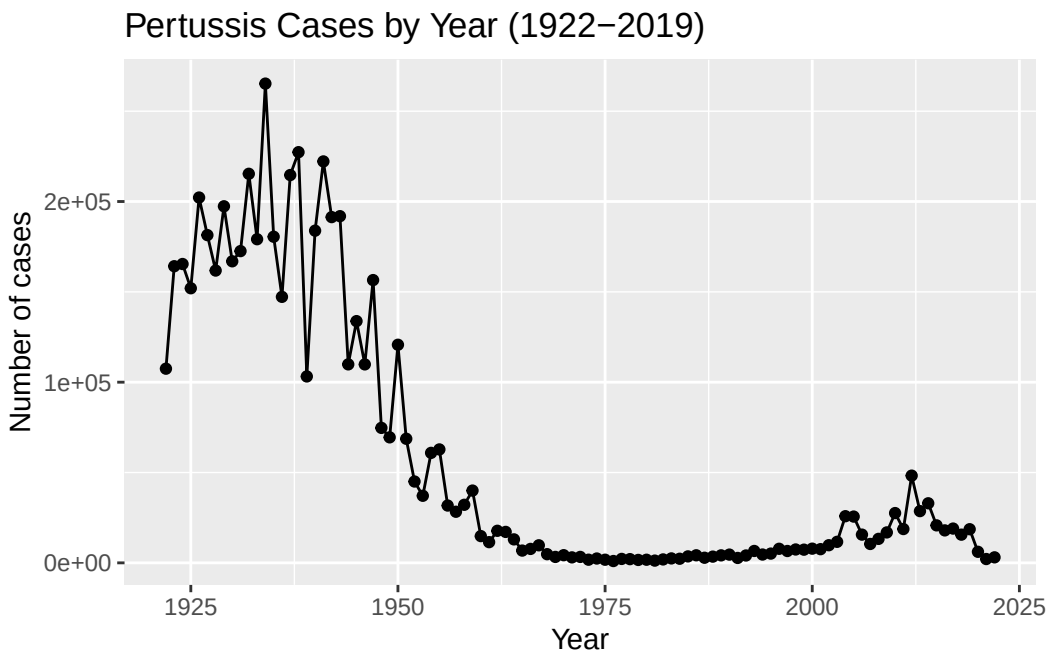
Class 18: Pertussis Mini Project

Carly Chang (A16843962)

Investigating pertusiss cases by year

> 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.

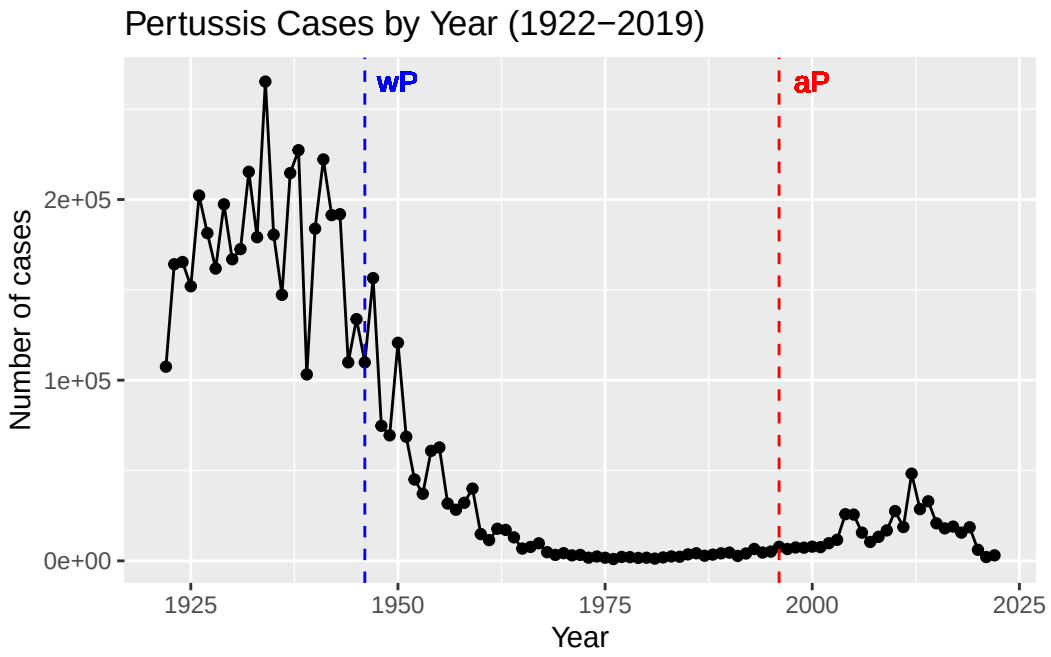
```
library(datapasta)

cdc <- data.frame(year = c(1922L,1923L,1924L,1925L,1926L,1927L,1928L,1929L,1930L,1931L,1932L
  cases = c(107473,164191,165418,152003,202210,181411,161799,197371,166914,172559,215343,179

library(ggplot2)

ggplot(cdc) +
  aes(year,cases) +
  geom_point() +
  geom_line() +
  labs(title="Pertussis Cases by Year (1922-2019)",x="Year",y="Number of cases")
```



