class19

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1. Investigating pertussis cases by year1. 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.

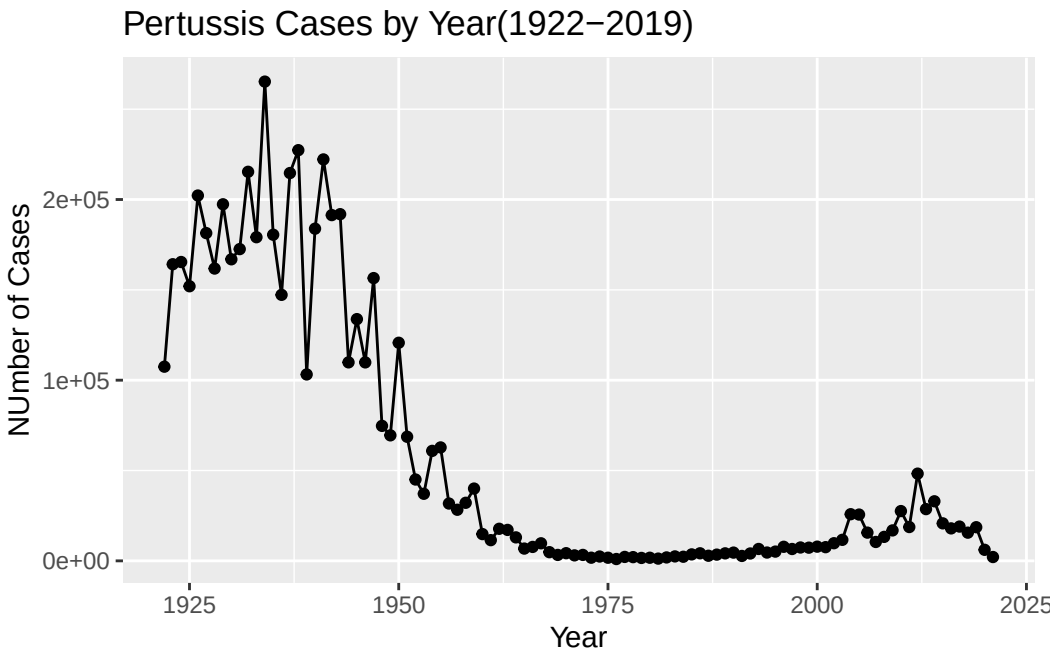
```
#install.packages("datapasta")
```

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

```
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,
```

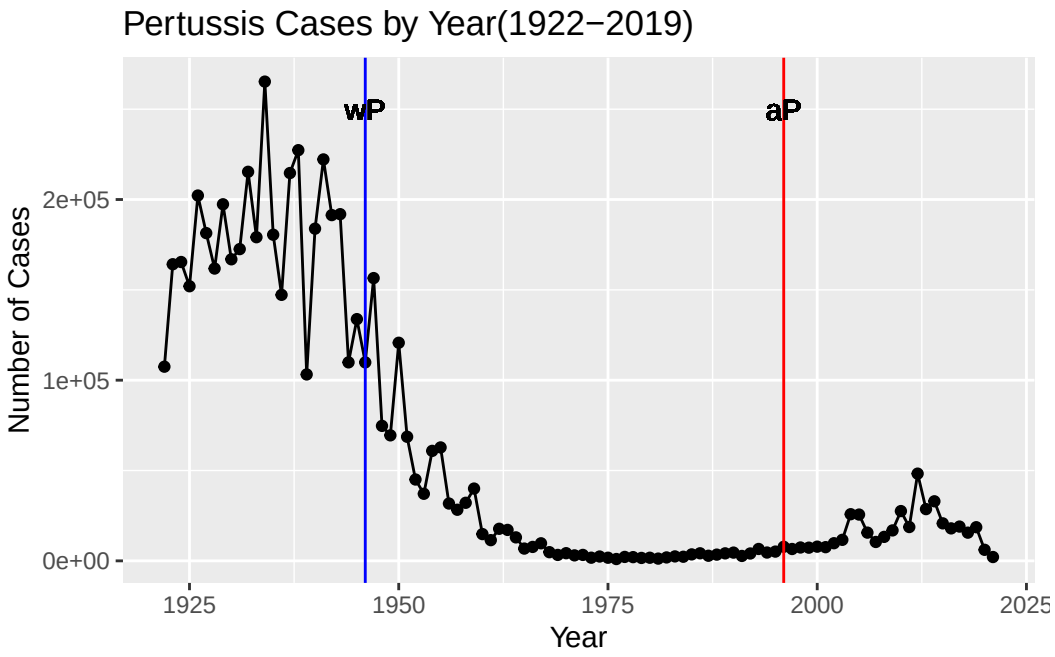
```
                                          2004L,2005L,2006L,2007L,2008L,
                                          2009L,2010L,2011L,2012L,2013L,
                                          2014L,2015L,2016L,2017L,
                                          2018L,2019L,2020L,2021L),
            No..Reported.Pertussis.Cases = 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, No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  labs(title="Pertussis Cases by Year(1922-2019)")+
  xlab("Year")+
  ylab("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?

