Class 19

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Class 19 Mini-Project

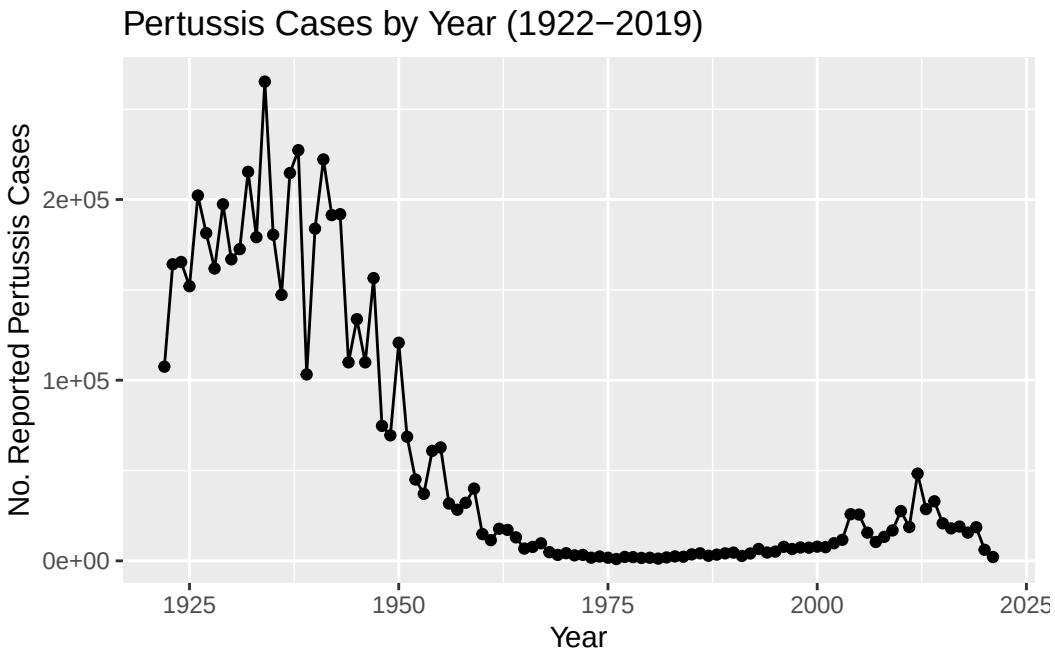
1. Investigating pertussis cases by year

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)

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)
)
```

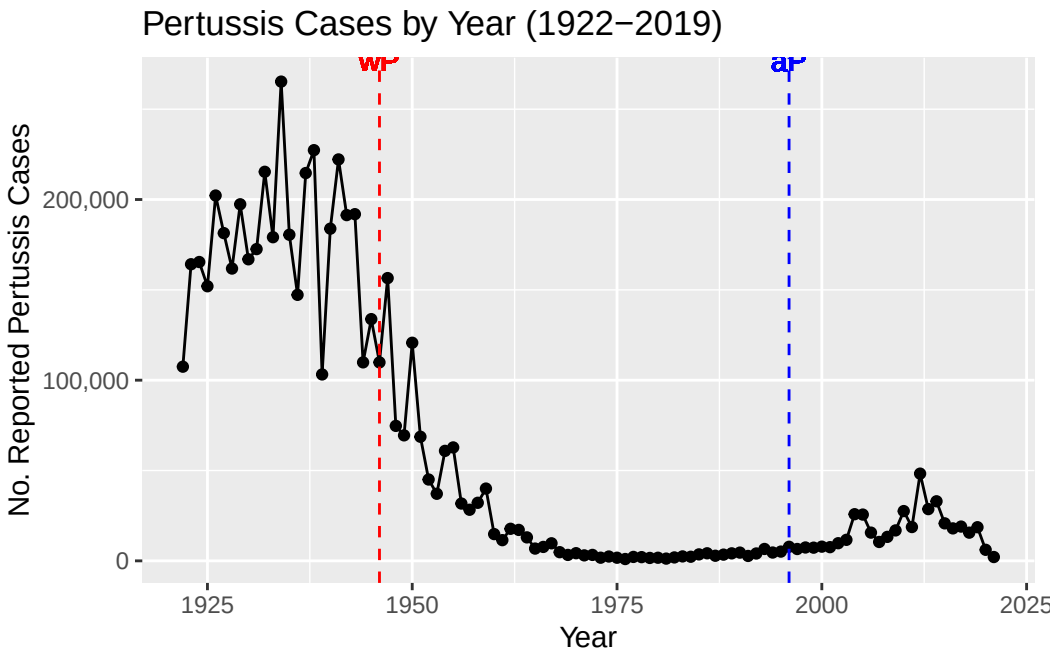
```
library(ggplot2)

