Class 15: Investigating Pertussis Resurgence

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#1. Investigating pertussis cases by year

Install the `datapasta` package in R brain:

```
#install.packages("datapasta")
```

> 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.

Importing Data (use "Paste as data.frame" under "Addins" to copy the data off websties)

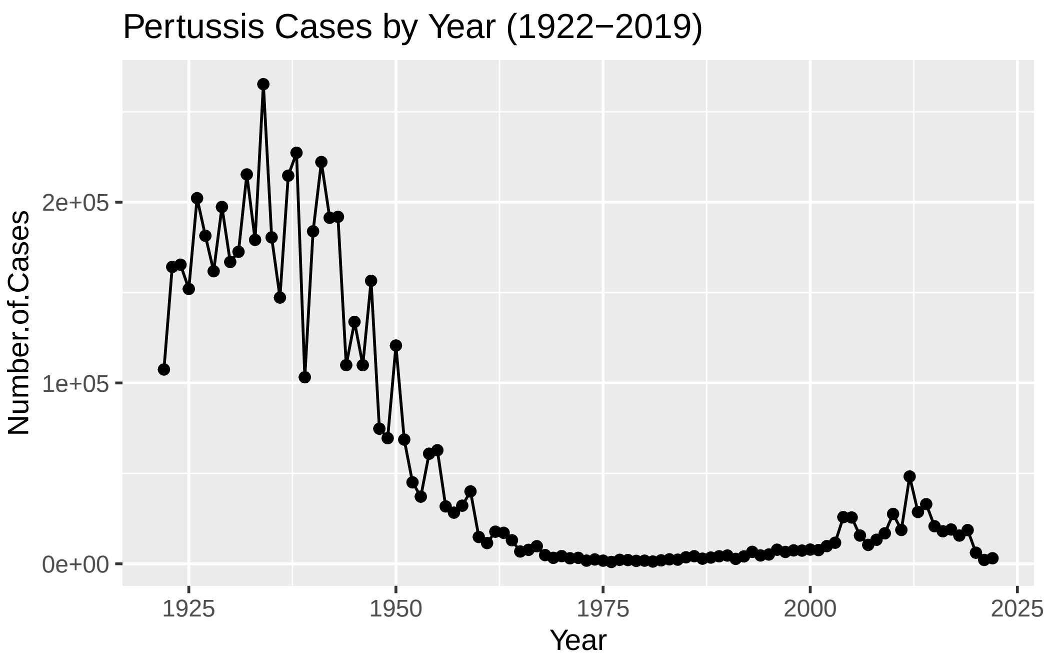
```
library(datapasta)
```

```
Warning: package 'datapasta' was built under R version 4.4.2
```

```
cdc <- data.frame(
                                 Year = c(1922L,1923L,1924L,1925L,
                                          1926L,1927L,1928L,1929L,1930L,1931L,
                                          1932L,1933L,1934L,1935L,1936L,
                                          1937L,1938L,1939L,1940L,1941L,1942L,
                                          1943L,1944L,1945L,1946L,1947L,
                                          1948L,1949L,1950L,1951L,1952L,
                                          1953L,1954L,1955L,1956L,1957L,1958L,
                                          1959L,1960L,1961L,1962L,1963L,
                                          1964L,1965L,1966L,1967L,1968L,1969L,
                                          1970L,1971L,1972L,1973L,1974L,
                                          1975L,1976L,1977L,1978L,1979L,1980L,
                                          1981L,1982L,1983L,1984L,1985L,
                                          1986L,1987L,1988L,1989L,1990L,
                                          1991L,1992L,1993L,1994L,1995L,1996L,
                                          1997L,1998L,1999L,2000L,2001L,
                                          2002L,2003L,2004L,2005L,2006L,2007L,
                                          2008L,2009L,2010L,2011L,2012L,
                                          2013L,2014L,2015L,2016L,2017L,2018L,
                                          2019L,2020L,2021L,2022L),
         Number.of.Cases = c(107473,164191,165418,152003,
                                          202210,181411,161799,197371,
                                          166914,172559,215343,179135,265269,
                                          180518,147237,214652,227319,103188,
                                          183866,222202,191383,191890,109873,
                                          133792,109860,156517,74715,69479,
                                          120718,68687,45030,37129,60886,
                                          62786,31732,28295,32148,40005,
                                          14809,11468,17749,17135,13005,6799,
                                          7717,9718,4810,3285,4249,3036,
                                          3287,1759,2402,1738,1010,2177,2063,
                                          1623,1730,1248,1895,2463,2276,
                                          3589,4195,2823,3450,4157,4570,
                                          2719,4083,6586,4617,5137,7796,6564,
                                          7405,7298,7867,7580,9771,11647,
                                          25827,25616,15632,10454,13278,
                                          16858,27550,18719,48277,28639,32971,
                                          20762,17972,18975,15609,18617,
                                          6124,2116,3044)
       )
```

