Class#19_Pertussis

Zainab Ashir

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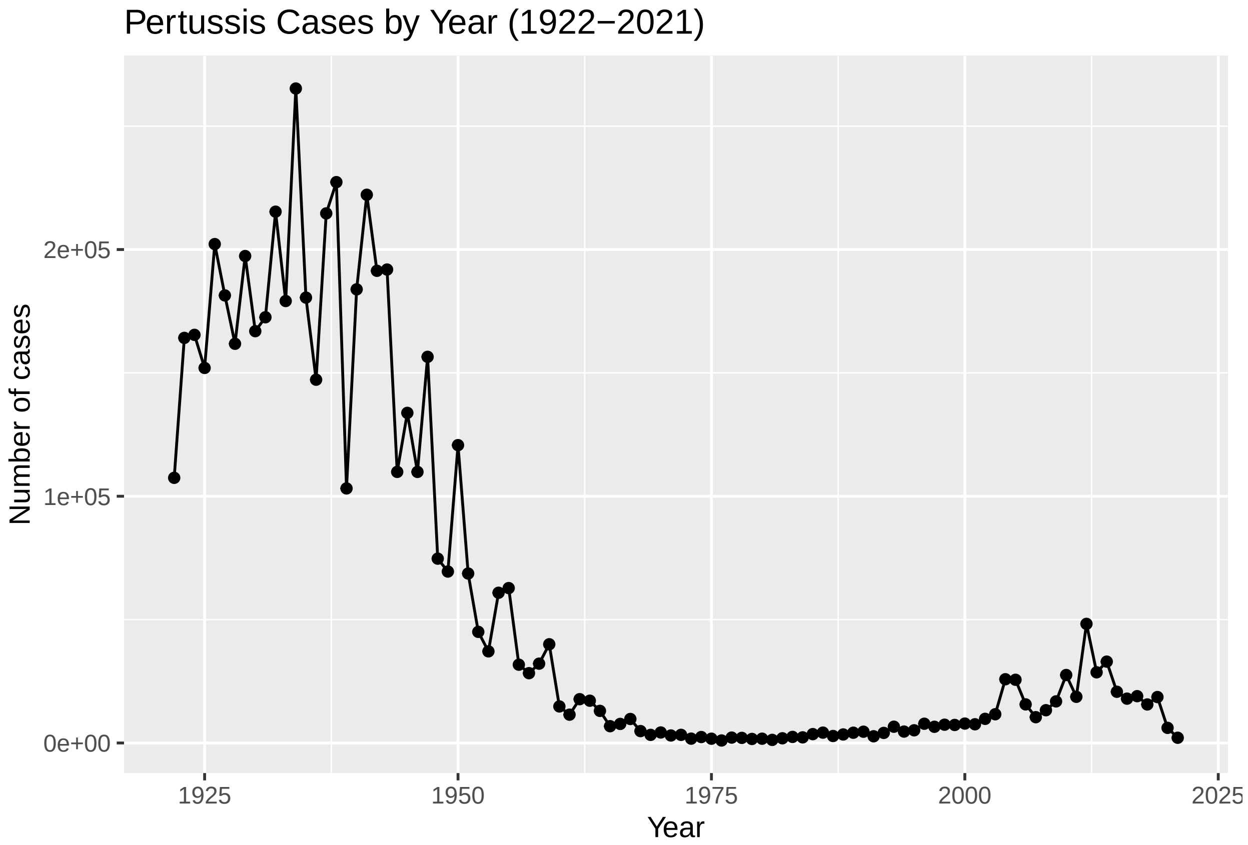
Q1. With the help of the R "addin" package datapasta assign the CDC pertussis case number data to a data frame called cdc and use ggplot to make a plot of cases numbers over time.