ggplot(cdc) +
  aes(x = Year, y = No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  labs(x = "Year", y = "No. Reported Pertussis Cases", title = "Pertussis Cases by Year (1
```



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(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") +
  geom_text(aes(x = 1946, y = max(No..Reported.Pertussis.Cases), label = "wP"), vjust = -0
  geom_text(aes(x = 1996, y = max(No..Reported.Pertussis.Cases), label = "aP"), vjust = -0
  labs(x = "Year", y = "No. Reported Pertussis Cases", title = "Pertussis Cases by Year (1
  scale_y_continuous(labels = scales::comma)
```



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 aP vaccine, an increase in the number of reported Pertussis cases noticeably increased. From only a few thousand cases, the Pertussis cases increased to almost 50,000 by 2010--making this the most amount of Pertussis cases since the early 1950s. Possible explanations for this trend include bacterial evolution and vaccine hesitancy.

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
```

There are 47 aP and 49 wP infancy vaccinated subjects in this dataset.

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

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

```
Female   Male
    66     30
```

There are 66 female and 30 male subjects in the dataset.

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
```

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?

```
library(lubridate)
```

```
Attaching package: 'lubridate'

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

    date, intersect, setdiff, union
```

```
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
```

```
# Calculate age in days for all subjects
subject$age <- today() - ymd(subject$year_of_birth)
```

```
# Filter data for aP individuals
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
```

```
# Filter data for wP individuals
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
```

```
# Perform a t-test to assess the significance of the difference in means
ttest_result <- t.test(time_length(ap$age, "years"), time_length(wp$age, "years"))
ttest_result$p.value
```

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

(i) The average age of wP individuals is 37.

(ii) The average of aP individuals is 26.

(iii) Since the p-value (1.316e-16) < 0.05, they are significantly different.

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

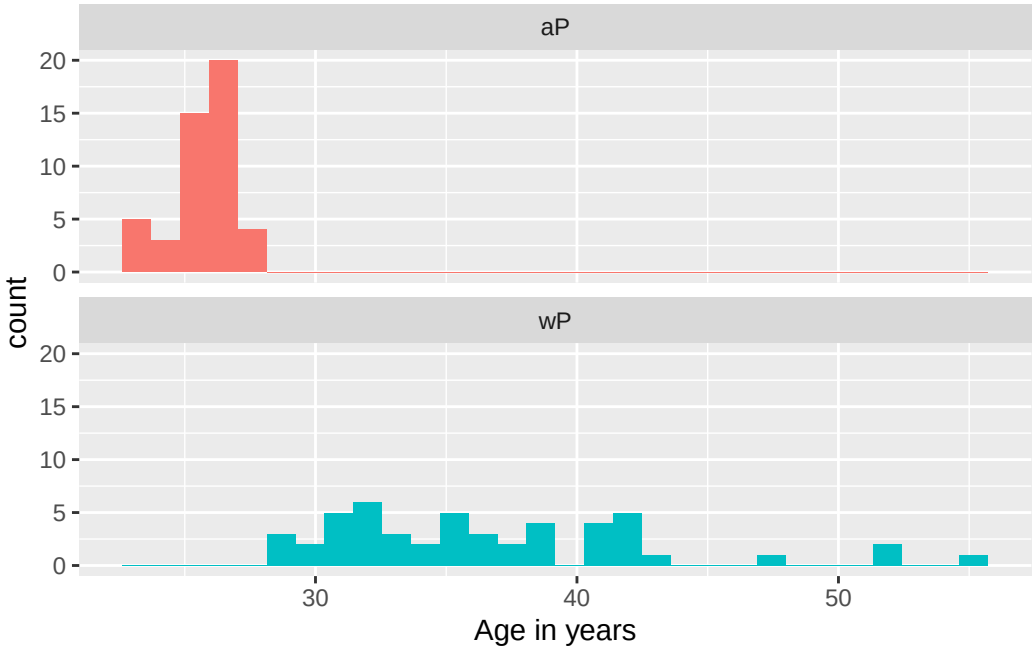
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
[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`.
```



Based on these plots, these two groups are clearly significantly different.

