Class19: Pertussis and the CMI-PB project

Aisha

Pertussis is a severe lung disease that is also known as whopping cough.

We will begin by investigating the number of Pertussis cases per year in the US.

The data is linked here: (https://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html)

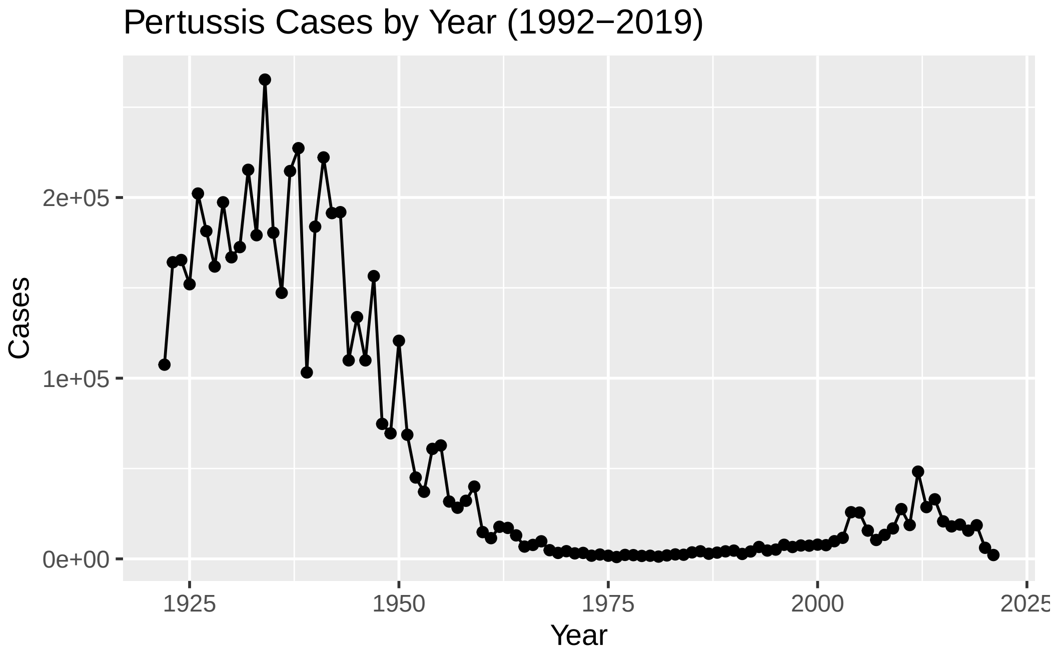
```
echo = FALSE

cdc <- data.frame(
```

```
head(cdc)
```

```
  Year  Cases
1 1922 107473
2 1923 164191
3 1924 165418
4 1925 152003
5 1926 202210
6 1927 181411
```

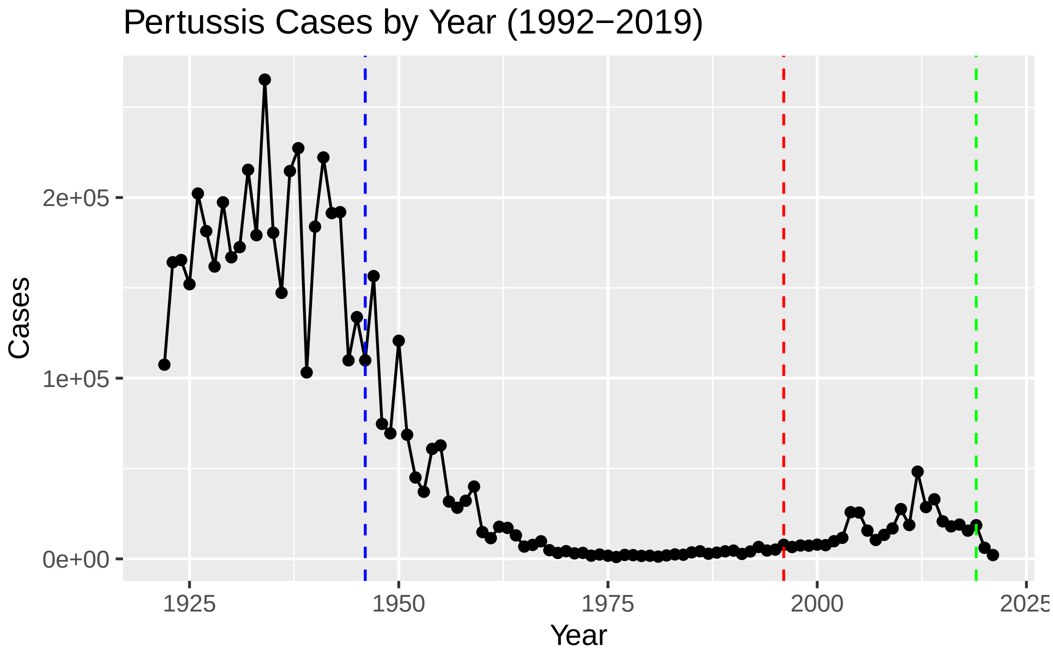
> Q1. With the help of the R “addin” package datapasta assign the CDC pertussis case number data to a data frame called cdc and use ggplot to make a plot of cases numbers over time.

```
library(ggplot2)

ggplot(cdc) + aes(Year, Cases) + geom_point() + geom_line() + labs(title = "Pertussis Case
```