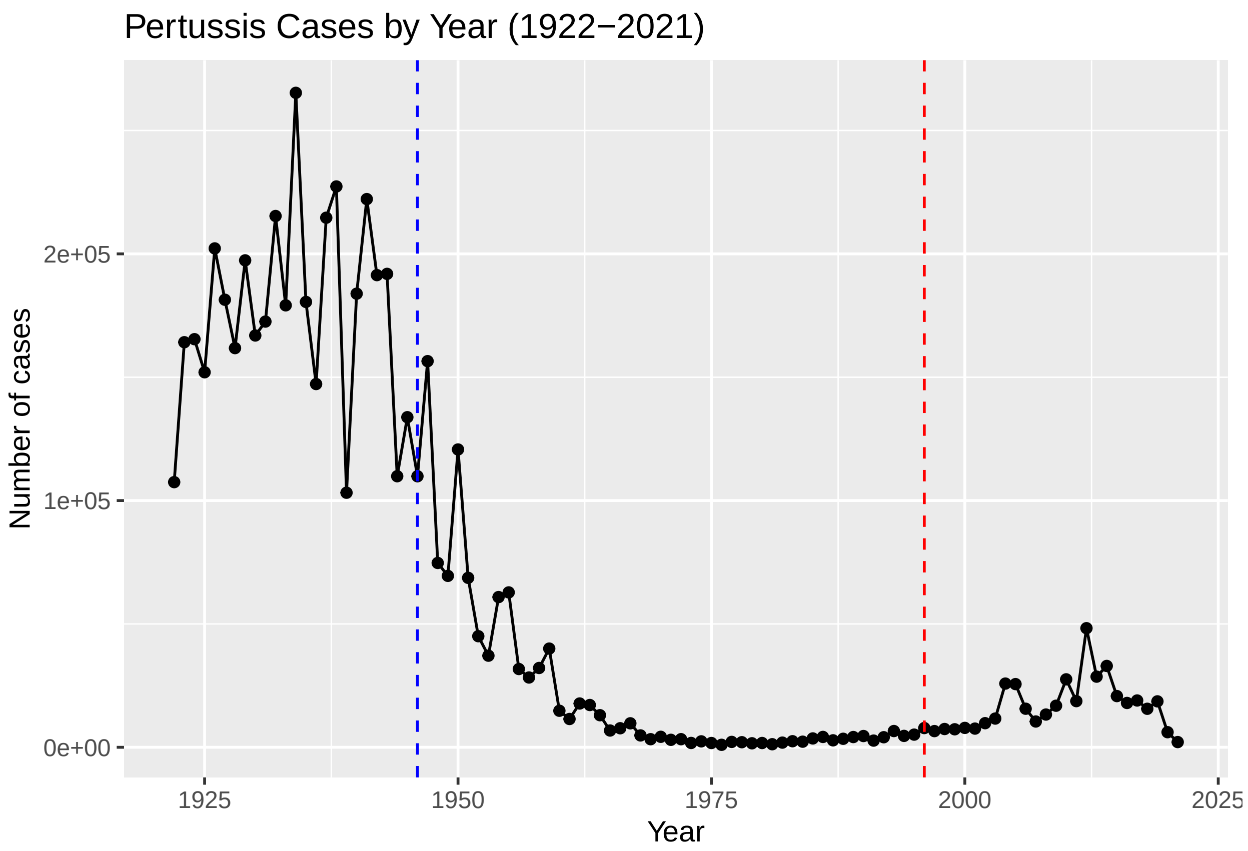
```
#Paste cdc data as data frame
cdc <- data.frame(
                                    Year = c(1922L,1923L,1924L,
                                             1925L,1926L,1927L,1928L,1929L,
                                             1930L,1931L,1932L,1933L,1934L,
                                             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)
          )
```

```
#Make a plot of data
library(ggplot2)
ggplot(cdc) +
  aes(x=Year, y= No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() + labs(
  title = "Pertussis Cases by Year (1922-2021)",
  x = "Year",
  y = "Number of cases")
```



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?

```
#Adding lines to our plot
library(ggplot2)
ggplot(cdc) +
  aes(x=Year, y= No..Reported.Pertussis.Cases) +
geom_point() + geom_line() + geom_vline(xintercept = c(1946, 1996), color= c("blue", "red"), linetype=
  title = "Pertussis Cases by Year (1922-2021)",
  x = "Year",
  y = "Number of cases")
```



#I notice that wehen the wP vaccine is instroduced, the pertussis cases had a significant drop, but when the aP vaccine is introduced, the cases have started to increase a bit. >Q3. Describe what happened after the introduction of the aP vaccine? Do you have a possible explanation for the observed trend?