A tale of two vaccines (wP & aP)

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?

```
ggplot(cdc) +
  aes(year,cases) +
  geom_point() +
  geom_line() +
  labs(title="Pertussis Cases by Year (1922-2019)",x="Year",y="Number of cases") +
  geom_vline(xintercept=1946,col="blue",linetype=2) +
  geom_vline(xintercept=1996,col="red",linetype=2) +
  geom_text(x=1950,y=max(cdc$cases),label="wP",color="blue") +
  geom_text(x=2000,y=max(cdc$cases),label="aP",color="red")
```



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

The introduction of the aP vaccine caused a slight increase in the number of cases especially around 2012. This could be due to more sensitive or widespread testing, pertussis bacterial evolution, anti-vaccination, etc.

Exploring CMI-PB data

```
# Allows us to read, write and process JSON data
library(jsonlite)
subject <- read_json("https://www.cmi-pb.org/api/subject", simplifyVector = TRUE)
head(subject, 3)
```

```
  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
  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
```

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

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

```
aP wP
87 85
```

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

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

```
Female   Male
   112     60
```

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

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

```
        American Indian/Alaska Native Asian Black or African American
  Female                             0    32                         2
  Male                               1    12                         3

        More Than One Race Native Hawaiian or Other Pacific Islander
  Female                 15                                       1
  Male                    4                                       1

        Unknown or Not Reported White
  Female                      14    48
  Male                         7    32
```

Working with dates:

```
library(lubridate)
```

```
Attaching package: 'lubridate'
```

```
The following objects are masked from 'package:base':

    date, intersect, setdiff, union
```

```
today() #What is today's date
```

```
[1] "2025-03-09"
```

```
today() - ymd("2000-01-01") #How many days have passed since new year 2000
```

```
Time difference of 9199 days
```

```
time_length( today() - ymd("2000-01-01"),  "years") #What is this in years?
```

```
[1] 25.18549
```

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?

```
# Use todays date to calculate age in days
subject$age <- today() - ymd(subject$year_of_birth)

library(dplyr)
```

```
Attaching package: 'dplyr'

The following objects are masked from 'package:stats':

    filter, lag

The following objects are masked from 'package:base':

    intersect, setdiff, setequal, union
```

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

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
     22      26      27      27      28      34
```

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

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
     22      32      34      36      39      57
```