Q2. Using the ggplot geom_vline() function add lines to your previous plot for the 1946 introduction of the wP vaccine and the 1996 switch to aP vaccine (see example in the hint below). What do you notice?

```r
ggplot(cdc) + aes(Year, Cases) + geom_point() + geom_line() +
  labs(title = "Pertussis Cases by Year (1992-2019)") +
  geom_vline(xintercept = 1946, linetype = "dashed", col = "blue") +
  geom_vline(xintercept = 1996, linetype = "dashed", col = "red") +
  geom_vline(xintercept = 2019, linetype = "dashed", col = "green")
```



We can see a drop of cases after the introduction of the 46' vaccine and a small increase with the 96' vaccine.

> Q3. Describe what happened after the introduction of the aP vaccine? Do you have a possible explanation for the observed trend?

After the introduction of the aP vaccine, there is a small lag and then we can see how the Pertussis cases have increasingly rose afterwards. The aP vaccine doesn't seem to be as effective/long-term such as the wP vaccine.

Exploring CMI-PB data

Why is this vaccine-preventable disease on the upswing? To answer this question we need to investigate the mechanisms underlying waning protection against pertussis. This requires evaluation of pertussis-specific immune responses over time in wP and aP vaccinated individuals.

This is the goals of the CMI-PB project: https://www.cmi-pb.org/

The CMI-PB project makes its data available via "API-endpoint" that return the JSON format.

We will use the **jsonlite** package to access this data. The main funciton in this package is called `read_json()`.

```
library(jsonlite)

# Subject table

subject <- read_json("http://cmi-pb.org/api/subject", simplifyVector = TRUE)
specimen <- read_json("http://cmi-pb.org/api/specimen", simplifyVector = TRUE)

titer <- read_json("http://cmi-pb.org/api/v4/plasma_ab_titer", simplifyVector = TRUE)
```

Lets have a look at these new objects:

```
head(subject)
```

```
  subject_id infancy_vac biological_sex              ethnicity  race
1          1          wP         Female Not Hispanic or Latino White
2          2          wP         Female Not Hispanic or Latino White
3          3          wP         Female                Unknown White
4          4          wP           Male Not Hispanic or Latino Asian
5          5          wP           Male Not Hispanic or Latino Asian
6          6          wP         Female Not Hispanic or Latino White
  year_of_birth date_of_boost      dataset
1    1986-01-01    2016-09-12 2020_dataset
2    1968-01-01    2019-01-28 2020_dataset
3    1983-01-01    2016-10-10 2020_dataset
4    1988-01-01    2016-08-29 2020_dataset
5    1991-01-01    2016-08-29 2020_dataset
6    1988-01-01    2016-10-10 2020_dataset
```

```
head(titer)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgE               FALSE   Total 1110.21154       2.493425
2           1     IgE               FALSE   Total 2708.91616       2.493425
3           1     IgG                TRUE      PT   68.56614       3.736992
4           1     IgG                TRUE     PRN  332.12718       2.602350
5           1     IgG                TRUE     FHA 1887.12263      34.050956
6           1     IgE                TRUE     ACT    0.10000       1.000000
   unit lower_limit_of_detection
```

```
1 UG/ML                 2.096133
2 IU/ML                29.170000
3 IU/ML                 0.530000
4 IU/ML                 6.205949
5 IU/ML                 4.679535
6 IU/ML                 2.816431
```

> Q4. How many aP and wP infancy vaccinated subjects are in the dataset?

```
table(subject$infancy_vac)
```

```

aP wP 
60 58 
```