#The cases started to increase when aP vaccine is introduced eventhough its a slight increase. One reason for this increase could be an increase in vacccination hesitancy.

```
# 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
```

#Thus, there are 47 aP infacy vaccinated subjects and 49 wP infacy vaccinated subjects.

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

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

```
## 
## Female   Male 
##     66     30
```

#Thus, the dataset has 66 females, and 30 males.

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

```r
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
```

```r
library(lubridate)
```

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

## The following objects are masked from 'package:base':
## 
##     date, intersect, setdiff, union
```

```r
today()
```

```
## [1] "2023-06-09"
```

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

```
## Time difference of 8560 days
```

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

```
## [1] 23.436
```

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

```r
#using dplyr for aP
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
```

```
#using dplyr for wP
library(dplyr)

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
```

#For i) the average of aP is 26. For ii) The average is 37 for wP. For iii) It seems that the two averages are significant.

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

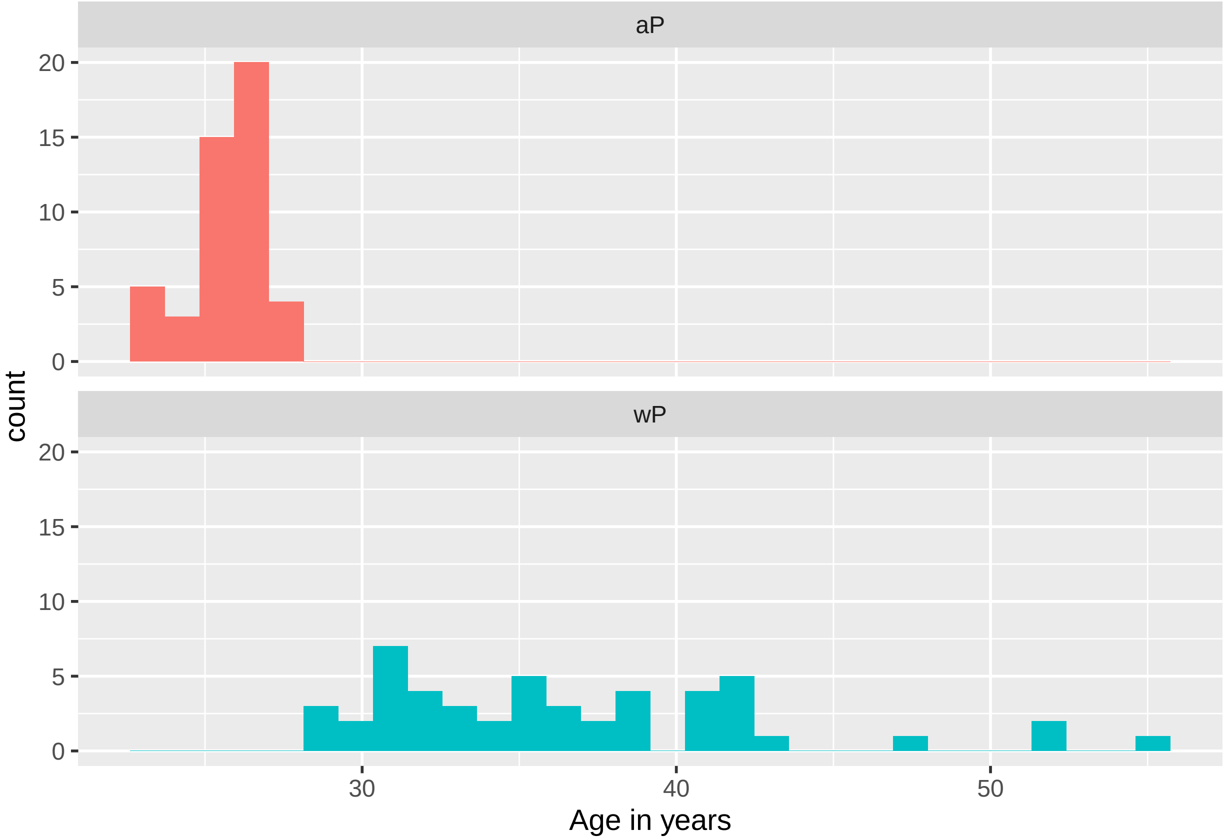
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
## [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?