Making ggplot

```
library(ggplot2)

plot <- ggplot(cdc) +
  aes(Year, Number.of.Cases) +
  geom_point() +
  geom_line() +
  labs(title="Pertussis Cases by Year (1922-2019)")
plot
```



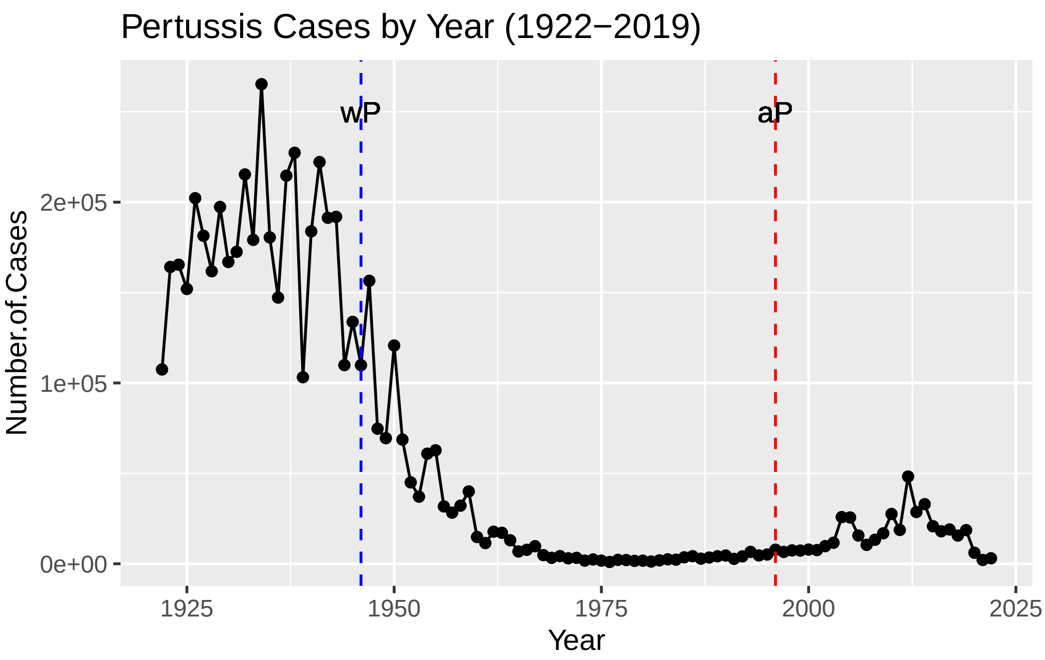
#2. A tale of two vaccines (wP & aP)

> 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?

```
plot +
  geom_vline(xintercept=1946, col="blue",lty="dashed")+
  geom_text(aes(x=1946, y=2.5*10^5, label="wP"))+
  geom_vline(xintercept=1996, col="red",lty="dashed") +
    geom_text(aes(x=1996, y=2.5*10^5, label="aP"))
```

```
Warning in geom_text(aes(x = 1946, y = 2.5 * 10^5, label = "wP")): All aesthetics have lengt
i Please consider using `annotate()` or provide this layer with data containing
  a single row.
```

```
Warning in geom_text(aes(x = 1996, y = 2.5 * 10^5, label = "aP")): All aesthetics have lengt
i Please consider using `annotate()` or provide this layer with data containing
  a single row.
```



There was a decrease and then plateau over time.

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

It is likely that the aP vaccine was not very effective as the number of cases did not decrease after the introduction. There may be many reasons for the truct such as increase sensitivity to PCR-based testing, vaccine hesitancy, and bacterial evolution.

#3. Exploring CMI-PB data

```
# Allows us to read, write and process JSON data
library(jsonlite)
```

Reading Data

```
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 
```

There are 87 aP subjects adn 85 wP subjects.

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

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

```

Female   Male 
   112     60 
```

There are 112 female and 60 males.

> 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
```

```r
library(lubridate)
```

```
Warning: package 'lubridate' was built under R version 4.4.2
```

```

Attaching package: 'lubridate'

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

    date, intersect, setdiff, union
```

```r
today()
```

```
[1] "2024-11-23"
```

```r
today() - ymd("2000-01-01")
```

```
Time difference of 9093 days
```

```r
time_length( today() - ymd("2000-01-01"),  "years")
```

```
[1] 24.89528
```

> 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?

