Class 19: Mini Project Investigating Pertussis Resurgence with CMI-PB

Aparajita Pranjal

6/7/23

1. Investigating pertussis cases by year

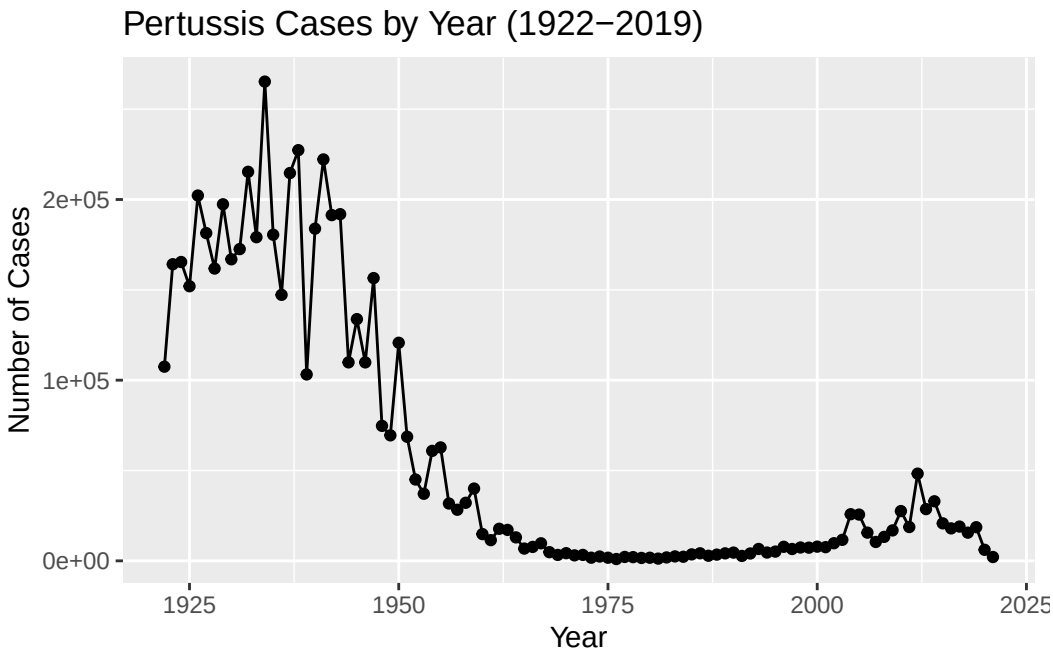
```
library(datapasta)
library(ggplot2)
```

- **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.

```
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),
```

```
  No..Reported.Pertussis.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)
)
```

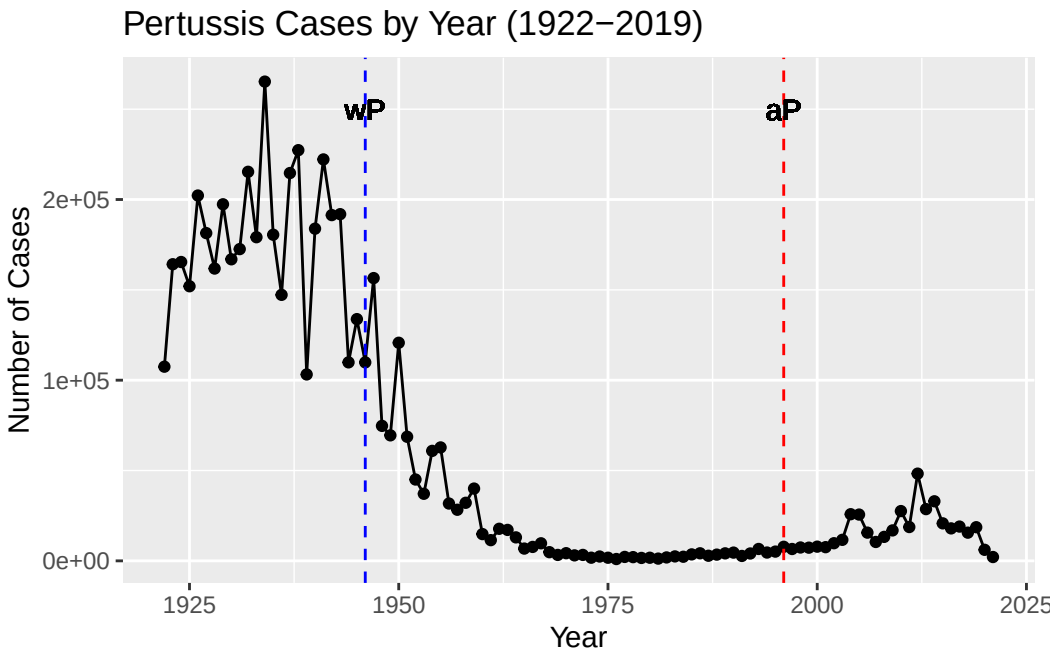
```
 ggplot(cdc) +
  aes(Year,No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  ggtitle("Pertussis Cases by Year (1922-2019)") +      xlab("Year") +
  ylab("Number of Cases")
```