> Q5. How many Male and Female subjects/patients are in the dataset?

```
table(subject$biological_sex)
```

```

Female   Male 
    79     39 
```

> Q6. What is the breakdown of race and biological sex (e.g. number of Asian females, White males etc…)?

```
table(subject$race, subject$biological_sex)
```

```
                                           
                                            Female Male
  American Indian/Alaska Native                  0    1
  Asian                                         21   11
  Black or African American                      2    0
  More Than One Race                             9    2
  Native Hawaiian or Other Pacific Islander      1    1
  Unknown or Not Reported                       11    4
  White                                         35   20
```

```
library(tidyverse)
```

```
-- Attaching core tidyverse packages ------------------------ tidyverse 2.0.0 --
v dplyr     1.1.3     v readr     2.1.4
v forcats   1.0.0     v stringr   1.5.0
v lubridate 1.9.3     v tibble    3.2.1
v purrr     1.0.2     v tidyr     1.3.0
-- Conflicts ------------------------------------------ tidyverse_conflicts() --
x dplyr::filter()  masks stats::filter()
x purrr::flatten() masks jsonlite::flatten()
x dplyr::lag()     masks stats::lag()
i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to becom
```

```
today()
```

```
[1] "2023-12-10"
```

```
today() - mdy("11-28-2001")
```

```
Time difference of 8047 days
```

```
time_length( today() - ymd("2002-08-18"), "years")
```

```
[1] 21.31143
```

> Q7. Using this approach determine (i) the average age of wP individuals, (ii) the average age of aP individuals; and (iii) are they significantly different?

```
subject$age <- today() - ymd(subject$year_of_birth)
```

```
library(dplyr)

 ap <- subject %>% filter(infancy_vac == "aP")

round( summary( time_length( ap$age, "years" ) ) )
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
     21      26      26      26      27      30
```

```
# wP

wp <- subject %>% filter(infancy_vac == "wP")
round( summary( time_length( wp$age, "years" ) ) )
```

```
 Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
   28      31      35      36      39      56 
```

The difference between both of their average ages is about 10 years.

Q8. Determine the age of all individuals at time of boost?

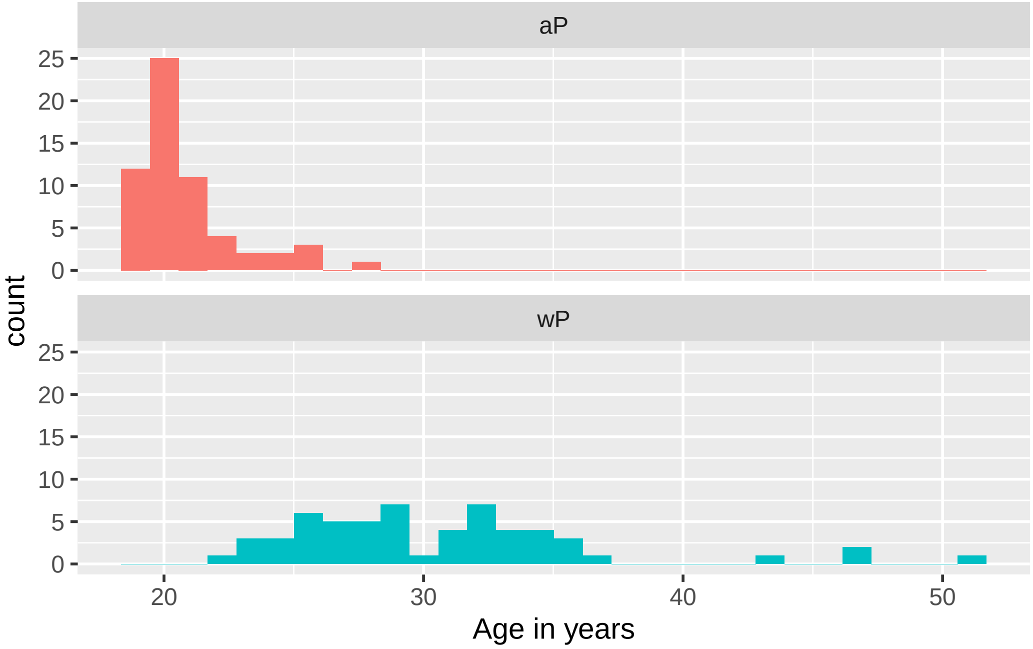
```
subject$age <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)

subject$age_year <- time_length(subject$age, "year")

head(subject)
```

```
  subject_id infancy_vac biological_sex              ethnicity  race
1          1          wP         Female Not Hispanic or Latino White
2          2          wP         Female Not Hispanic or Latino White
3          3          wP         Female                Unknown White
4          4          wP           Male Not Hispanic or Latino Asian
5          5          wP           Male Not Hispanic or Latino Asian
6          6          wP         Female Not Hispanic or Latino White
  year_of_birth date_of_boost      dataset        age age_year
1    1986-01-01    2016-09-12 2020_dataset 11212 days 30.69678
2    1968-01-01    2019-01-28 2020_dataset 18655 days 51.07461
3    1983-01-01    2016-10-10 2020_dataset 12336 days 33.77413
4    1988-01-01    2016-08-29 2020_dataset 10468 days 28.65982
5    1991-01-01    2016-08-29 2020_dataset  9372 days 25.65914
6    1988-01-01    2016-10-10 2020_dataset 10510 days 28.77481
```