```
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] 1.316045e-16
```

#Yes, by just looking at the two graphs, it does seem that the two groups are significally different. In addition, the p-value reported is also showing that the two groups are significally different since it is <0.01.

```
# 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/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 13673 days
## 2 13673 days
## 3 13673 days
## 4 13673 days
## 5 13673 days
## 6 13673 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
```

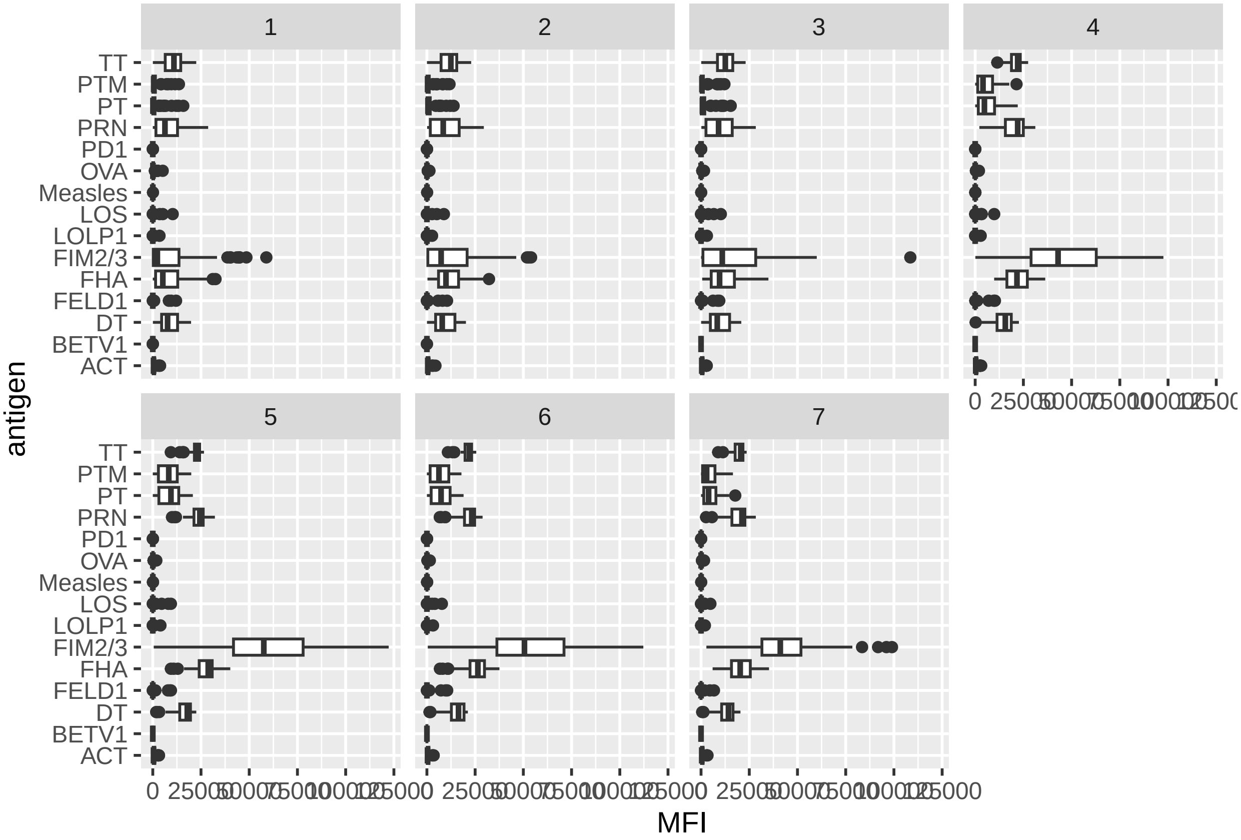
#The number of visit 8 specieman seems to be significally smaller than the other ones.