```
# 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 13682 days
2 13682 days
3 13682 days
4 13682 days
```

```
5 13682 days
6 13682 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
```

```
head(abdata)
```

```
  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 subject_id actual_day_relative_to_boost
1 UG/ML                 2.096133          1                           -3
2 IU/ML                29.170000          1                           -3
3 IU/ML                 0.530000          1                           -3
4 IU/ML                 6.205949          1                           -3
5 IU/ML                 4.679535          1                           -3
6 IU/ML                 2.816431          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 13682 days
2 13682 days
3 13682 days
4 13682 days
5 13682 days
6 13682 days
```

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
```

While the other visits range from 3920-5795, visit 8 specimens only have 80, which is significantly less than the other visits.

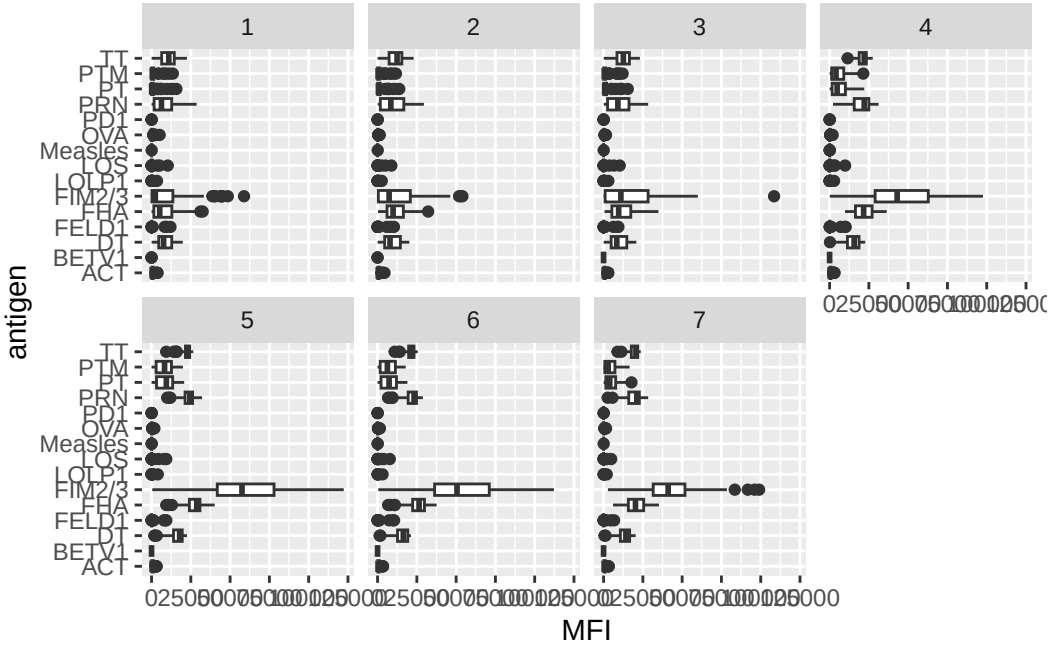
4. Examine IgG1 Ab titer levels

```
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 13682 days
2 13682 days
3 13682 days
4 13682 days
5 13682 days
6 13682 days
```