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?

```
ggplot(cdc) +
 aes(Year,No..Reported.Pertussis.Cases) +
 geom_point() +
 geom_line() +
 geom_vline(xintercept = 1946, col = "blue", linetype= "dashed") +
 geom_vline(xintercept = 1996, col = "red", linetype = "dashed") +
 geom_text(x=1946, y=250000, label = "wP") +
 geom_text(x=1996, y=250000, label = "aP") +
 ggtitle("Pertussis Cases by Year (1922-2019)") +      xlab("Year") +
 ylab("Number of Cases")
```



- **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 new aP vaccine there is a slight increase in the number of Pertussis cases. This could be due to bacterial evolution which reduces the efficacy of the new vaccine, hesitancy to get the new vaccine, or a more sensitive PCR-based testing among other potential hypotheses.

3. 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
47 49
```

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

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

```
Female   Male
    66     30
```

- **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    18                         2
  Male                               1     9                         0

        More Than One Race Native Hawaiian or Other Pacific Islander
  Female                  8                                        1
  Male                    2                                        1

        Unknown or Not Reported White
  Female                      10    27
  Male                         4    13
```

Working with dates:

```
library(lubridate)
```

```
Attaching package: 'lubridate'

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

    date, intersect, setdiff, union
```

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

```
Time difference of 8558 days
```

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

```
[1] 23.43053
```

- **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)
head(subject$age)
```

```
Time differences in days
[1] 13671 20246 14767 12941 11845 12941
```

```
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.
   23      25      26      26      26      27
```

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

```
 Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
   28      32      35      37      40      55
```

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

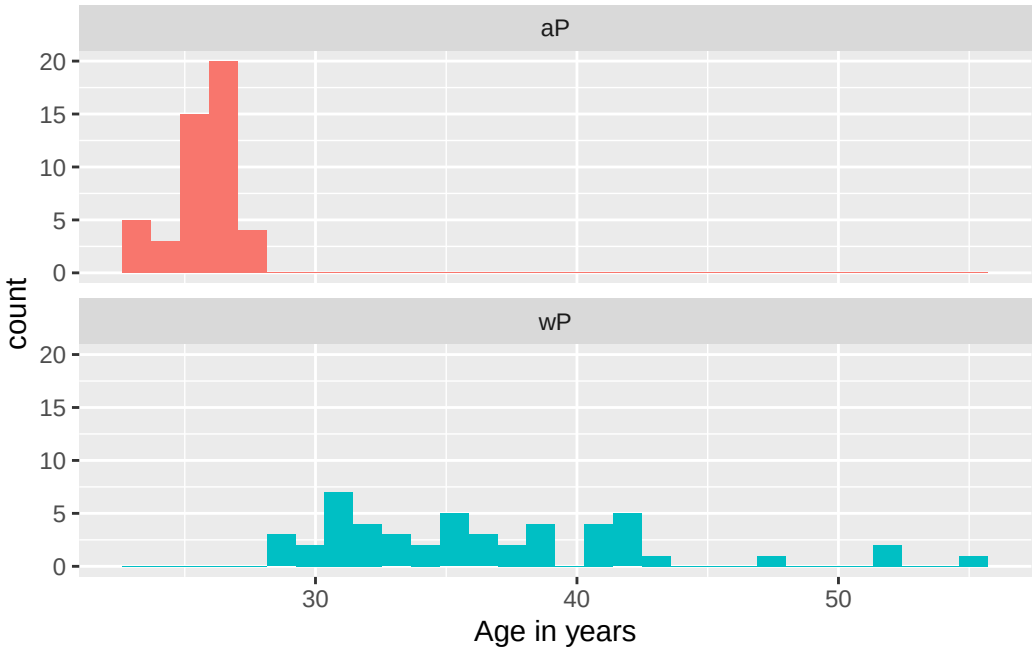
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
[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`.
```