```
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 13673 days
## 2 13673 days
## 3 13673 days
## 4 13673 days
## 5 13673 days
## 6 13673 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)
```

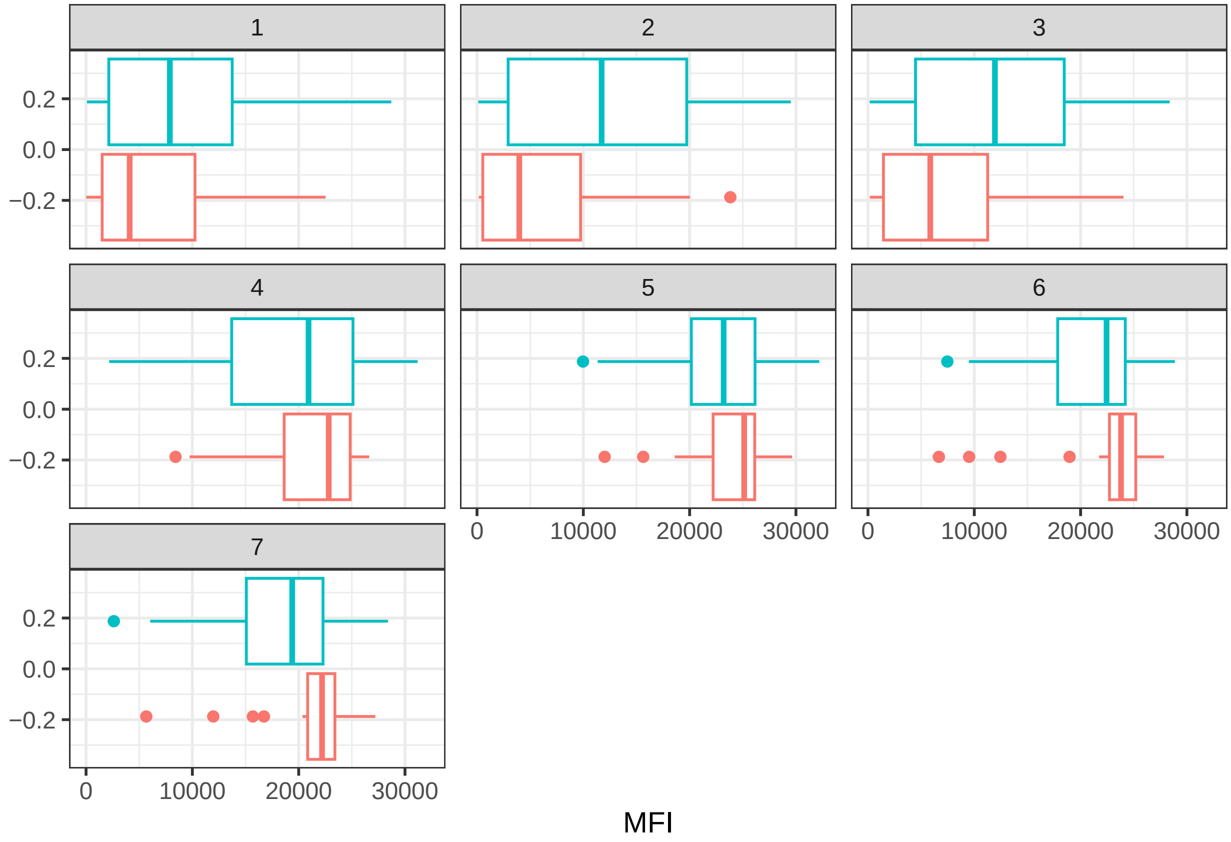


Q15. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

