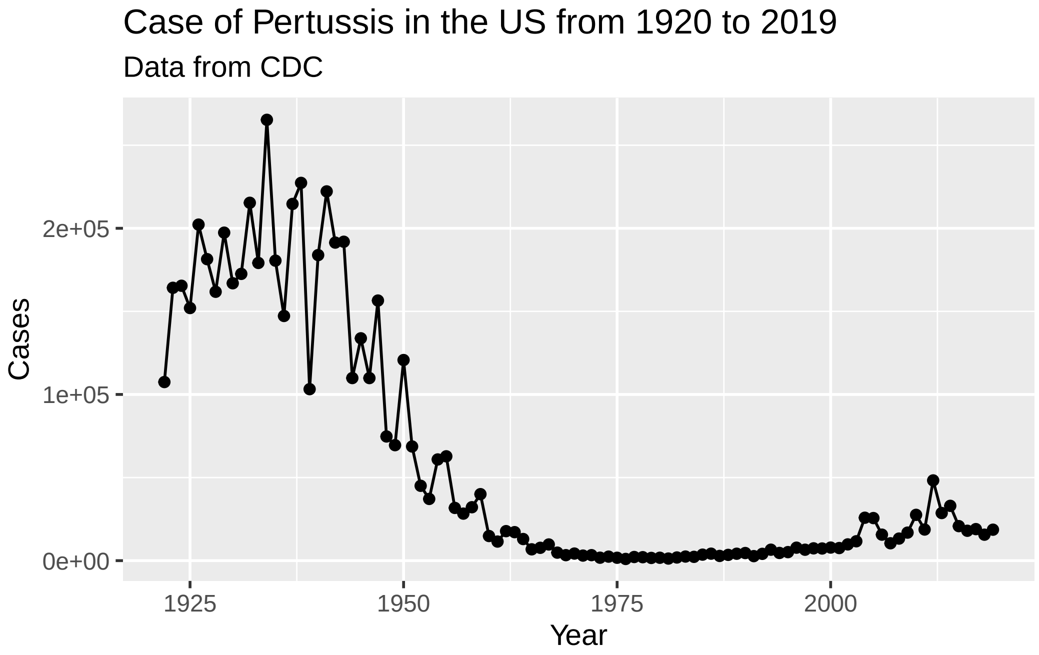
class19

Investigating pertussis cases by year

The CDC tracks cases of pertussisi the US, we can get their data via web-scrapping.

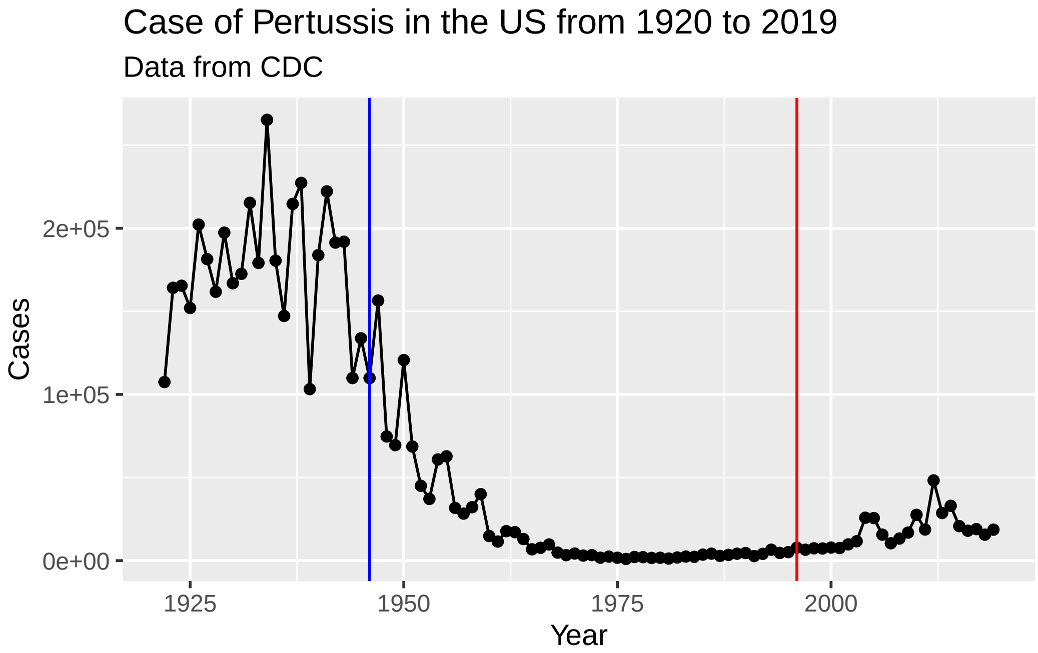
Q1. With the help of the R “addin” package datapasta assign the CDC pertussis case number data to a data frame called cdc and use ggplot to make a plot of cases numbers over time.

```
library(ggplot2)
baseplot <- ggplot(cdc) +
  aes(Year, Cases) +
  geom_point() +
  geom_line() +
  labs(title = "Case of Pertussis in the US from 1920 to 2019", subtitle="Data from CDC")

baseplot
```