```
ggplot(cdc)+
  aes(x=Year, No..Reported.Pertussis.Cases)+
  geom_point()+
  geom_line()+
  geom_vline(xintercept=1946,col="blue")+
  geom_vline(xintercept=1996,col="red")+
  geom_text(x=1946, y=250000, label="wP")+
  geom_text(x=1996, y=250000, label="aP")+
  labs(title="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 aP vaccination, the pertussis cases started to increase again. It is likely that the bacteria have evolved again.

3. Exploring CMI-PB data

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

```
library(jsonlite)
```

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

```
head(subject)
```

```
  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
4          4          wP           Male Not Hispanic or Latino Asian
5          5          wP           Male Not Hispanic or Latino Asian
6          6          wP         Female Not Hispanic or Latino 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
4    1988-01-01    2016-08-29 2020_dataset
5    1991-01-01    2016-08-29 2020_dataset
6    1988-01-01    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$race,subject$biological_sex)
```

```
                                   Female Male
  American Indian/Alaska Native         0    1
  Asian                                18    9
  Black or African American             2    0
  More Than One Race                    8    2
```

```
  Native Hawaiian or Other Pacific Islander      1    1
  Unknown or Not Reported                       10    4
  White                                         27   13
```

Side-Note: Working with dates

```
library(lubridate)
```

```
Attaching package: 'lubridate'

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

    date, intersect, setdiff, union
```

```
today()
```

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

```
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?

```
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
```

```
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
```

```
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
```

```
t.test(time_length(ap$age,"years"), time_length(wp$age,"years"))
```

```
    Welch Two Sample t-test

data:  time_length(ap$age, "years") and time_length(wp$age, "years")
t = -12.092, df = 51.082, p-value < 2.2e-16
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -12.644857  -9.044045
sample estimates:
mean of x mean of y
 25.75380  36.59825
```

p-value is smaller than 0.05. They are significantly different.

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

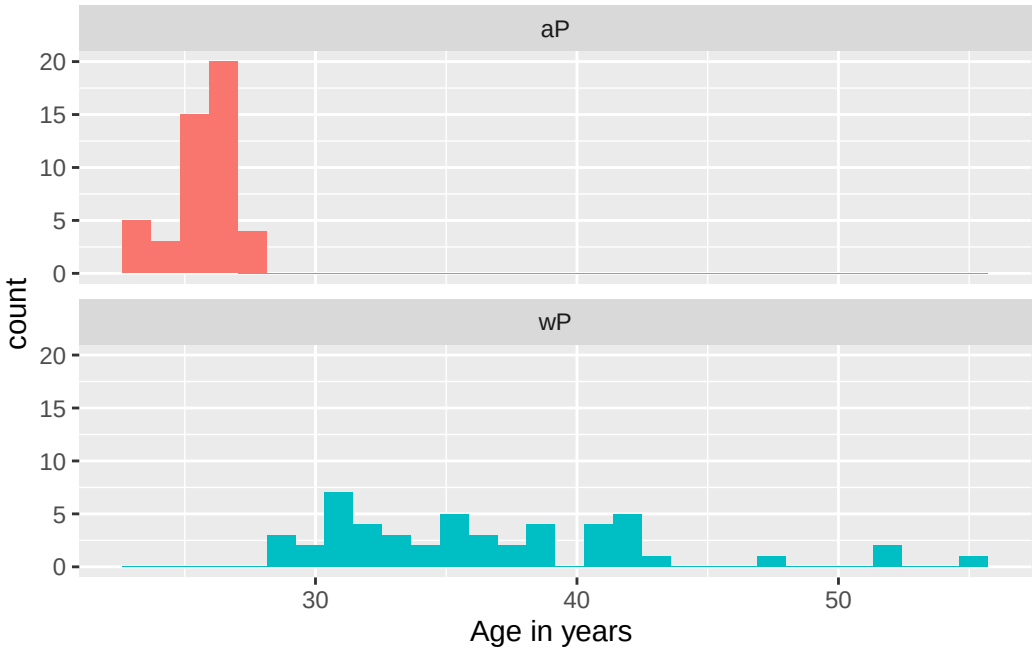
```
int <- (ymd(subject$date_of_boost)-ymd(subject$year_of_birth))
age_at_boost <- time_length(int, "years")
head(age_at_boost)
```