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

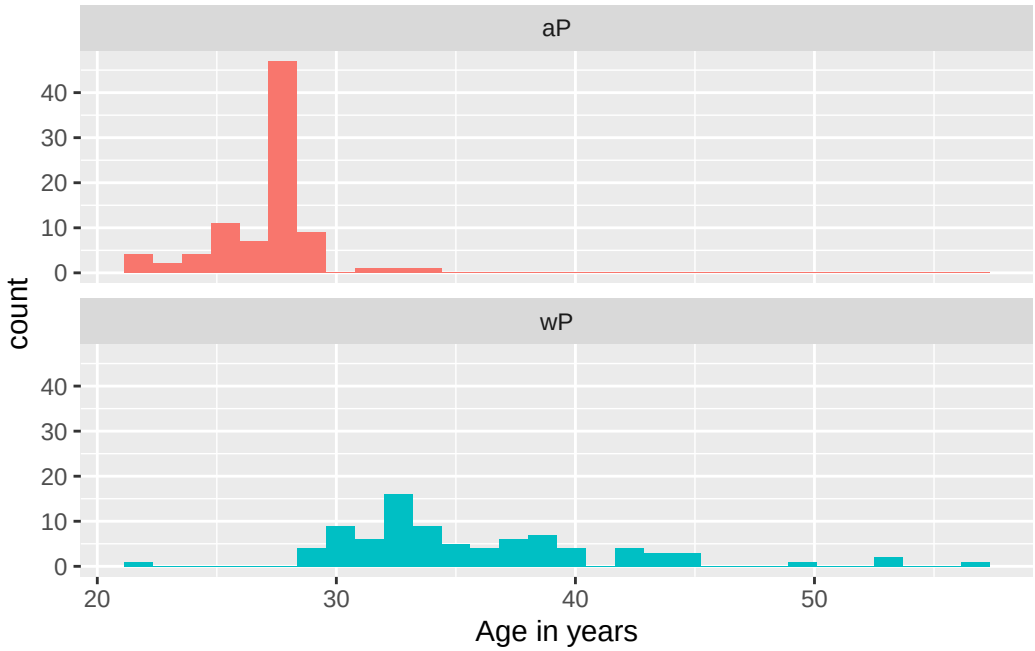
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
[1] 30.69678 51.07461 33.77413 28.65982 25.65914 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`.
```



The groups are clearly different based on the histograms.

> 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:

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

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

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

```
dim(meta)
```

```
[1] 1503   14
```

```
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
1 14312 days
2 14312 days
3 14312 days
4 14312 days
5 14312 days
6 14312 days
```

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.

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

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

```
dim(abdata)
```

```
[1] 52576    21
```

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

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

```
  IgE   IgG  IgG1  IgG2  IgG3  IgG4
 6698  5389 10117 10124 10124 10124
```

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

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

```
2020_dataset 2021_dataset 2022_dataset 2023_dataset
       31520         8085         7301         5670
```

Examine IgG Ab titer levels

Now using our joined/merged/linked abdata dataset filter() for IgG isotype:

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

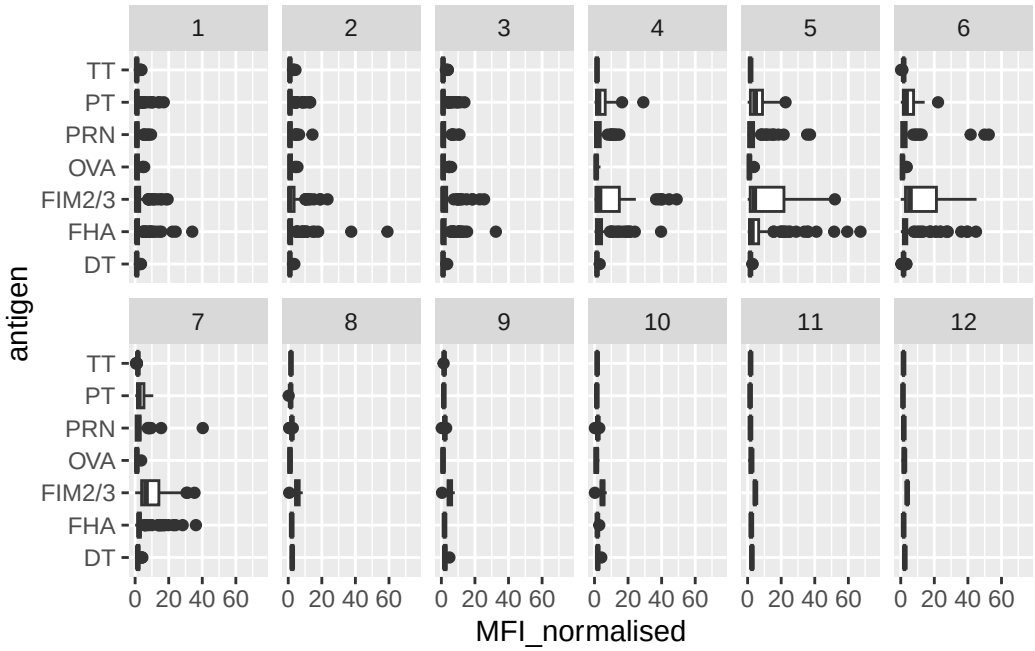
```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgG                TRUE      PT   68.56614       3.736992
2           1     IgG                TRUE     PRN  332.12718       2.602350
3           1     IgG                TRUE     FHA 1887.12263      34.050956
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
1 14312 days
2 14312 days
3 14312 days
4 15408 days
5 15408 days
6 15408 days
```

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 outside the scale range
(`stat_boxplot()`).
```