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

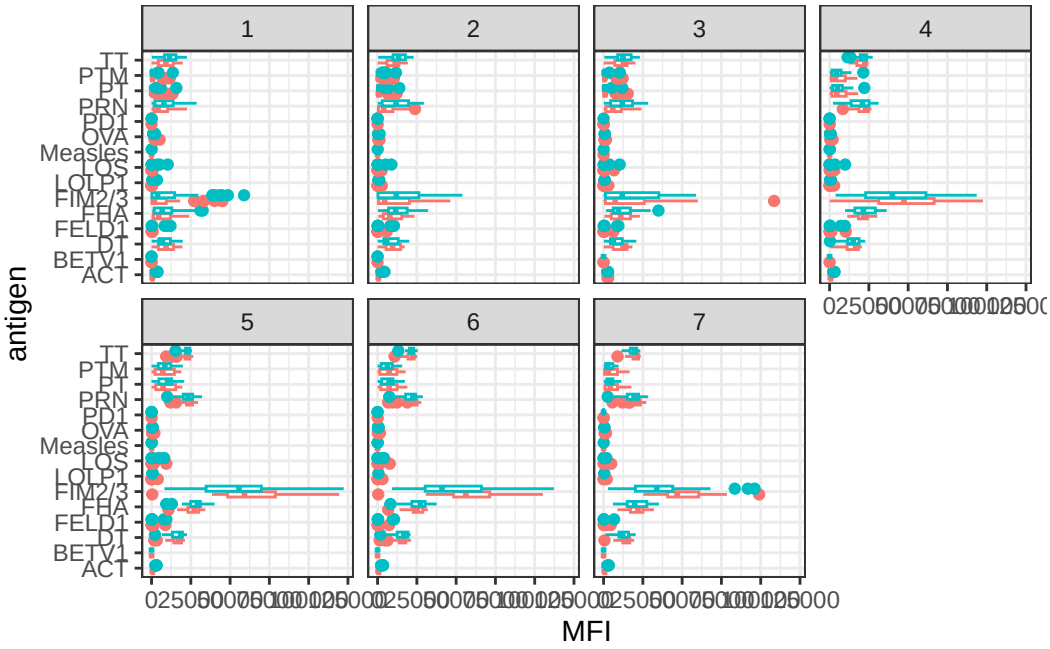
```
ggplot(ig1) +
  aes(antigen, MFI) +
  geom_boxplot() +
  facet_wrap(vars(visit), nrow = 2) +
  coord_flip()
```



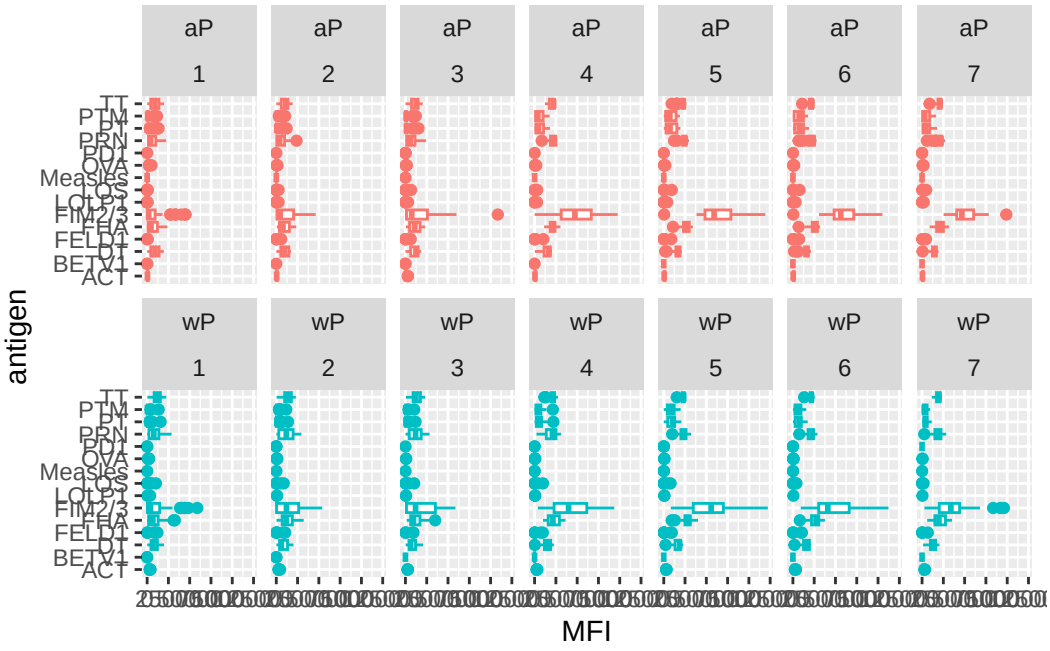
Q15. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

