# Class19

Shayan Malekpoor

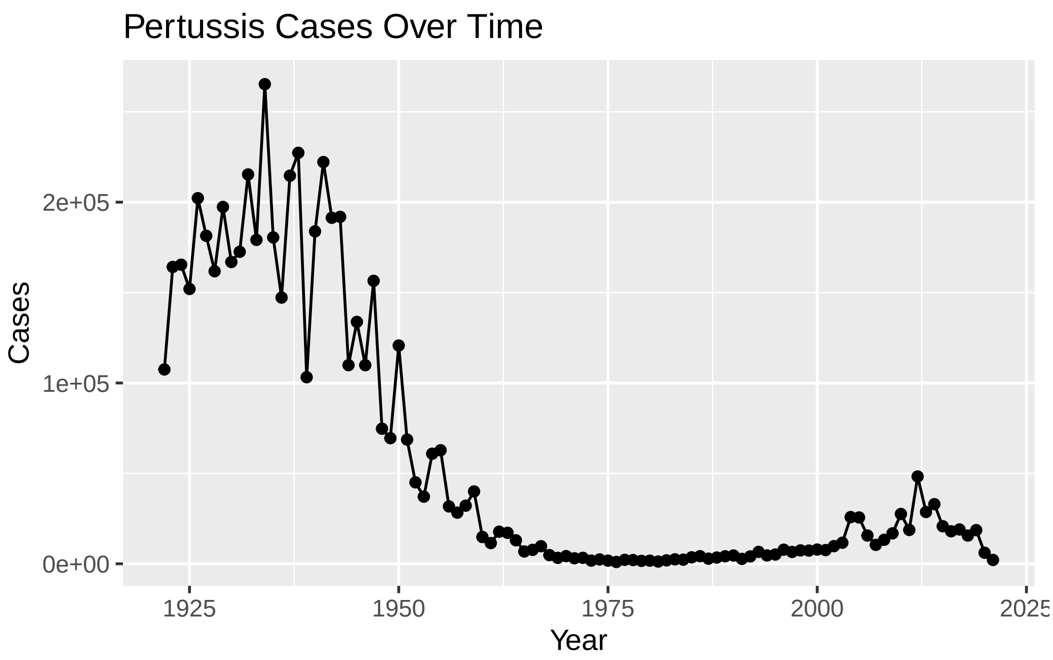
## Investigating pertussis cases by year

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)
library(ggplot2)
```

```
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)
       )
```

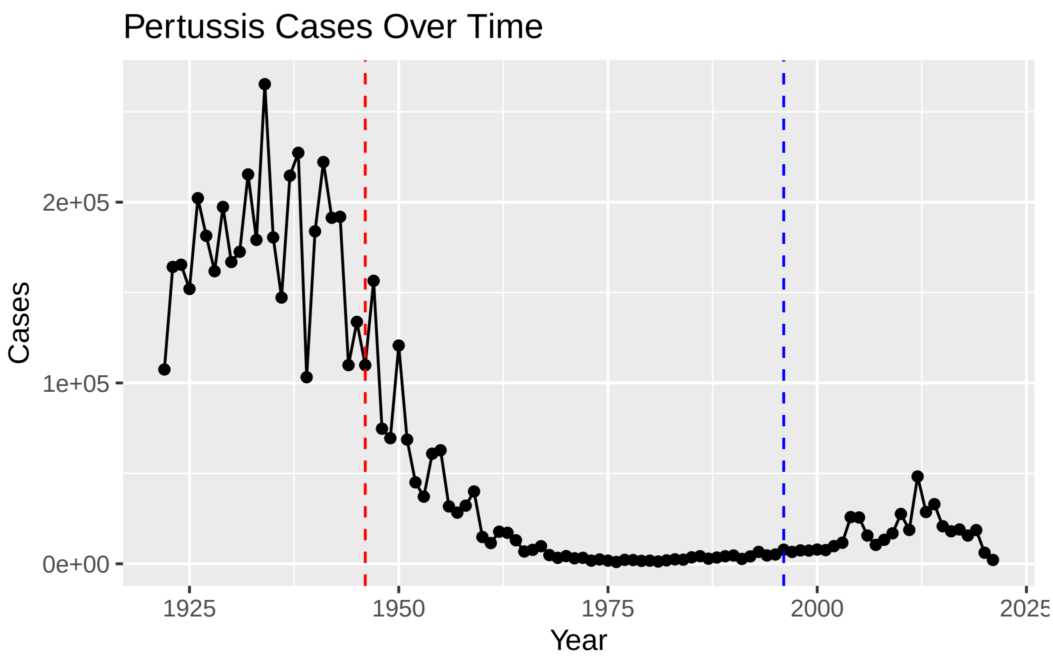
```
ggplot(cdc) +
  aes(x = Year, y = No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  labs(x = "Year", y = "Cases", title = "Pertussis Cases Over Time")
```



### 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?