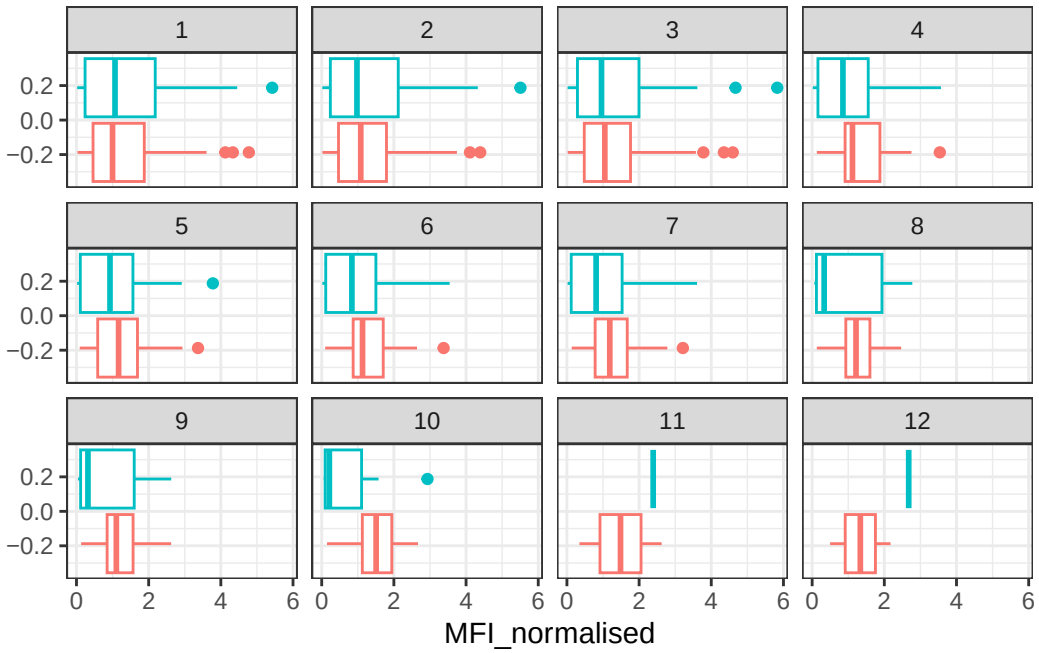
Q14. What antigens show differences in the level of IgG antibody titers recognizing them over time? Why these and not others?

PT, PRN, FIM2/3, FHA. These antigens are related to pertussis and are proteins produced by pertussis bacteria, which makes sense why there would be differences in the level of IgG antibody titers recognizing them over time.

Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("OVA", that is not in our vaccines) and a clear antigen of interest ("PT", Pertussis Toxin, one of the key virulence factors produced by the bacterium B. pertussis).

