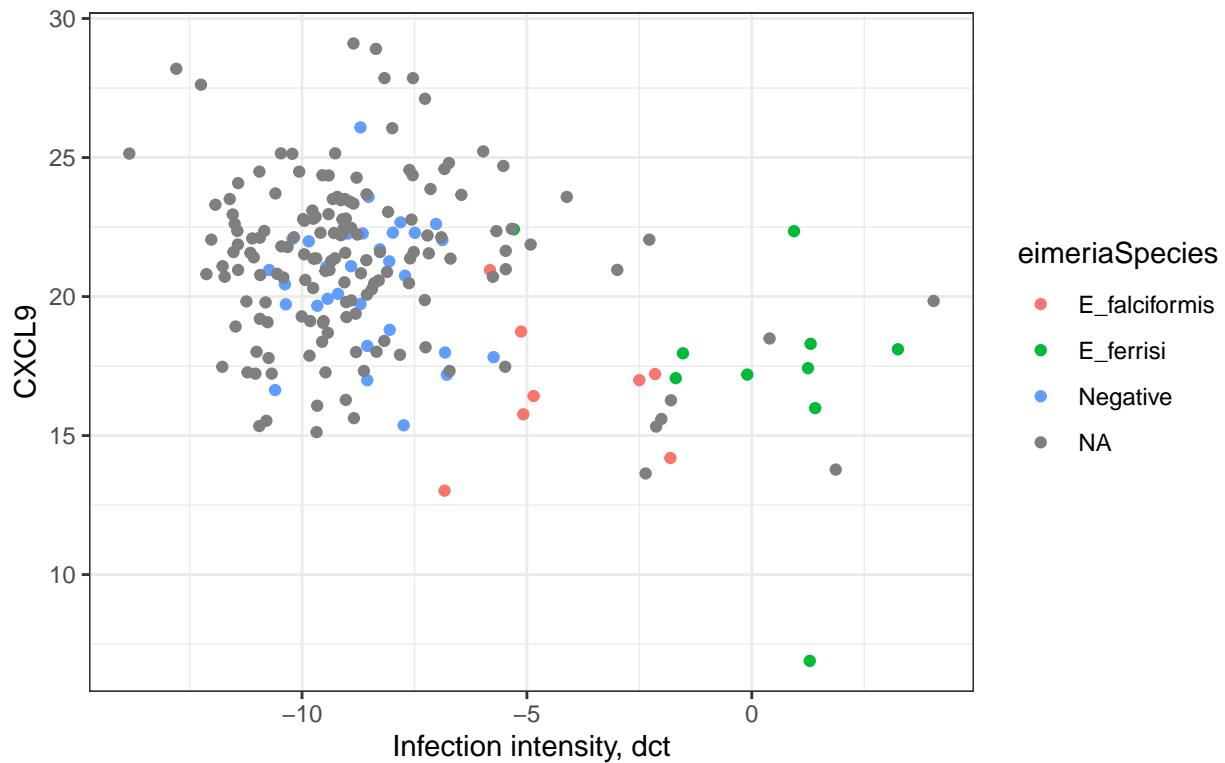
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = CXCL9, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
       samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

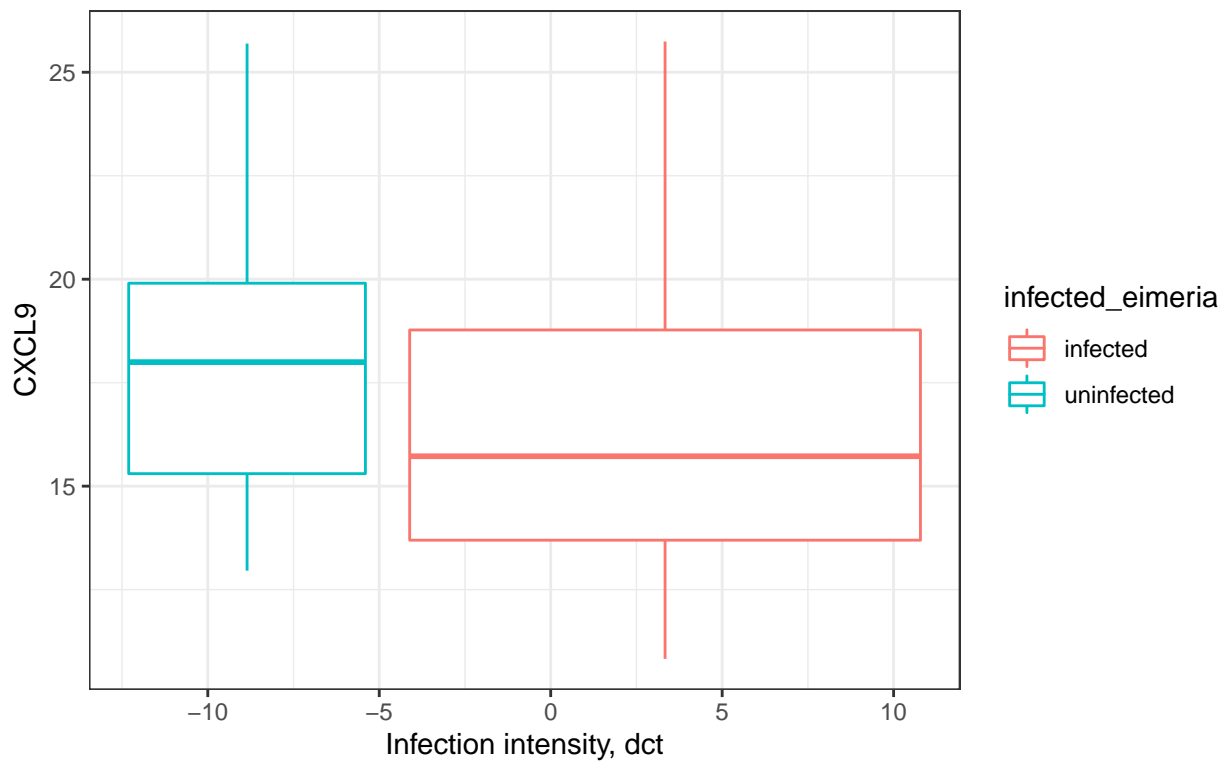
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = CXCL9, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

```
## Warning: Removed 6 rows containing missing values (stat_boxplot).
```

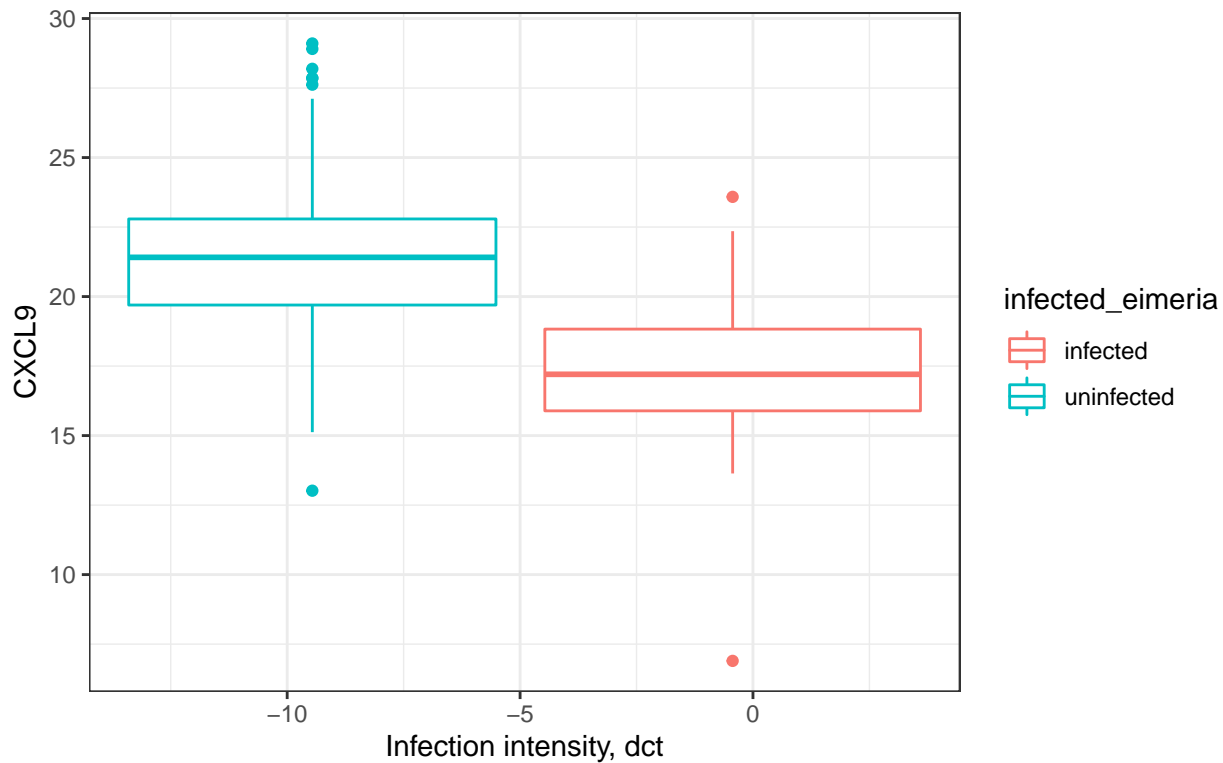
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = CXCL9,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples



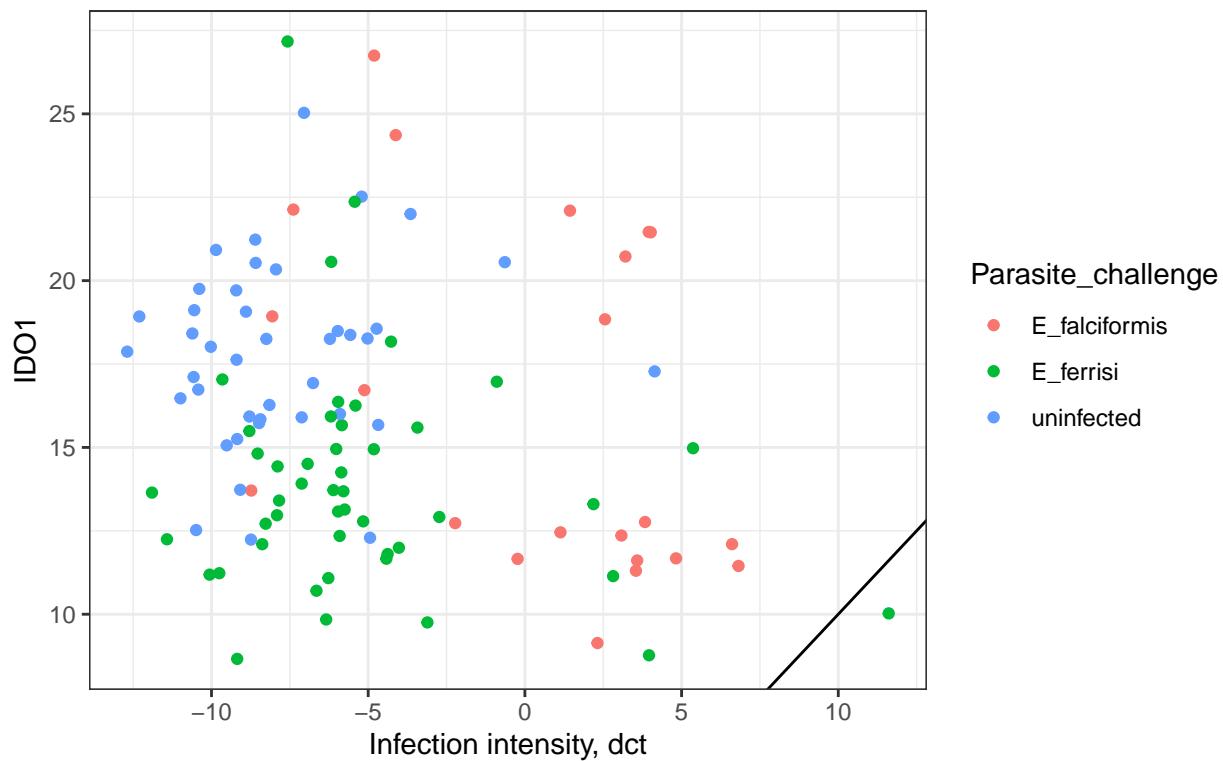
### 9. IDO1

```
ggplot(lab, aes(x = delta, y = IDO1, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).



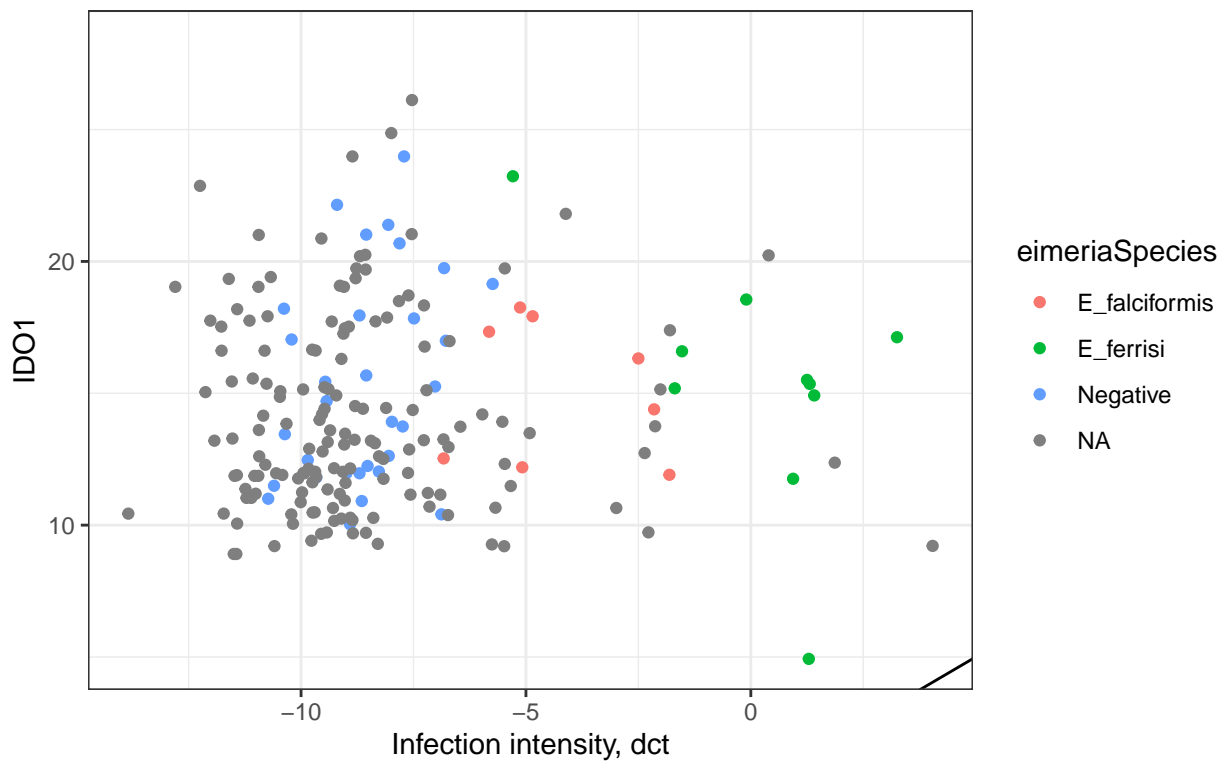
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IDO1, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
       samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

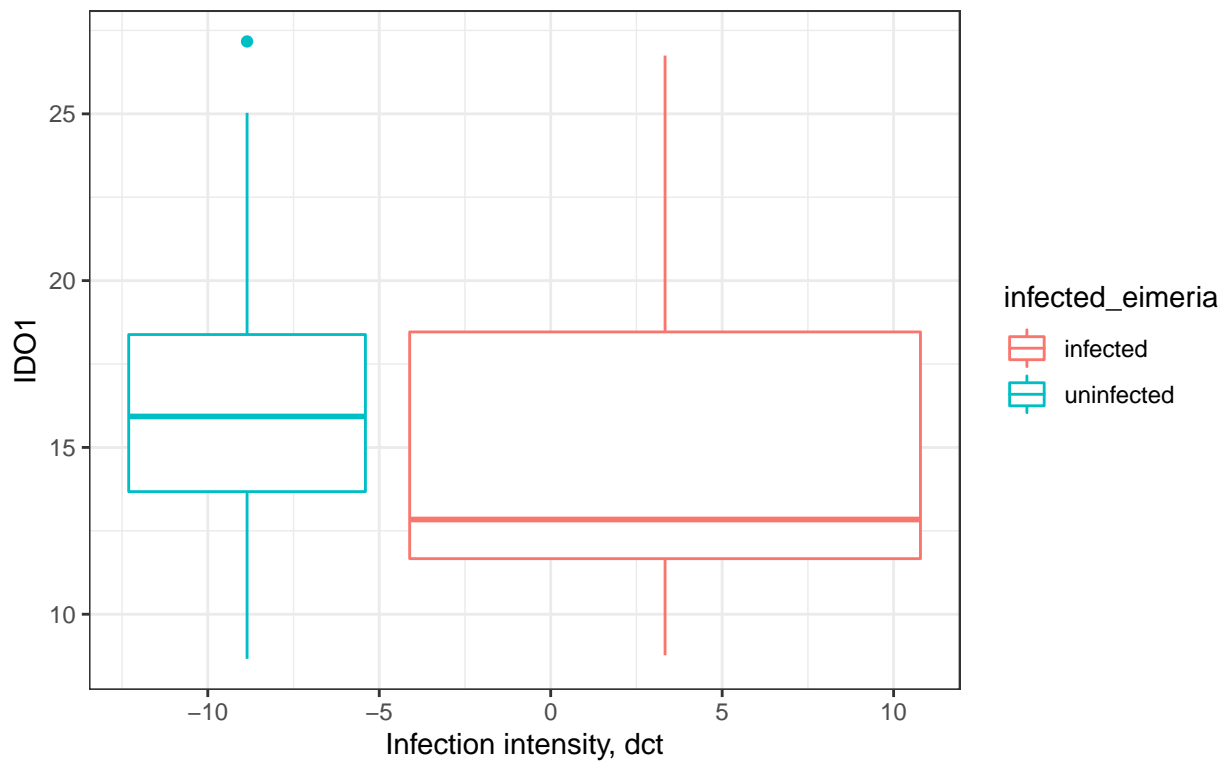
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = IDO1, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

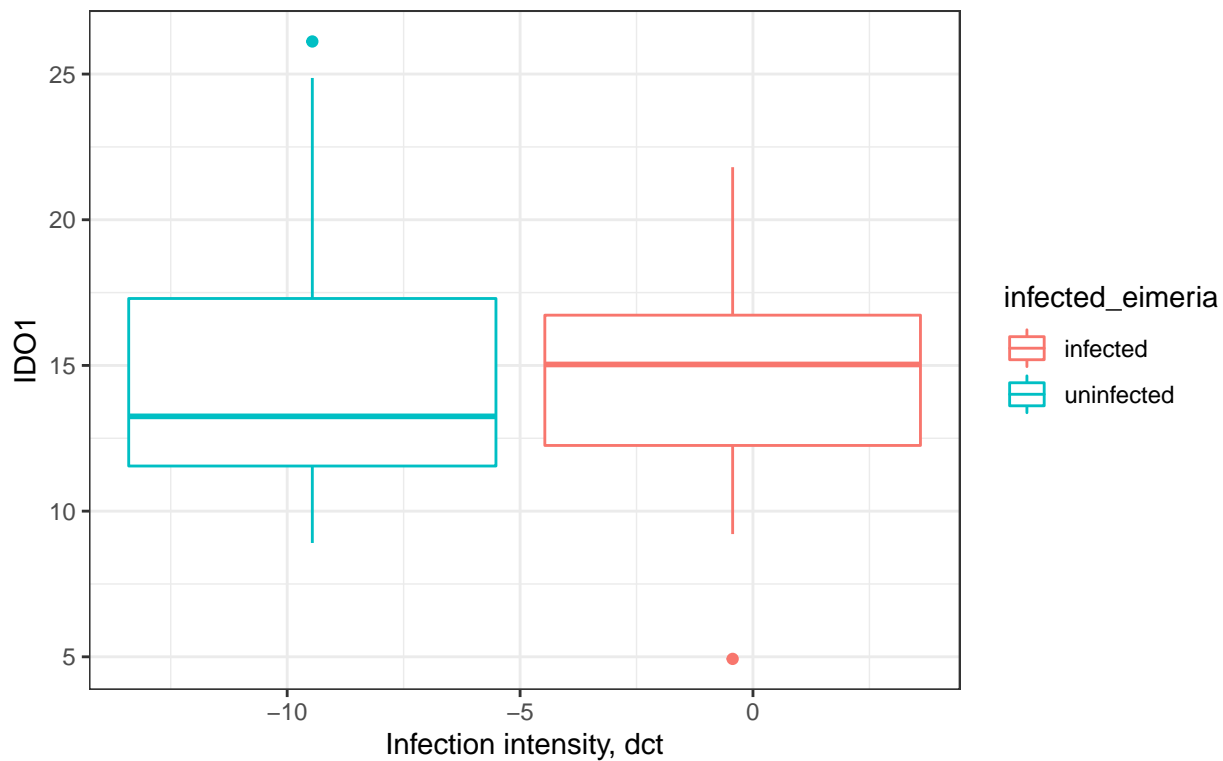
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IDO1,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples

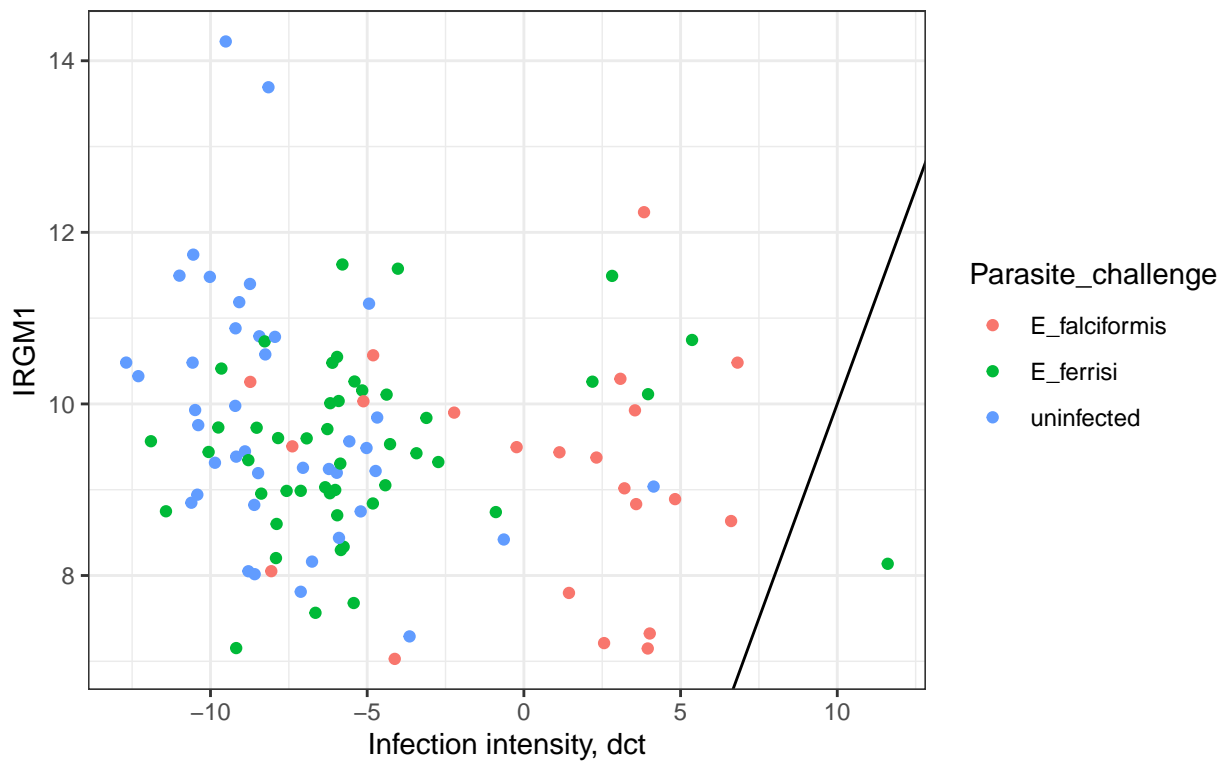


### 10. IRGM1