```
[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,"years"),
      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`.
```



They are significantly different.

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 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
```

4. Examine IgG1 Ab titer levels

Now using our joined/merged/linked `abdata` dataset `filter()` for IgG1 `isotype` and exclude the small number of visit 8 entries.

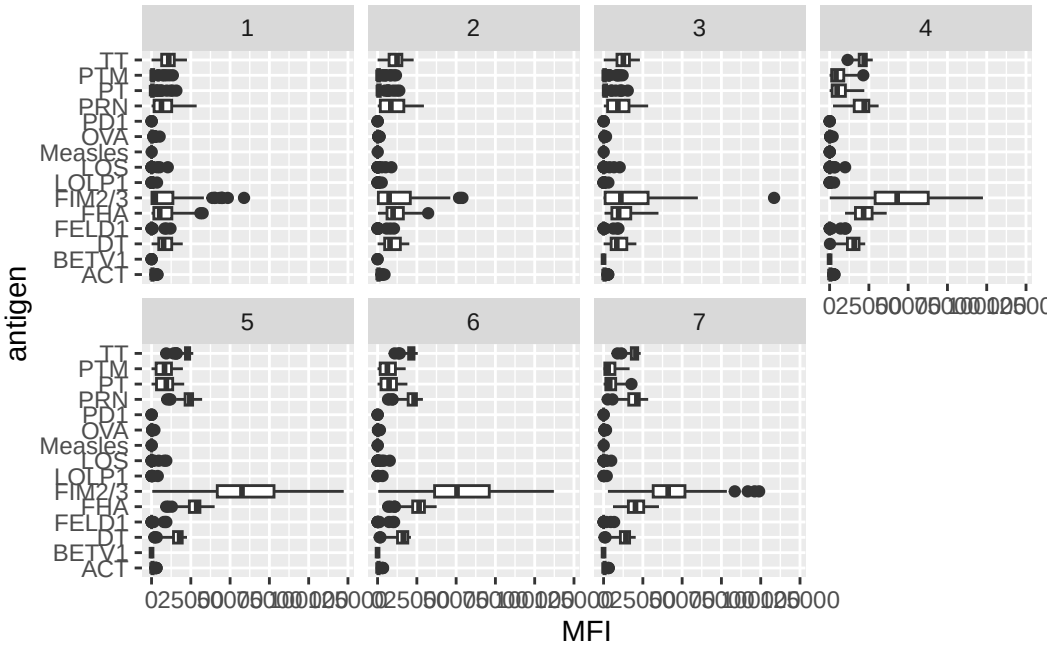
```
ig1 <- abdata %>% filter(isotype == "IgG1", visit!=8)
head(ig1)
```

```
  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)
```