Q9. With the help of a faceted boxplot or histogram (see below), do you think these two groups are significantly different?

```
ggplot(subject) +
  aes(time_length(age, "year"),
      fill=as.factor(infancy_vac)) +
  geom_histogram(show.legend=FALSE) +
  facet_wrap(vars(infancy_vac), nrow=2) +
  xlab("Age in years")
```

```
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



These two groups are very significantly differently.

Merge or join tables

Q9. Complete the code to join specimen and subject tables to make a new merged data frame containing all specimen records along with their associated subject details:

```
meta <- inner_join(specimen, subject)
```

```
Joining with `by = join_by(subject_id)`
```

```
dim(meta)
```

```
[1] 939  15
```

```
head(meta)
```

```
  specimen_id subject_id actual_day_relative_to_boost
1           1          1                           -3
2           2          1                            1
3           3          1                            3
4           4          1                            7
5           5          1                           11
6           6          1                           32
  planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
1                             0         Blood     1          wP         Female
2                             1         Blood     2          wP         Female
3                             3         Blood     3          wP         Female
4                             7         Blood     4          wP         Female
5                            14         Blood     5          wP         Female
6                            30         Blood     6          wP         Female
               ethnicity  race year_of_birth date_of_boost      dataset
1 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
2 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
3 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
4 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
5 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
6 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
         age age_year
1 11212 days 30.69678
2 11212 days 30.69678
3 11212 days 30.69678
4 11212 days 30.69678
5 11212 days 30.69678
6 11212 days 30.69678
```

Antibody measurements in the blood.

```
head(titer)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgE               FALSE   Total 1110.21154       2.493425
2           1     IgE               FALSE   Total 2708.91616       2.493425
```

```
3           1     IgG                TRUE      PT   68.56614       3.736992
4           1     IgG                TRUE     PRN  332.12718       2.602350
5           1     IgG                TRUE     FHA 1887.12263      34.050956
6           1     IgE                TRUE     ACT    0.10000       1.000000
   unit lower_limit_of_detection
1 UG/ML                 2.096133
2 IU/ML                29.170000
3 IU/ML                 0.530000
4 IU/ML                 6.205949
5 IU/ML                 4.679535
6 IU/ML                 2.816431
```

> Q10. Now using the same procedure join meta with titer data so we can further analyze this data in terms of time of visit aP/wP, male/female etc.

```r
abdata <- inner_join(titer, meta)
```

```
Joining with `by = join_by(specimen_id)`
```

```r
dim(abdata)
```

```
[1] 41810    22
```

```r
head(abdata)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgE               FALSE   Total 1110.21154       2.493425
2           1     IgE               FALSE   Total 2708.91616       2.493425
3           1     IgG                TRUE      PT   68.56614       3.736992
4           1     IgG                TRUE     PRN  332.12718       2.602350
5           1     IgG                TRUE     FHA 1887.12263      34.050956
6           1     IgE                TRUE     ACT    0.10000       1.000000
   unit lower_limit_of_detection subject_id actual_day_relative_to_boost
1 UG/ML                 2.096133          1                           -3
2 IU/ML                29.170000          1                           -3
3 IU/ML                 0.530000          1                           -3
4 IU/ML                 6.205949          1                           -3
5 IU/ML                 4.679535          1                           -3
6 IU/ML                 2.816431          1                           -3
```

```
  planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
1                              0         Blood     1          wP         Female
2                              0         Blood     1          wP         Female
3                              0         Blood     1          wP         Female
4                              0         Blood     1          wP         Female
5                              0         Blood     1          wP         Female
6                              0         Blood     1          wP         Female
               ethnicity  race year_of_birth date_of_boost      dataset
1 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
2 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
3 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
4 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
5 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
6 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
         age age_year
1 11212 days 30.69678
2 11212 days 30.69678
3 11212 days 30.69678
4 11212 days 30.69678
5 11212 days 30.69678
6 11212 days 30.69678
```

> Q11. How many specimens (i.e. entries in abdata) do we have for each isotype?