```
ggplot(lab, aes(x = delta, y = IRGM1, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).

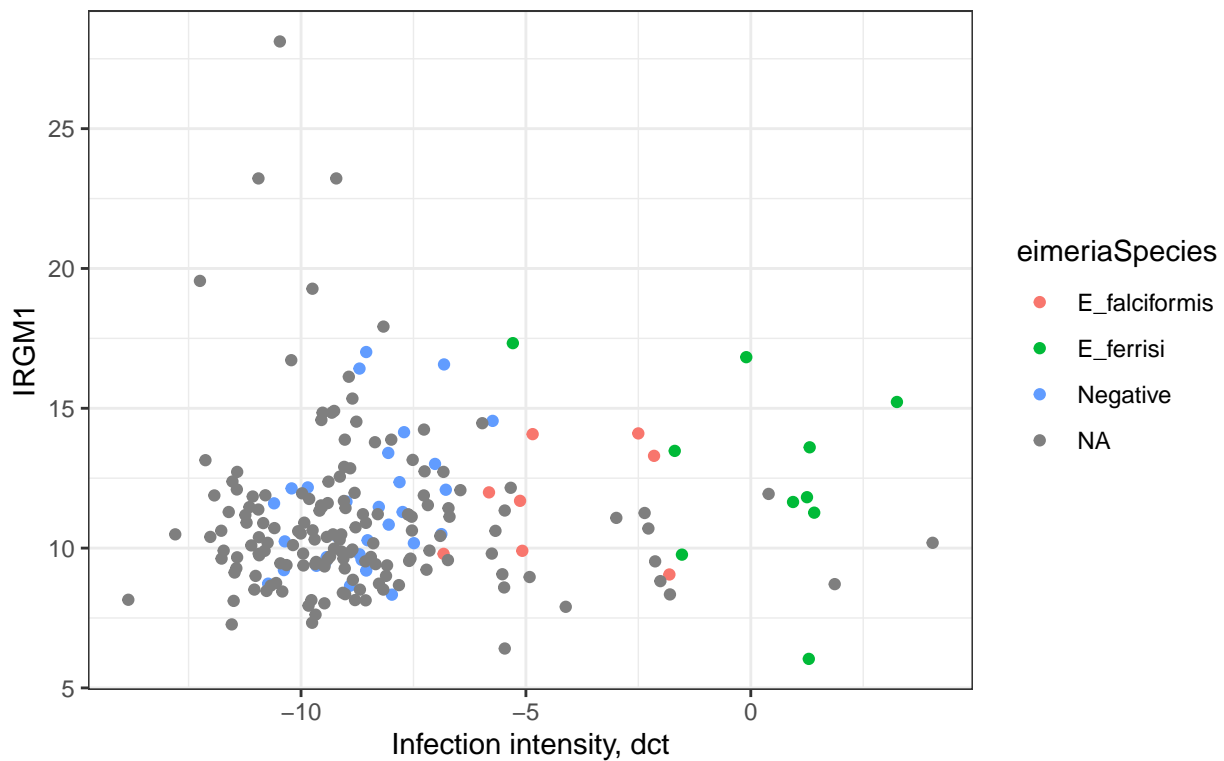
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IRGM1, color = eimeriaSpecies)) +  
  geom_jitter() +  
  geom_abline() +  
  labs(x = "Infection intensity, dct",  
        title = "Immune gene expression against infection intensity in field  
        samples") +  
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

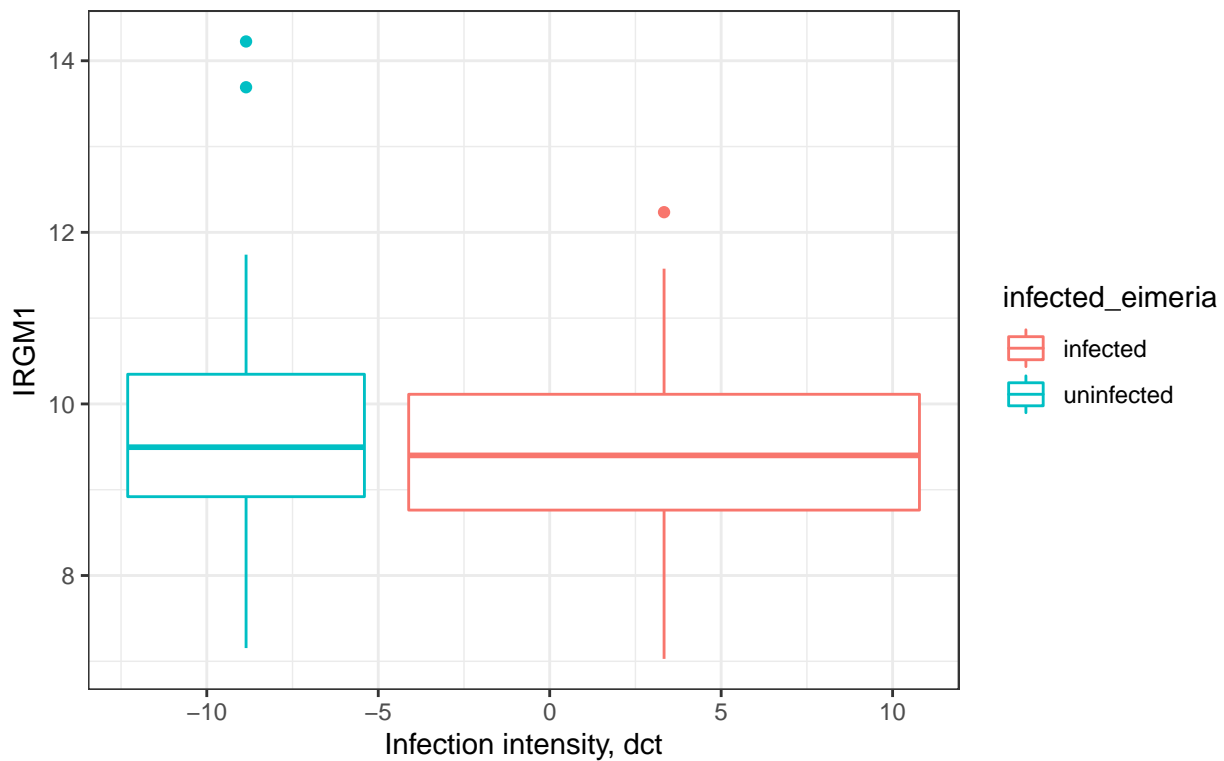
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = IRGM1, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

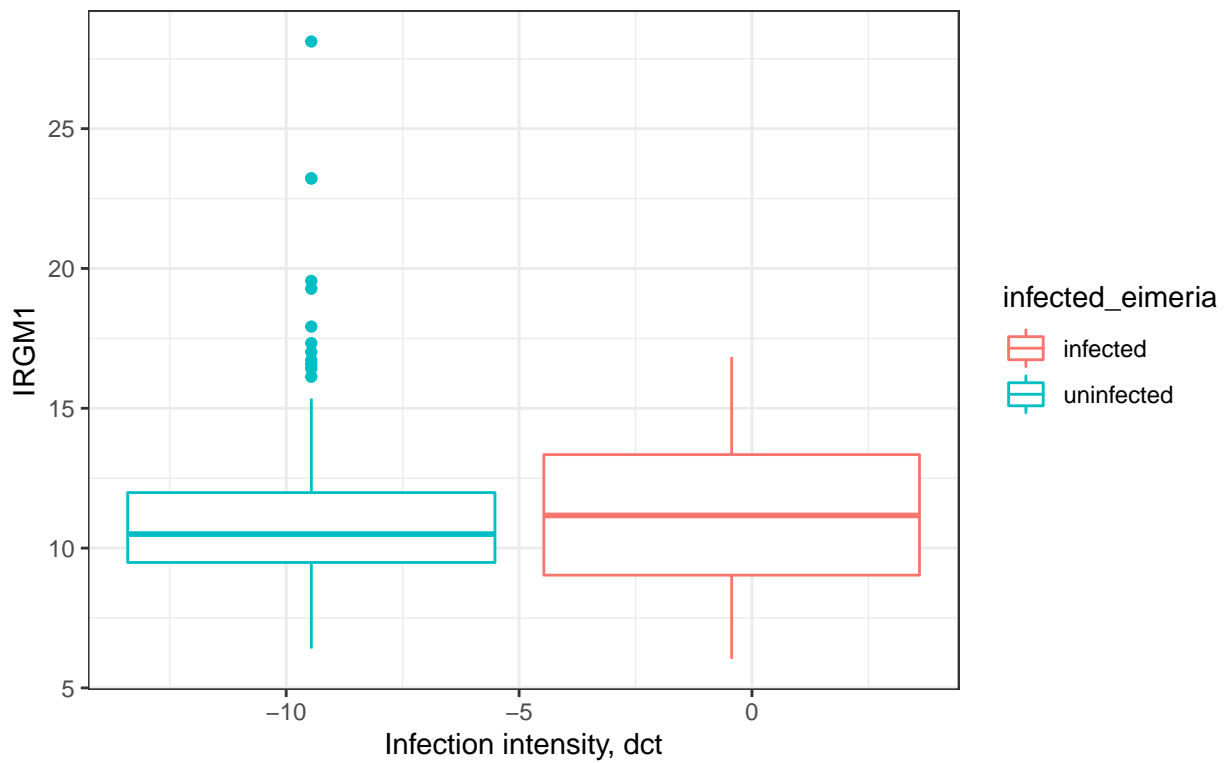
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = IRGM1,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
               samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples



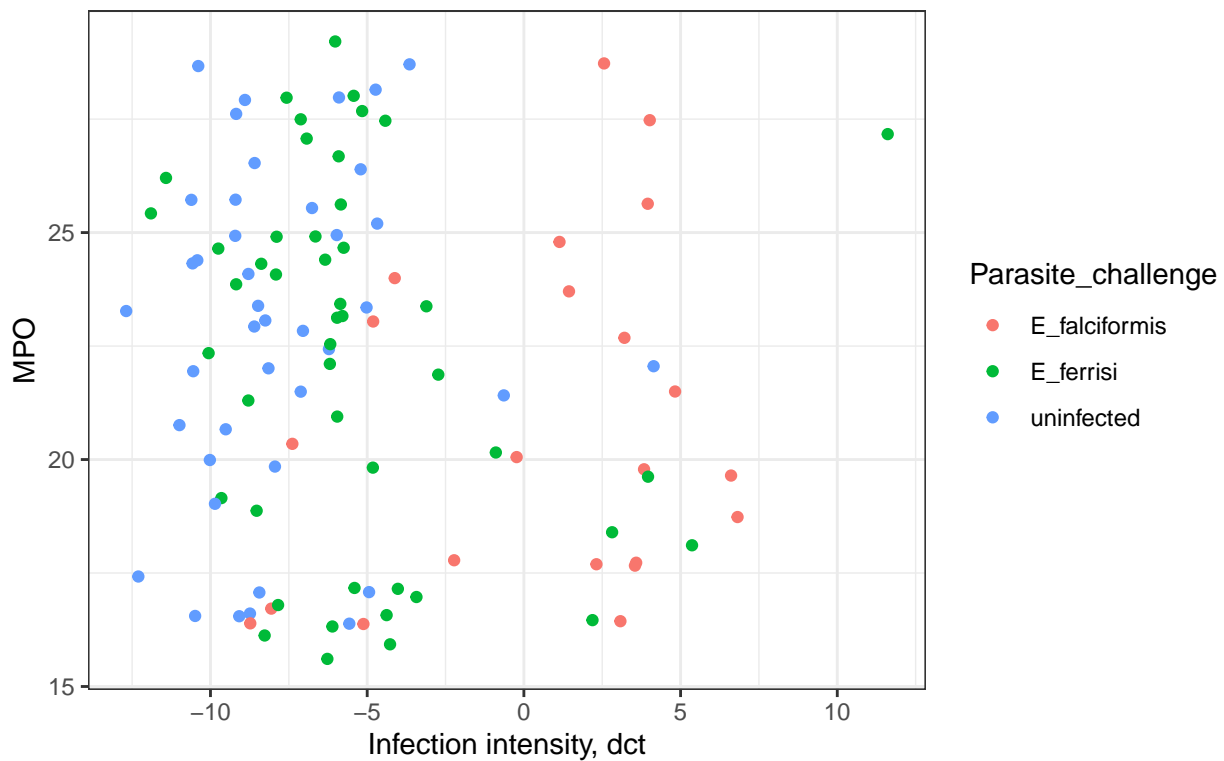
### 11. MPO

```
ggplot(lab, aes(x = delta, y = MPO, color = Parasite_challenge)) +  
  geom_jitter() +  
  geom_abline() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity  
in lab infections") +  
  theme_bw()
```

```
## Warning: Removed 6 rows containing missing values (geom_point).
```



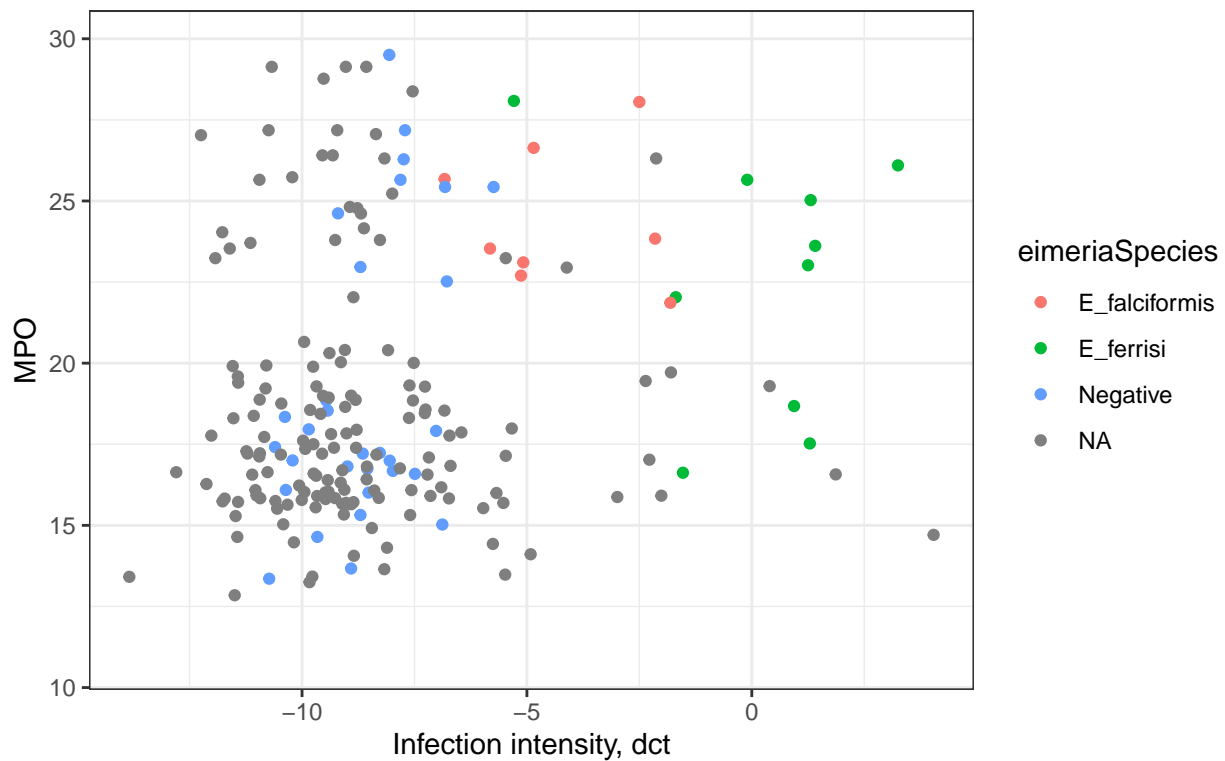
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = MPO, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