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

x$p.value
```

```
[1] 1.316045e-16
```

```
y <- wilcox.test(time_length( wp$age, "years" ),
       time_length( ap$age, "years" ))
```

```
Warning in wilcox.test.default(time_length(wp$age, "years"),
time_length(ap$age, : cannot compute exact p-value with ties
```

```
y$p.value
```

```
[1] 1.992926e-17
```

Joining multiple tables:

```
# Complete the API URLs...
specimen <- read_json("https://www.cmi-pb.org/api/specimen", simplifyVector = TRUE)
titer <- read_json("https://www.cmi-pb.org/api/ab_titer", simplifyVector = TRUE)
```

- **Q10.** 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] 729  14
```

```
head(meta)
```

```
  specimen_id subject_id actual_day_relative_to_boost
1           1          1                           -3
2           2          1                          736
3           3          1                            1
4           4          1                            3
5           5          1                            7
6           6          1                           11
  planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
1                             0         Blood     1          wP         Female
2                           736         Blood    10          wP         Female
3                             1         Blood     2          wP         Female
4                             3         Blood     3          wP         Female
5                             7         Blood     4          wP         Female
6                            14         Blood     5          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 13671 days
2 13671 days
3 13671 days
```

```
4 13671 days
5 13671 days
6 13671 days
```

- **Q11.** 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] 32675    21
```

- **Q12.** How many specimens (i.e. entries in `abdata`) do we have for each `isotype`?

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

```
 IgE  IgG IgG1 IgG2 IgG3 IgG4
6698 1413 6141 6141 6141 6141
```

- **Q13.** What do you notice about the number of visit 8 specimens compared to other visits?

```
table(abdata$visit)
```

```
   1    2    3    4    5    6    7    8
5795 4640 4640 4640 4640 4320 3920   80
```

The number of specimens in visit 8 are significantly lower than all the other visits.

4. Examine IgG1 Ab titer levels

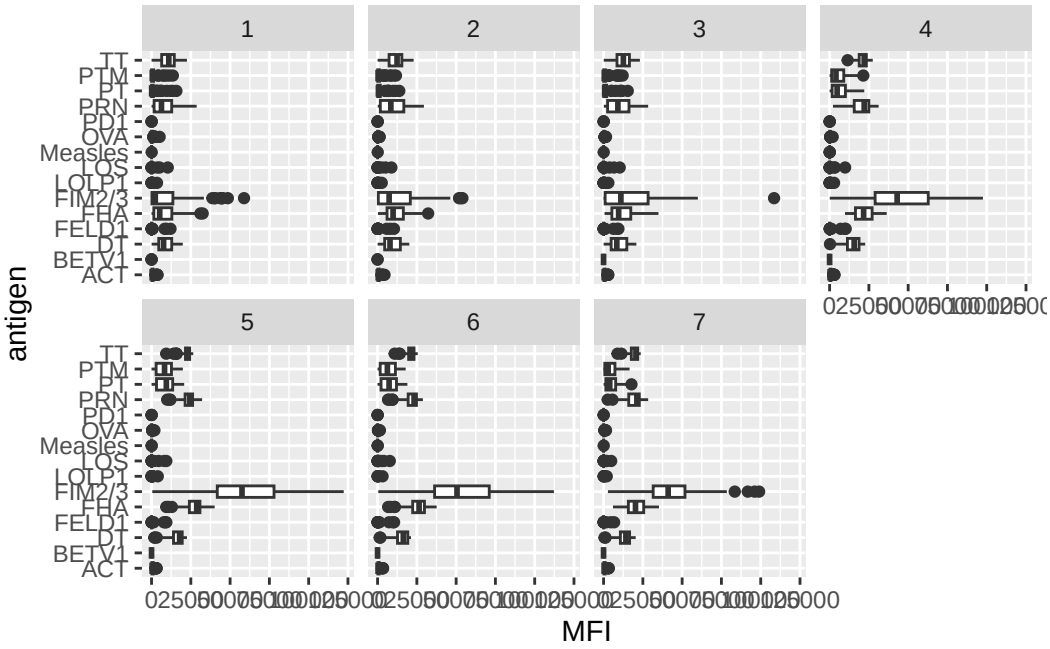
```
ig1 <- abdata %>% filter(isotype == "IgG1", visit!=8)
head(ig1)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1    IgG1                TRUE     ACT 274.355068      0.6928058
2           1    IgG1                TRUE     LOS  10.974026      2.1645083
3           1    IgG1                TRUE   FELD1   1.448796      0.8080941
4           1    IgG1                TRUE   BETV1   0.100000      1.0000000
5           1    IgG1                TRUE   LOLP1   0.100000      1.0000000
6           1    IgG1                TRUE Measles  36.277417      1.6638332
   unit lower_limit_of_detection subject_id actual_day_relative_to_boost
1 IU/ML                3.848750          1                           -3
2 IU/ML                4.357917          1                           -3
3 IU/ML                2.699944          1                           -3
4 IU/ML                1.734784          1                           -3
5 IU/ML                2.550606          1                           -3
6 IU/ML                4.438966          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
1 13671 days
2 13671 days
3 13671 days
4 13671 days
5 13671 days
6 13671 days
```

- **Q14.** Complete the following code to make a summary boxplot of Ab titer levels (MFI) for all antigens:

```
ggplot(ig1) +
  aes(MFI, antigen) +
  geom_boxplot() +
```

```
  facet_wrap(vars(visit), nrow=2)
```

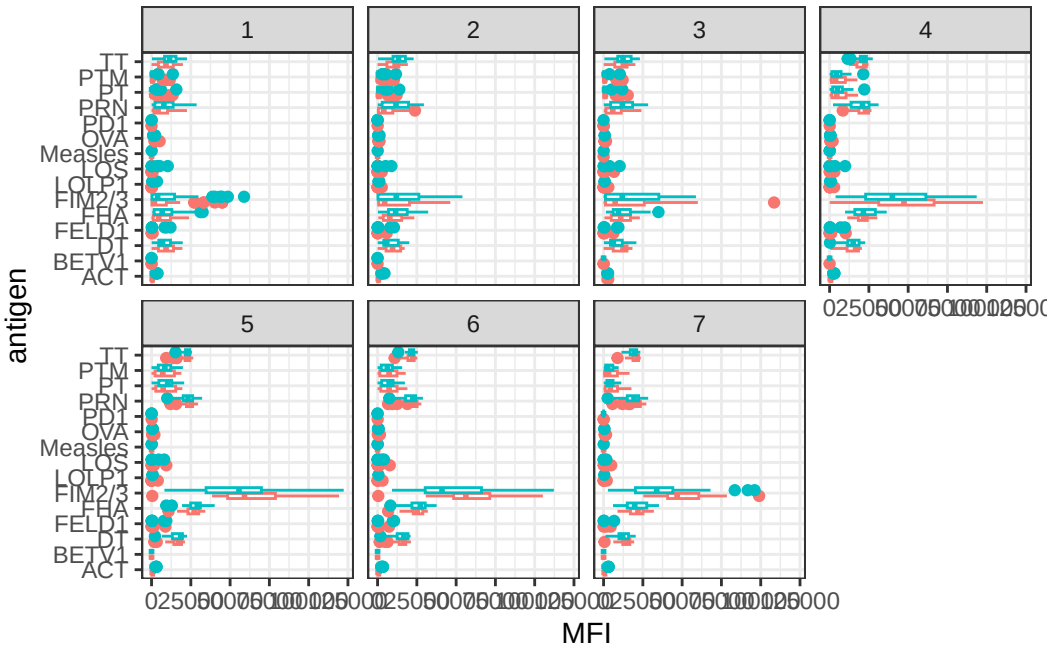


- **Q15.** What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

Most antigens remain constant but only FIM2/3 significantly increases.

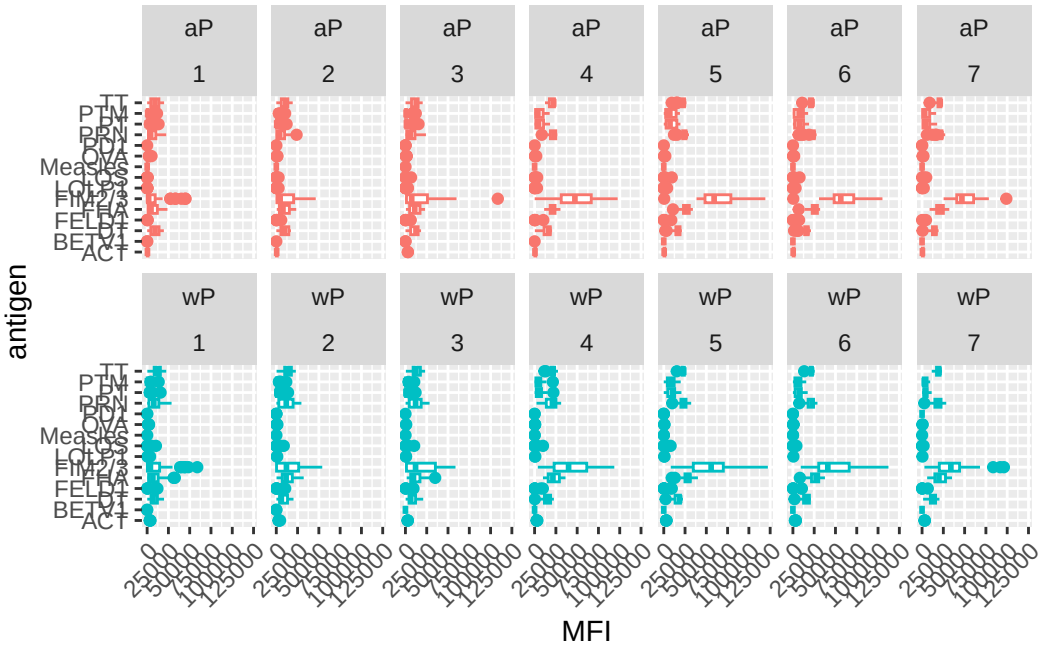
Adding color to plot:

```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  theme_bw()
```



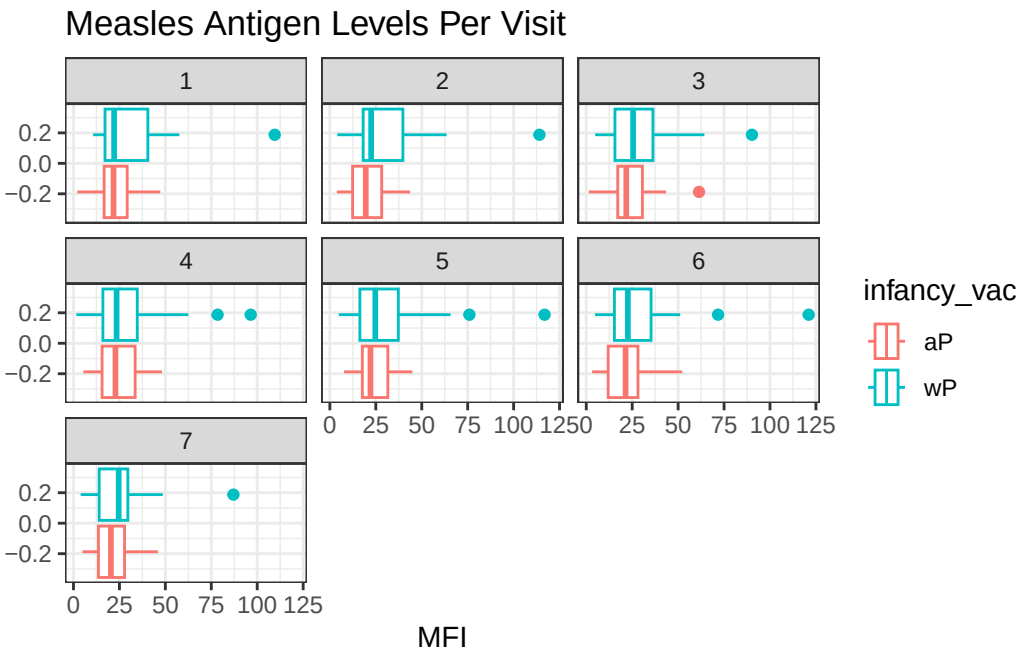
Another version of plot adding `infancy_vac` to the faceting:

```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(infancy_vac, visit), nrow=2) +
  theme(axis.text.x = element_text(angle = 45, hjust=1))
```



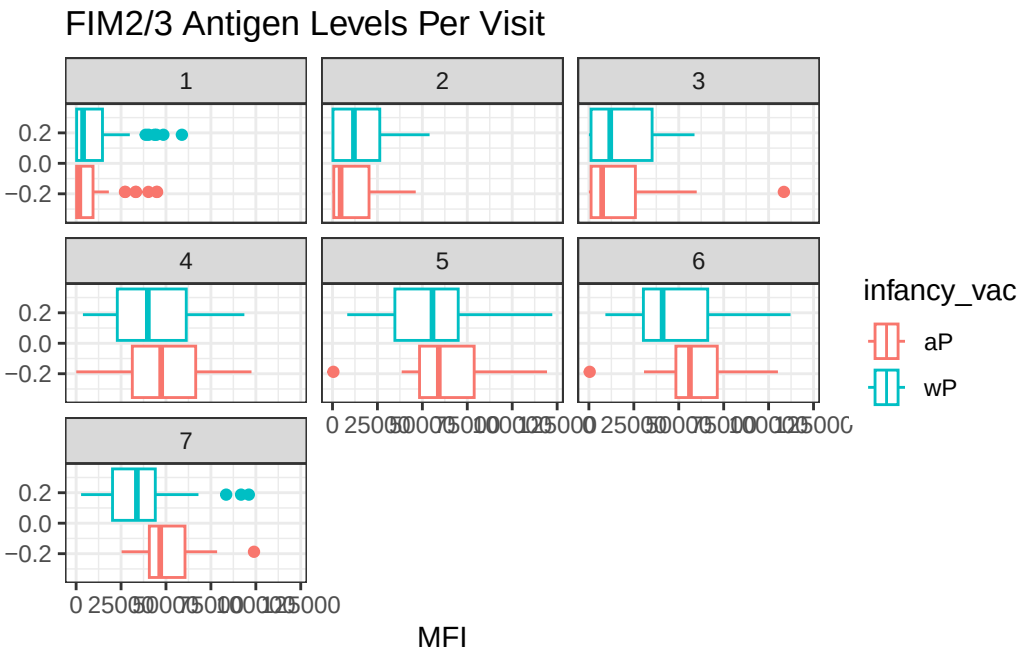
- **Q16.** Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen (**"Measles"**, that is not in our vaccines) and a clear antigen of interest (**"FIM2/3"**, extra-cellular fimbriae proteins from *B. pertussis* that participate in substrate attachment).

```
filter(ig1, antigen=="Measles") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = T) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  ggtitle("Measles Antigen Levels Per Visit")
```



Similar plot for FIM2/3 antigen:

```
filter(ig1, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = T) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  ggtitle("FIM2/3 Antigen Levels Per Visit")
```



- **Q17.** What do you notice about these two antigens time courses and the FIM2/3 data in particular?

Over time, the levels of FIM2/3 increase and surpass those of Measles by a significant margin. These levels seem to reach their highest point during visit 5 and subsequently decline. This pattern seems to be consistent for both wP and aP subjects.

- **Q18.** Do you see any clear difference in aP vs. wP responses?

No, there is no clear difference between the two vaccines, they both follow a similar trend. The wP seems to have a larger range of MFI values than aP overall.

5. Obtaining CMI-PB RNASeq data

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

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

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

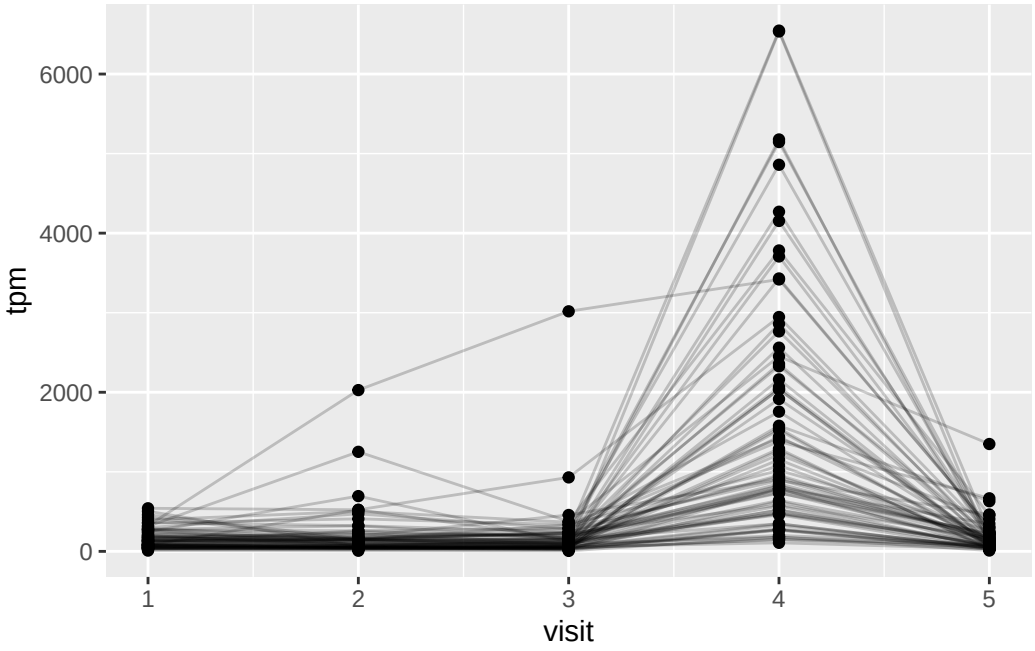
```
Joining with `by = join_by(specimen_id)`
```