```r
table(abdata$isotype)
```

```

 IgE  IgG IgG1 IgG2 IgG3 IgG4 
6698 3240 7968 7968 7968 7968 
```

> Q12. What are the different $dataset values in abdata and what do you notice about the number of rows for the most “recent” dataset?

```r
table(abdata$dataset)
```

```

2020_dataset 2021_dataset 2022_dataset 
       31520         8085         2205 
```

The number of rows for the 2022_data is far less than the other datasets. The number of rows seems to decrease as the years go by.

Examine IgG Ab titer levels

Let’s focus on one of these IgG.

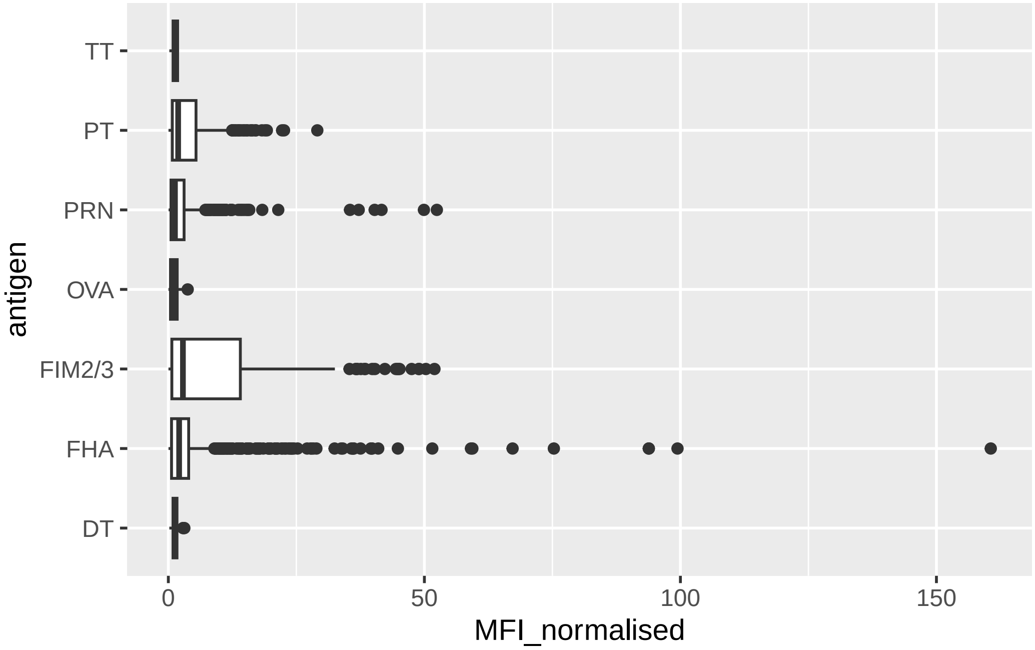
```r
igg <- abdata %>% filter(isotype == "IgG")