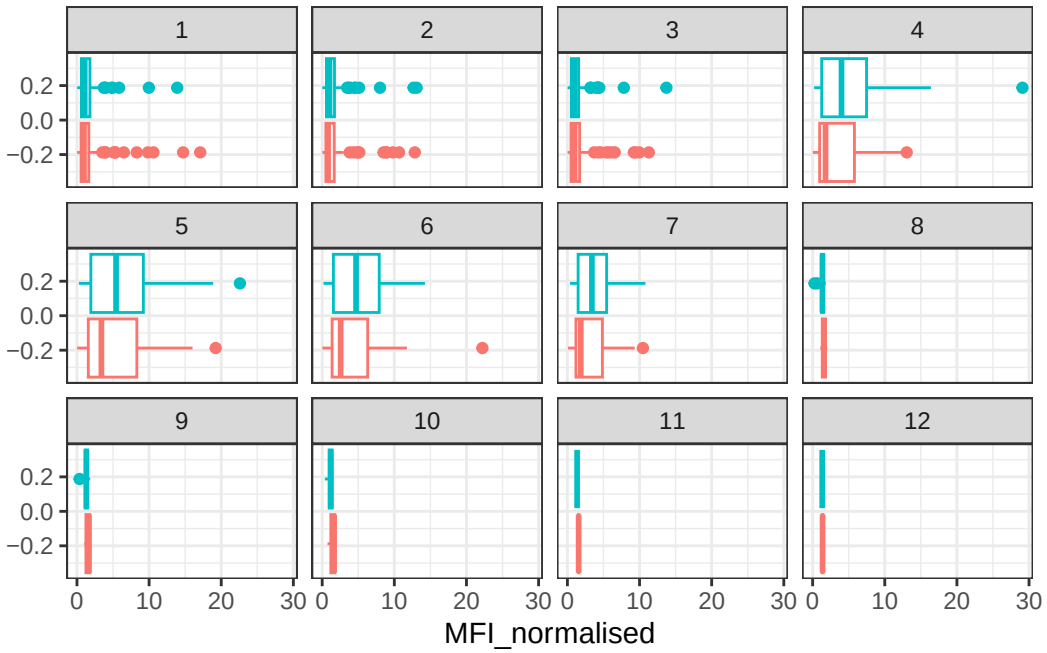
OVA antigen levels per visit (aP red, wP teal)

```
filter(igg, antigen=="OVA") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = F) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



PT antigen levels per visit (aP red, wP teal)

```
filter(igg, antigen=="PT") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = F) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



Q16. What do you notice about these two antigens time courses and the PT data in particular?

PT levels rises until visit 5 and then declines, while OVA levels stay pretty similar throughout the visits.

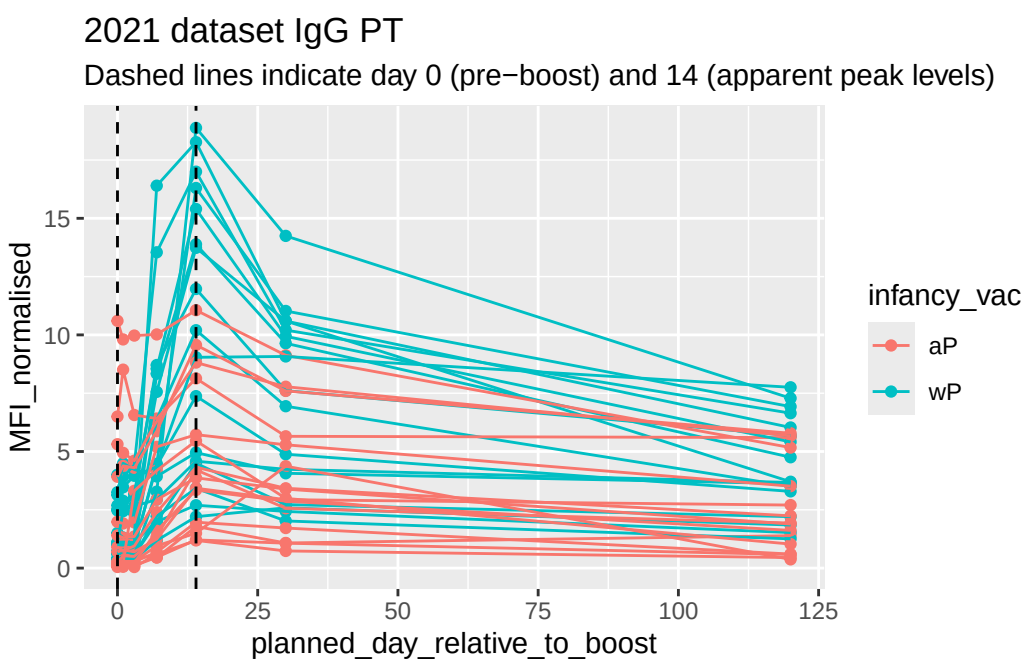
Q17. Do you see any clear difference in aP vs. wP responses?