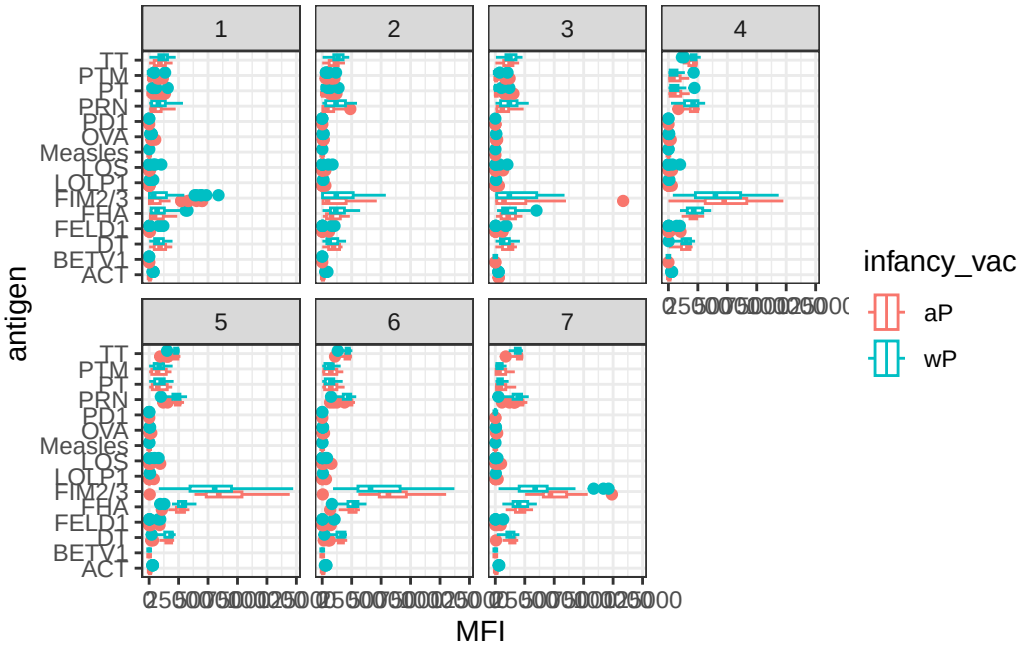


Q15. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

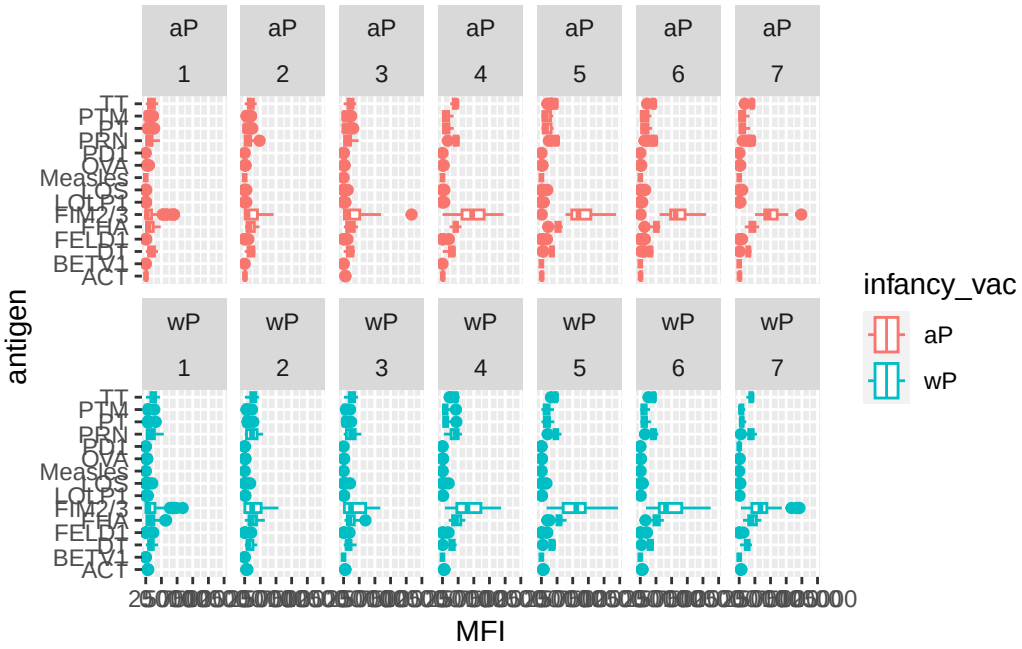
FIM2/3 show differences in the level of IgG1 antibody titers over time. Some papers indicate that FIM2/3 inprove the vaccination-efficacy, and some papers suggest that B. pertussis expresses both Fim2 and Fim3 during infection. So Fim2 and Fim3 would raise anti-fim2 and anti-fim3 antibody level after vaccination.