FIM2/3, FHA, and PRN show differences in the level of IgG1 antibody titers recognizing them over time. They are specific antigens that are known to elicit immune responses in individuals, potentially having unique properties that result in variations in the production and persistence of IgG1 antibodies over time. Additionally, it is possible that FIM2/3, FHA, and PRN are particularly immunogenic, leading to higher or more sustained IgG1 antibody titers compared to other antigens.

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

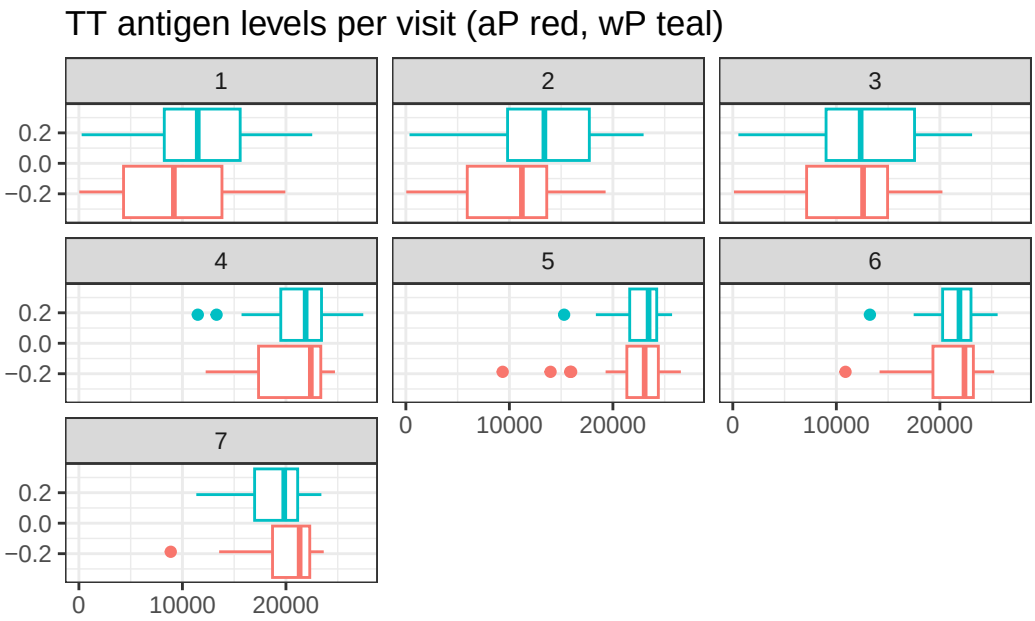