```r
ggplot(cdc) +
  aes(x = Year, y = No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  geom_vline(xintercept = 1946, linetype = "dashed", color = "red") +
  geom_vline(xintercept = 1996, linetype = "dashed", color = "blue") +

  labs(x = "Year", y = "Cases", title = "Pertussis Cases Over Time")
```



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

### Exploring CMI-PB data

#### The CMI-PB API returns 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


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
```

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

```
[1] 23.45243
```

Q7. Using this approach determine (i) the average age of wP individuals, (ii) the average age of aP individuals;

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

```
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
```

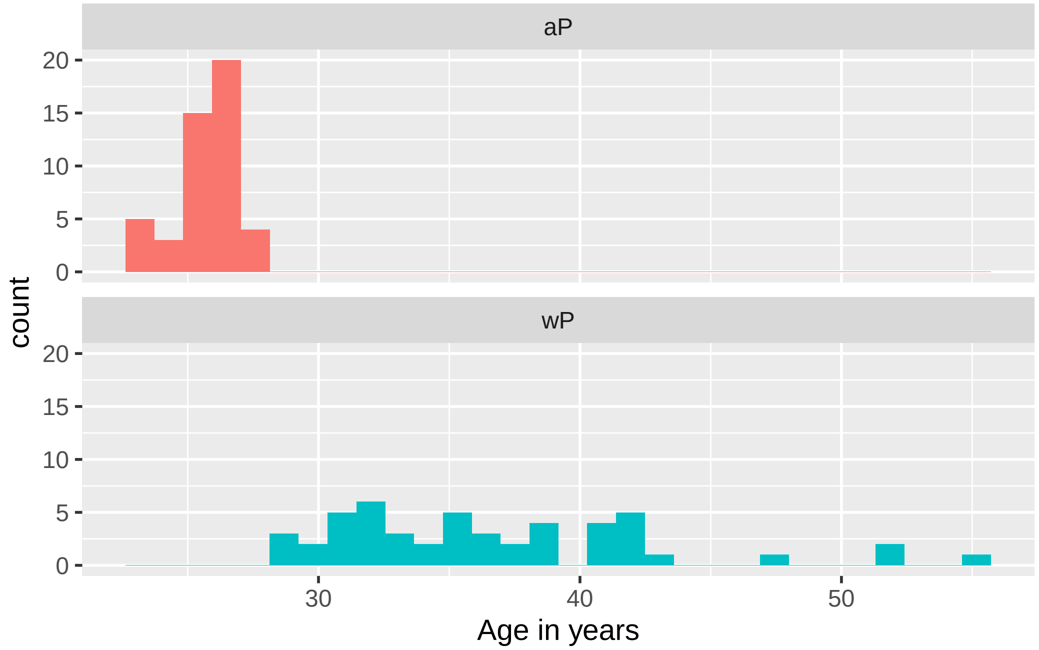
Q9. With the help of a faceted boxplot or histogram (see below), do you think these two groups are significantly different? not significant difference.