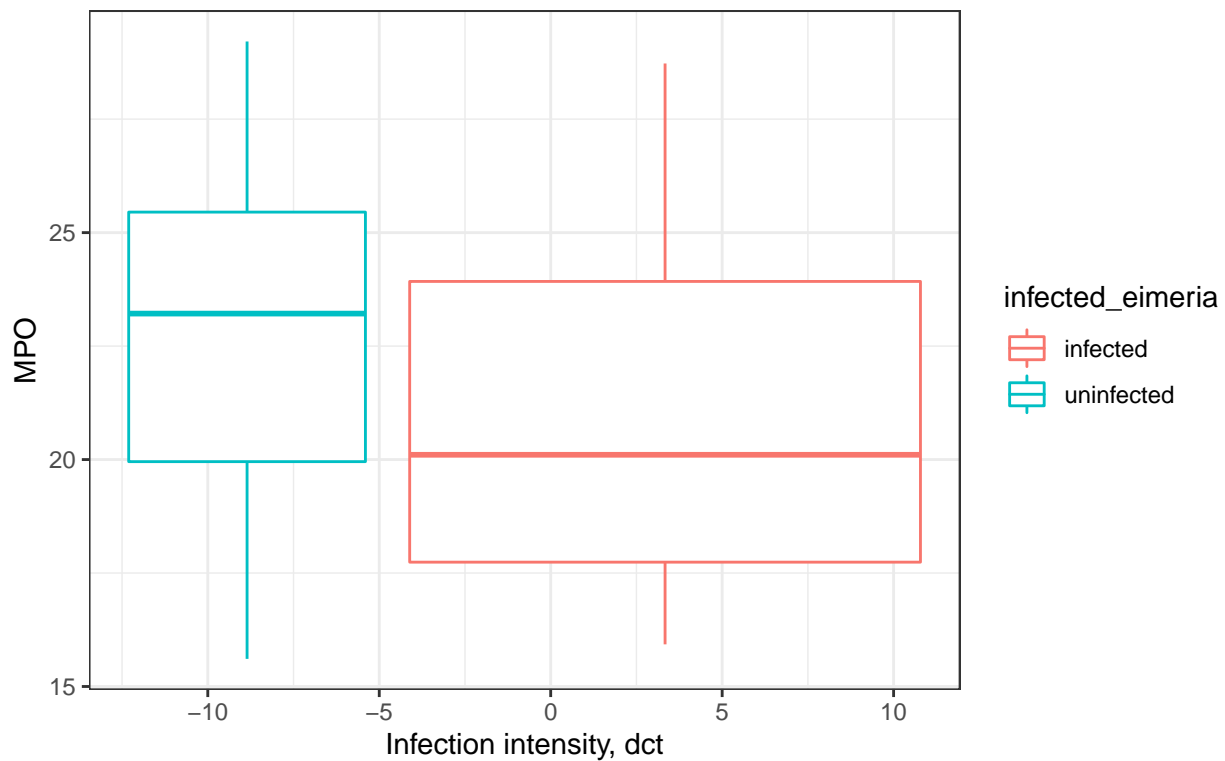
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = MPO, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

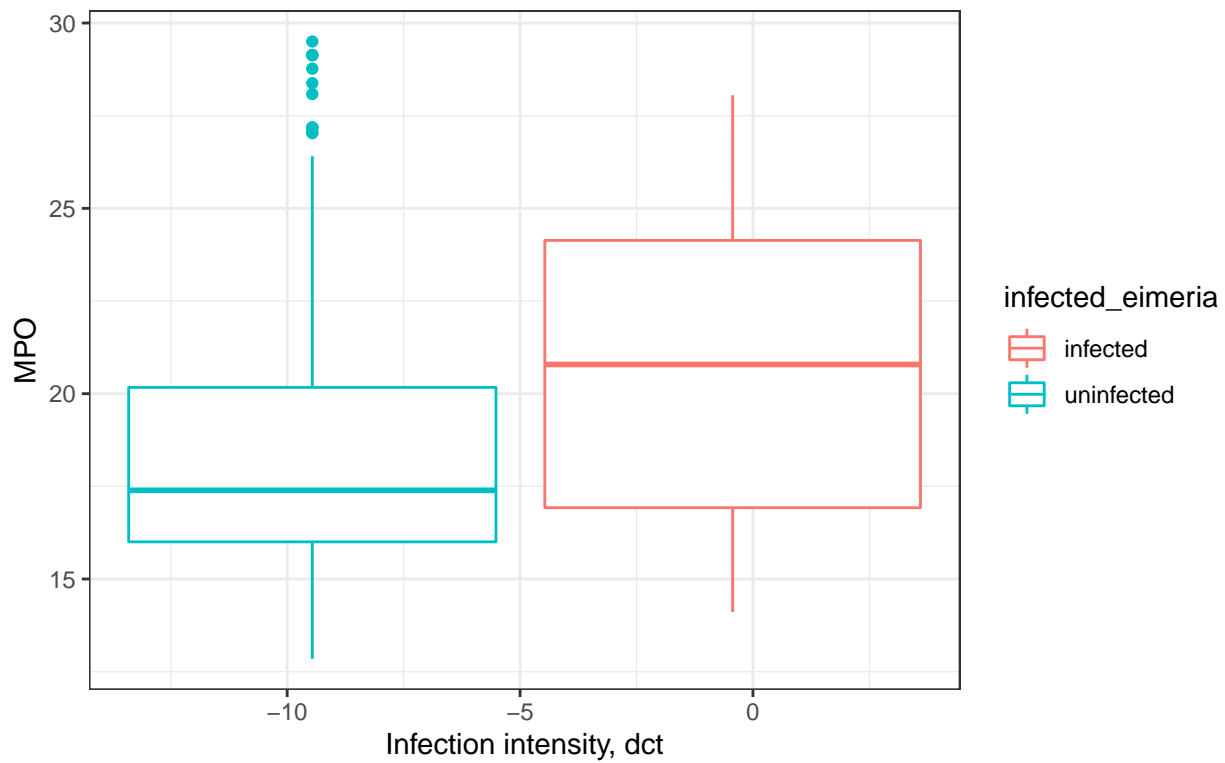
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = MPO,  
                  color = infected_eimeria)) +  
  geom_boxplot() +  
  labs(x = "Infection intensity, dct",  
        title = "Immune gene expression against infection intensity in field  
samples") +  
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples

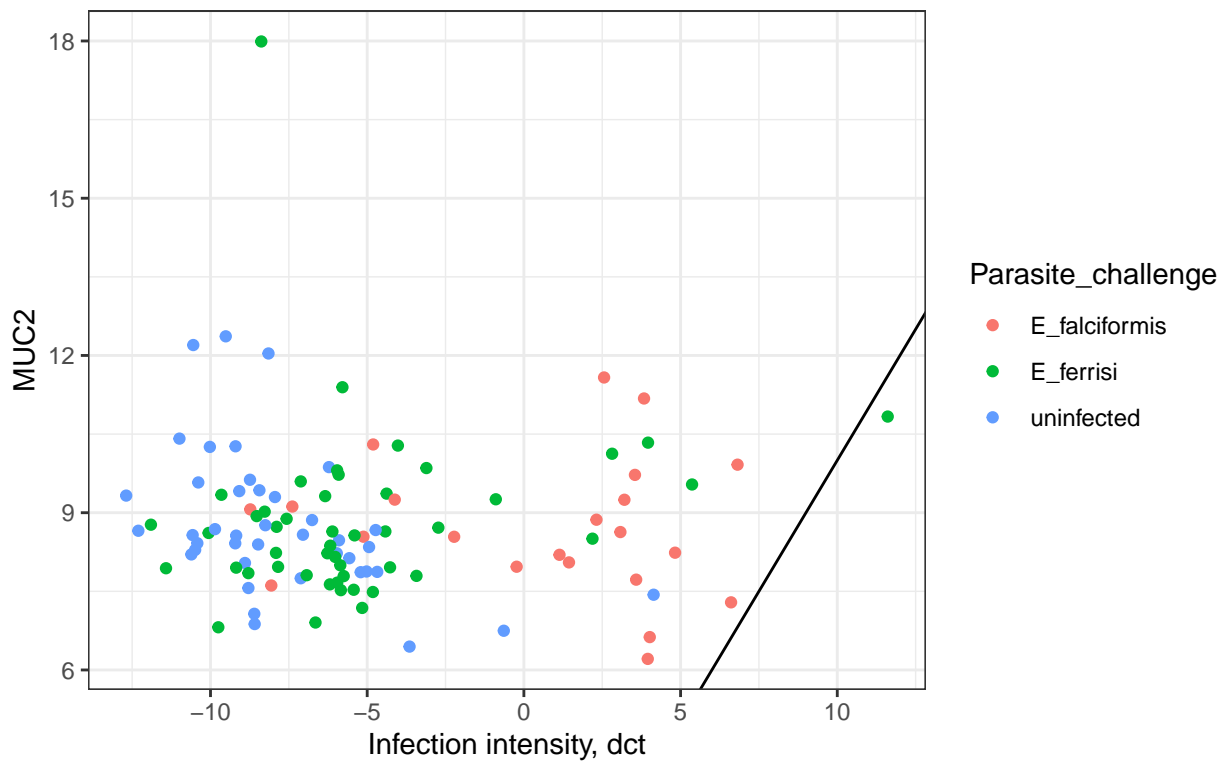


## 12. MUC2

```
ggplot(lab, aes(x = delta, y = MUC2, color = Parasite_challenge)) +  
  geom_jitter() +  
  geom_abline() +  
  labs(x = "Infection intensity, dct",  
        title = "Immune gene expression against infection intensity  
in lab infections") +  
  theme_bw()
```

```
## Warning: Removed 6 rows containing missing values (geom_point).
```

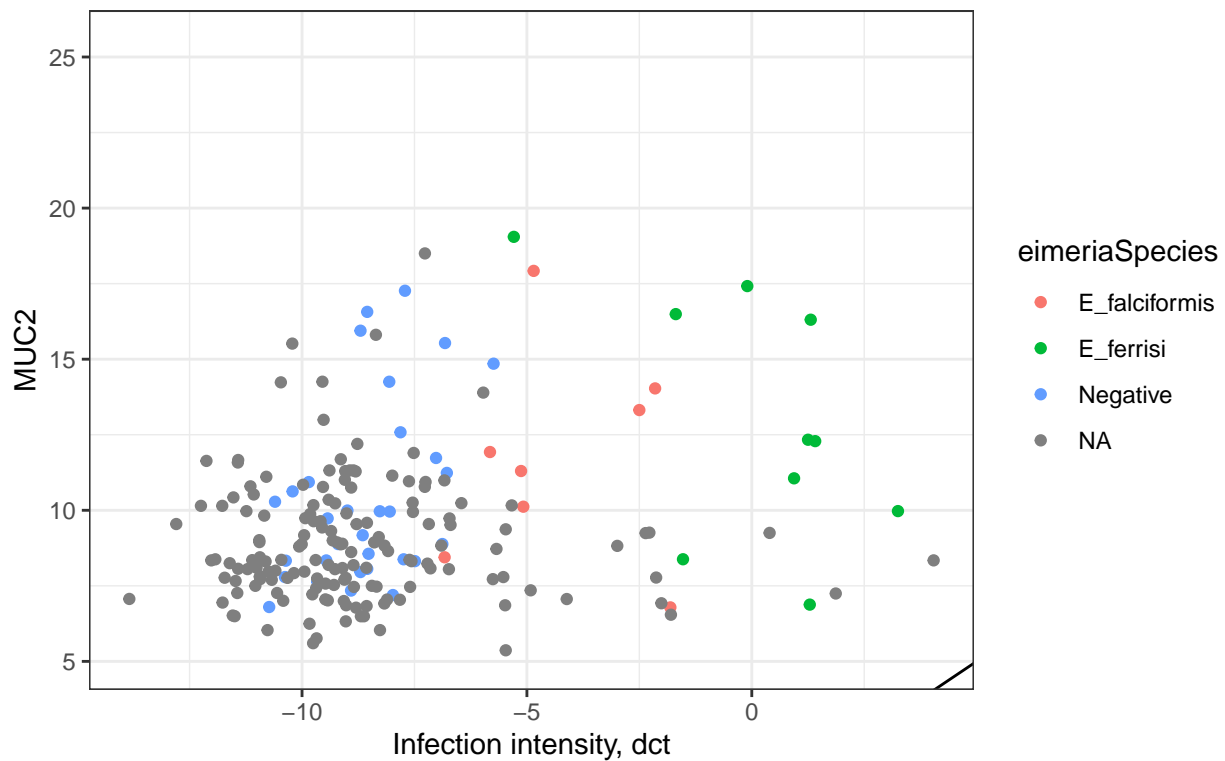
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = MUC2, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
               samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

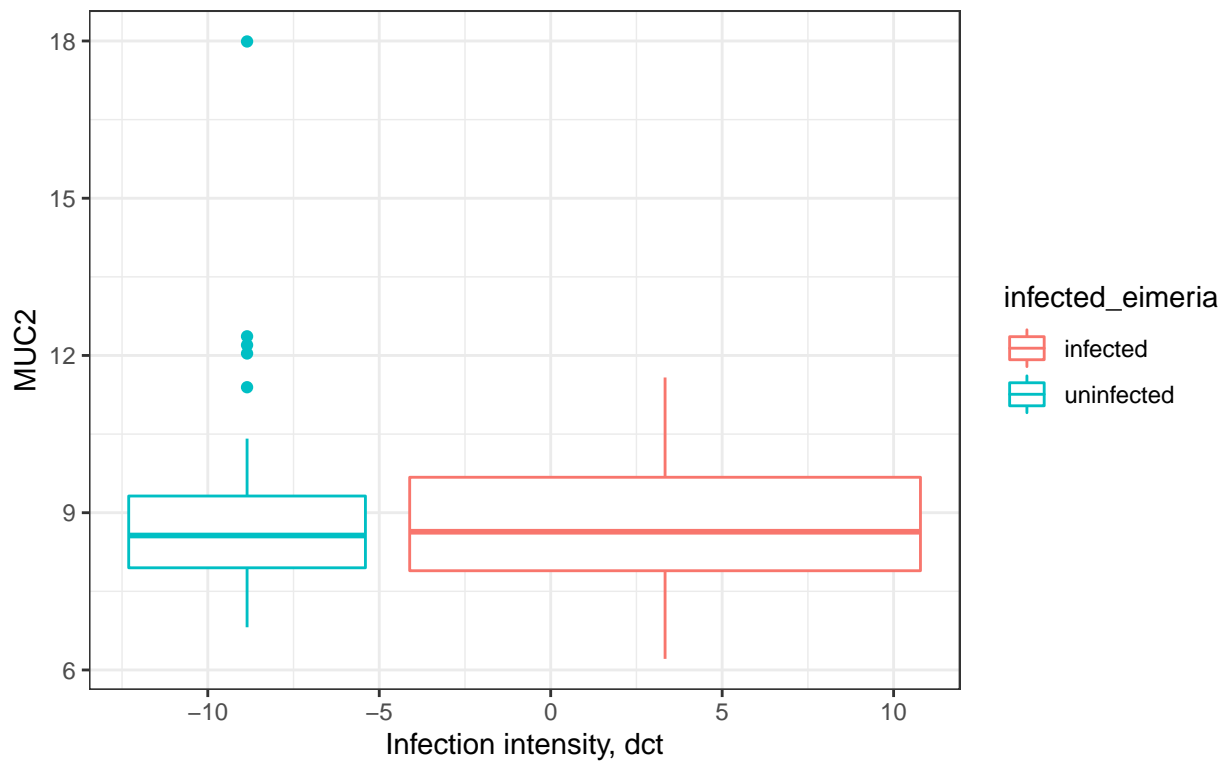
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = MUC2, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

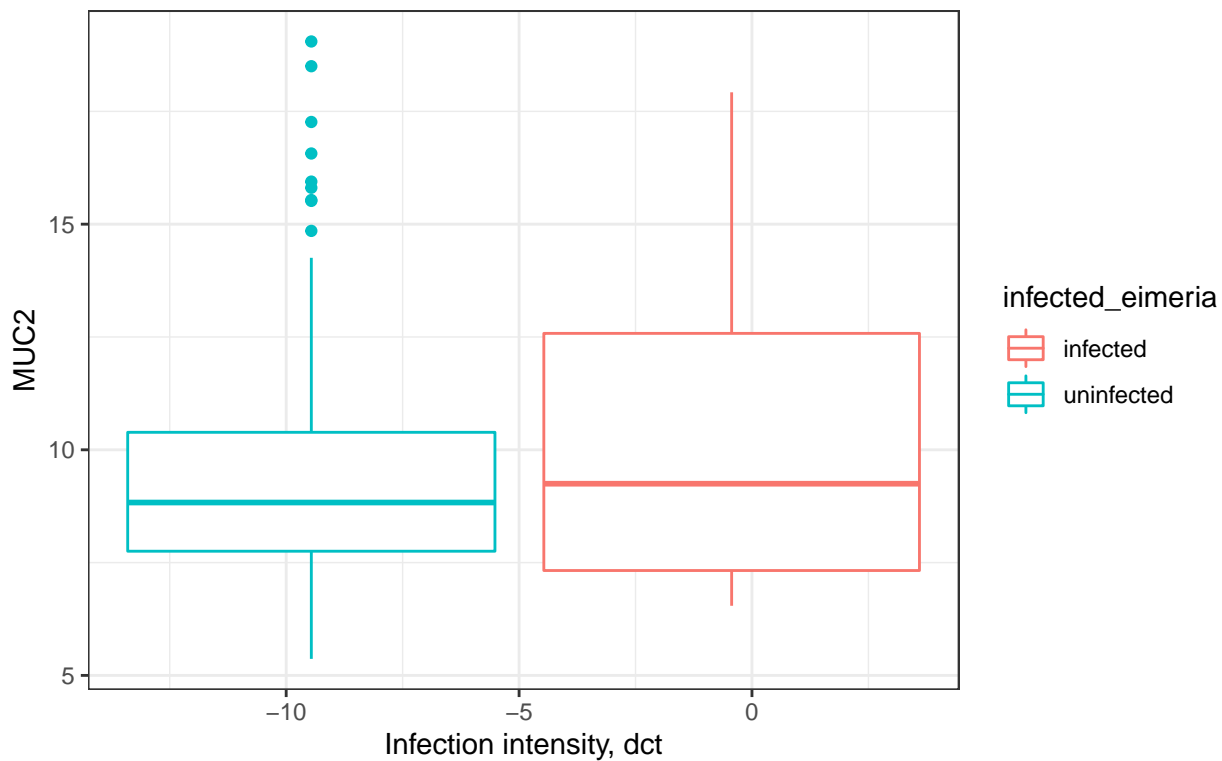
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = MUC2,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples



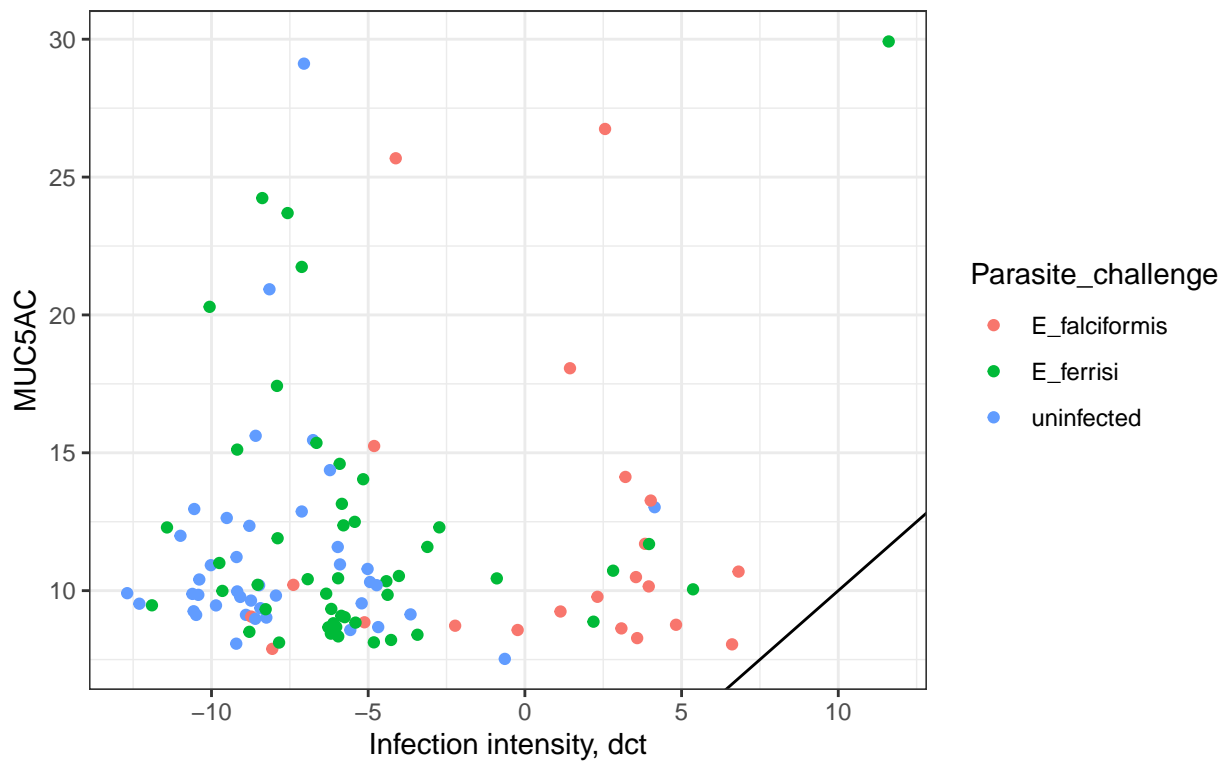
### 13. MUC5AC

```
ggplot(lab, aes(x = delta, y = MUC5AC, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).



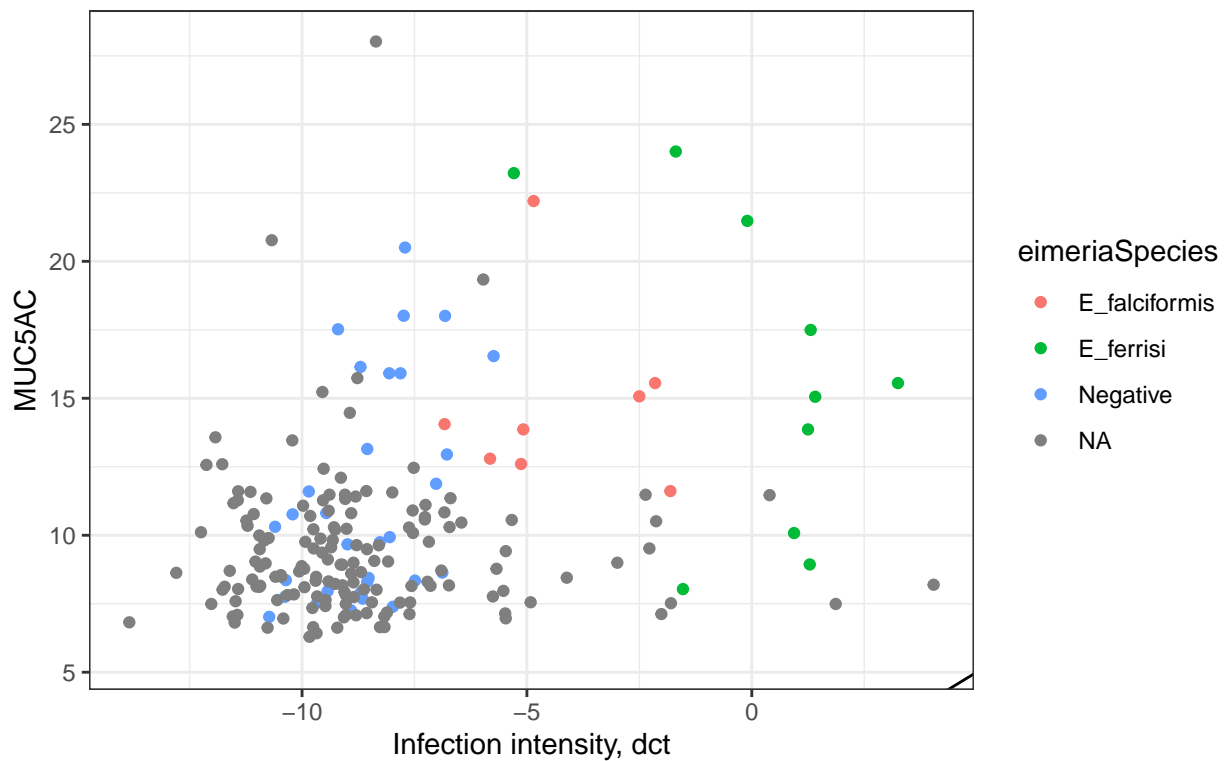
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = MUC5AC, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
               samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

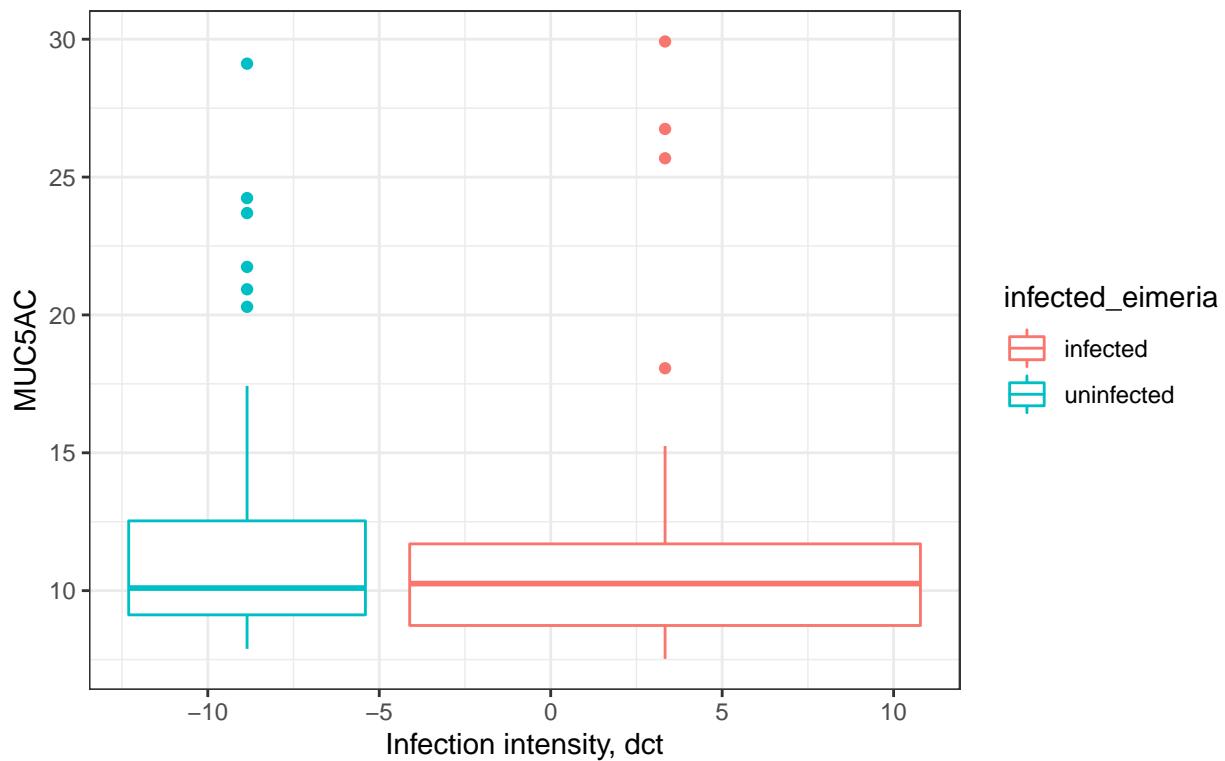
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = MUC5AC, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

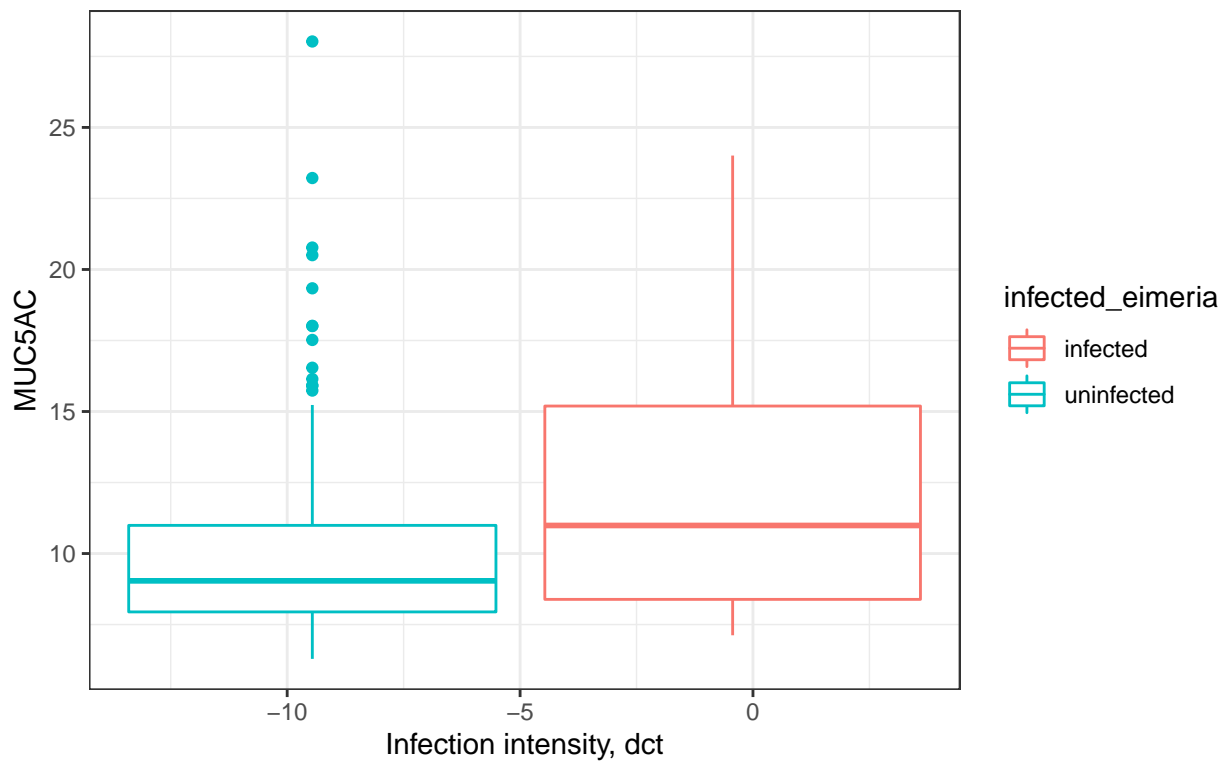
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = MUC5AC,  
                  color = infected_eimeria)) +  
  geom_boxplot() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity in field  
samples") +  
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples

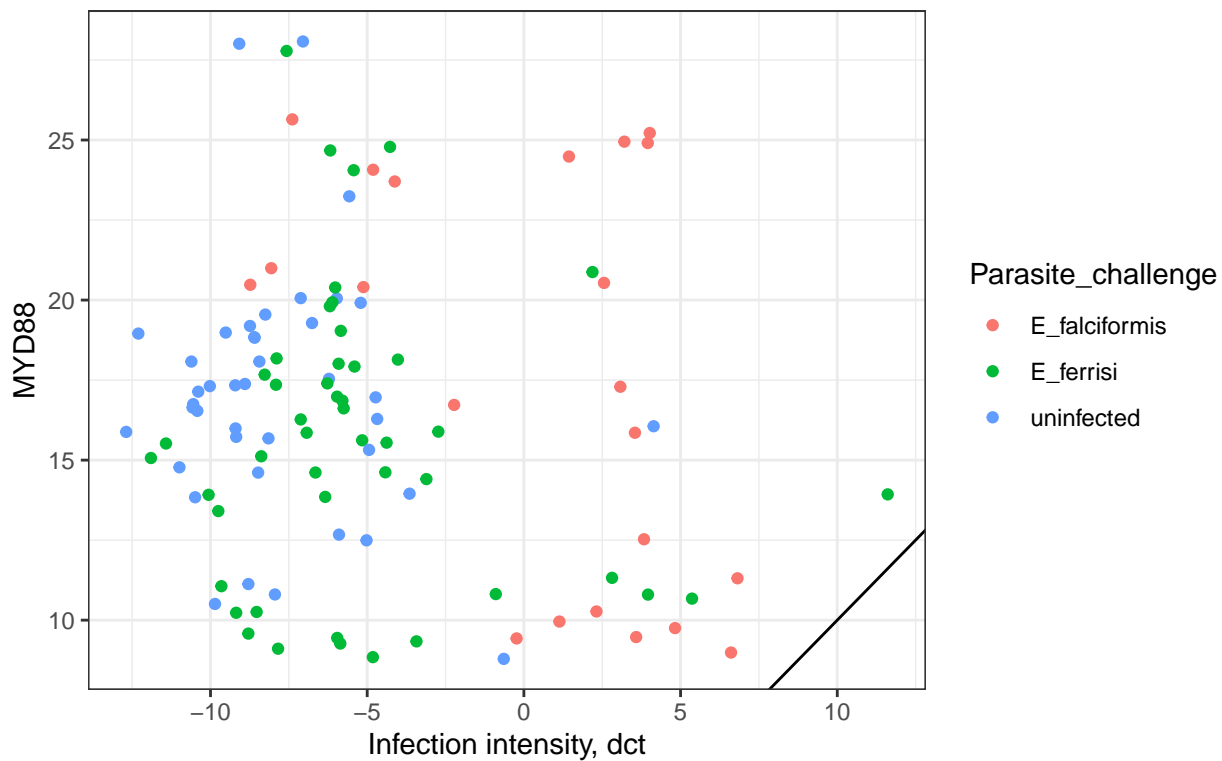


### 14. MYD88

```
ggplot(lab, aes(x = delta, y = MYD88, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).

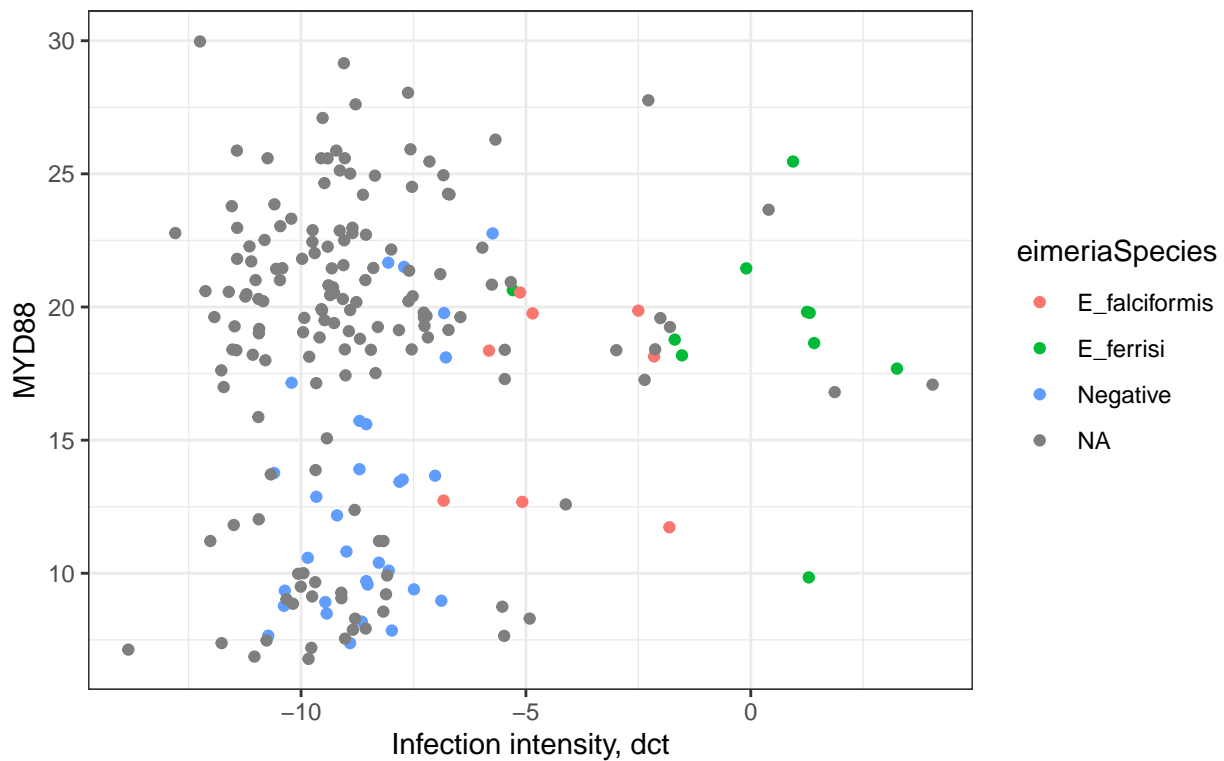
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = MYD88, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

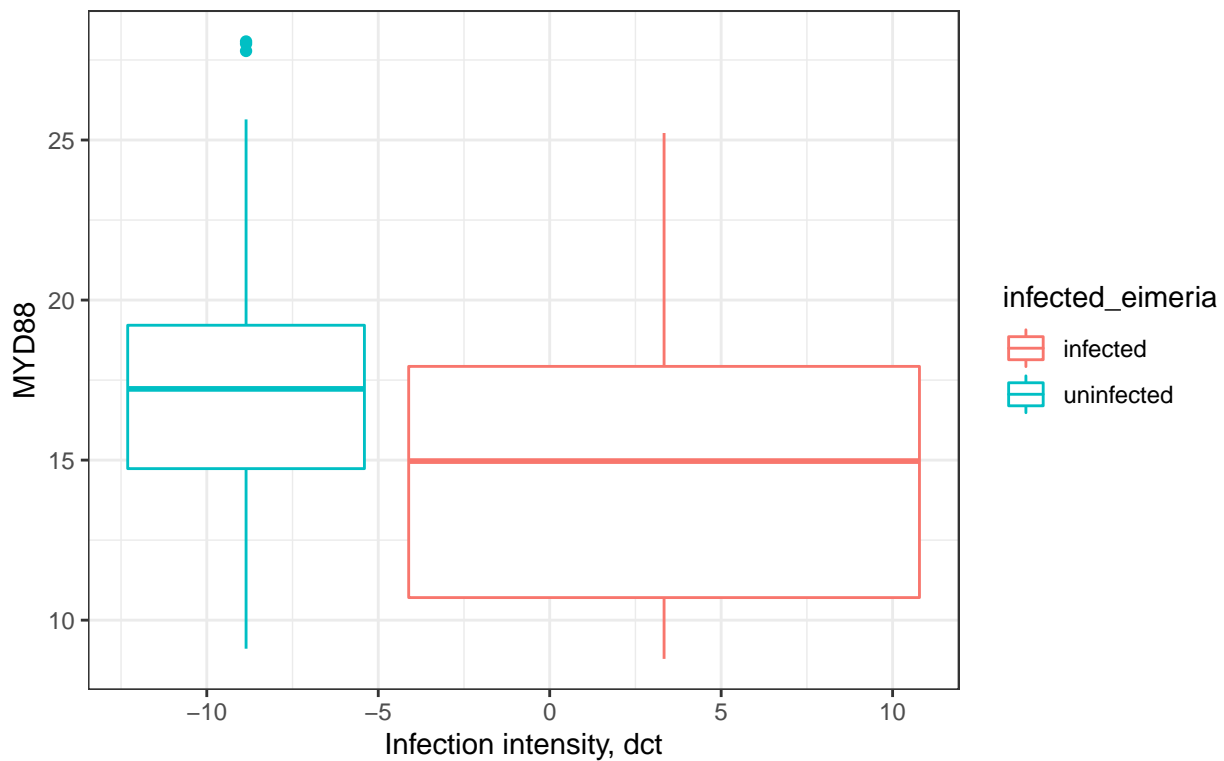
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = MYD88, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