```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

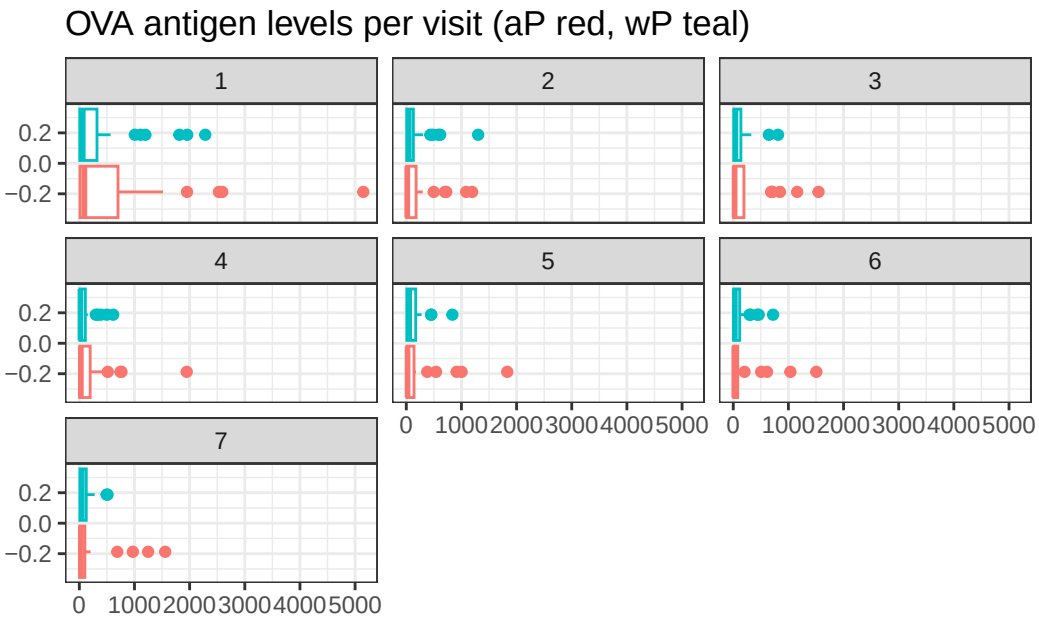


Q16. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can choose any you like. Below I picked a "control" antigen ("Measles", that is not in our vaccines) and a clear antigen of interest ("FIM2/3", extra-cellular fimbriae proteins from *B. pertussis* that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

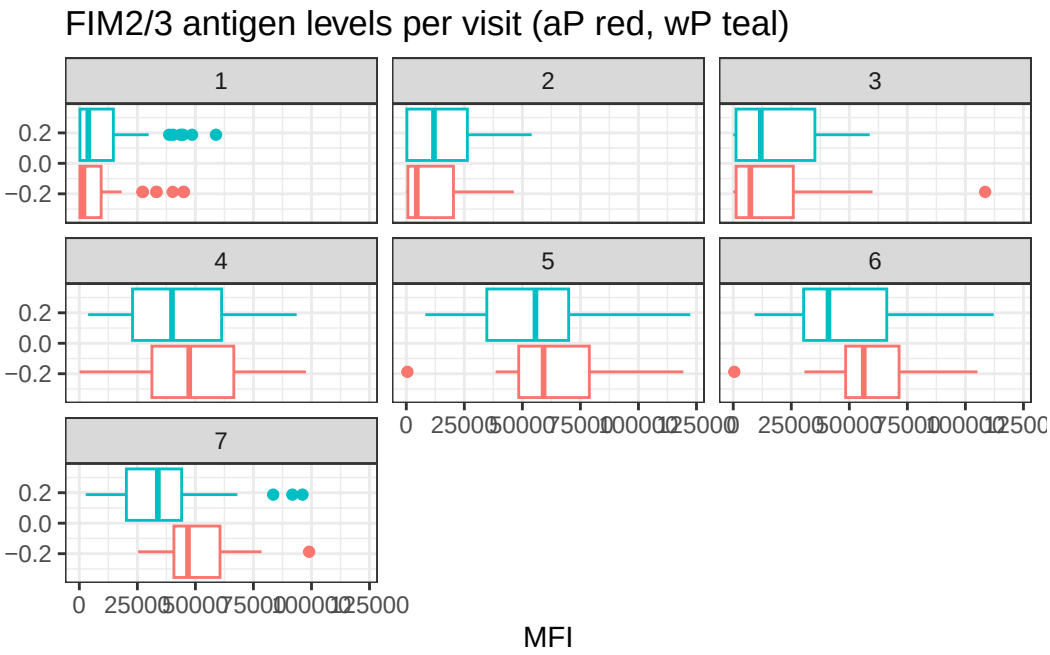
```
filter(ig1, antigen=="TT") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  labs(title = "TT antigen levels per visit (aP red, wP teal)")
```