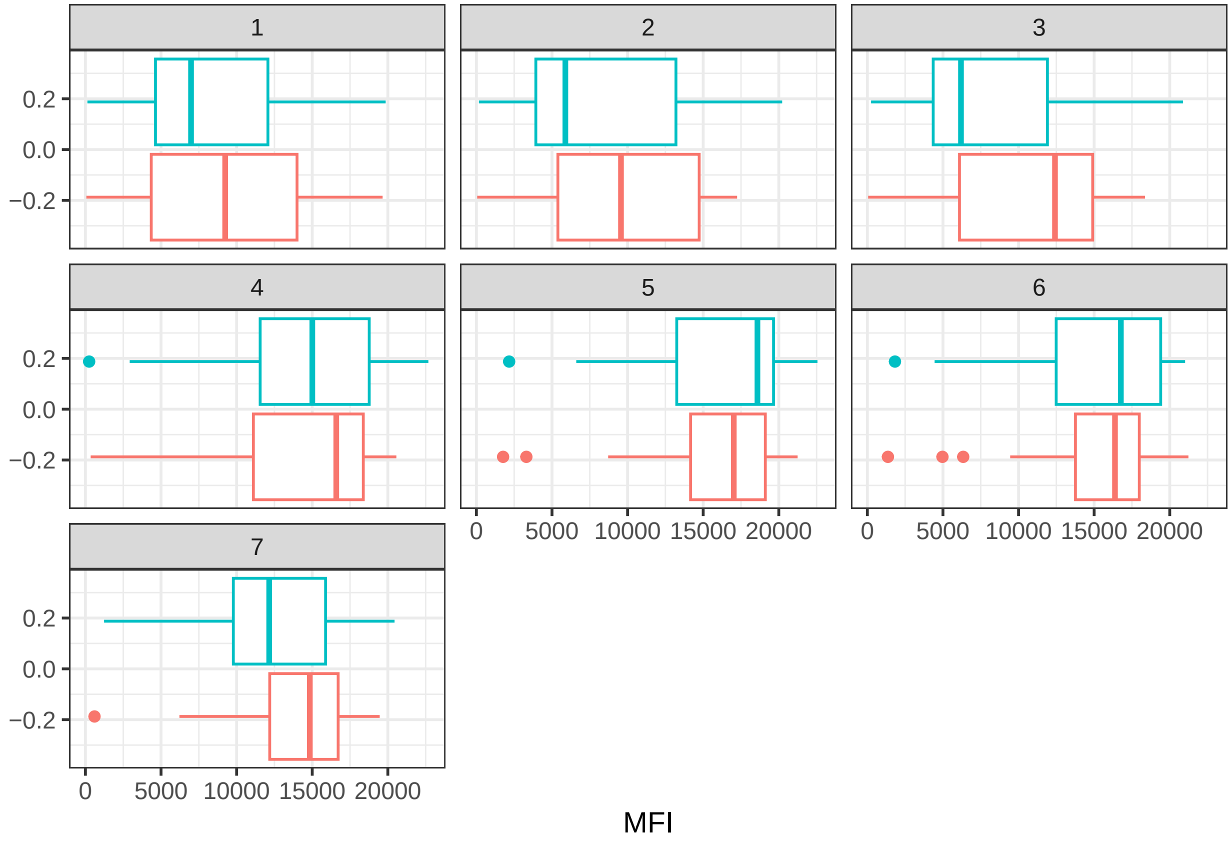
#It seems that FIM2/3 and some FHA have noticeably higher level of IgG1 titer antibody over time. Both of these antigens tend to participate in attachemt subtrate from B.pertussis proteins since they are a clear anigen of interest. The other antigens