```r
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
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 
```

```
t.test(ap$age,wp$age)
```

```


    Welch Two Sample t-test

data:  ap$age and wp$age
t = -12.918 days, df = 104.03, p-value < 2.2e-16
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -3686.855 days -2705.535 days
sample estimates:
Time differences in days
mean of x mean of y 
 9785.276 12981.471 
```

There are significant difference as the p-value is less than 0.05.

> 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)
```

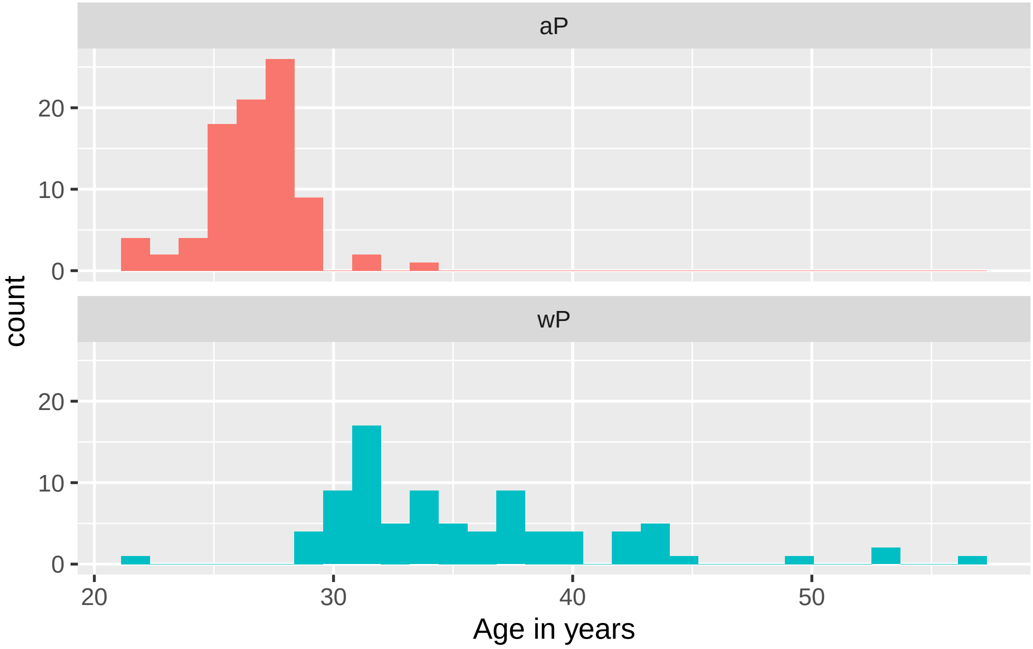
```
[1] 30.69678 51.07461 33.77413 28.65982 25.65914 28.77481
```

Age of all individual at time of boost is stored in age-at-boost.

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

```
library(ggplot2)
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`.
```



```
# Or use wilcox.test()
x <- t.test(time_length( wp$age, "years" ),
       time_length( ap$age, "years" ))

x$p.value
```

```
[1] 2.372101e-23
```

There is clearly a difference between the two groups as the graphs are very different with a very small p-value.

Joining multiple tables

```
# Complete the API URLs...
library(jsonlite)
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)
```

> 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] 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 14206 days
2 14206 days
3 14206 days
4 14206 days
5 14206 days
6 14206 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.

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

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

```r
dim(abdata)
```

```
[1] 52576    21
```

> 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  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?

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

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

#4. Examine IgG Ab titer levels

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

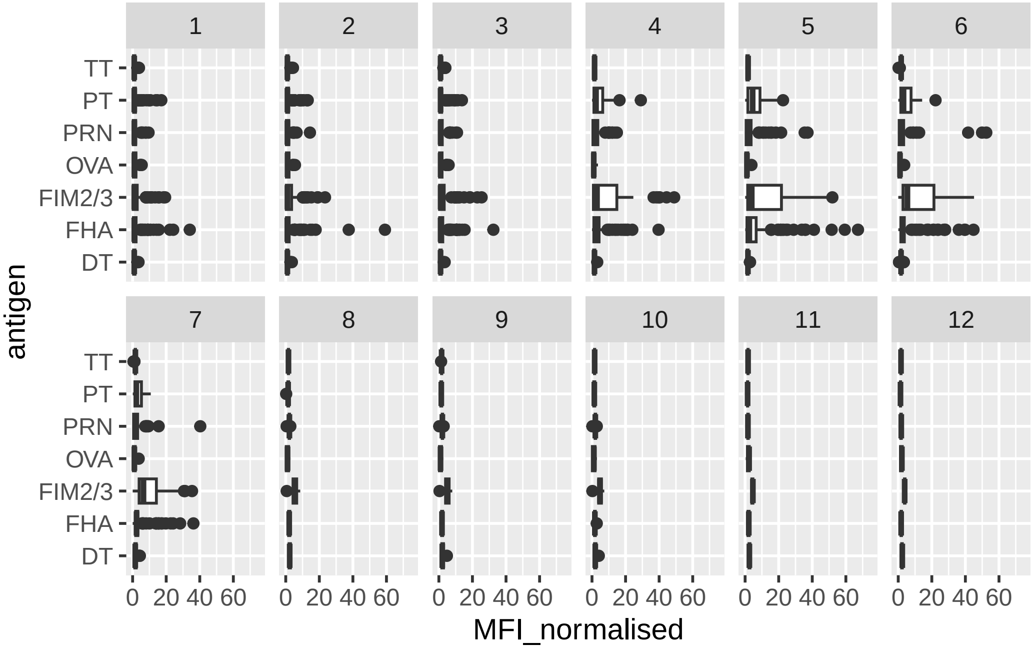
```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgG                TRUE      PT   68.56614       3.736992
2           1     IgG                TRUE     PRN  332.12718       2.602350
3           1     IgG                TRUE     FHA 1887.12263      34.050956
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
1 14206 days
```

```
2 14206 days
3 14206 days
4 15302 days
5 15302 days
6 15302 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()`).
```