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

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

```
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`.
```



### Joining multiple tables

```r
# 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:

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

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

```r
dim(meta)
```

```
[1] 729  14
```

```r
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 13679 days
2 13679 days
3 13679 days
4 13679 days
5 13679 days
6 13679 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? lower than the other visits

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

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

### Examine IgG1 Ab titer levels

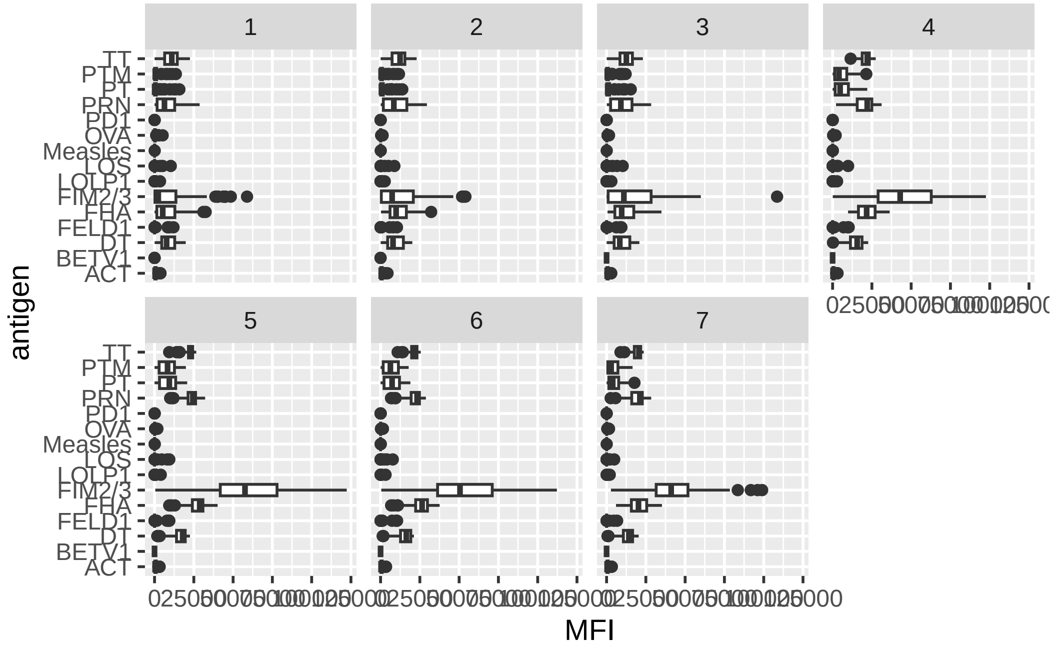
```
ig1 <- abdata %>% filter(isotype == "IgG1", visit!=8)
head(ig1)
```

```
  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 13679 days
2 13679 days
3 13679 days
4 13679 days
5 13679 days
6 13679 days
```

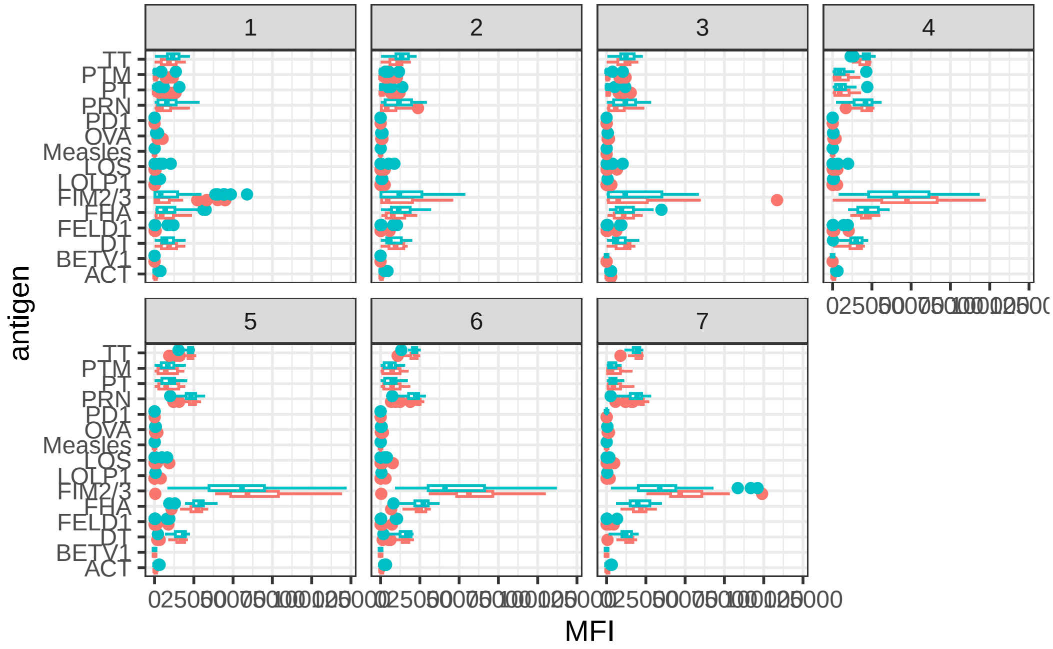
**Q14.** Complete the following code to make a summary boxplot of Ab titer levels (MFI) for all antigens:

```
ggplot(ig1) +
  aes(x = MFI, y = antigen) +
  geom_boxplot() +
  facet_wrap(vars(visit), nrow = 2)
```