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? After the wP vaccine was introduced, there was a sharp decrease in the number of new cases of pertussis. But, after the aP vaccine was introduced, there was a moderate increase of new cases.

```
baseplot + geom_vline(xintercept=1946, col = "blue") +
  geom_vline(xintercept = 1996, col = "red")
```



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

The cases remained at a low amount for a few years, but then seemed to increase once again after the switch to the aP vaccine.

#CMI-PB project

The CMI_PB project is collecting data on aP and wP indiviuals and their immune response to infection and or booster shots.

CMI-PB returns data from its API in JSON format (like most APIs). We will use the hsonlite package to get data gtom this API.

```
# 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)
```

```
  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 may aP and wP infancy vaccinated subjects are in the dataset? aP = 47 subjects wP = 49 subjects

```
table(subject$infancy_vac)
```

```

aP wP 
47 49 
```

Q5. How many Male and Female subjects/patients are in the dataset? Female = 66 Male = 30

```
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
```

```r
library(lubridate)
```

```

Attaching package: 'lubridate'

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

    date, intersect, setdiff, union
```

```r
today()
```

```
[1] "2023-03-14"
```

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

```
Time difference of 8473 days
```

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

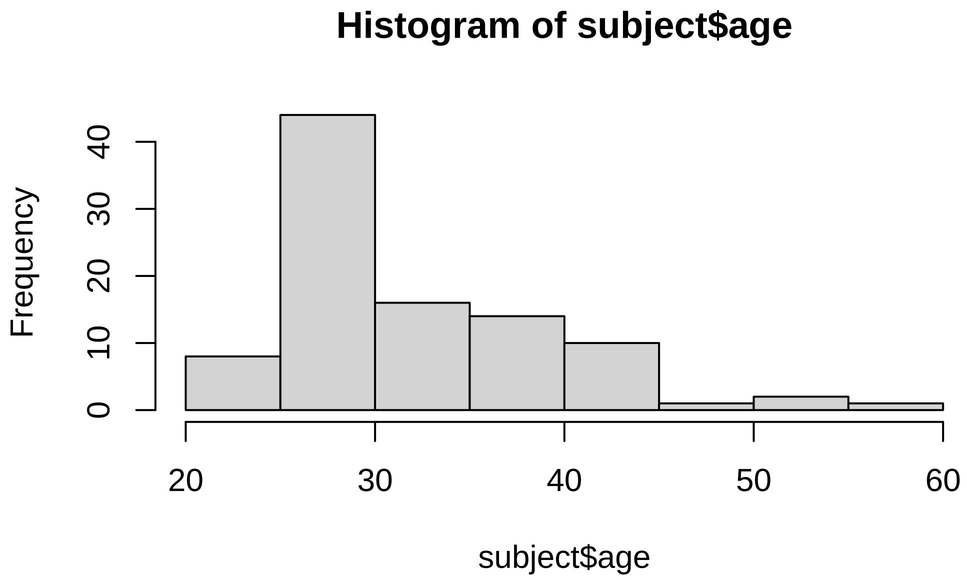
```
[1] 23.19781
```

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? average wp age = 36.36006 average ap age = 25.5156 They are significantly different–> p-value < 2.2e-16 which is less than 0.05.

Calculate the age in years of all subjects:

```r
age_days <- today() - ymd(subject$year_of_birth)
age_years <- time_length(age_days, "years")
subject$age <-age_years
```

```
hist(subject$age)
```



Now find the average of all individuals:

```
mean(subject$age)
```

```
[1] 31.05079
```

Now use dplyer to subset to wP or aP subjects:

```
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
```

```r
ap.age <- filter(subject,infancy_vac == "aP")$age
wp.age <- filter(subject,infancy_vac == "wP")$age
```

```r
mean(ap.age)
```

```
[1] 25.5156
```

```r
mean(wp.age)
```

```
[1] 36.36006
```

```r
#T-test
t.test(ap.age,wp.age)
```

```

    Welch Two Sample t-test

data:  ap.age and wp.age
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
 25.51560  36.36006
```

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

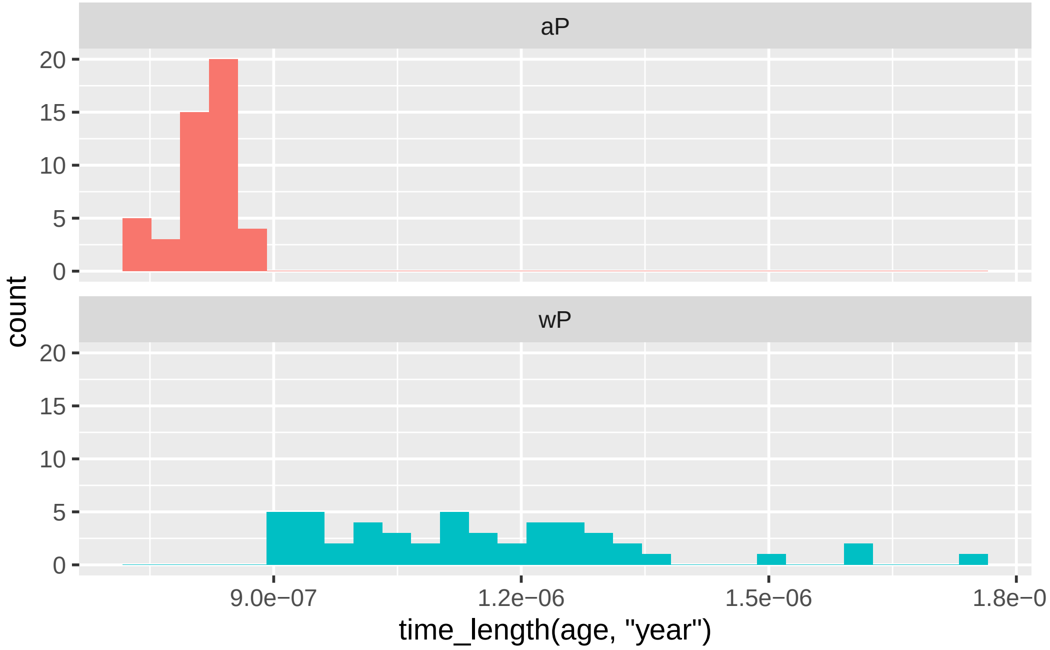
```r
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

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

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

```
ggplot(subject) +
  aes(time_length(age, "year"),
      fill=as.factor(infancy_vac)) +
  geom_histogram(show.legend=FALSE) +
  facet_wrap(vars(infancy_vac), nrow=2)
```

```
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



Joining multiple tables

Read the specimen and ab_titer tables into R and store the data as specimen and titer named data frames.

```
# 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)
```

```r
head(specimen)
```

```
  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
1                             0         Blood     1
2                           736         Blood    10
3                             1         Blood     2
4                             3         Blood     3
5                             7         Blood     4
6                            14         Blood     5
```

```r
head(titer)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgE               FALSE   Total 1110.21154       2.493425
2           1     IgE               FALSE   Total 2708.91616       2.493425
3           1     IgG                TRUE      PT   68.56614       3.736992
4           1     IgG                TRUE     PRN  332.12718       2.602350
5           1     IgG                TRUE     FHA 1887.12263      34.050956
6           1     IgE                TRUE     ACT    0.10000       1.000000
   unit lower_limit_of_detection
1 UG/ML                 2.096133
2 IU/ML                29.170000
3 IU/ML                 0.530000
4 IU/ML                 6.205949
5 IU/ML                 4.679535
6 IU/ML                 2.816431
```

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:

```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 37.19644
2 37.19644
3 37.19644
4 37.19644
5 37.19644
6 37.19644
```

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)`
```

```
dim(abdata)
```

```
[1] 32675    21
```

Q11. 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 
```

Q12. What do you notice about the number of visit 8 specimens compared to other visits? Visit 8 had much less speciment compared to 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

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

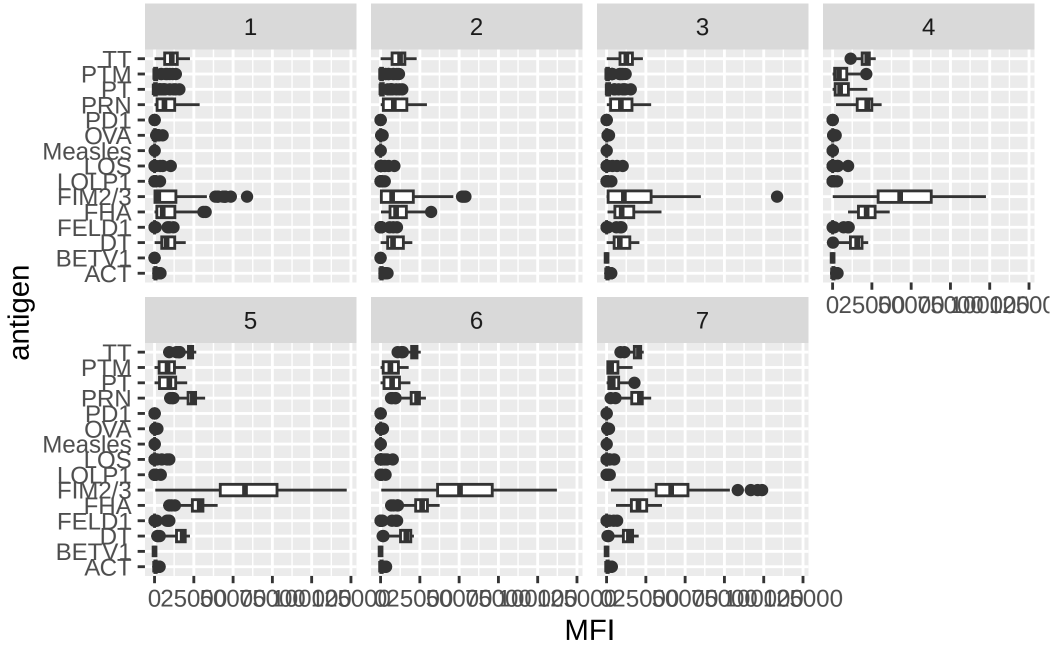
```
ig1 <- abdata %>% filter(isotype == "IgG1", visit!=8)
head(ig1)
```