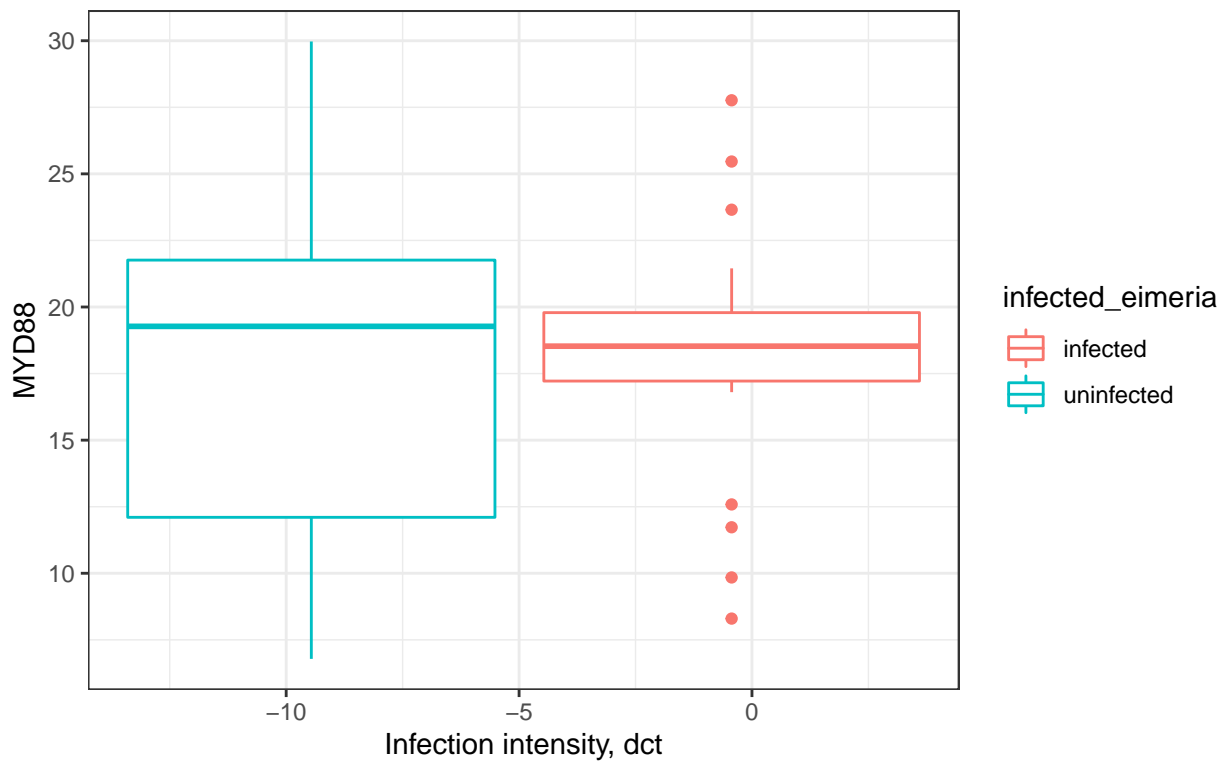
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = MYD88,  
                  color = infected_eimeria)) +  
  geom_boxplot() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity in field  
samples") +  
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples



## 15.

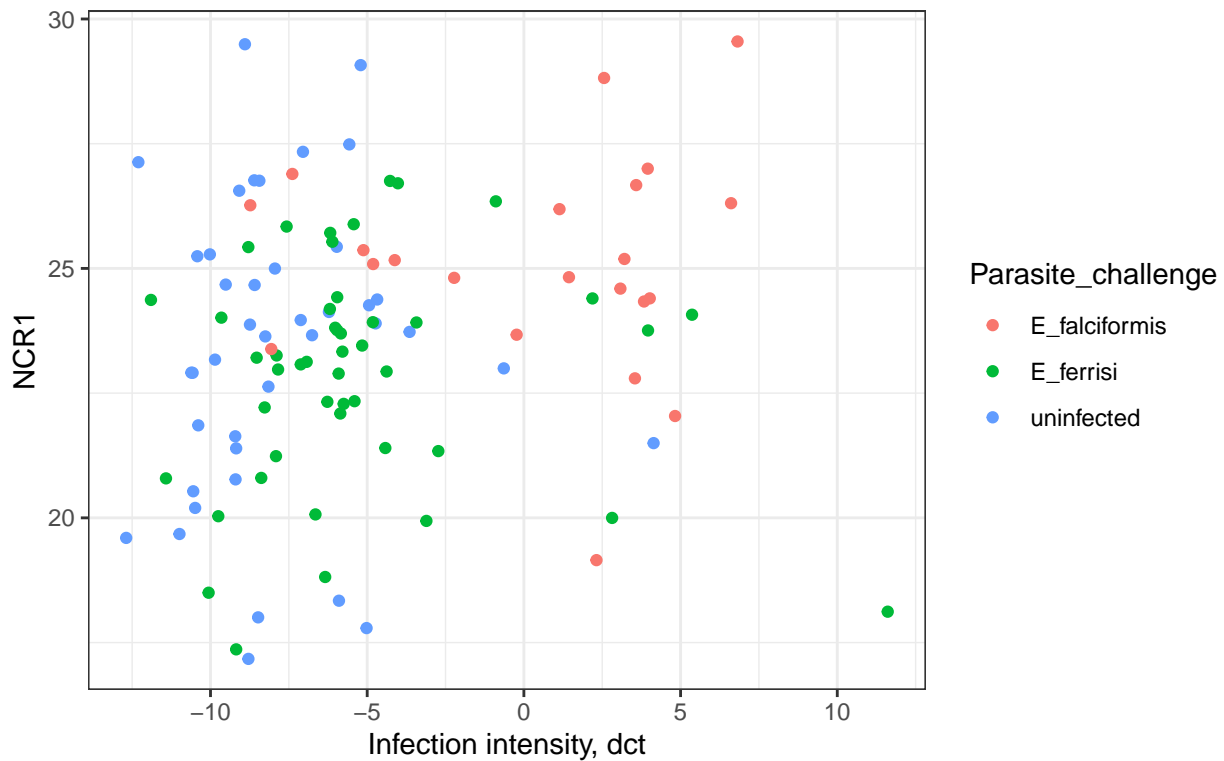
NCR1

```
ggplot(lab, aes(x = delta, y = NCR1, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).



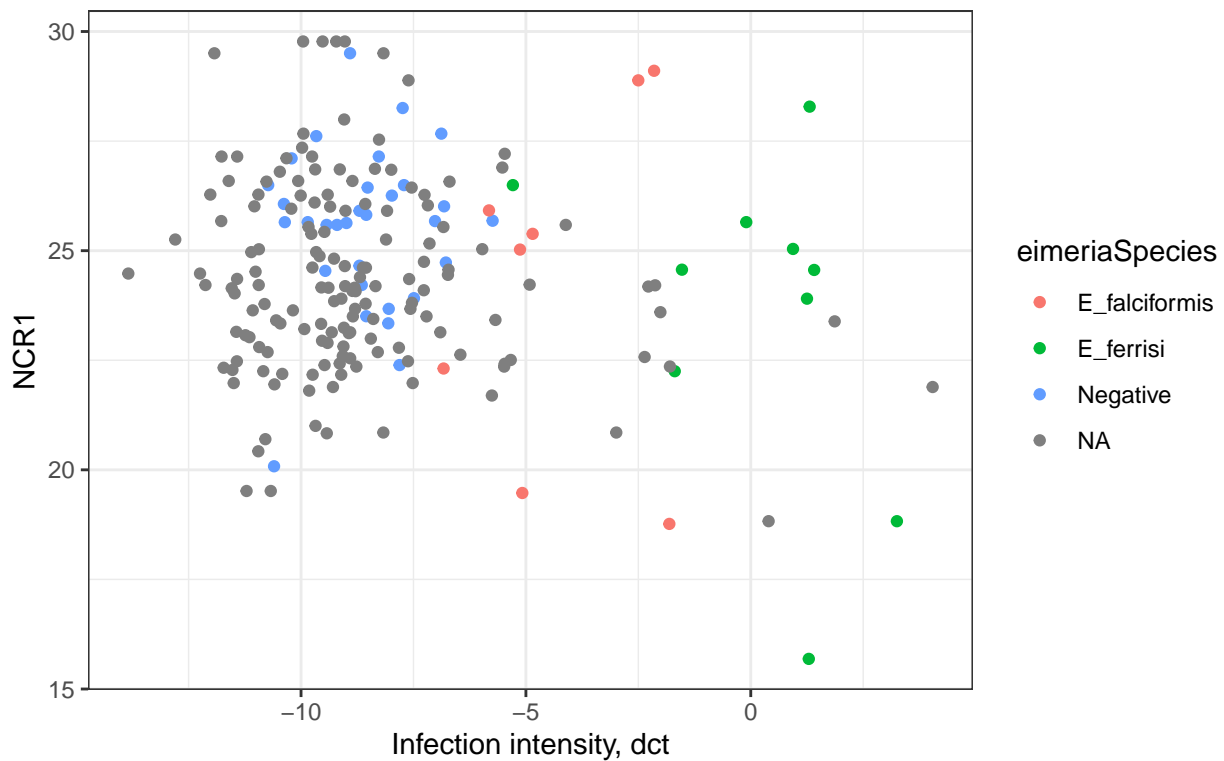
# Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = NCR1, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
       samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

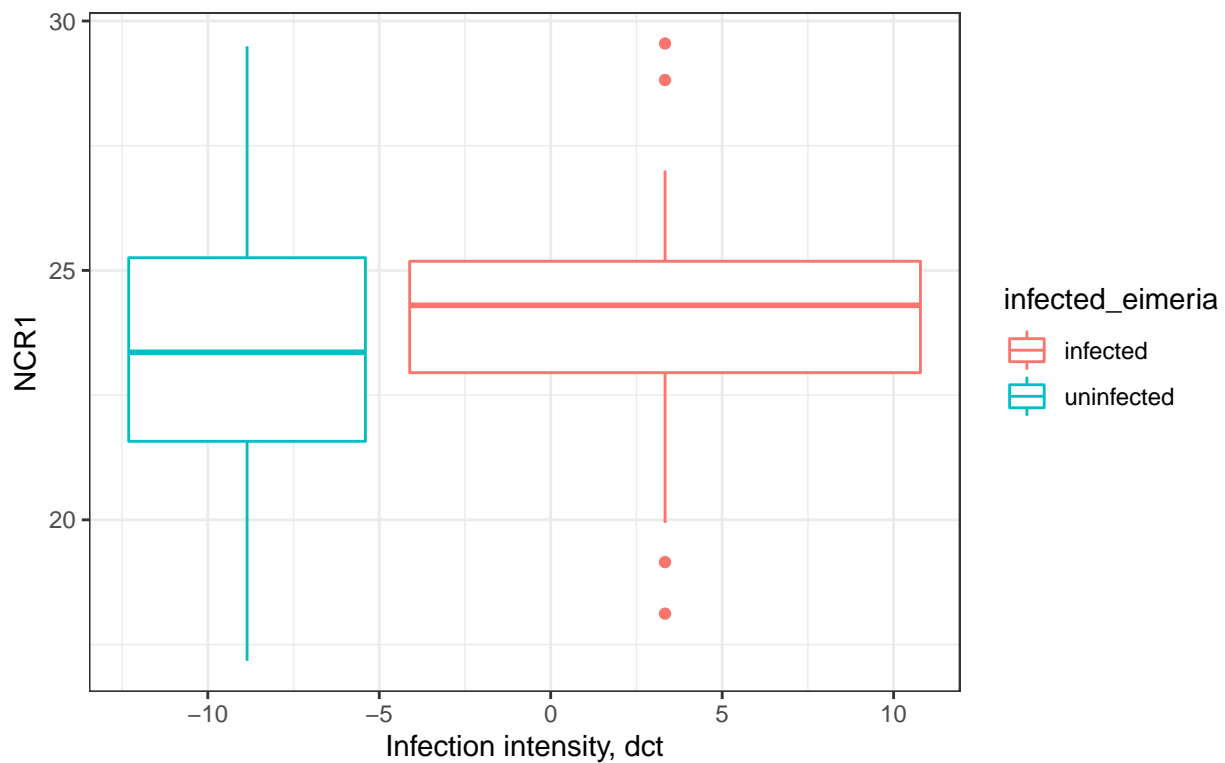
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = NCR1, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

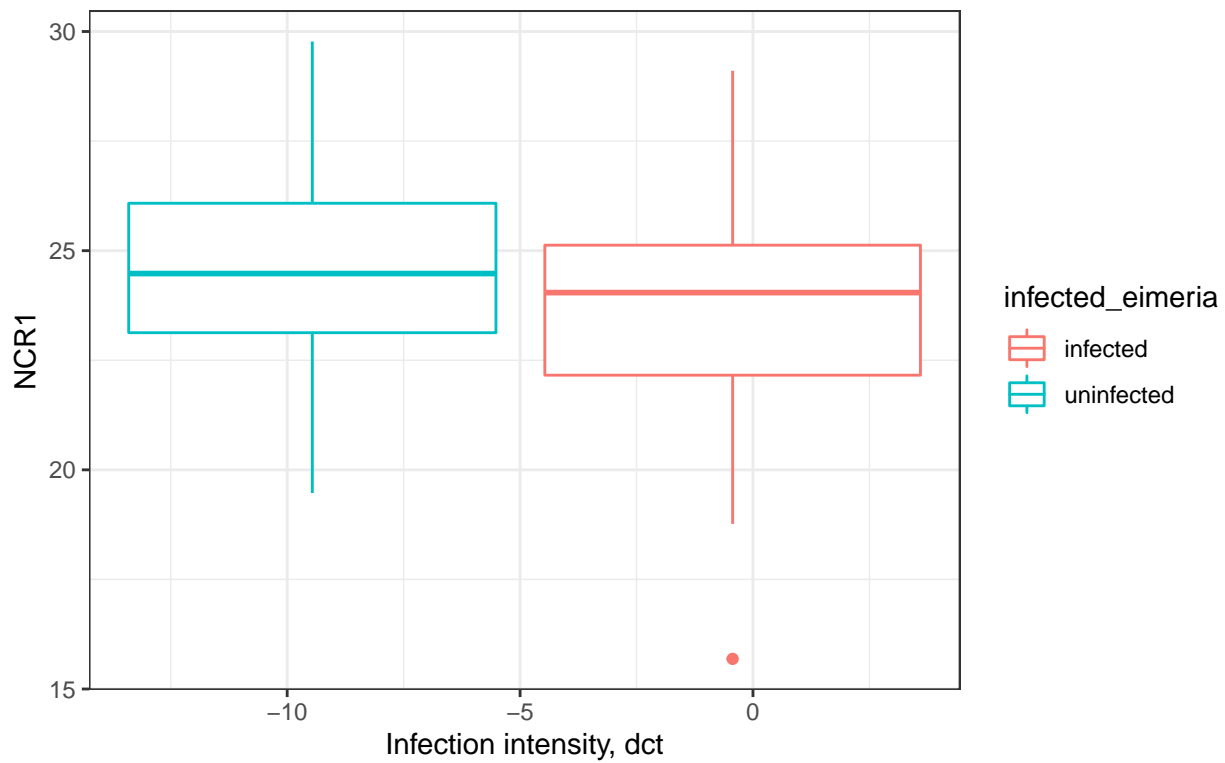
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = NCR1,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples

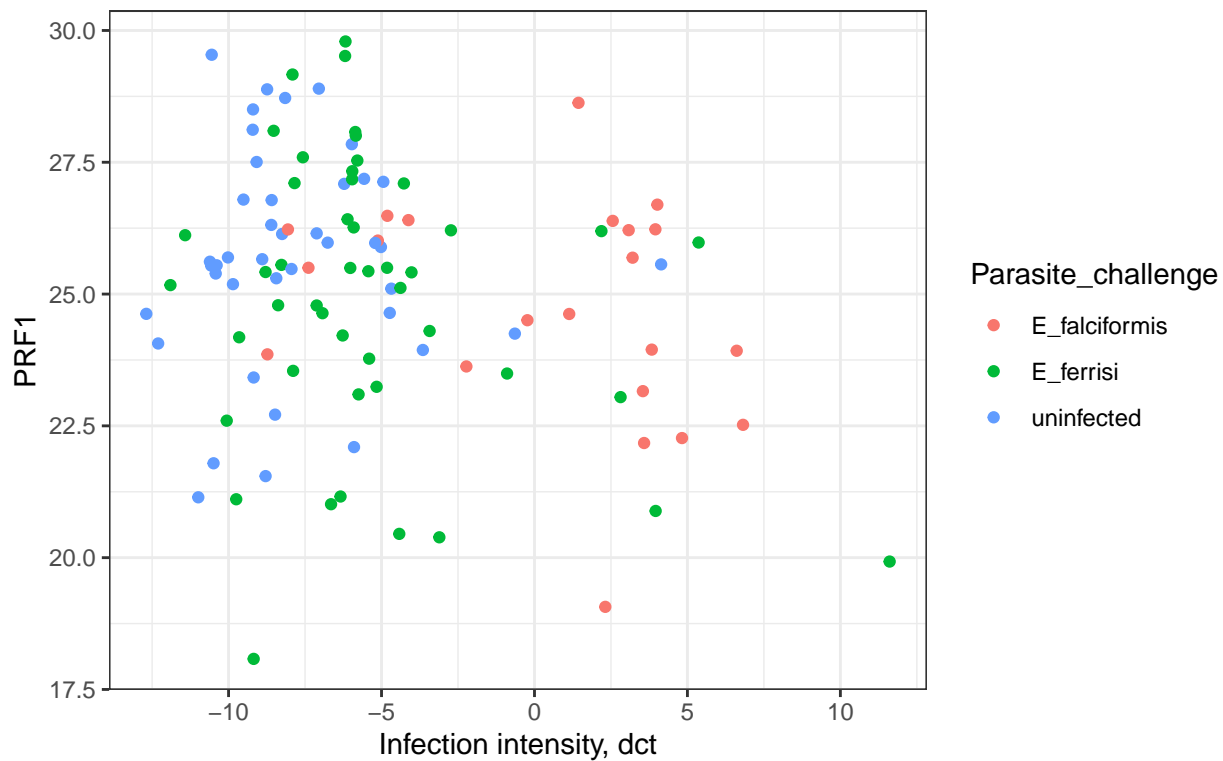


### 16. PRF1

```
ggplot(lab, aes(x = delta, y = PRF1, color = Parasite_challenge)) +  
  geom_jitter() +  
  geom_abline() +  
  labs(x = "Infection intensity, dct",  
        title = "Immune gene expression against infection intensity  
in lab infections") +  
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).