We can attempt to examine differences between wP and aP here by setting color and/or facet values of the plot to include `infancy_vac` status (see below). However these plots tend to be rather busy and thus hard to interpret easily.

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

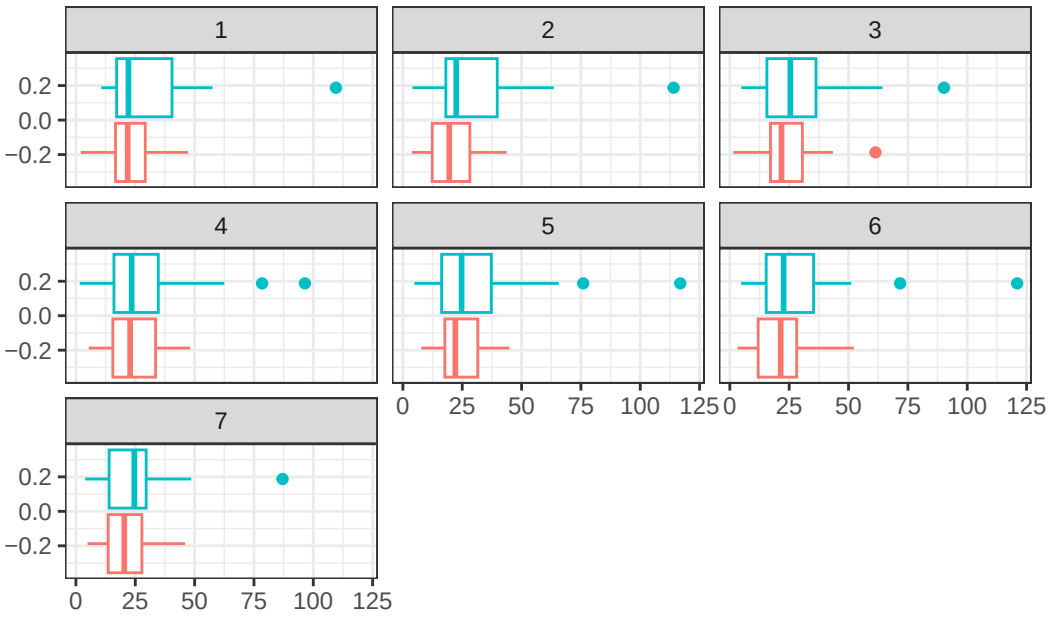



```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot() +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

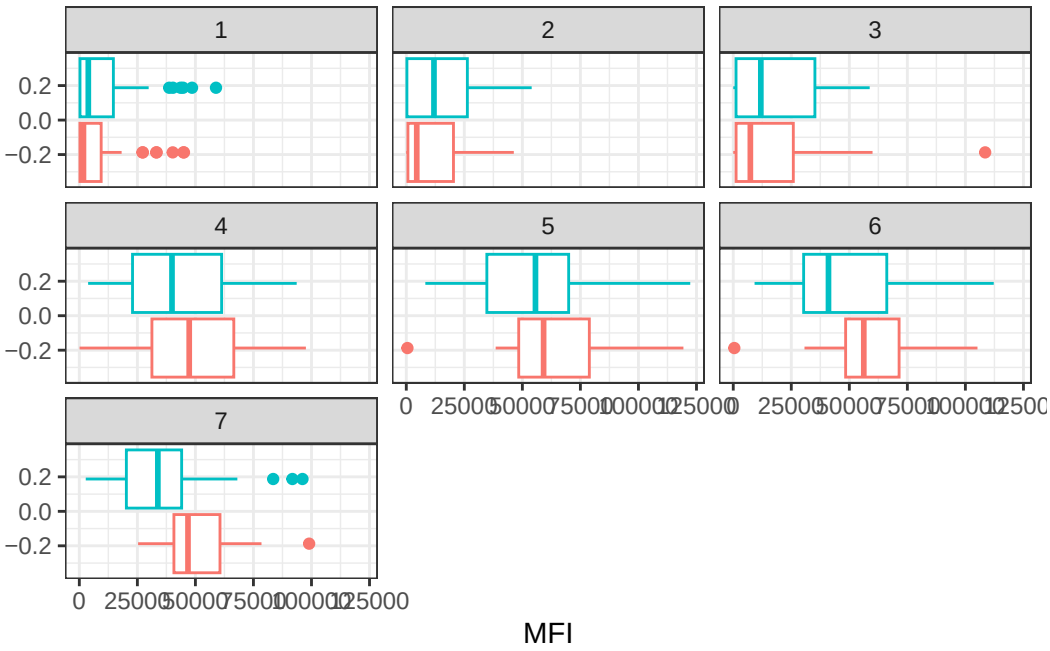


Q16. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen (**"Measles"**, that is not in our vaccines) and a clear antigen of interest (**"FIM2/3"**, extra-cellular fimbriae proteins from *B. pertussis*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

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