```
filter(ig1, antigen=="OVA") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  labs(title = "OVA antigen levels per visit (aP red, wP teal)")
```




```
filter(ig1, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  labs(title = "FIM2/3 antigen levels per visit (aP red, wP teal)")
```



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

TT levels clearly rise over time, peaking at visit 6 and declining slightly at 7. OVA levels peak at visit 1 and decrease over time. FIM2/3 levels also rise over time, peaking at visit 5 and then declining after. All these trends appear similar for wP and aP subjects.

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

Not really, the results for both aP and wP responses are generally similar.

5. Obtaining CMI-PB RNASeq data

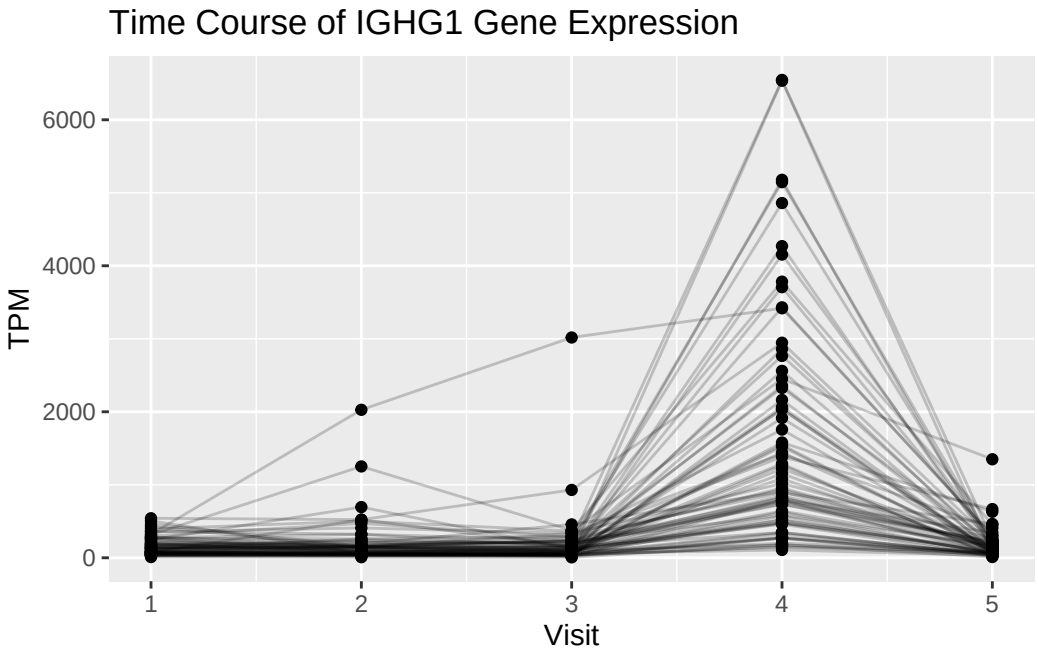
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.

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(x = visit, y = tpm, group = subject_id) +
  geom_point() +
  geom_line(alpha = 0.2) +
  labs(x = "Visit", y = "TPM", title = "Time Course of IGHG1 Gene Expression")
```