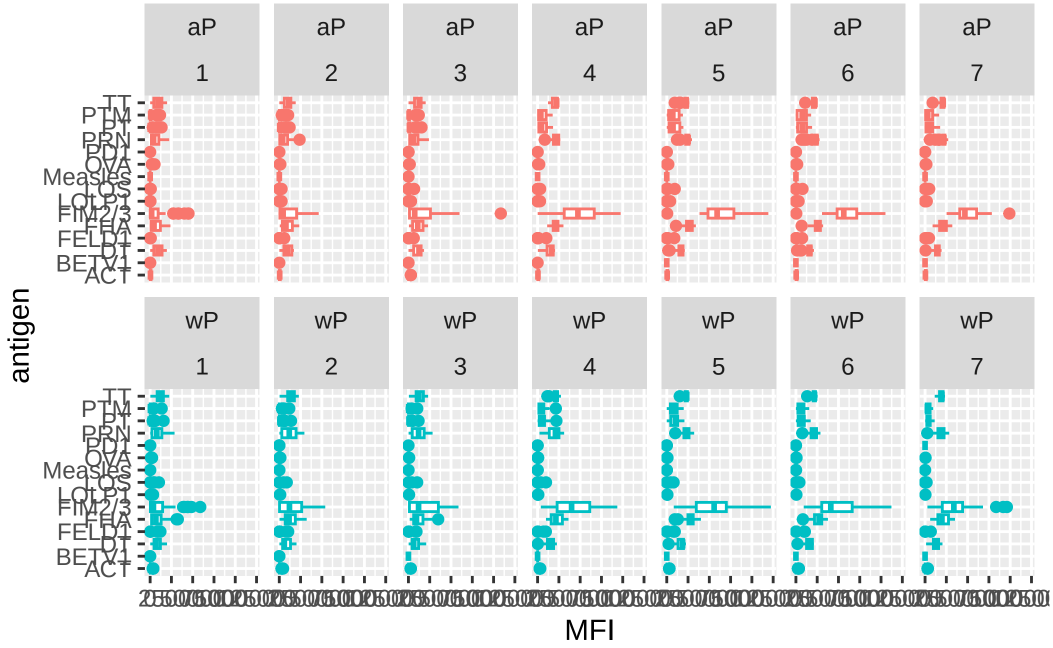
**Q15.** What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others? antigen 5 has significant difference and FIM2/3 are different.