```
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?

THe Measles antibody level is stable over time. FIM2/3 antibody level increases over time.

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

aP have increased Ab titer level than wP after the 4th visit. But before the 4 th visit, it was vice versa.

5. Obtaining CMI-PB RNASeq data

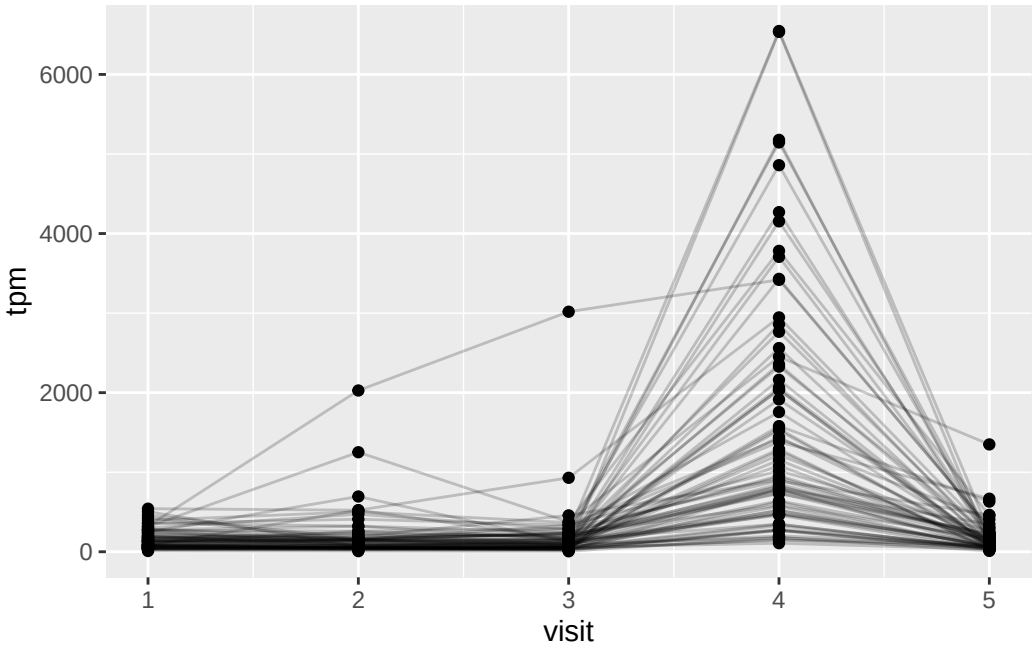
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.

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

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

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

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)?

