Lab 19 - Mini Project: Investigating Pertussis Resurgence

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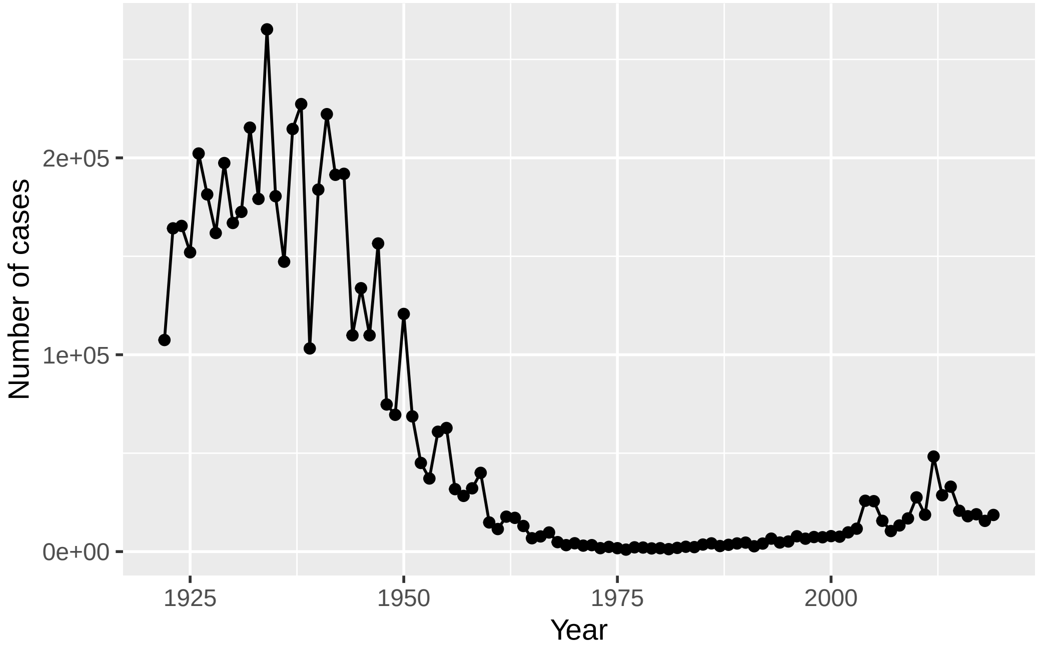
Q1. With the help of the R "addin" package datapasta assign the CDC pertussis case number data to a data frame called cdc and use ggplot to make a plot of cases numbers over time.

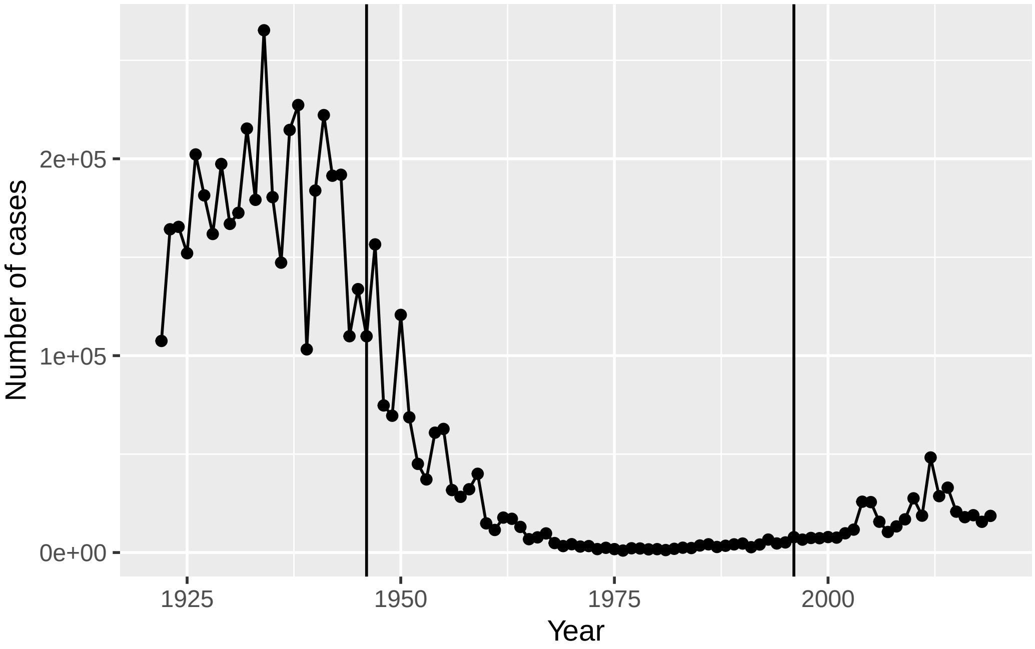
```
library(datapasta)

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),
         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)
       )
View(cdc)
```

```
library(ggplot2)
ggplot(cdc) +
  aes(Year, No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  labs(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?

```
ggplot(cdc) +
  aes(Year, No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  labs(x = "Year", y = "Number of cases") + geom_vline(xintercept = 1946) + geom_vline(xin
```



Q2A. I notice that the amount of pertussis cases decreased after the introduction of the wP vacccine and increased after the switch to the aP vaccine.

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

Q3A. After the introduction of the aP vaccine, pertussis cases increased. A possible explanation for the observed trend is hesitation in getting vaccines and increasing distrust in them by the public.

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

```
View(subject)
```

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

Q4A.

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

```

aP wP 
47 49 
```

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

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

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

```

Female   Male 
    66     30 
```

Q5A. There are 66 female and 30 male patients.

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

Q6A.