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



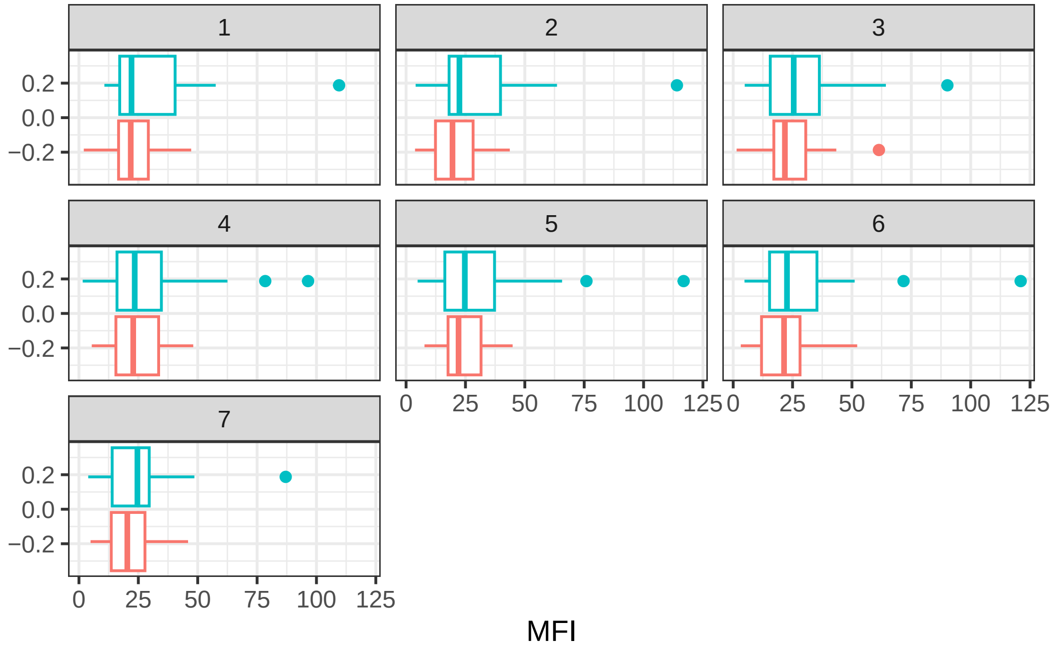
Another version :

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

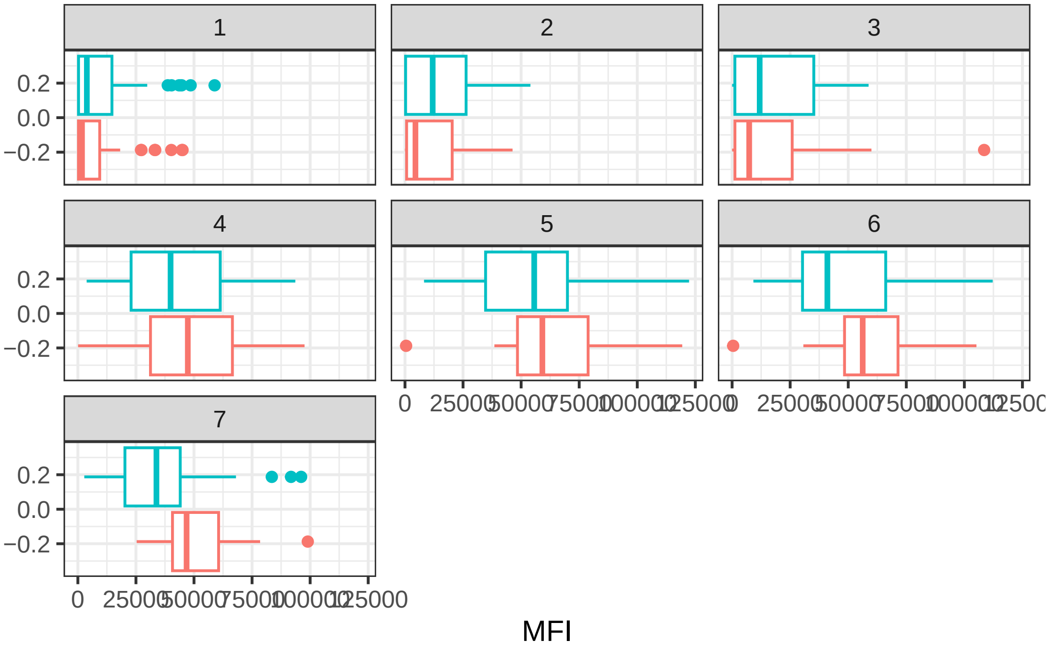


**Q16.** Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen (**"Measles"**, that is not in our vaccines) and a clear antigen of interest (**"FIM2/3"**, extra-cellular fimbriae proteins from *B. pertussis*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

```
filter(ig1, antigen=="Measles") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = F ) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