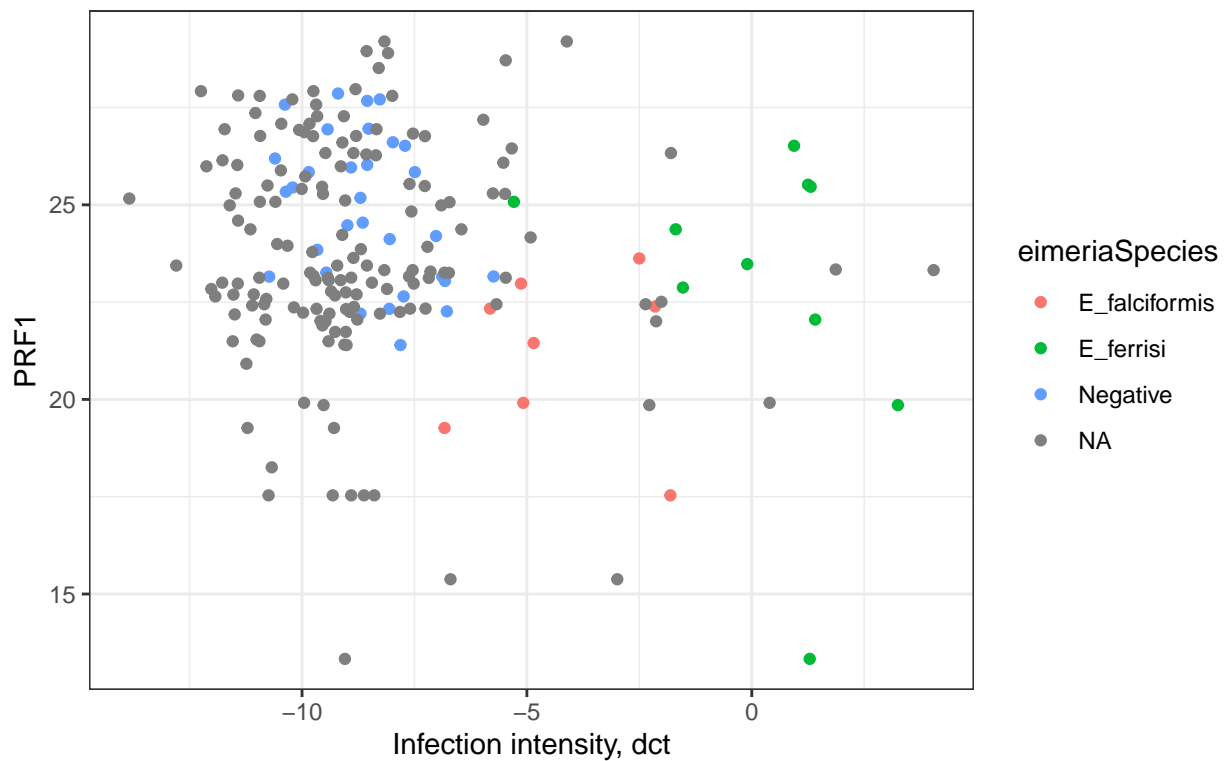
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = PRF1, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
               samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

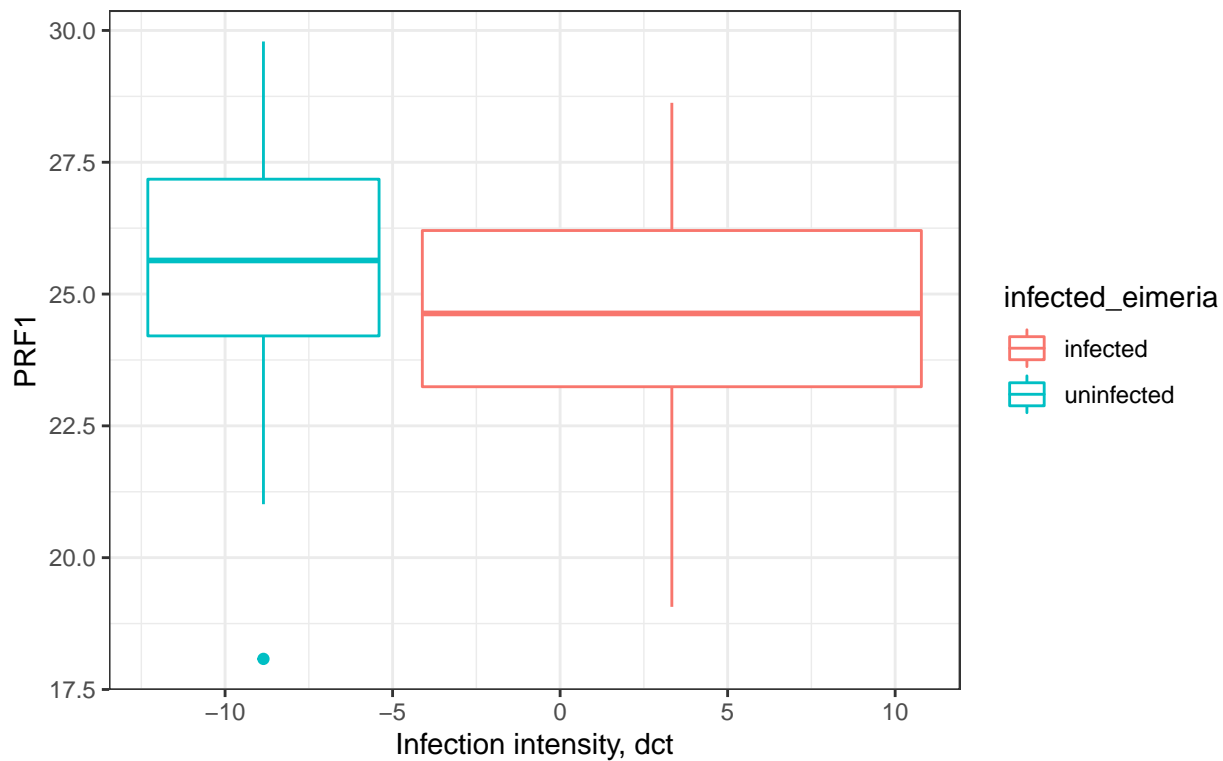
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = PRF1, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

```
## Warning: Removed 6 rows containing missing values (stat_boxplot).
```

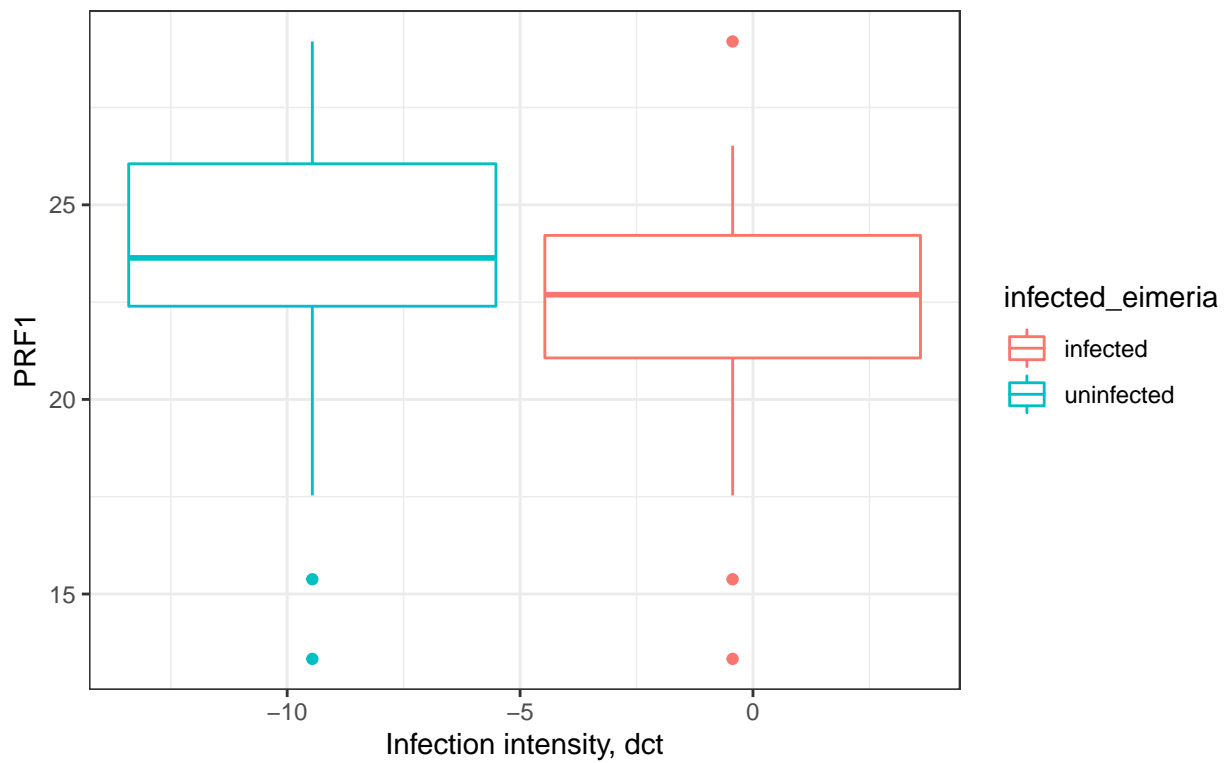
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = PRF1,  
                  color = infected_eimeria)) +  
  geom_boxplot() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity in field  
samples") +  
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples



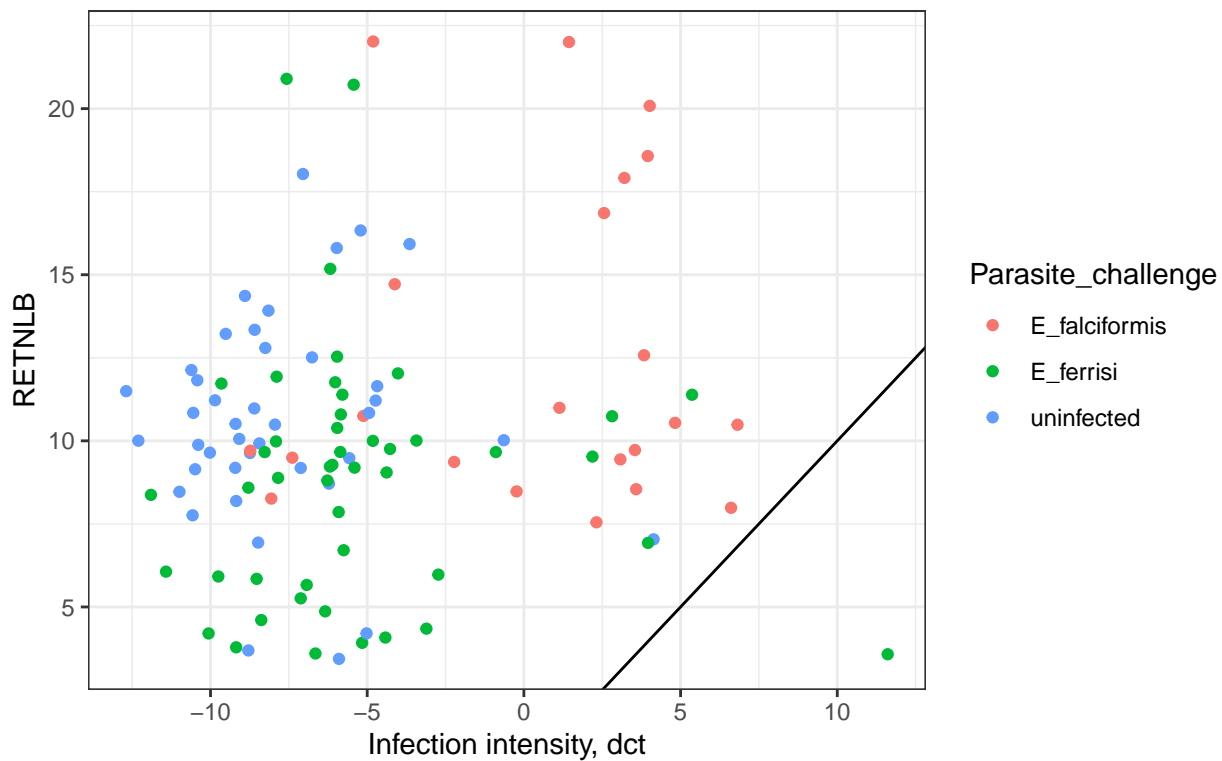
### 17. RETNLB

```
ggplot(lab, aes(x = delta, y = RETNLB, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).



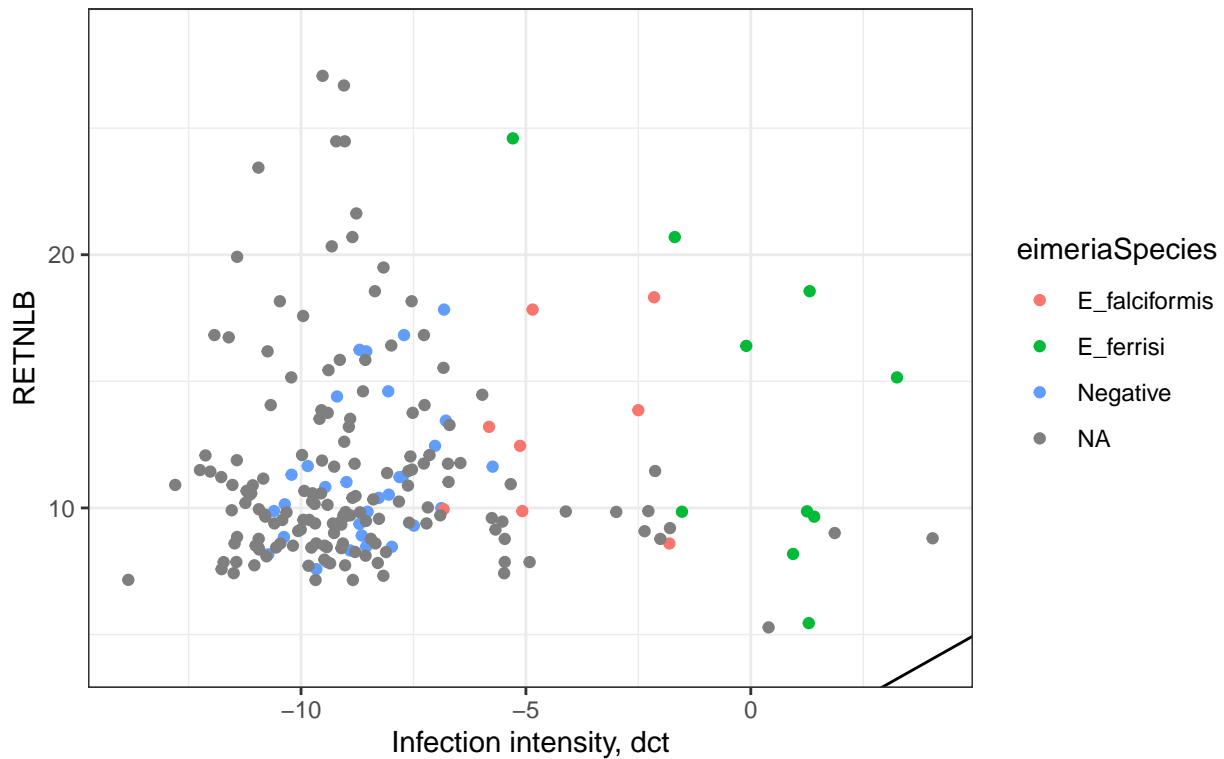
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = RETNLB, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
               samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

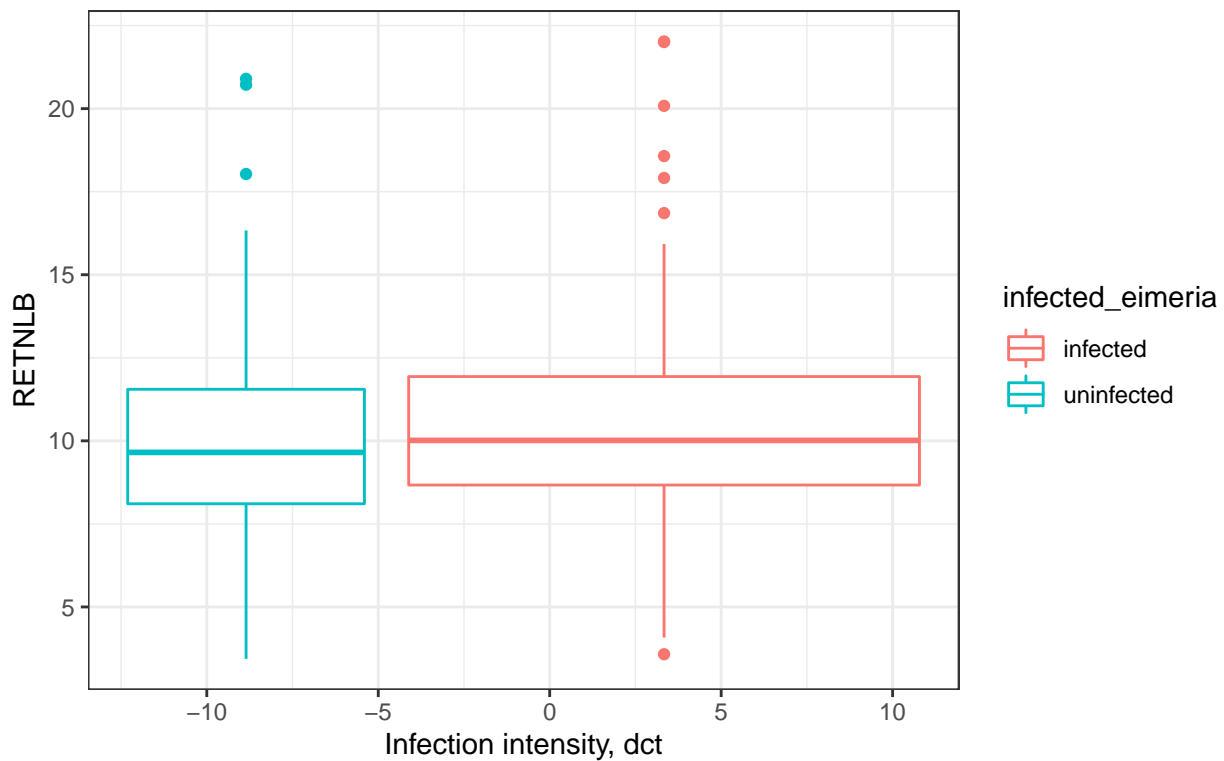
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = RETNLB, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

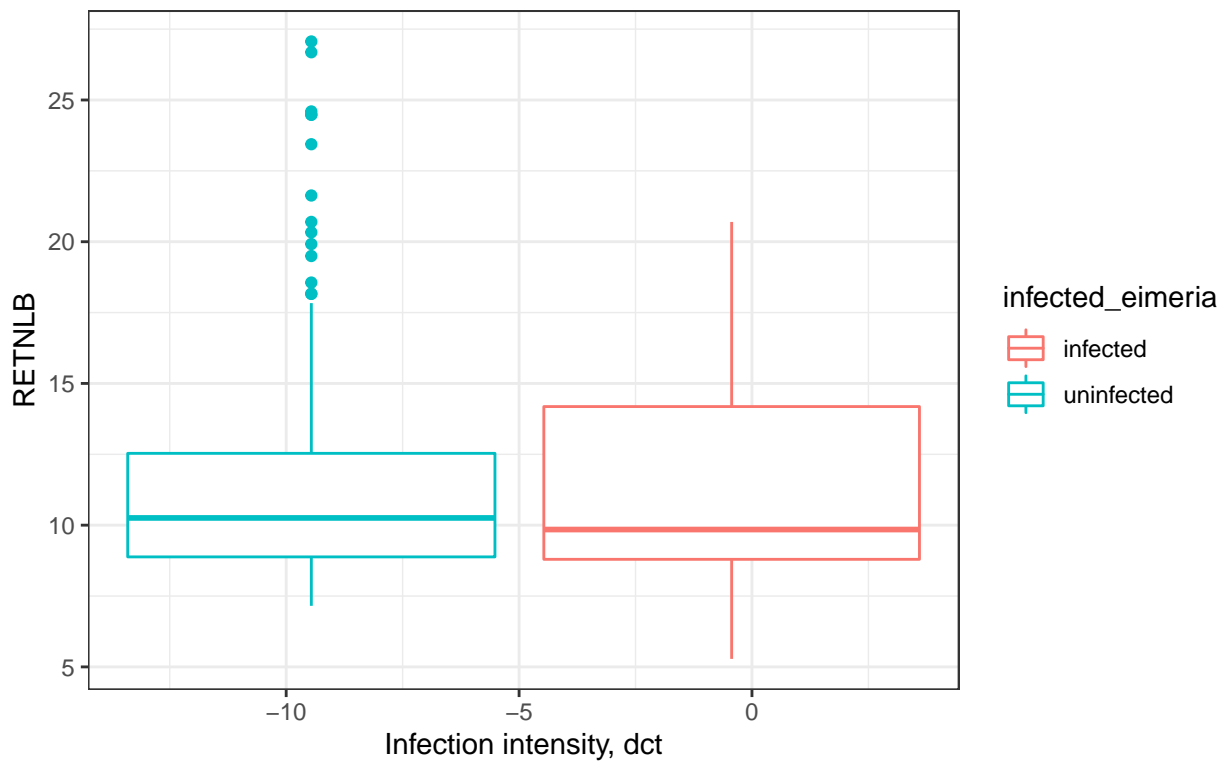
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = RETNLB,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples

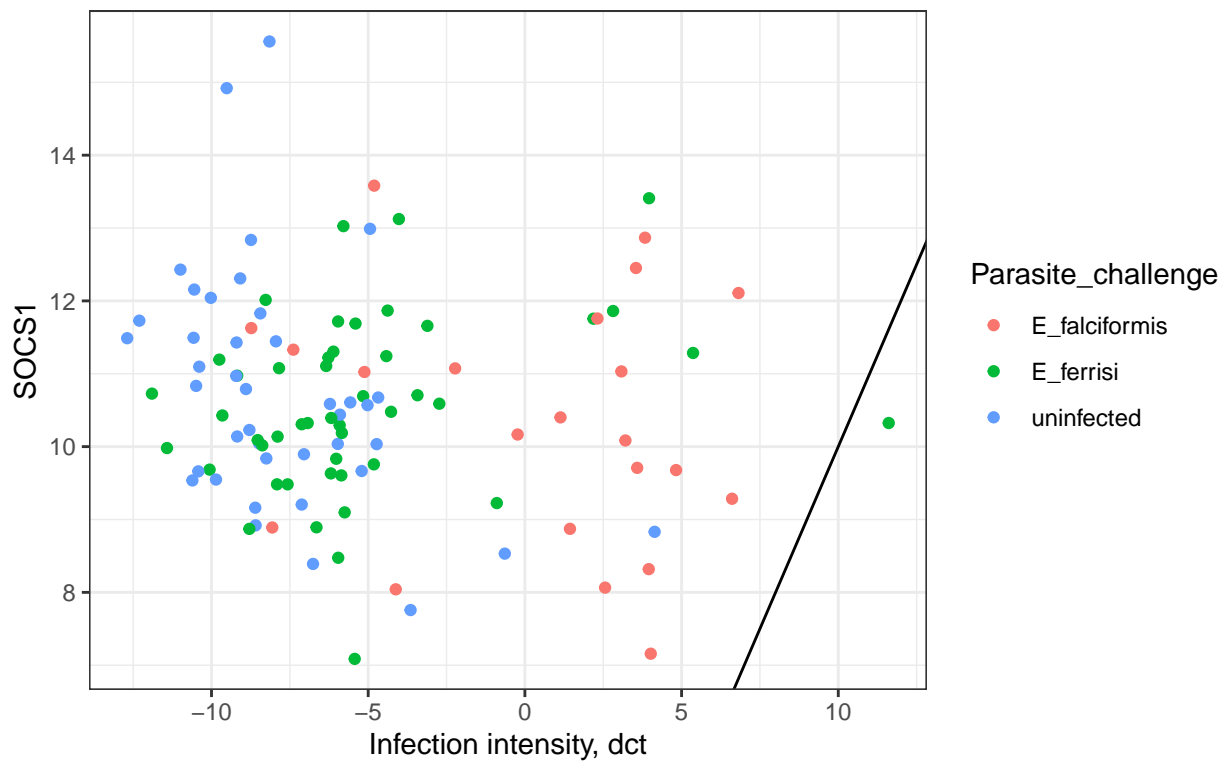


### 18. SOCS1

```
ggplot(lab, aes(x = delta, y = SOCS1, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).