```
head(ssrna)
```

```
  versioned_ensembl_gene_id specimen_id raw_count      tpm subject_id
1         ENSG00000211896.7         344     18613  929.640         44
2         ENSG00000211896.7         243      2011  112.584         31
3         ENSG00000211896.7         261      2161  124.759         33
4         ENSG00000211896.7         282      2428  138.292         36
5         ENSG00000211896.7         345     51963 2946.136         44
6         ENSG00000211896.7         244     49652 2356.749         31
  actual_day_relative_to_boost planned_day_relative_to_boost specimen_type
1                            3                             3         Blood
2                            3                             3         Blood
3                           15                            14         Blood
4                            1                             1         Blood
5                            7                             7         Blood
6                            7                             7         Blood
  visit infancy_vac biological_sex              ethnicity                race
1     3          aP         Female     Hispanic or Latino More Than One Race
2     3          wP         Female Not Hispanic or Latino               Asian
3     5          wP           Male     Hispanic or Latino More Than One Race
4     2          aP         Female     Hispanic or Latino               White
5     4          aP         Female     Hispanic or Latino More Than One Race
6     4          wP         Female Not Hispanic or Latino               Asian
  year_of_birth date_of_boost      dataset        age
1    1998-01-01    2016-11-07 2020_dataset  9288 days
2    1989-01-01    2016-09-26 2020_dataset 12575 days
3    1990-01-01    2016-10-10 2020_dataset 12210 days
4    1997-01-01    2016-10-24 2020_dataset  9653 days
5    1998-01-01    2016-11-07 2020_dataset  9288 days
6    1989-01-01    2016-09-26 2020_dataset 12575 days
```

- **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)?

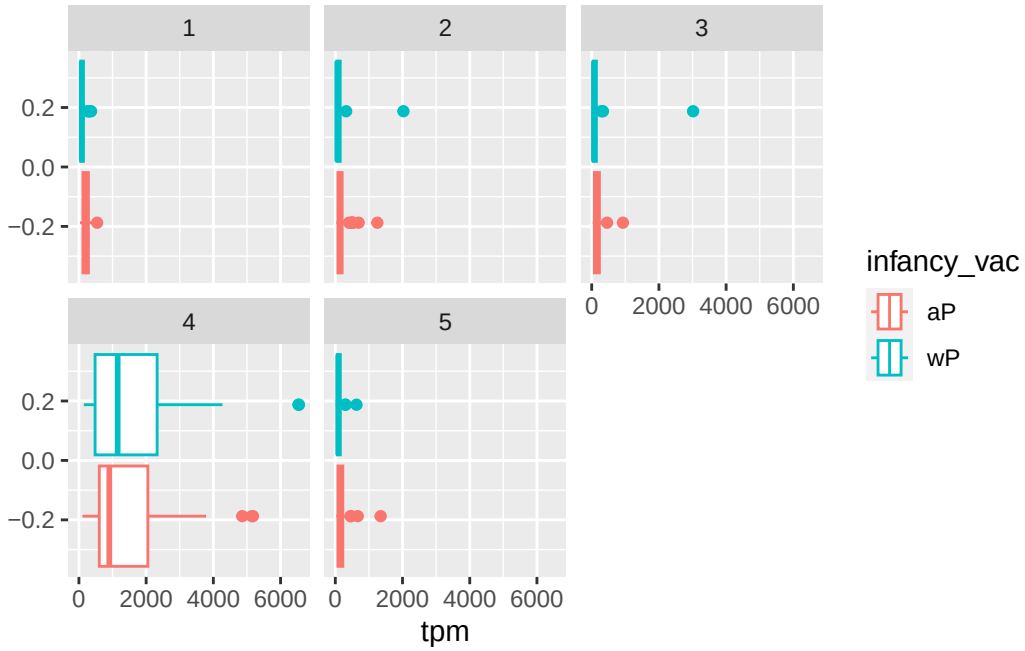
The gene expression is highest in visit 4.

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

Yes, the trend does match our plot in Q15 as visit 4 has a much larger peak than the other visits.

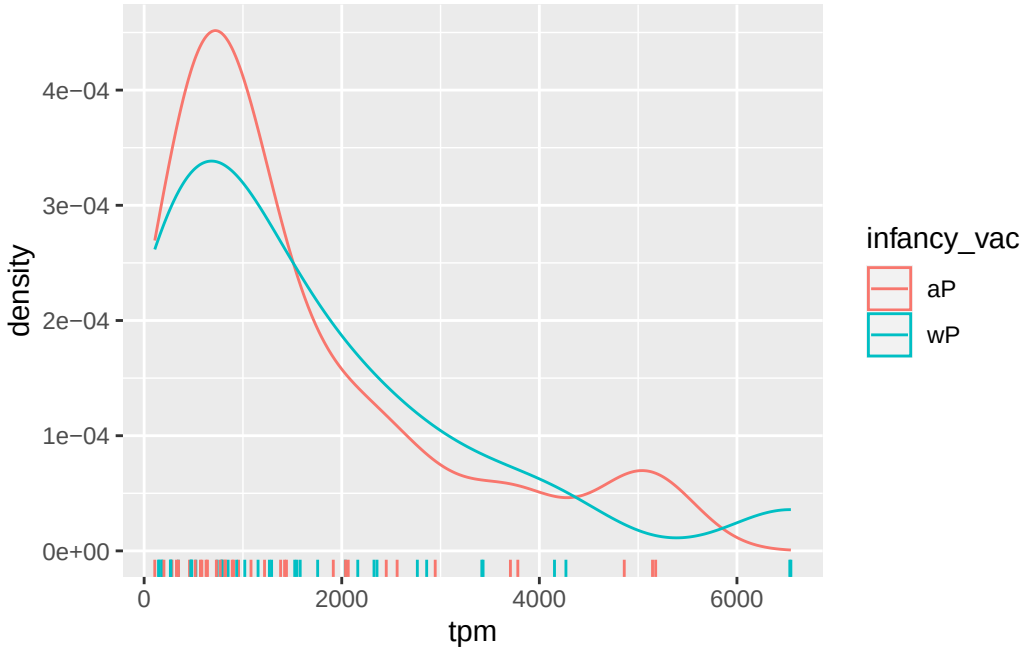
Modifying plot with faceting by `infancy_vac` status:

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



Only plotting data for visit 4 (there is however no obvious wP vs. aP differences even in this one):

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



6. Working with larger datasets [OPTIONAL]

```
# 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
```

Working with long format data:

```
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
```

```
length(n_genes)
```

```
[1] 180
```

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

```
[1] TRUE
```

Converting 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
```