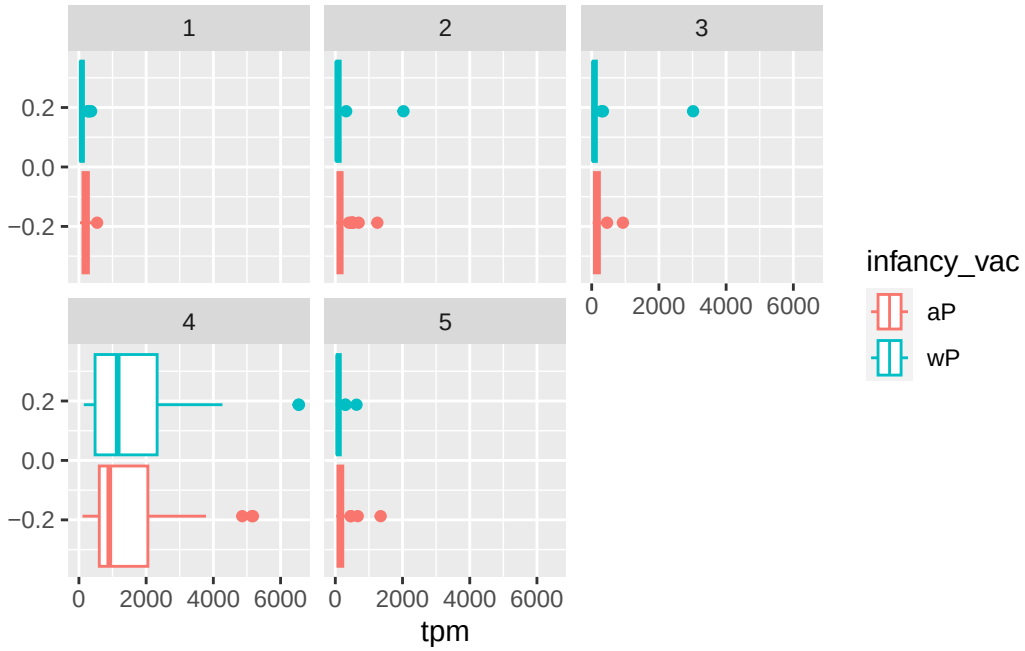
It goes to its peak at visit 4.

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

It matches the trend of antibody titer data.

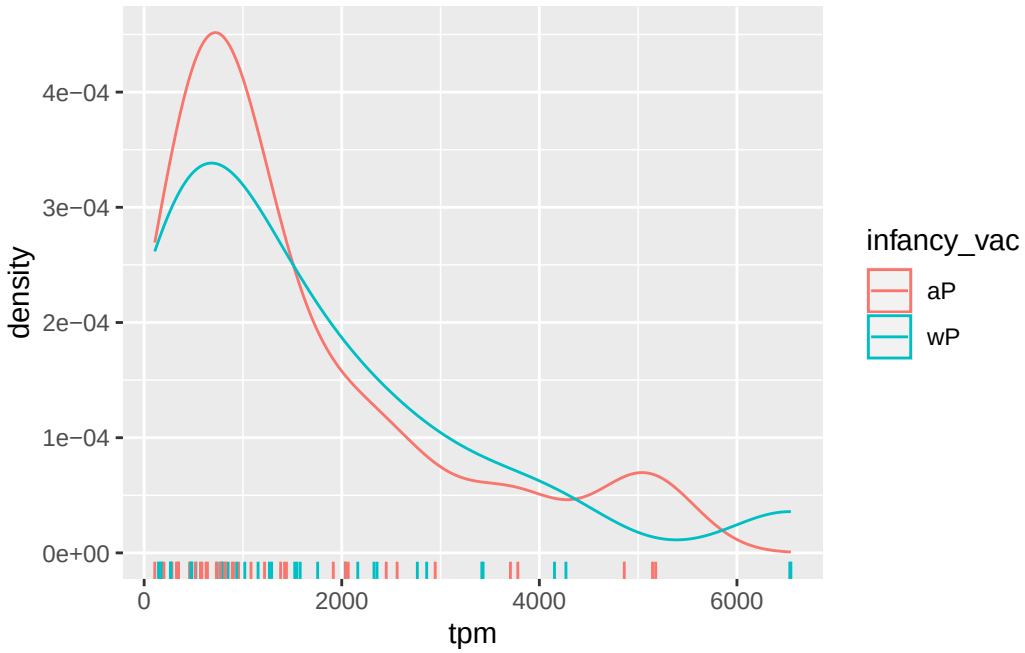
We can dig deeper and color and/or facet by `infancy_vac` status:

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



There is however 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()
```


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