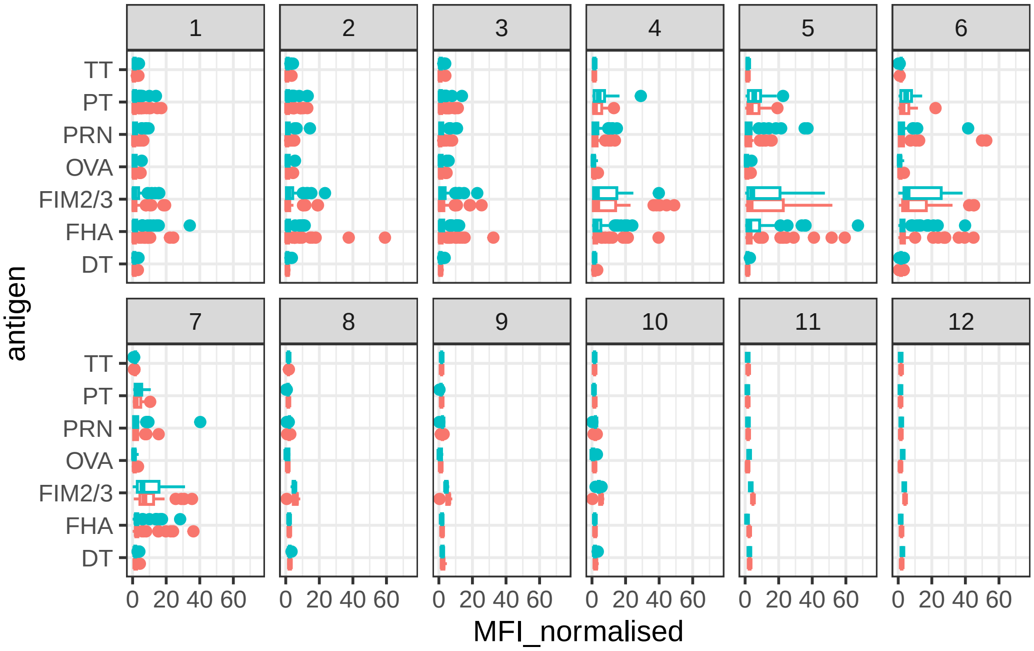


> Q14. What antigens show differences in the level of IgG antibody titers recognizing them over time? Why these and not others?

FIM2/3 show differences in the level of IgG antibody titer recognizing them over time.

```
ggplot(igg) +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  xlim(0,75) +
  theme_bw()
```