```r
## and the same for antigen=="FIM2/3"
filter(ig1, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = F) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



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

FIM2/3 has longer time course compared to Measles

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

No clear difference

## Obtaining CMI-PB RNASeq data

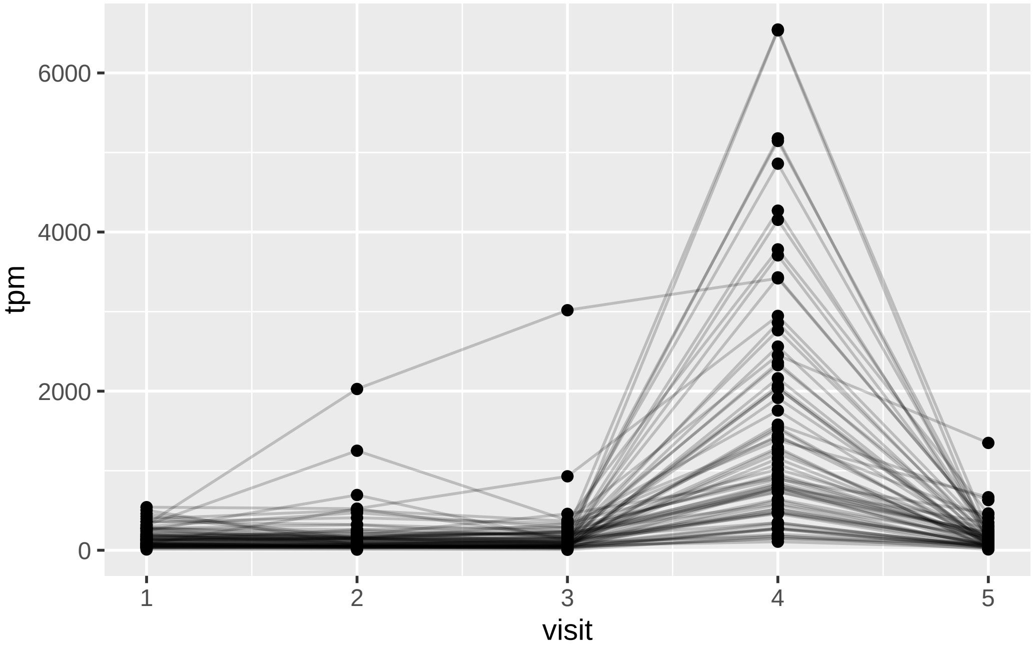
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.