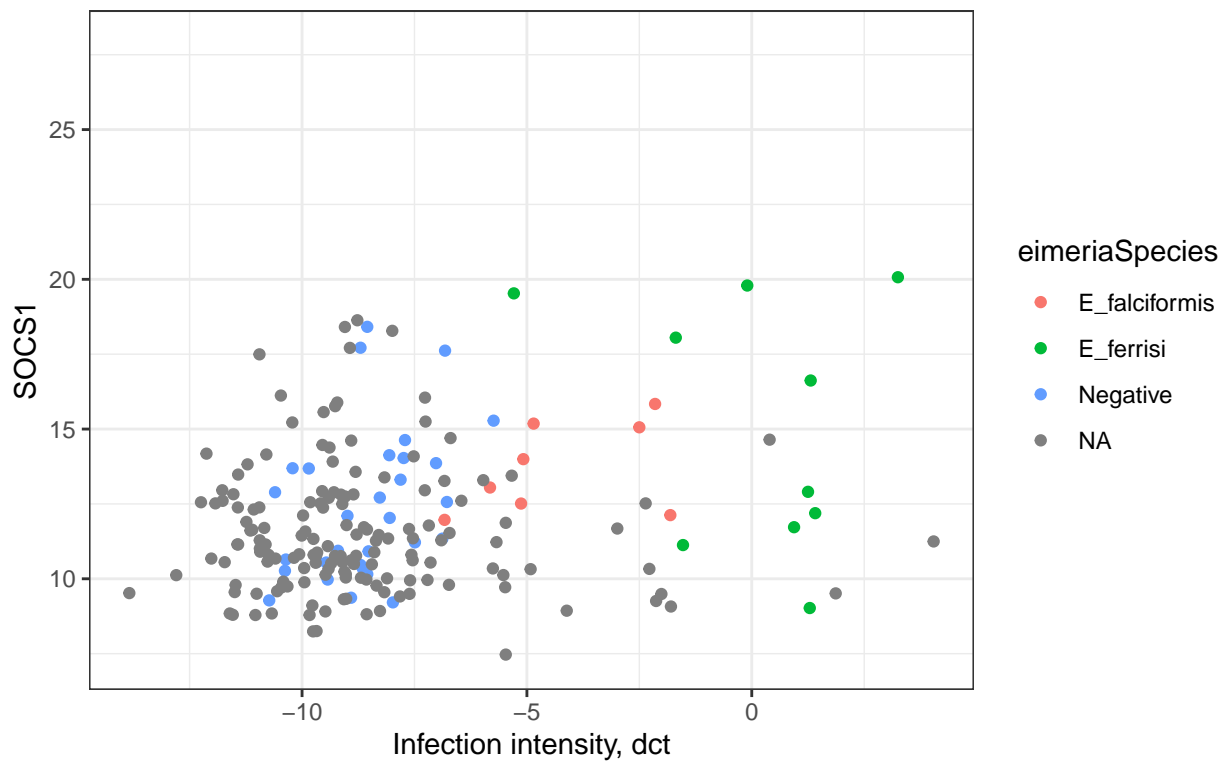
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = SOCS1, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
               samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

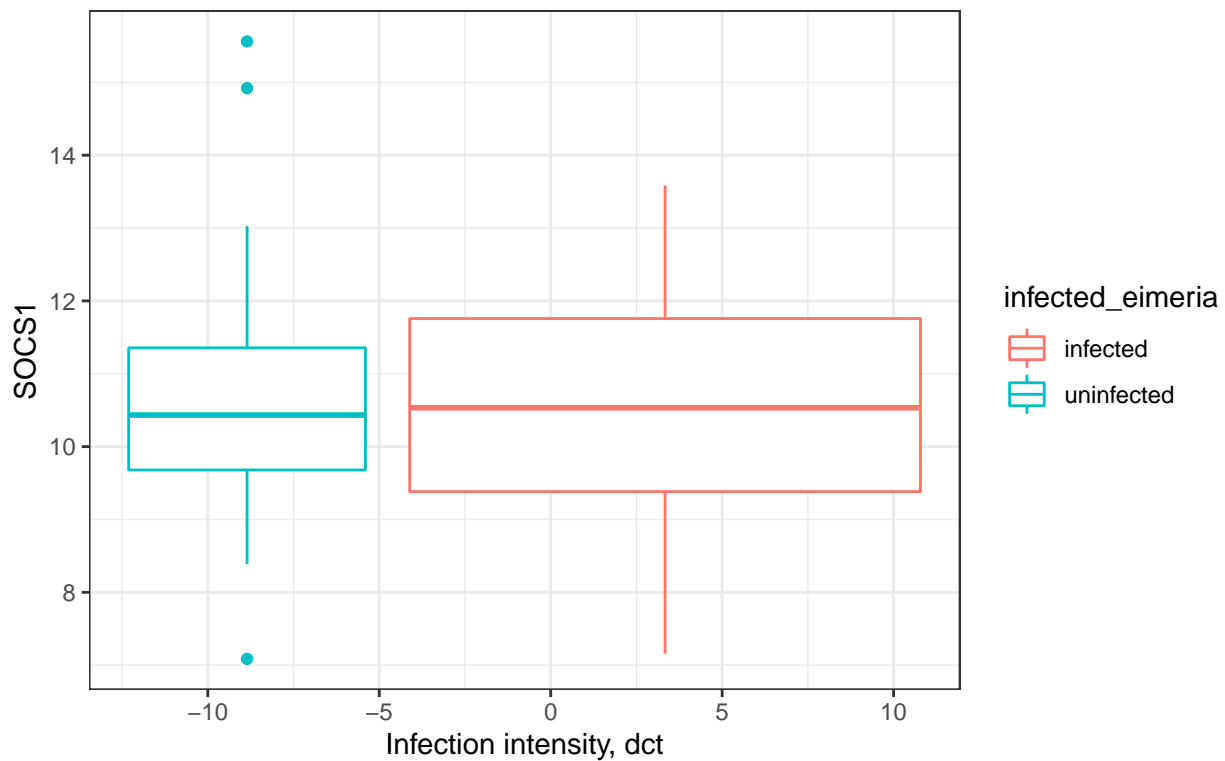
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = SOCS1, color = infected_eimeria)) +  
  geom_boxplot() +  
  labs(x = "Infection intensity, dct",  
       title = "Immune gene expression against infection intensity in lab  
infections") +  
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

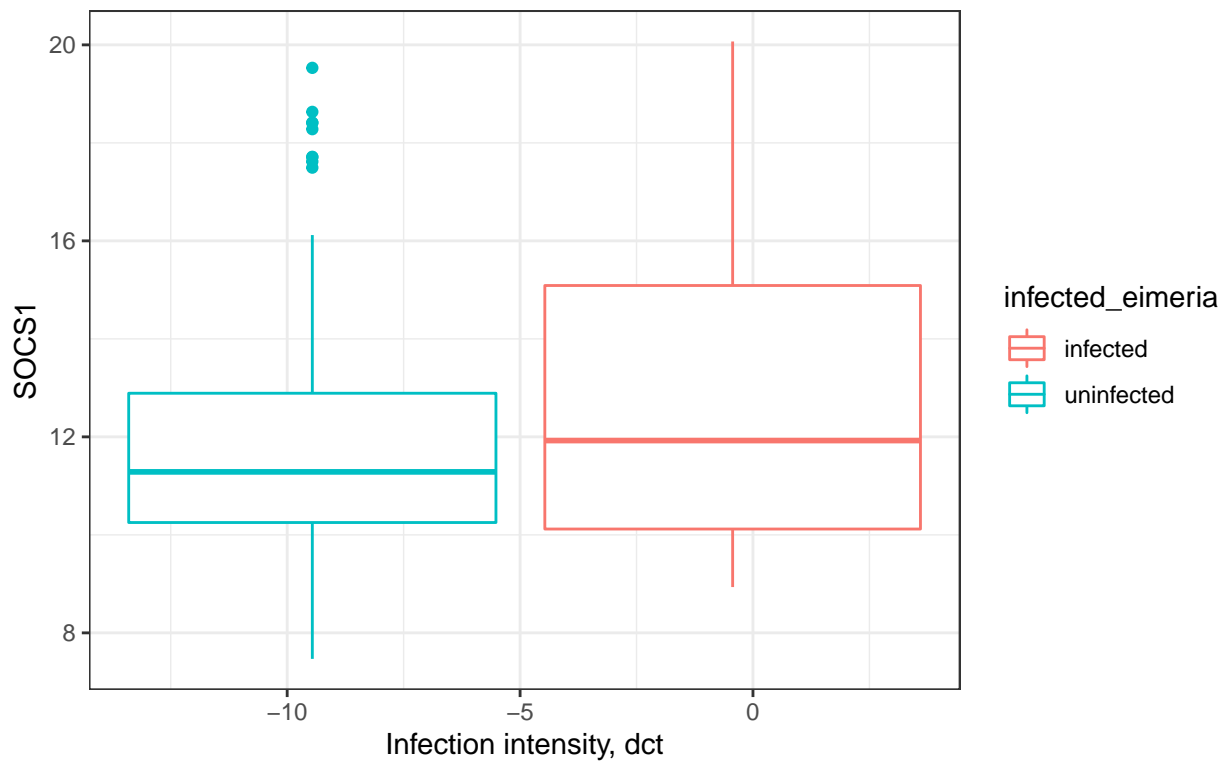
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = SOCS1,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples



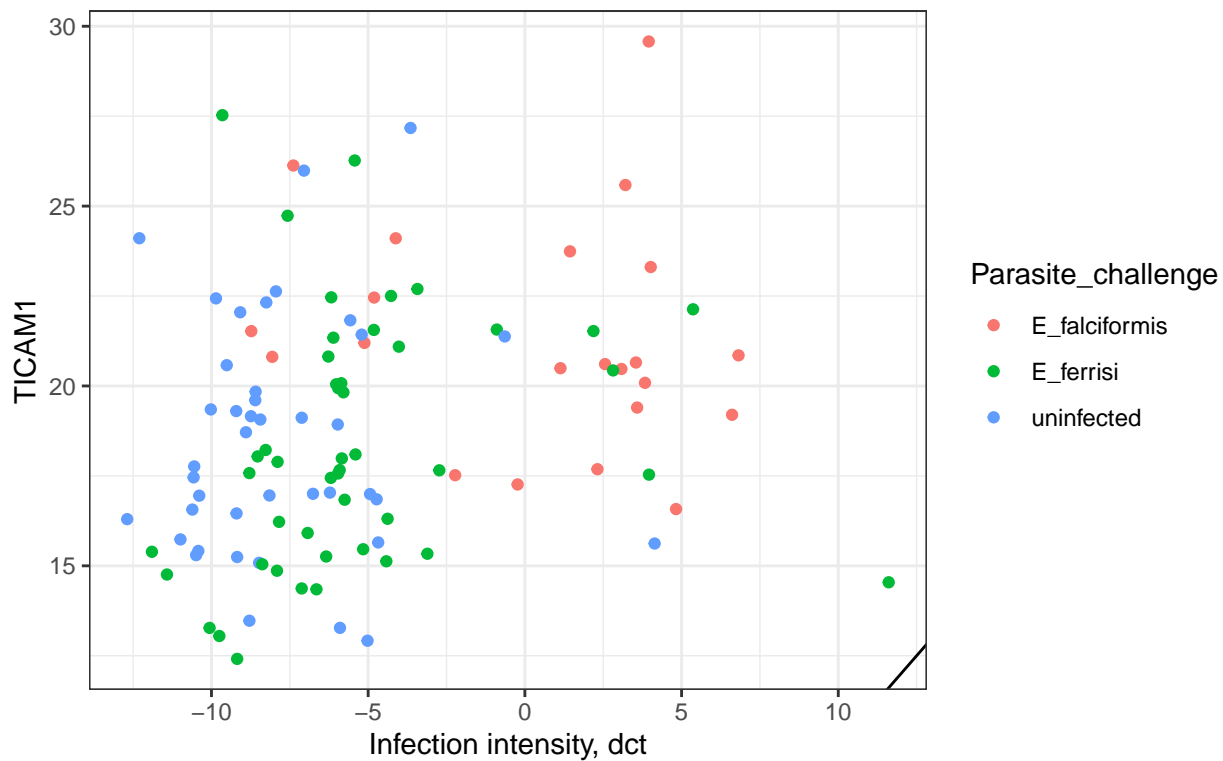
### 19. TICAM1

```
ggplot(lab, aes(x = delta, y = TICAM1, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
               in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).



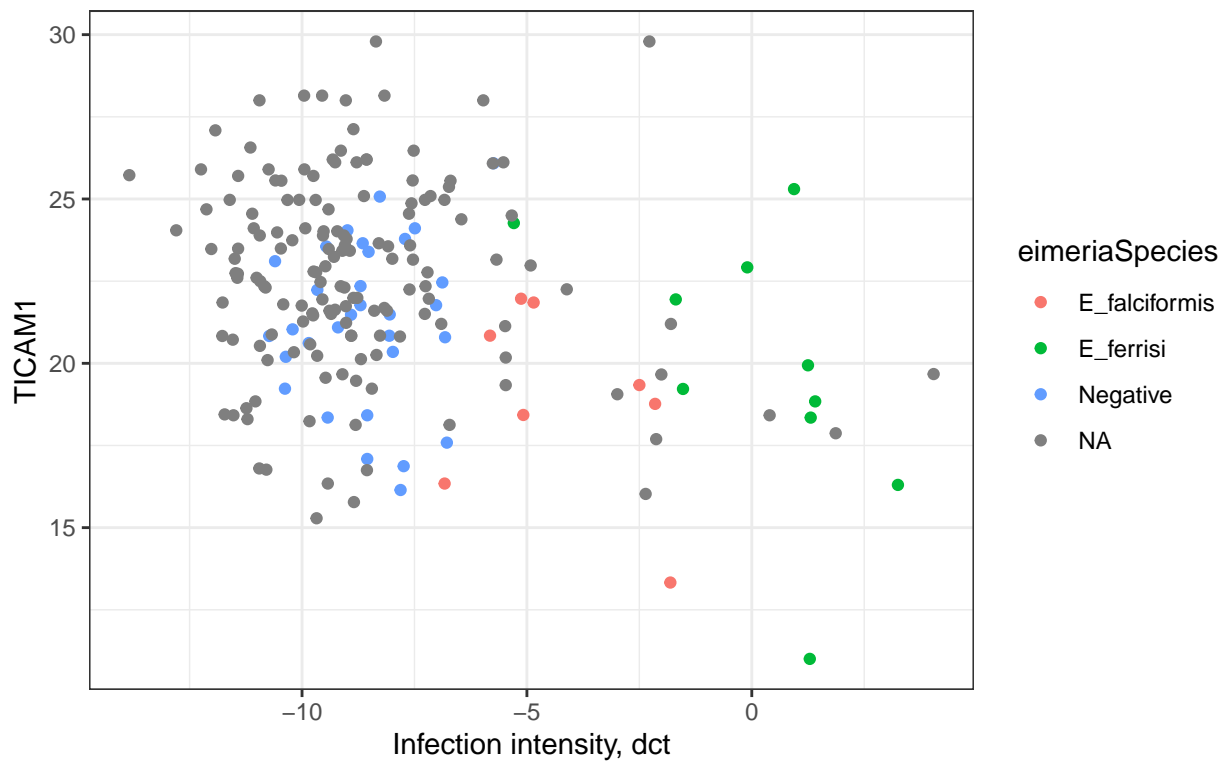
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = TICAM1, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

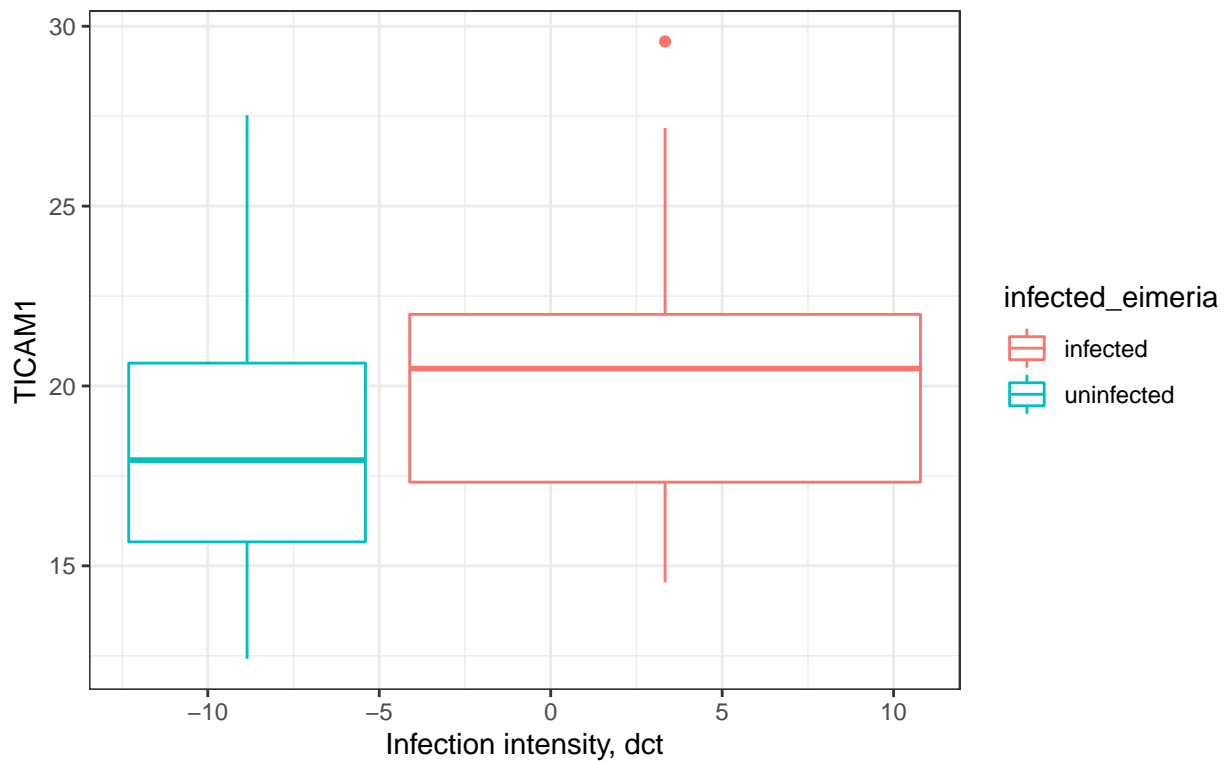
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = TICAM1, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (stat\_boxplot).

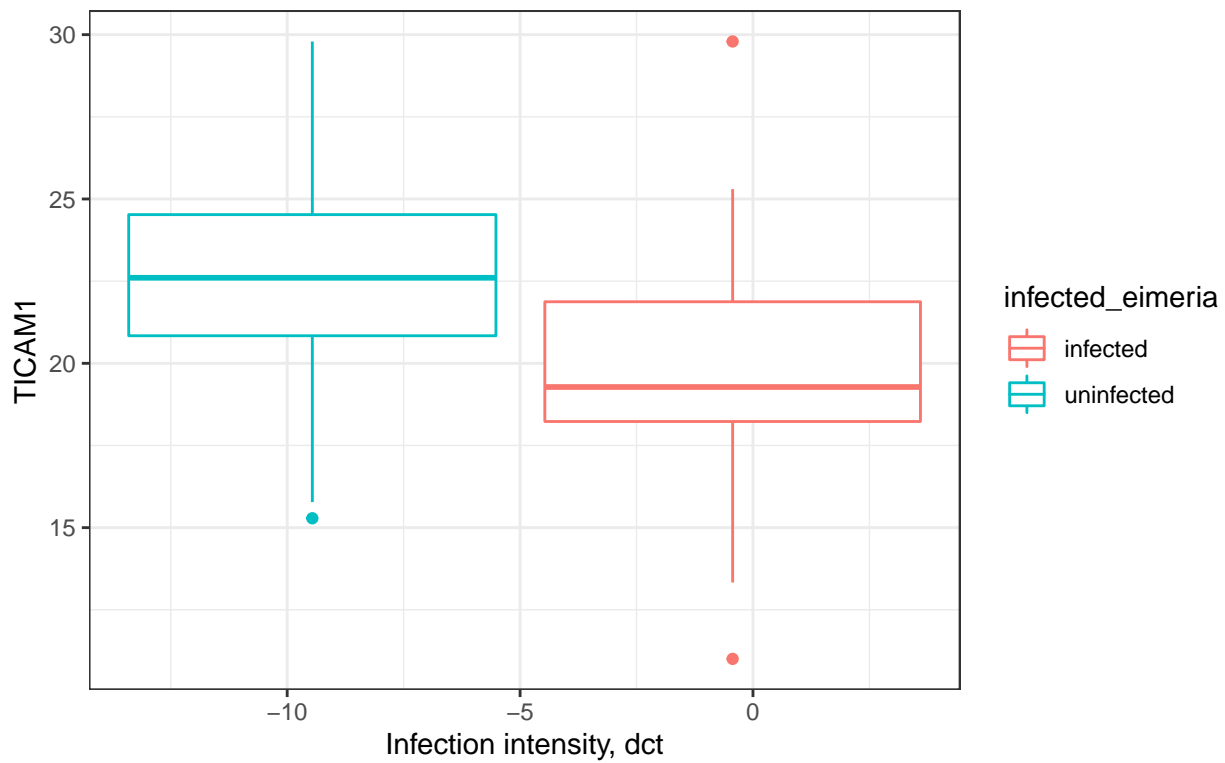
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = TICAM1,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples

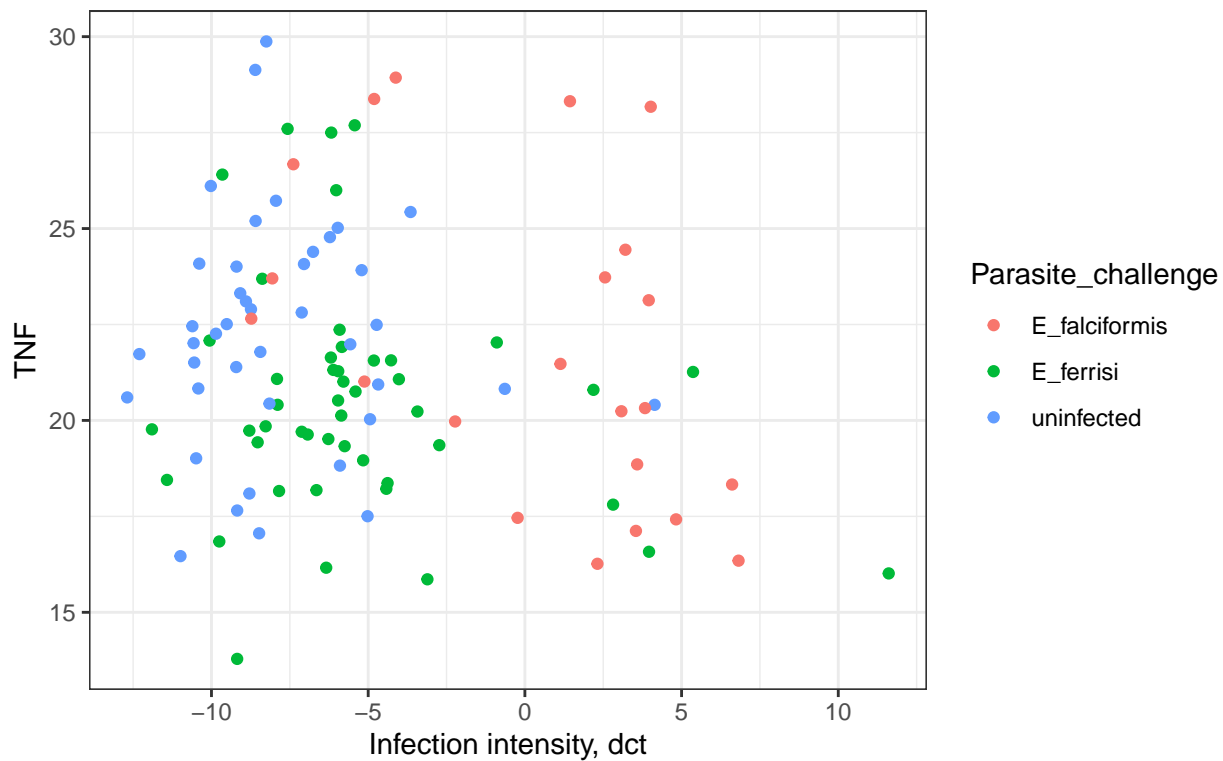


## 20. TNF