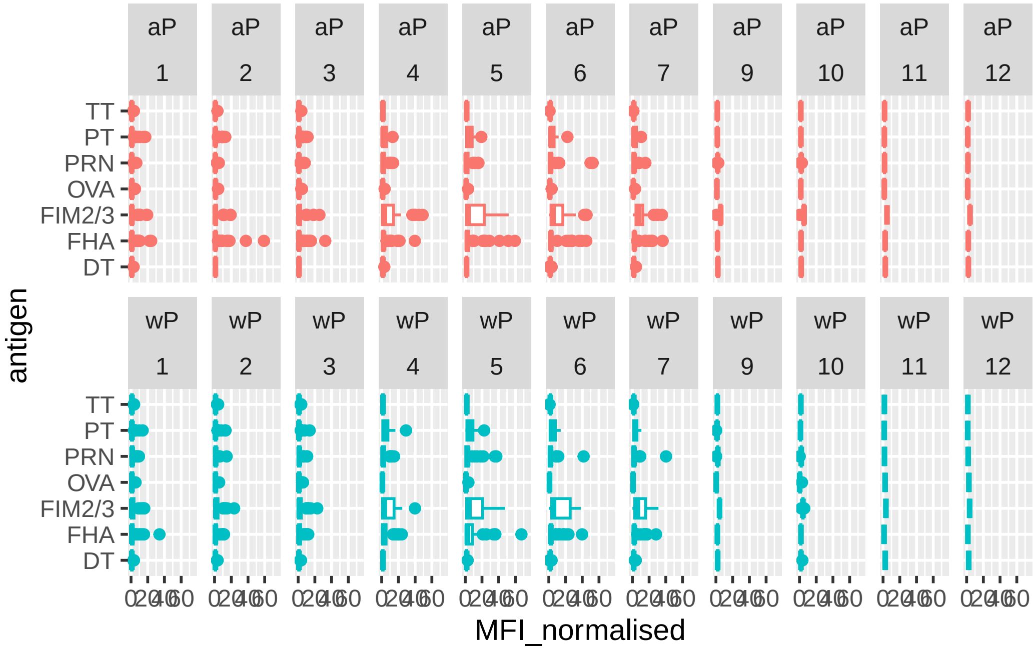
```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```



Adding `infancy_vac`

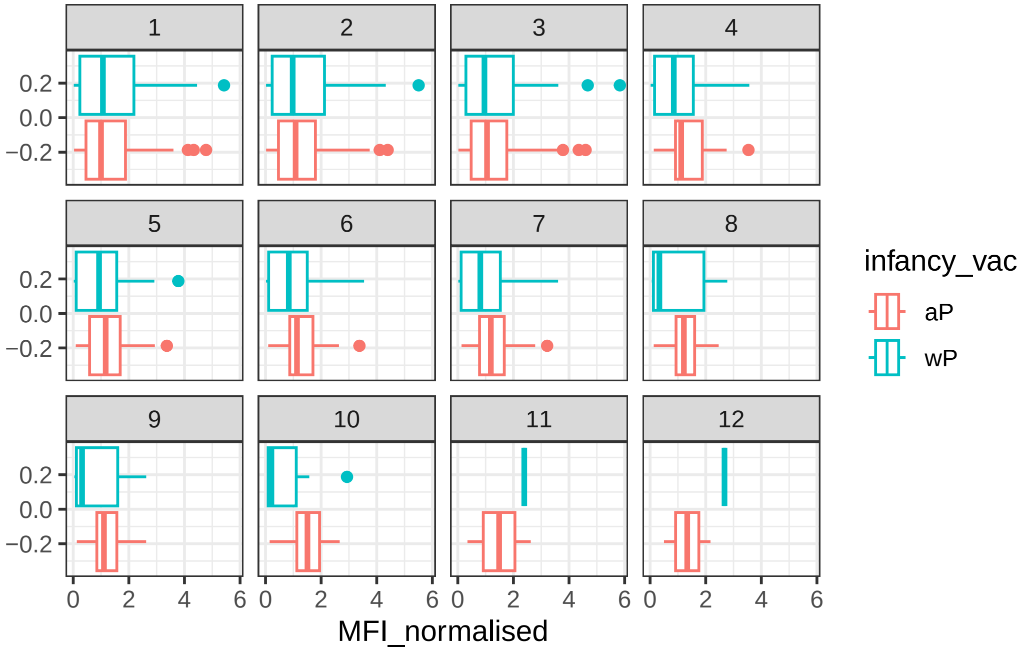
```
igg %>% filter(visit != 8) %>%
ggplot() +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  xlim(0,75) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```



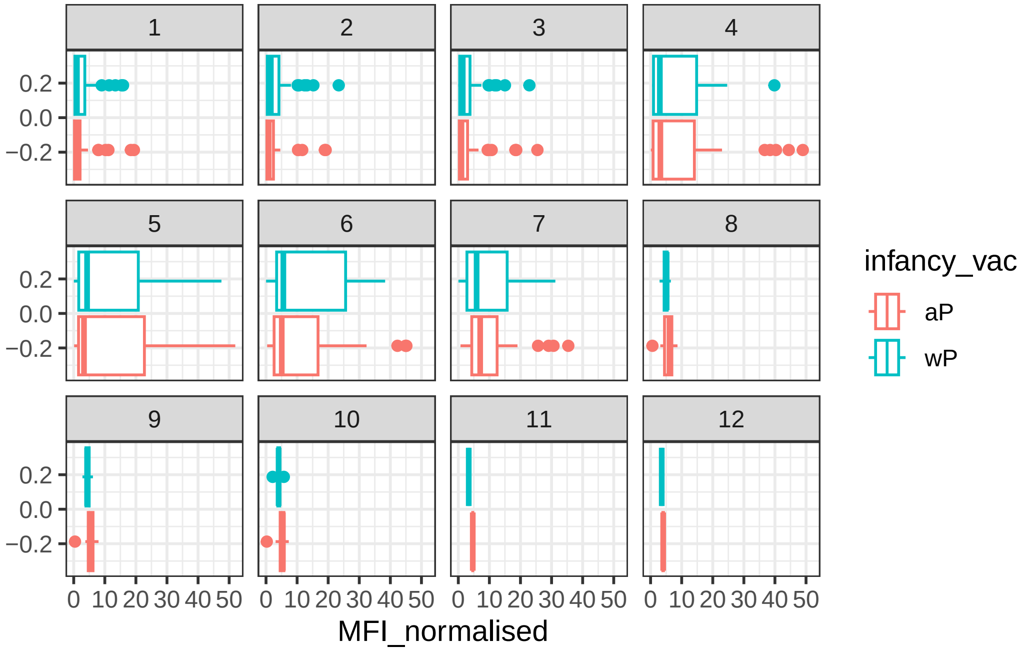
Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("OVA", that is not in our vaccines) and a clear antigen of interest ("PT", Pertussis Toxin, one of the key virulence factors produced by the bacterium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