head(igg)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgG                TRUE      PT   68.56614       3.736992
2           1     IgG                TRUE     PRN  332.12718       2.602350
3           1     IgG                TRUE     FHA 1887.12263      34.050956
4          19     IgG                TRUE      PT   20.11607       1.096366
5          19     IgG                TRUE     PRN  976.67419       7.652635
6          19     IgG                TRUE     FHA   60.76626       1.096457
   unit lower_limit_of_detection subject_id actual_day_relative_to_boost
1 IU/ML                 0.530000          1                           -3
2 IU/ML                 6.205949          1                           -3
3 IU/ML                 4.679535          1                           -3
4 IU/ML                 0.530000          3                           -3
5 IU/ML                 6.205949          3                           -3
6 IU/ML                 4.679535          3                           -3
  planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
1                             0         Blood     1          wP         Female
2                             0         Blood     1          wP         Female
3                             0         Blood     1          wP         Female
4                             0         Blood     1          wP         Female
5                             0         Blood     1          wP         Female
6                             0         Blood     1          wP         Female
               ethnicity  race year_of_birth date_of_boost      dataset
1 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
2 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
3 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
4                Unknown White    1983-01-01    2016-10-10 2020_dataset
5                Unknown White    1983-01-01    2016-10-10 2020_dataset
6                Unknown White    1983-01-01    2016-10-10 2020_dataset
         age age_year
1 11212 days 30.69678
2 11212 days 30.69678
3 11212 days 30.69678
4 12336 days 33.77413
5 12336 days 33.77413
```

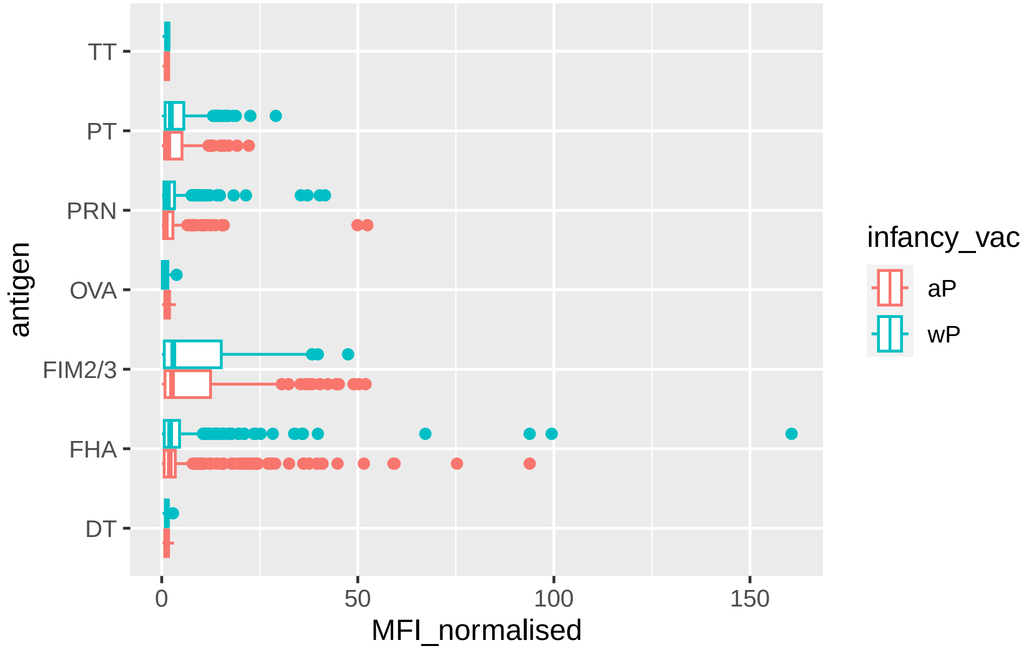
```
6 12336 days 33.77413
```

Boxplot of MFI_normalised vs antigen.

```
ggplot(igg) + aes(MFI_normalised, antigen) + geom_boxplot()
```



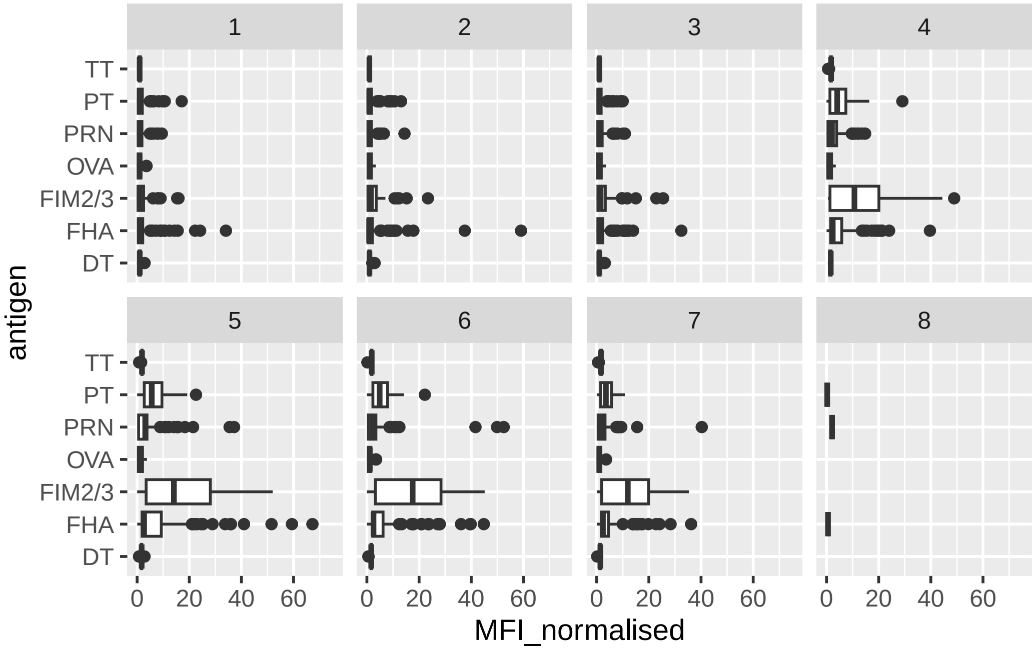
```
ggplot(igg) +
  aes(MFI_normalised, antigen, col = infancy_vac) +
  geom_boxplot()
```



Q13. Complete the following code to make a summary boxplot of Ab titer levels (MFI) for all antigens:

```
ggplot(igg) +
  aes(MFI_normalised, antigen) +
  geom_boxplot() + xlim(0,75) + facet_wrap(vars(visit), nrow=2)
```

```
Warning: Removed 5 rows containing non-finite values (`stat_boxplot()`).
```



```r
head(igg)
```

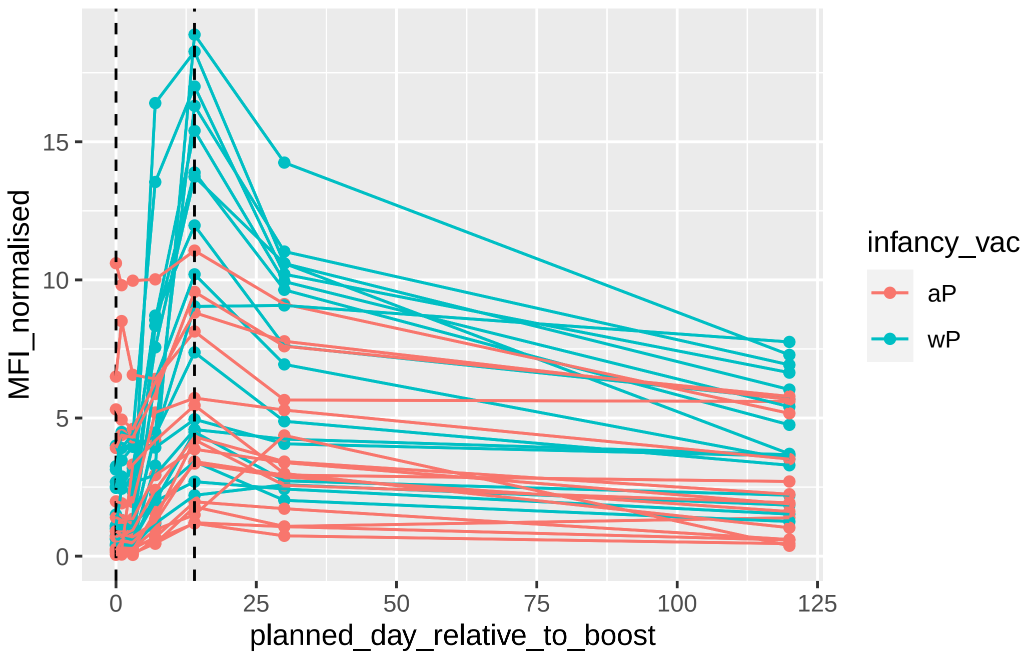
```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgG                TRUE      PT   68.56614       3.736992
2           1     IgG                TRUE     PRN  332.12718       2.602350
3           1     IgG                TRUE     FHA 1887.12263      34.050956
4          19     IgG                TRUE      PT   20.11607       1.096366
5          19     IgG                TRUE     PRN  976.67419       7.652635
6          19     IgG                TRUE     FHA   60.76626       1.096457
   unit lower_limit_of_detection subject_id actual_day_relative_to_boost
1 IU/ML                 0.530000          1                           -3
2 IU/ML                 6.205949          1                           -3
3 IU/ML                 4.679535          1                           -3
4 IU/ML                 0.530000          3                           -3
5 IU/ML                 6.205949          3                           -3
6 IU/ML                 4.679535          3                           -3
  planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
1                             0         Blood     1          wP         Female
2                             0         Blood     1          wP         Female
3                             0         Blood     1          wP         Female
4                             0         Blood     1          wP         Female
5                             0         Blood     1          wP         Female
```

```
6                              0         Blood     1           wP         Female
               ethnicity  race year_of_birth date_of_boost      dataset
1 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
2 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
3 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
4                Unknown White    1983-01-01    2016-10-10 2020_dataset
5                Unknown White    1983-01-01    2016-10-10 2020_dataset
6                Unknown White    1983-01-01    2016-10-10 2020_dataset
         age age_year
1 11212 days 30.69678
2 11212 days 30.69678
3 11212 days 30.69678
4 12336 days 33.77413
5 12336 days 33.77413
6 12336 days 33.77413
```

Focus in on IgG to the Pertussis Toxin (PT) antigen.