```
  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 37.19644
2 37.19644
3 37.19644
4 37.19644
5 37.19644
6 37.19644
```

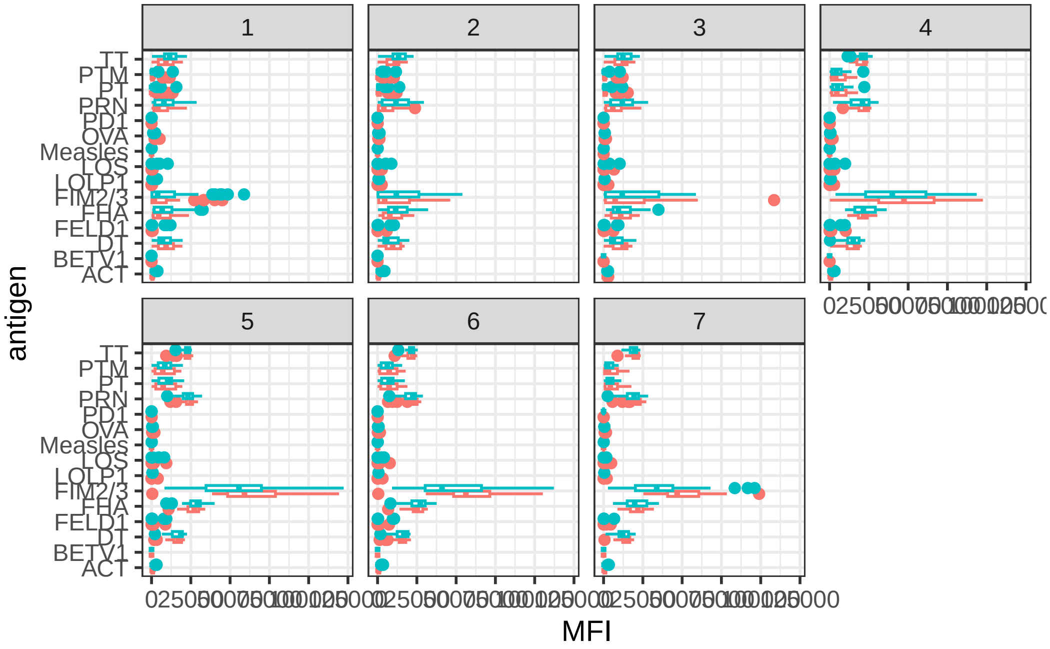
Q13. Complete the following code to make a summary boxplot of Ab titer levels for all antigens:

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

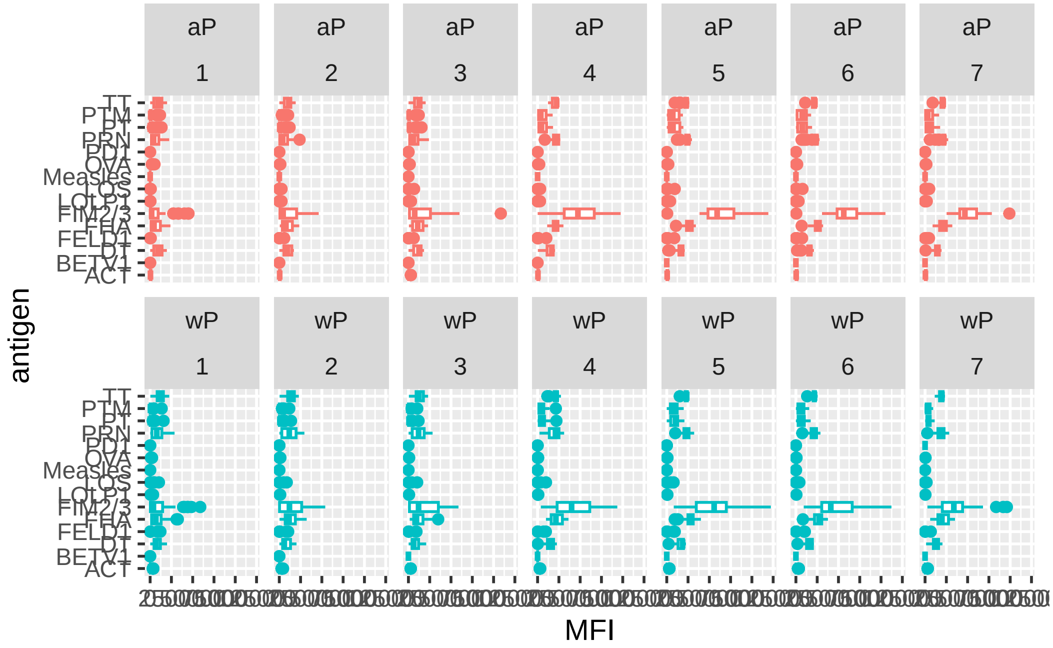


Q14. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others? FIM 2/3 –> cell adhesion
FHA –> role in host-cell binding and infection TT –> negative regulation of neurotransmitter secretion DT–> diphtheria toxin PRN –> binds to eukaryotic cells All of these antigens are involved in the infection, so the body will produce more antibodies to recognize these antigens after the vaccine is administered.

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

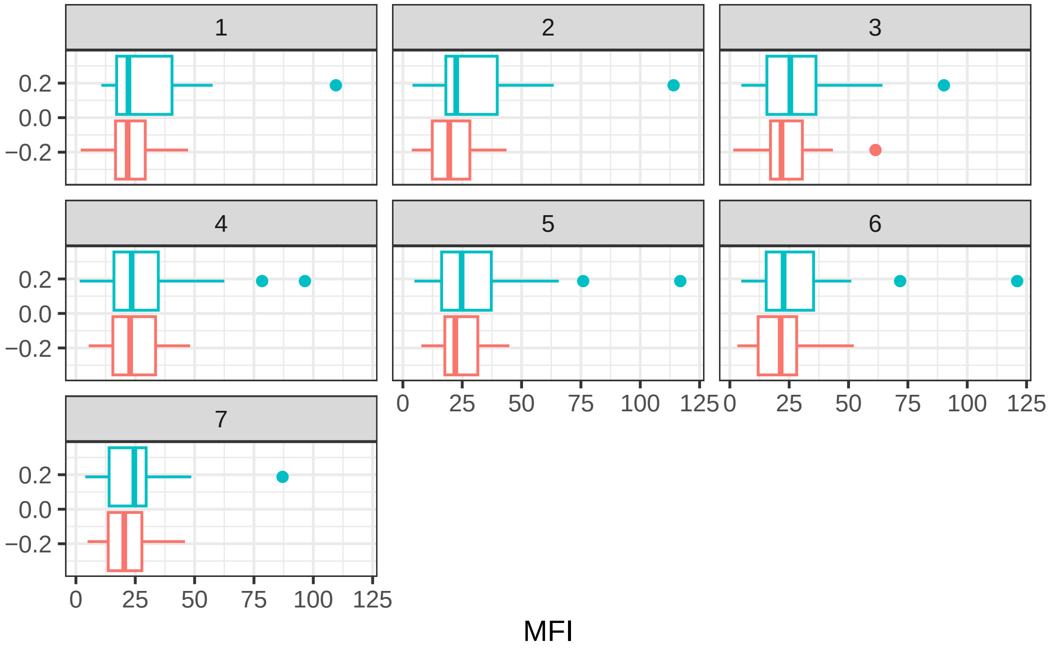