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



for antigen FIM2/3

```
filter(igg, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = T) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



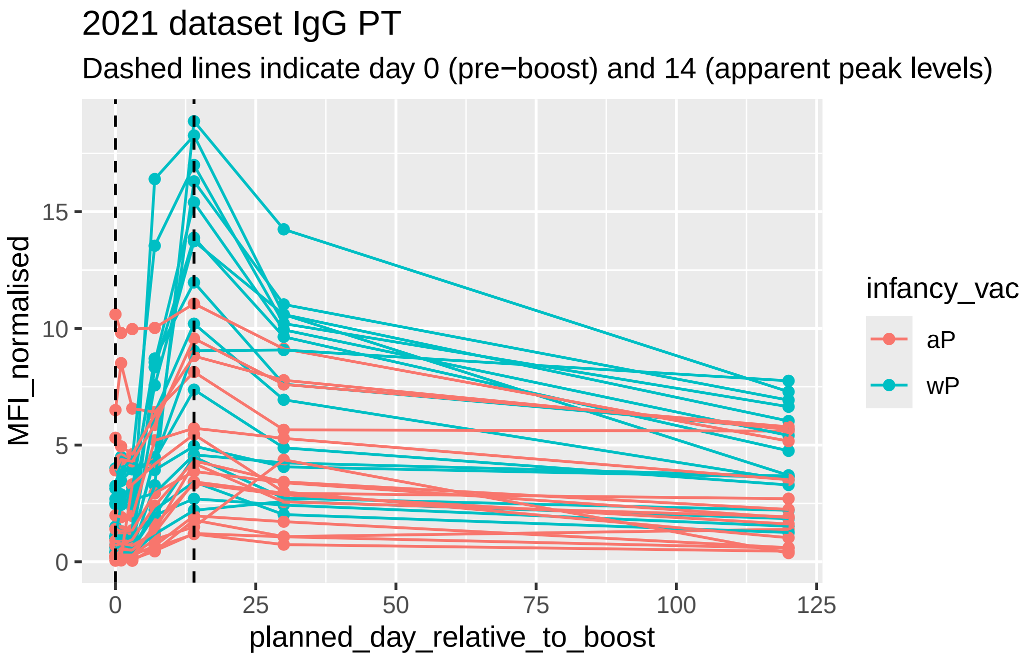
> Q16. What do you notice about these two antigens time courses and the PT data in particular?

The PT levels rises over time, while the OVA doesn't change that much.

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

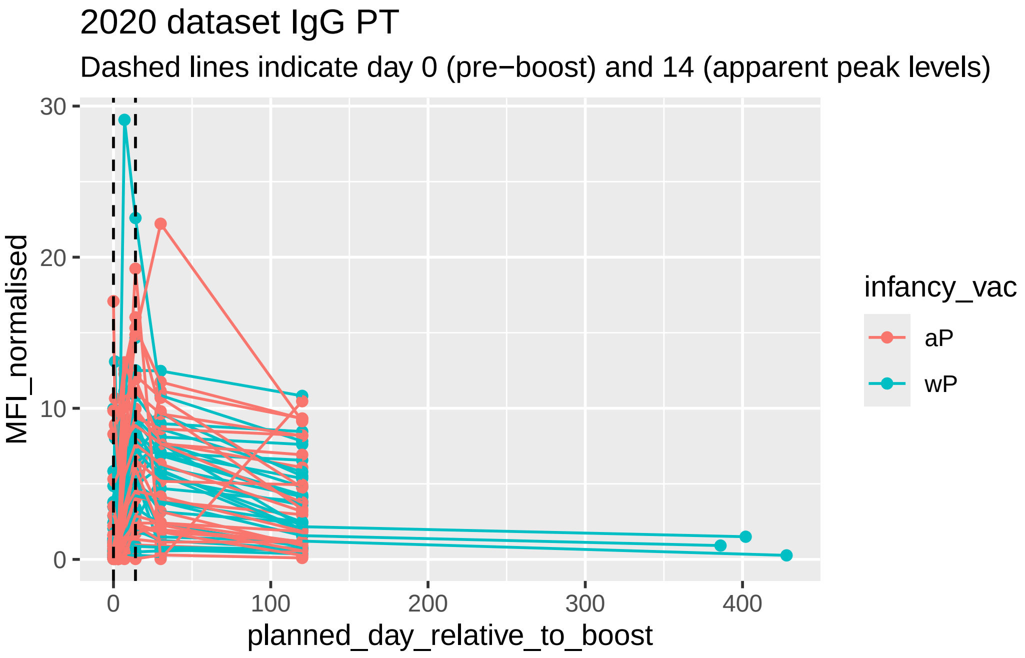
There is no clear difference in aP vs WP responses as the two colors aligns fairly similarly. 2021 dataset

```
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)")
```



2020 dataset

```
abdata.20 <- abdata %>% filter(dataset == "2020_dataset")

abdata.20 %>%
  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="2020 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?