```
igg.pt <- igg %>% filter(antigen == "PT", dataset == "2021_dataset")
```

```
ggplot(igg.pt) +
  aes(planned_day_relative_to_boost, MFI_normalised,
      col = infancy_vac, group = subject_id) +
  geom_point() + geom_line() + geom_vline(xintercept = 0, linetype = "dashed", col = "blac
  geom_line() + geom_vline(xintercept = 14, linetype = "dashed", col = "black")
```



Obtaining CMI-PB RNASeq data

For RNA-Seq data the API query mechanism quickly hits the web browser interface limit for file size. We will present alternative download mechanisms for larger CMI-PB datasets in the next section. However, we can still do "targeted" RNA-Seq querys via the web accessible API.

```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.

rna <- read_json(url, simplifyVector = TRUE)
```

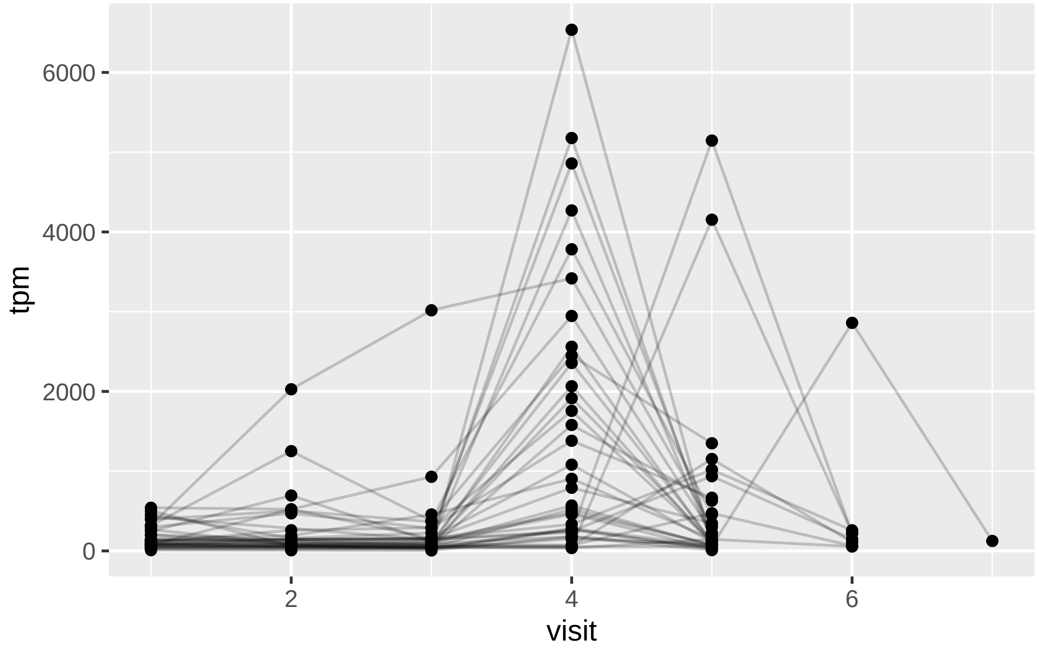
To facilitate further analysis we need to "join" the rna expression data with our metadata meta, which is itself a join of sample and specimen data.

```
#meta <- inner_join(specimen, subject)
ssrna <- inner_join(rna, meta)
```

```
Joining with `by = join_by(specimen_id)`
```

> Q19. Make a plot of the time course of gene expression for IGHG1 gene (i.e. a plot of visit vs. tpm).

```
ggplot(ssrna) +
  aes(x = visit, y = tpm, group=subject_id) +
  geom_point() +
  geom_line(alpha=0.2)
```



Q20.: What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

The expression of this gene when it is at its' maximum level has a TPM value of over 6000. But the maximum level doesn't endure for long as it drops down very drastically.