```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

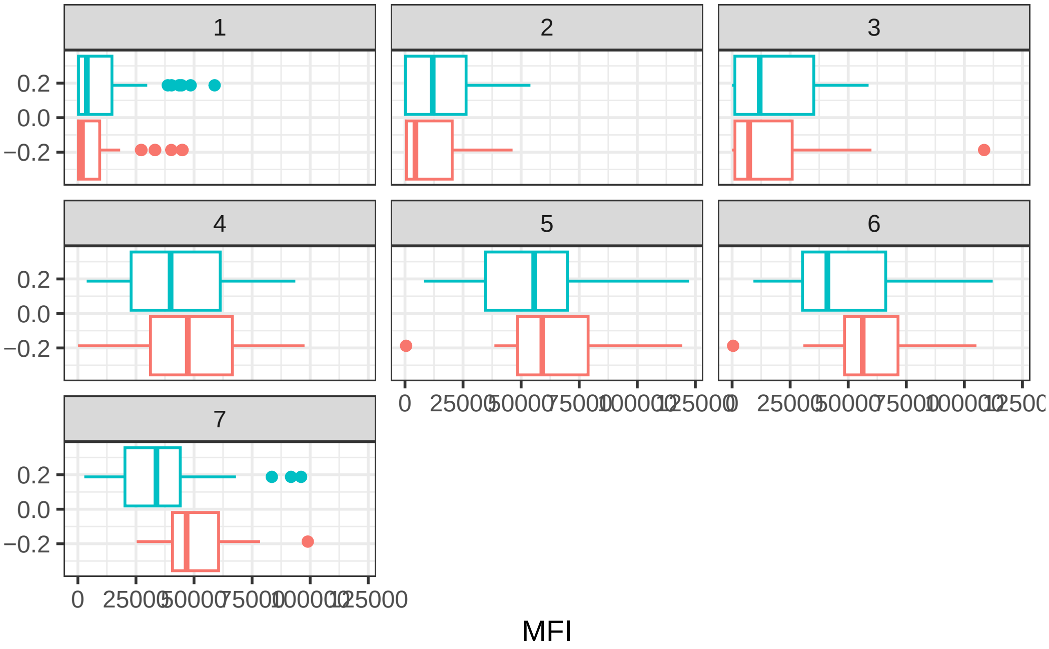


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("Measles", that is not in our vaccines) and a clear antigen of interest ("FIM2/3", extra-cellular fimbriae proteins from B. pertussis that participate in substrate attachment).

```r
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()
```



Q16. What do you notice about these two antigens time course and the FIM2/3 data in particular? FIM2/3 antibody titers are increasing over time up to visit 7, while the measles antibody titers stay consistent throughout the 7 visits. This trend is similar in both aP and wP patients. In the FIM2/3 data, the titers peak at visit 5 and start to decline through visit 7.