seem that they have a weak interaction with B.pertussis protein substrate.

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).

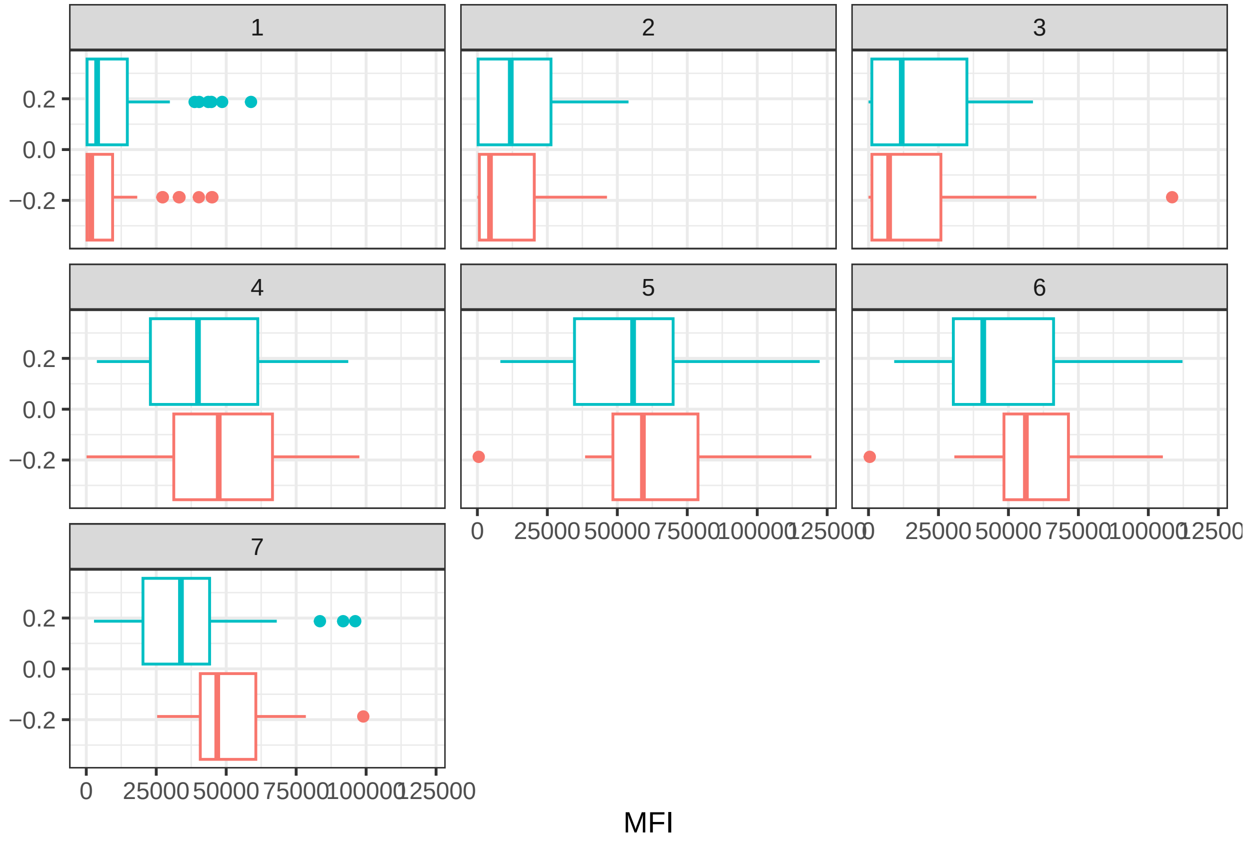
```
#Doing it for PRN first antigen
filter(ig1, antigen=="PRN") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