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

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

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

**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)
```



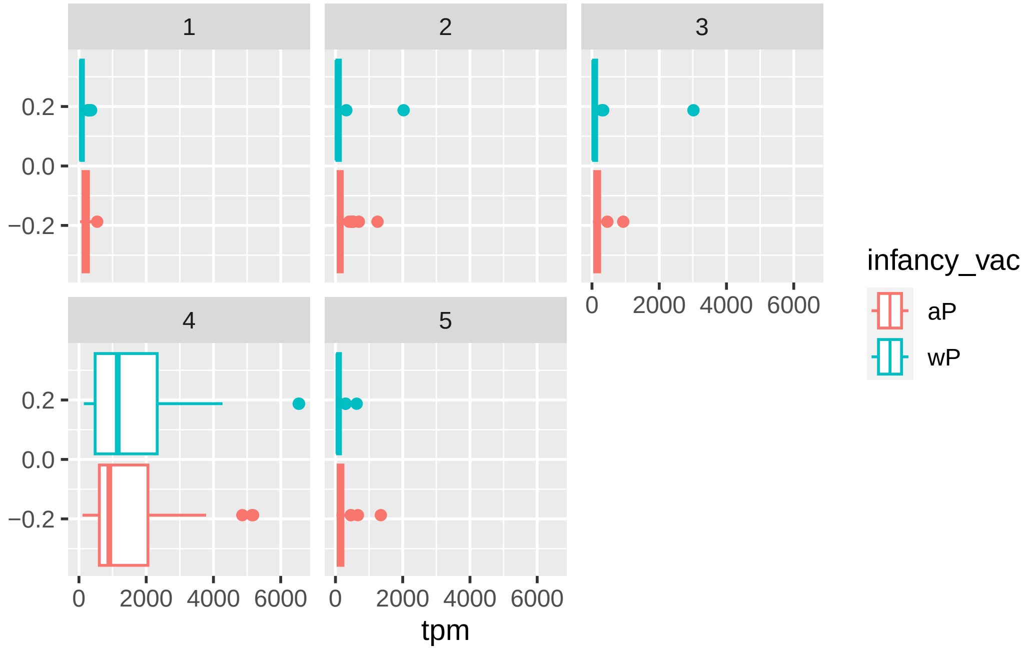
**Q20.**: What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

the expression is at maximum in 4th visit.

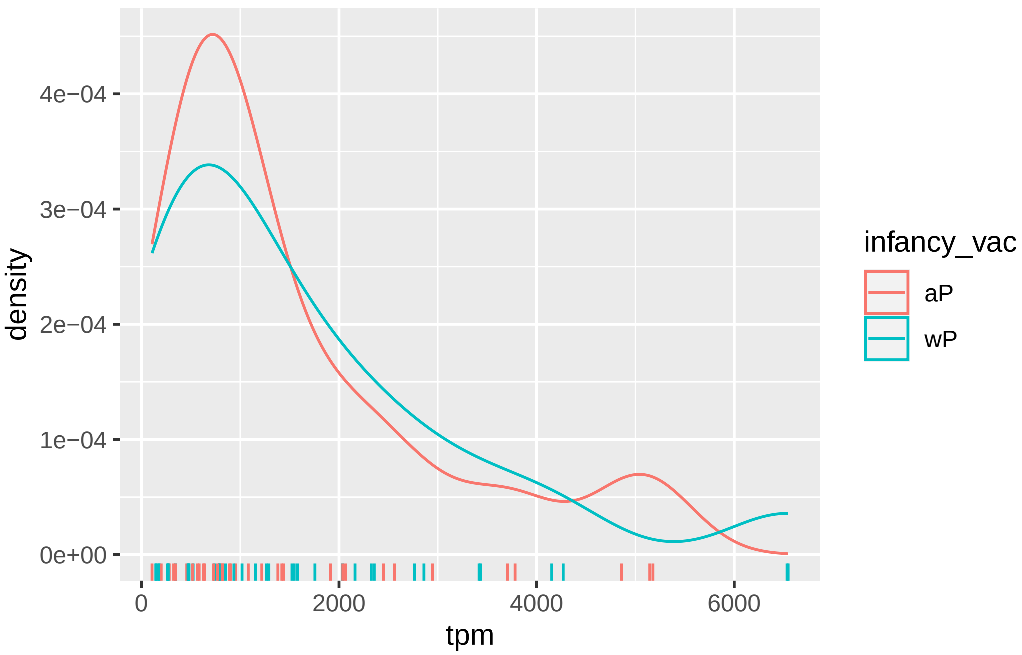
**Q21.** Does this pattern in time match the trend of antibody titer data?

yes it matches the plot.

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



```
## no obvious wP vs. aP differences here even if we focus in on a particular visit:
ssrna %>%
  filter(visit==4) %>%
  ggplot() +
    aes(tpm, col=infancy_vac) + geom_density() +
    geom_rug()
```



4e−04
3e−04
2e−04
1e−04
0e+00
density
0
2000
4000
6000
tpm
infancy_vac
aP
wP