The trends are slightly different. There is a clearer difference between aP and wP, but the general trend of large increase in the first 14 days is consistent.

#5. Obtaining CMI-PB RNASeq data

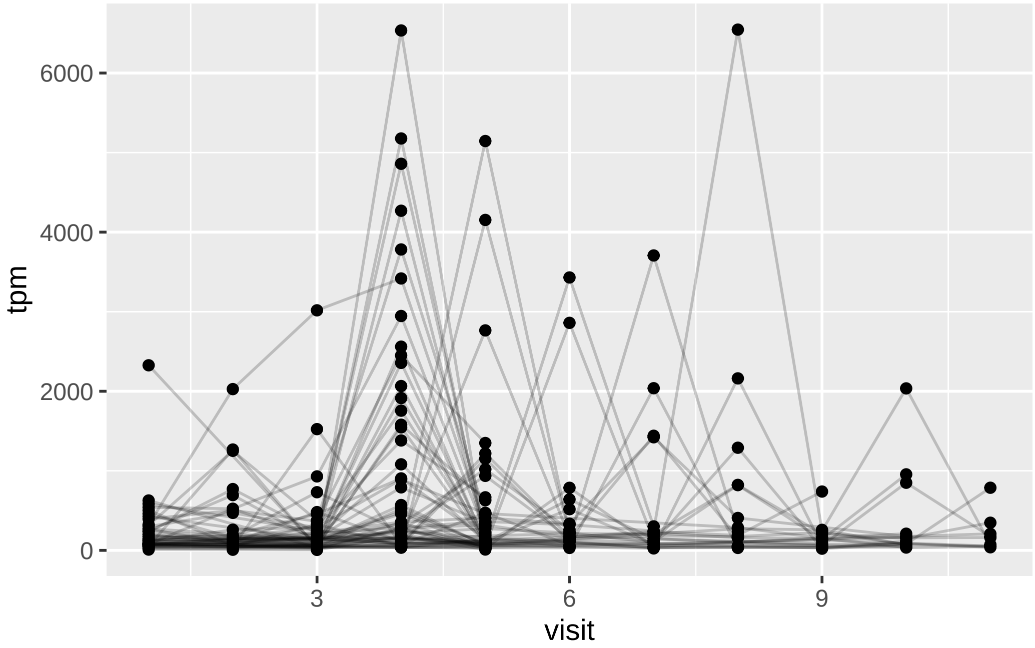
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.7"

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

```
#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(visit, tpm, group=subject_id) +
  geom_point() +
  geom_line(alpha=0.2)
```



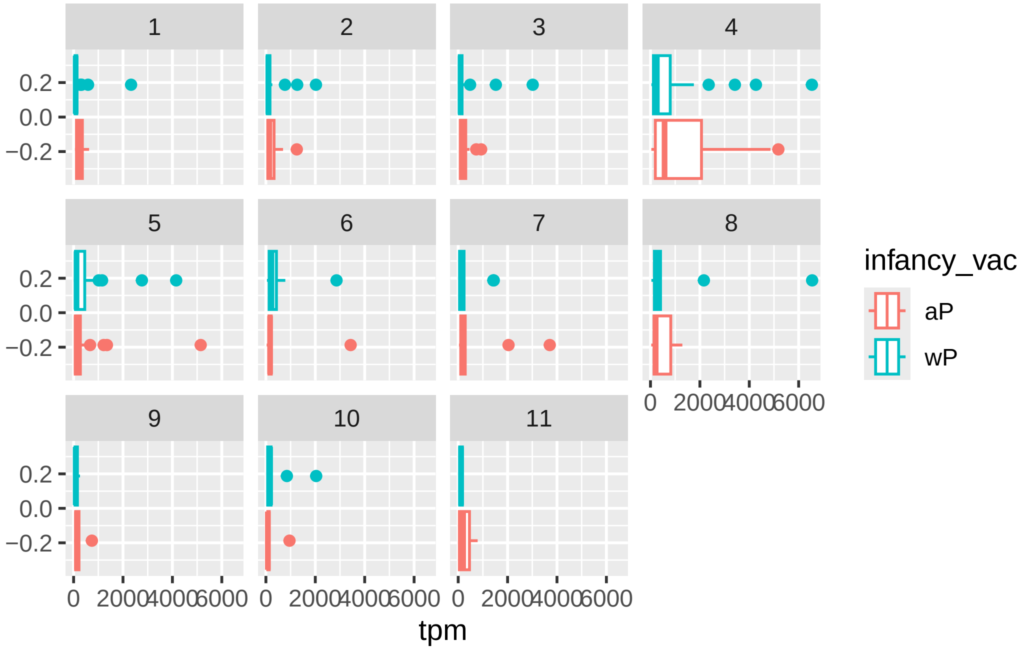
> Q20.: What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

It is at it's maximum level at 4 visits.