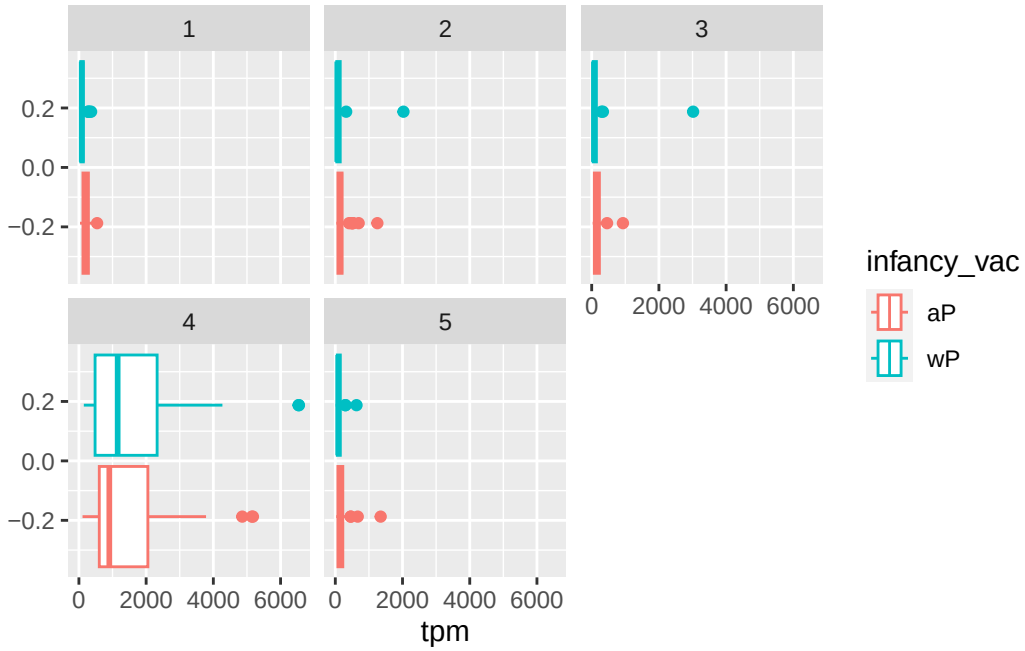
Q20. What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

The expression of this gene is at its maximum at visit 4.

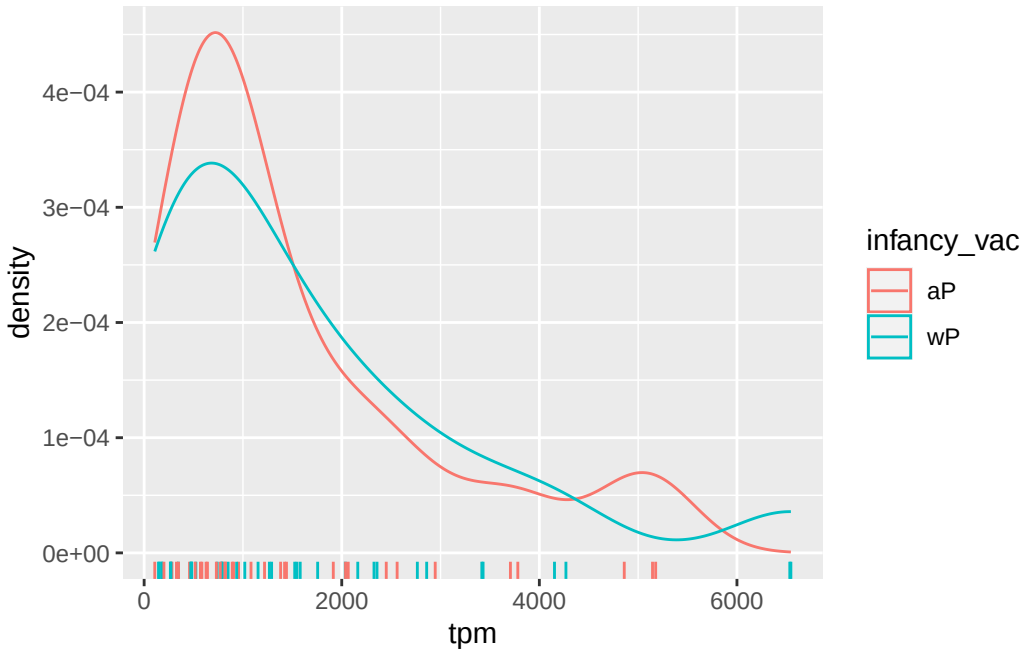
Q21. Does this pattern in time match the trend of antibody titer data? If not, why not?

Since the cell makes antibodies that are long-lived, then yes this pattern in time matches the antibody titer trend. At visit 4, TPM reaches its peak. We can see (referring back to Q15) that the antigens tend to peak around visit 4 or 5 as well, which is when the antibodies are needed.

```
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()
```


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