```
ggplot(lab, aes(x = delta, y = TNF, color = Parasite_challenge)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity
in lab infections") +
  theme_bw()
```

## Warning: Removed 6 rows containing missing values (geom\_point).

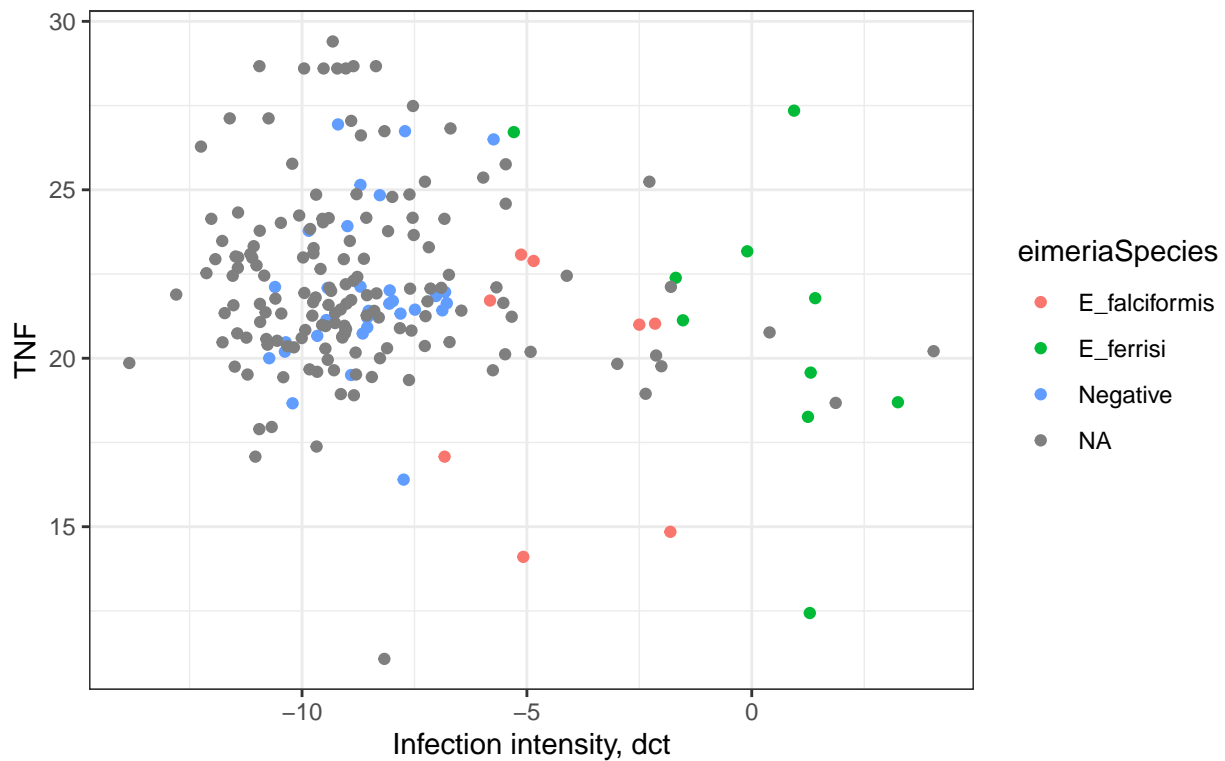
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = TNF, color = eimeriaSpecies)) +
  geom_jitter() +
  geom_abline() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
               samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (geom\_point).

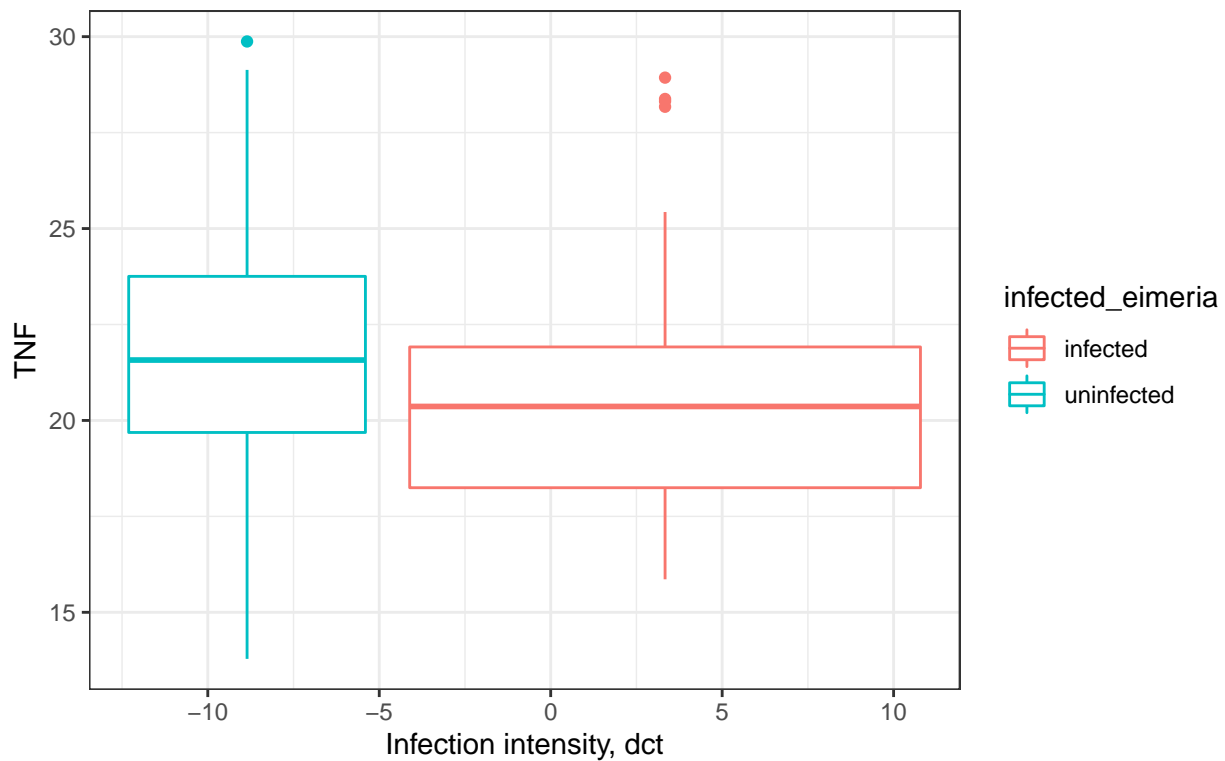
## Immune gene expression against infection intensity in field samples



```
ggplot(lab, aes(x = delta, y = TNF, color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in lab
infections") +
  theme_bw()
```

```
## Warning: Removed 6 rows containing missing values (stat_boxplot).
```

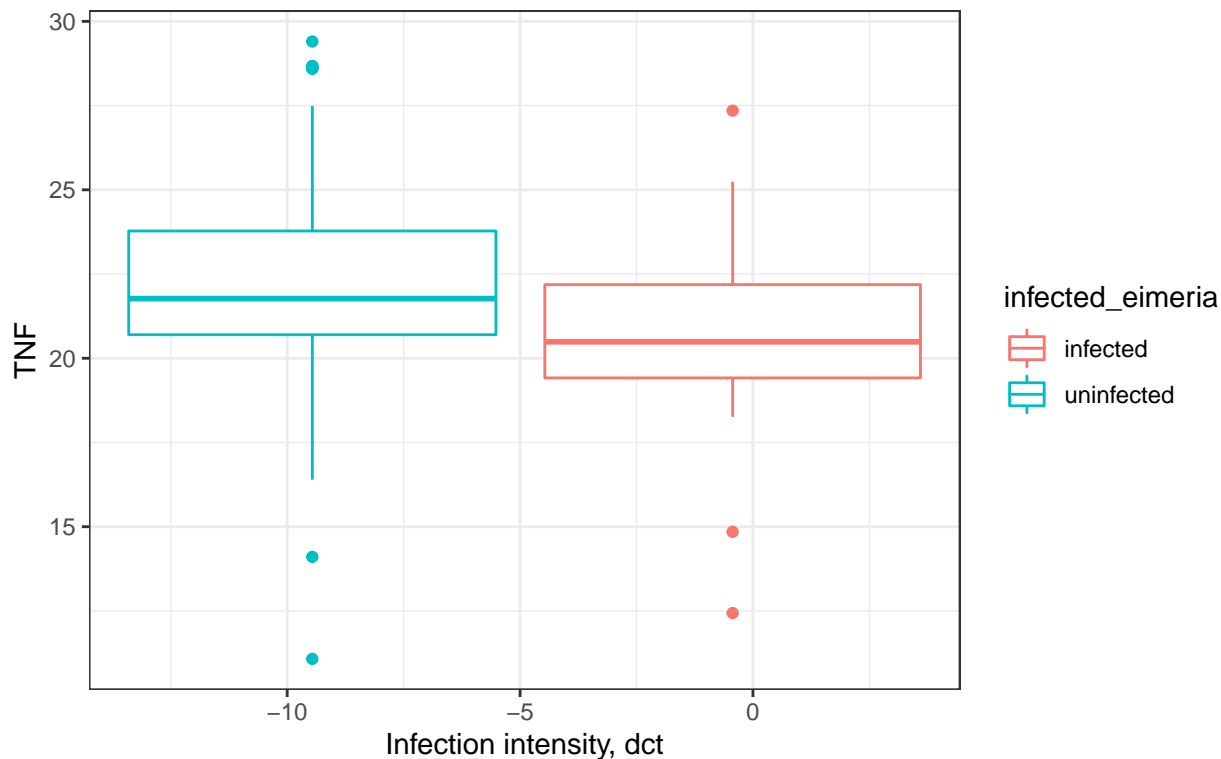
## Immune gene expression against infection intensity in lab infections



```
ggplot(field, aes(x = delta_ct_cewe_MminusE, y = TNF,
                  color = infected_eimeria)) +
  geom_boxplot() +
  labs(x = "Infection intensity, dct",
       title = "Immune gene expression against infection intensity in field
               samples") +
  theme_bw()
```

## Warning: Removed 154 rows containing missing values (stat\_boxplot).

## Immune gene expression against infection intensity in field samples



Compare the differences in uninfected and infected data and field / lab

Write a function which does a t test to quantify the differences of gene expression between uninfected and infected mice in the lab and field infections and also compares the uninfected lab and field and the infected lab and field

Function to print the p values for each t tet

```
eimeria_t_test_pvalue <- function(x) {
  # select the uninfected (delta ct under -5 mice)
  lab_uni <- lab %>%
    dplyr::filter(infected_eimeria == "uninfected")
  # select the infected lab mice
  lab_inf <- lab %>%
    dplyr::filter(infected_eimeria == "infected")
  # select the uninfected field mice (delta ct under -5 mice)
  field_uni <- field %>%
    dplyr::filter(infected_eimeria == "uninfected")
  # select the infected lab mice
  field_inf <- field %>%
    dplyr::filter(infected_eimeria == "infected")

  lab.uni_lab.inf <- t.test(lab_uni %>% dplyr::select(all_of(x)),
                           lab_inf %>% dplyr::select(all_of(x)))$p.value

  field.uni_field.inf <- t.test(field_uni %>% dplyr::select(all_of(x)),
```



```

        field_inf %>% dplyr::select(all_of(x)))$p.value

lab.uni_field_uni <- t.test(lab_uni %>% dplyr::select(all_of(x)),
        field_uni %>% dplyr::select(all_of(x)))$p.value

lab.inf_field.inf <- t.test(lab_inf %>% dplyr::select(all_of(x)),
        field_inf %>% dplyr::select(all_of(x)))$p.value

A <- data.frame(lab.uni_lab.inf,field.uni_field.inf,lab.uni_field_uni,
        lab.inf_field.inf)

rownames(A) <- toString(x)

print(A)
}

P <- lapply(intersect(Genes_field, Genes_lab), eimeria_t_test_pvalue)

##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IFNy      0.0009216811      0.000143669      0.007010837      0.03026008
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## CXCR3      0.576159      0.3506344      1.250472e-40      3.172915e-15
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL.6      0.5229923      0.01868255      1.345761e-05      0.8663768
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL.10      0.337847      0.2332154      0.0002005672      0.2231791
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL.13      0.01715789      0.01632698      3.649847e-14      9.452494e-06
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IL1RN      0.1469074      0.005467876      4.236683e-13      0.7809683
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## CASP1      0.3618237      0.8933641      0.0690306      0.6992377
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## CXCL9      0.05529236      1.901304e-05      3.825641e-12      0.3738375
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IDO1      0.2027322      0.9202276      0.0004362425      0.5587576
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## IRGM1      0.2697121      0.9310034      1.200882e-08      0.003644446
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MPO      0.09292337      0.03816342      1.434529e-10      0.6948777
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MUC2      0.7321875      0.1653492      0.04420135      0.03566309
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MUC5AC      0.9921441      0.03009515      0.001938751      0.6263826
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## MYD88      0.1178855      0.5363764      0.394004      0.0293453
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## NCR1      0.09757129      0.09390258      0.0001615751      0.4161399
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## PRF1      0.01292102      0.03772826      1.225351e-05      0.0164215
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## RETNLB      0.124246      0.7854483      0.0007932721      0.8498007
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## SOCS1      0.845947      0.188172      6.343844e-07      0.005368094

```

```
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## TICAM1      0.01821586      0.001249948      7.610351e-15      0.6535402
##      lab.uni_lab.inf field.uni_field.inf lab.uni_field_uni lab.inf_field.inf
## TNF      0.1559428      0.01251746      0.2561835      0.7687748

t.test.p.value <- reduce(P, rbind)

write.csv(t.test.p.value,
          "output_data/gene_expression/data_products/t.test.p.values_gene_exp.csv",
          row.names = FALSE)
```

## Results

1. Significant difference between means of lab uni and lab inf:

- IFNy
- IL13
- PRF1
- TICAM1

2. Significant difference between means of field uni and field inf:

- IFNy
- IL6
- IL13
- IL1RN
- CXCL9
- MPO
- MUC5AC
- PRF1
- TICAM1
- TNF

3. Significant difference between means of lab uni and field uni: IFNy CXCR3 IL6 IL10 IL13 IL1RN CXCL9 IDO1 IRGM1 MPO MUC2 MUC5AC NCR1 PRF1 RETNLB SOCS1 TICAM1

4. No Significant difference between means of lab uni and field uni:

- CASP1
- MYD88 -TNF

5. Significant difference between means of lab inf and field inf: IFNy CXCR3 IL13 IRGM1 MUC2 MYD88 PRF1 SOCS1

6. No Significant difference between means of lab inf and field inf: IL6 IL10 IL1RN CASP1 CXCL9 IDO1 MPO MUC5AC NCR1 RETNLB TICAM1 TNF

[https://docs.google.com/spreadsheets/d/1JWjsyzlQxWlSttZ5KwxYSsZapjZCgMCO1OcF0Yz9\\_ZQ/edit?usp=ssharing](https://docs.google.com/spreadsheets/d/1JWjsyzlQxWlSttZ5KwxYSsZapjZCgMCO1OcF0Yz9_ZQ/edit?usp=ssharing)