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

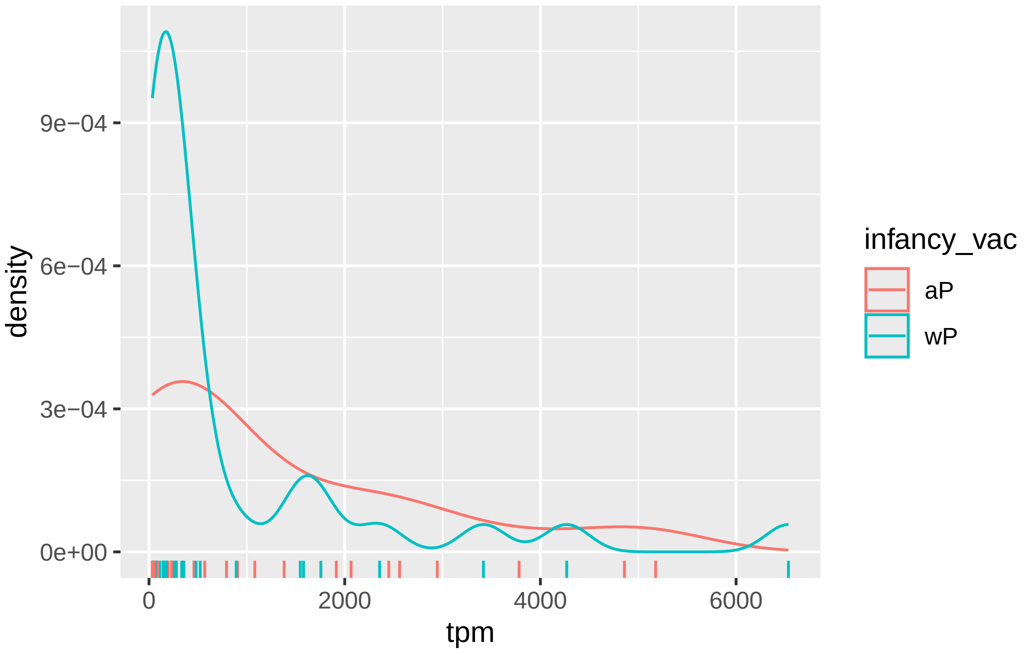
Both graph shows a significant increase at 4, but antibodies are more long lived.

```
ggplot(ssrna) +
  aes(tpm, col=infancy_vac) +
  geom_boxplot() +
  facet_wrap(vars(visit))
```



Focus on particular visit

```
ssrna %>%
  filter(visit==4) %>%
  ggplot() +
    aes(tpm, col=infancy_vac) + geom_density() +
    geom_rug()
```



#6. Working with larger datasets

```
# Change for your downloaded file path
rnaseq <- read.csv("2020LD_rnaseq.csv")

head(rnaseq,3)
```

```
  versioned_ensembl_gene_id specimen_id raw_count tpm
1         ENSG00000229704.1         209         0   0
2         ENSG00000229707.1         209         0   0
3         ENSG00000229708.1         209         0   0
```

```
dim(rnaseq)
```

```
[1] 10502460        4
```

How many genes reported for each specimen

```
n_genes <- table(rnaseq$specimen_id)
head( n_genes , 10)
```

```
    1     3     4     5     6    19    20    21    22    23
58347 58347 58347 58347 58347 58347 58347 58347 58347 58347
```

How many specimens?

```
length(n_genes)
```

```
[1] 180
```

Same number of genes per specimen?

```
all(n_genes[1]==n_genes)
```

```
[1] TRUE
```

Convert to wide format

```
library(tidyr)

rna_wide <- rnaseq %>%
  select(versioned_ensembl_gene_id, specimen_id, tpm) %>%
  pivot_wider(names_from = specimen_id, values_from=tpm)

dim(rna_wide)
```

```
[1] 58347   181
```

```
head(rna_wide[,1:7], 3)
```

```
# A tibble: 3 x 7
  versioned_ensembl_gene_id `209`  `74` `160`  `81` `102` `163`
  <chr>                     <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
1 ENSG00000229704.1             0     0     0     0     0     0
2 ENSG00000229707.1             0     0     0     0     0     0
3 ENSG00000229708.1             0     0     0     0     0     0
```