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



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



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

#It seems that PRN and DT antigons do not really change in thier time cources as opposed to FIM2/3 data in particular, it shows that they highly increase overtime and beats all the other antigens, ranking itself in the lead. They also appear to peak at visit 5 and then decline.

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

#Yes, similar to the previous trend, it seems that wP vaccines antigens exceeds thayt of aP, meanwhile, over time, aP vaccines tends to catch up to wP a bit by bit, untill it exceeds it slightly.

Obtaining CMI-PB RNASeq data

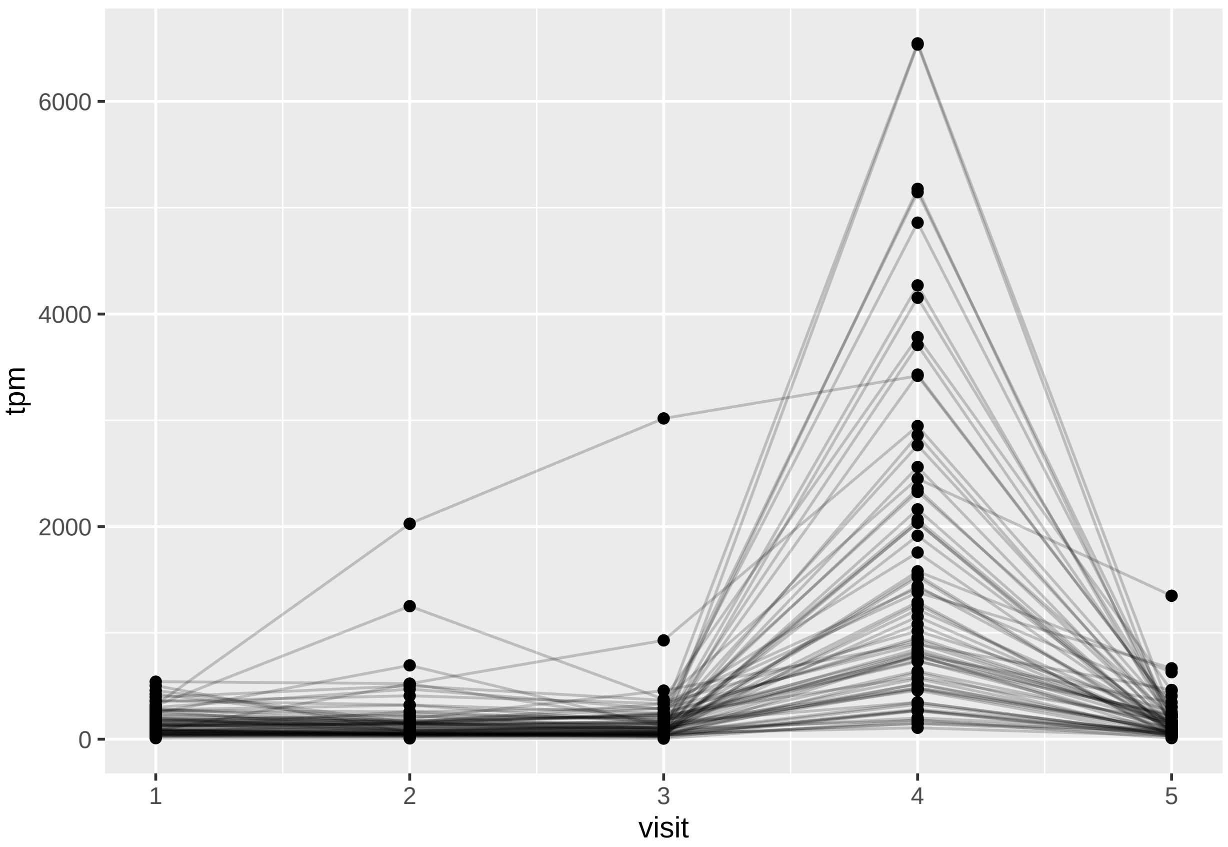
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.7"

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 seems that the maximum expression level occurs at visit 4.

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

#Yes, it does seem that it matches the trenf of FIM2/3 antigen data since this antigen has the highest (6000) tpm mostly at visit 5 since this is where the means of both wP and aP falls close to 6000.