No, both seem to have similar PT l evels, however, aP consistently has lower median despite similar ranges.

Looking at the 2021 dataset IgG PT antigen levels time-course:

```
abdata.21 <- abdata %>% filter(dataset == "2021_dataset")

abdata.21 %>%
  filter(isotype == "IgG",  antigen == "PT") %>%
  ggplot() +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
        col=infancy_vac,
        group=subject_id) +
    geom_point() +
    geom_line() +
```

```
    geom_vline(xintercept=0, linetype="dashed") +
    geom_vline(xintercept=14, linetype="dashed") +
  labs(title="2021 dataset IgG PT",
       subtitle = "Dashed lines indicate day 0 (pre-boost) and 14 (apparent peak levels)")
```



Q18. Does this trend look similar for the 2020 dataset?

Yes, it rises to a peak and then declines to 0.

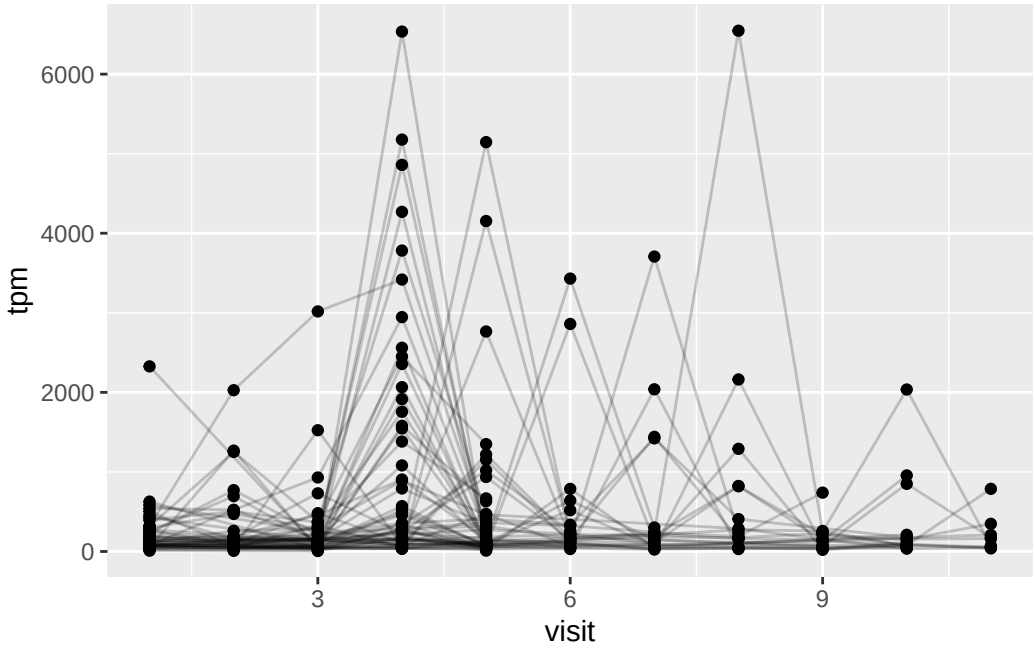
Obtaining CMI-PB RNASeq data

```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.7"

rna <- read_json(url, simplifyVector = TRUE)
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(visit, tpm, group=subject_id) +
  geom_point() +
  geom_line(alpha=0.2)
```



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

The gene is usually at its peak expression during visit 4 (sometimes visit 5).

Q21. Does this pattern in time match the trend of antibody titer data? If not, why not?

Yes, because the pertussis antigen levels decrease during visit 5 on average, right after when antibody titers peak.