Q17. Do you see any clear difference in aP vs. wP responses? It seems that in the FIM2/3 graphs, the aP patients' titers are initially lower, but experience a larger growth over time than the wP subjects' titers.

Obtaining CMI-PB RNASeq data

For RNA-Seq data the API query mechanism quickly hits the web browser interface limit for file size. We will present alternative download mechanisms for larger CMI-PB datasets in the next section. However, we can still do "targeted" RNA-Seq querys via the web accessible API.

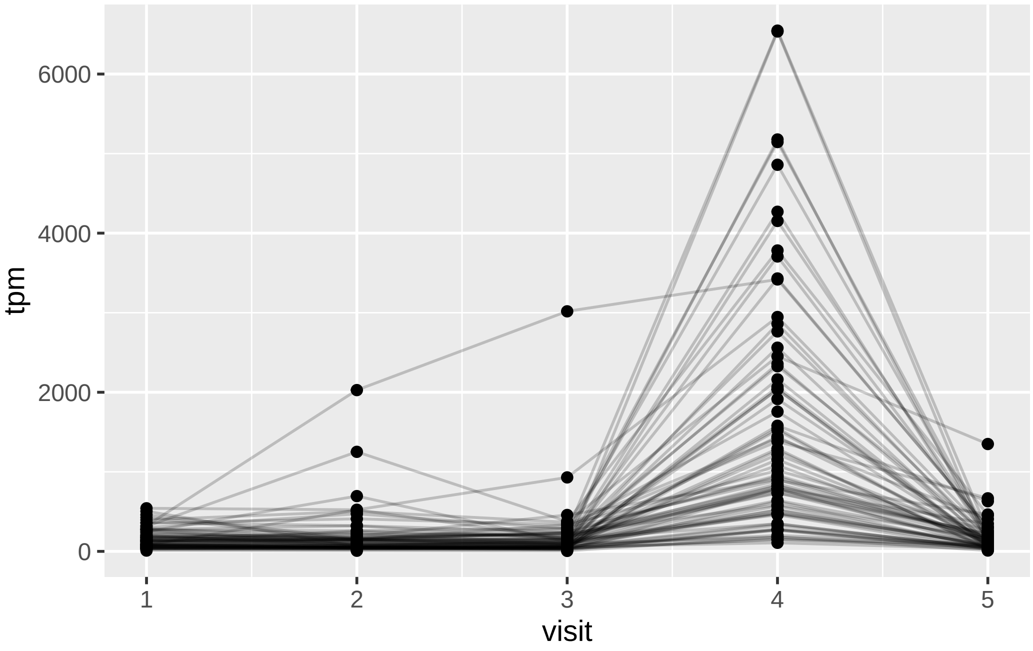
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.

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

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

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

Q18. Make a plot of the time course of gene expression for IGHG1 gene (i.e. a plot of visit vs. tpm).

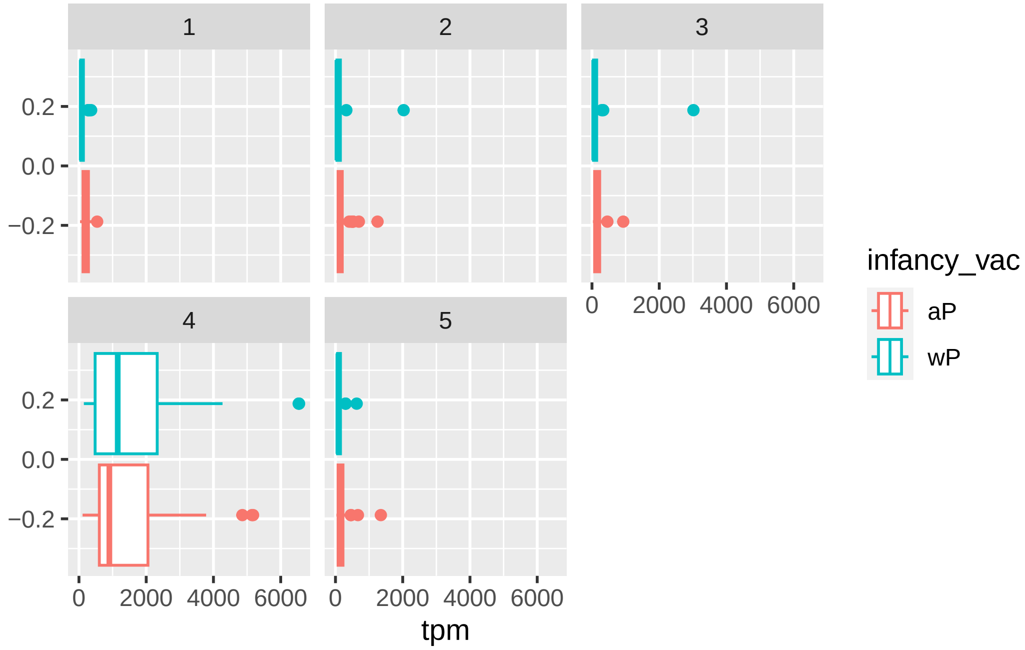
```
ggplot(ssrna) +
  aes(visit, tpm, group=subject_id) +
  geom_point() +
  geom_line(alpha=0.2)
```



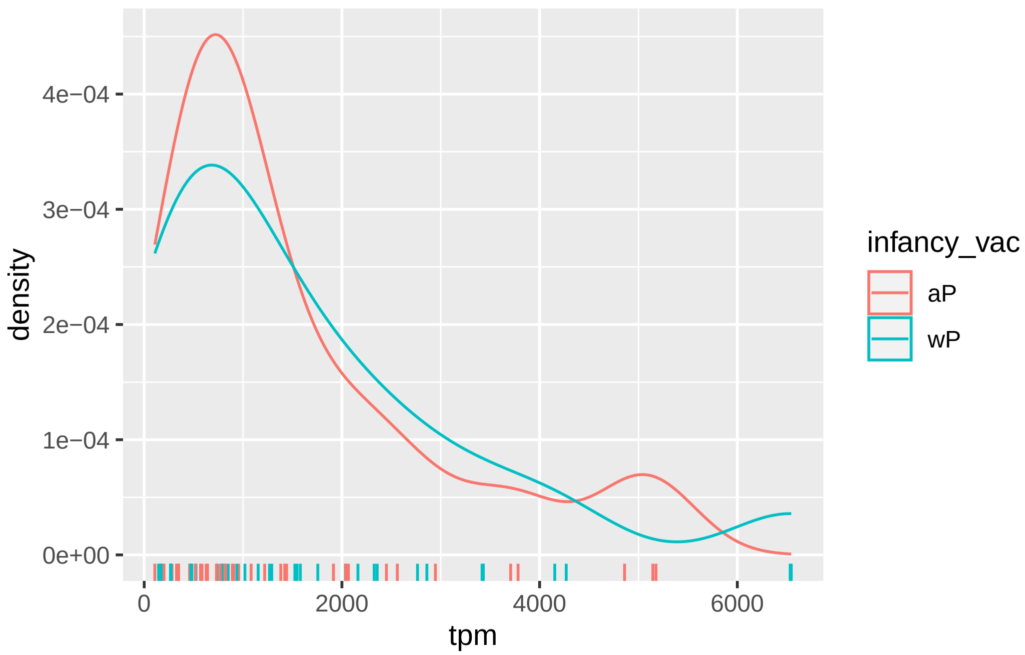
Q19. What do you notice about the expression of this gene (i.e. when is it at it's maximum level)? The gene expression peaks at visit 4.

Q20. Does this pattern in time match the trend of antibody titer data? If not, why not? This pattern does not match the antibody titer data, as the peak for the gene expression is at visit 4 while the antibody titer peak is at visit 5. This is because the gene is expressed to make the antibody, but the antibody itself will live for a long time, being more detectable after the initial gene expression.

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