```r
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] "2023-03-20"
```

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

```
Time difference of 8479 days
```

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

```
[1] 23.21424
```

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
subject$age <- today() - ymd(subject$year_of_birth)
```

```r
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 <- 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 
```

```r
wp <- subject %>% filter(infancy_vac == "wP")
round( summary( time_length( wp$age, "years" ) ) )
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
     28      32      35      36      40      55 
```

Q7A. i. The average age of wP individuals is 36. ii. The average age of aP individuals is 26. iii. Yes, these ages are signifcantly different.

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

Q8A.

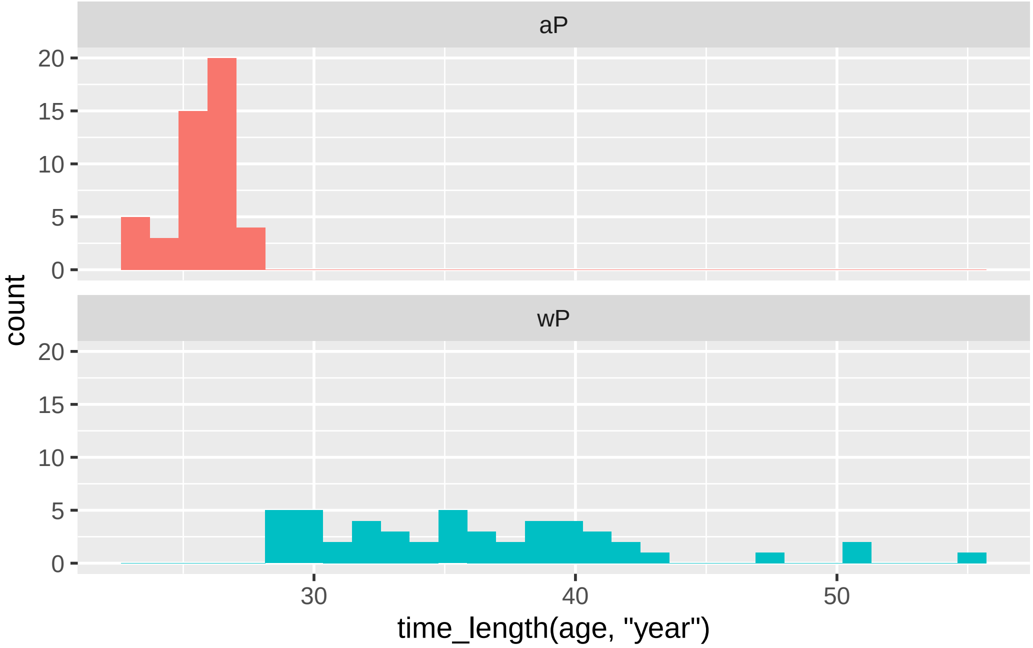
```r
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
[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?

```r
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`.
```



Q9A. Yes, these two groups are significantly different. AP vaccinated people had their vaccines at a much earlier age on average than wP vaccinated people.

```r
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)
```

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:

```
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 13592 days
2 13592 days
3 13592 days
4 13592 days
5 13592 days
6 13592 days
```

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)`
```

```r
dim(abdata)
```

```
[1] 32675    21
```

Q11. How many specimens (i.e. entries in abdata) do we have for each isotype?

```r
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?

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

```

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

Q12A. There is a lower amount of visit 8 specimens compared to other visits.

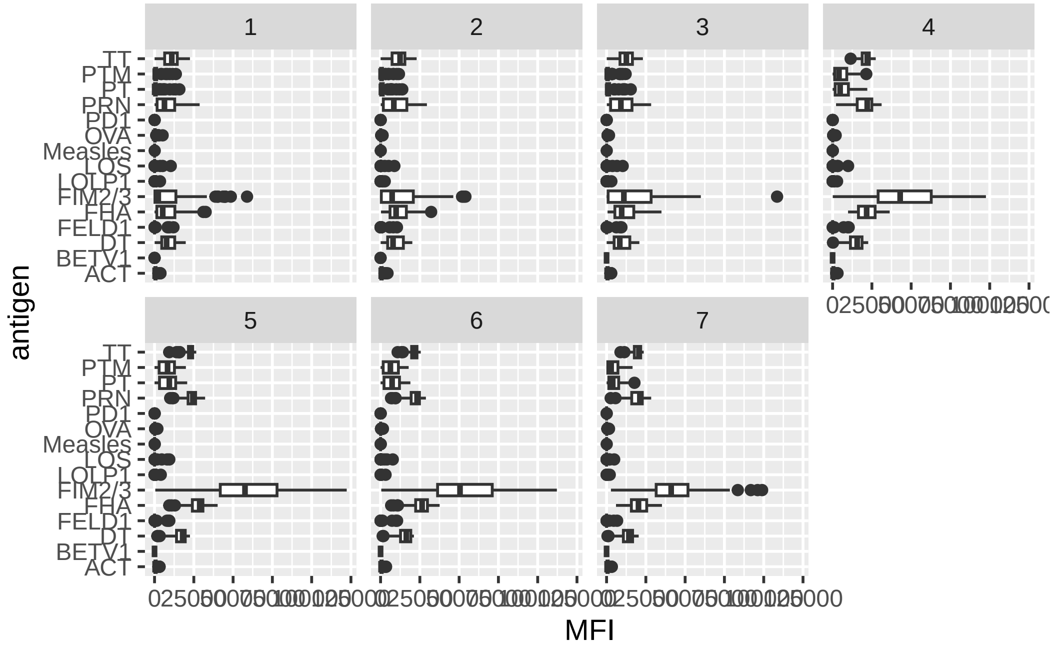
```r
ig1 <- abdata %>% filter(isotype == "IgG1", visit!=8)
head(ig1)
```

```
  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 13592 days
2 13592 days
3 13592 days
4 13592 days
5 13592 days
6 13592 days
```

```r
View(ig1)
```

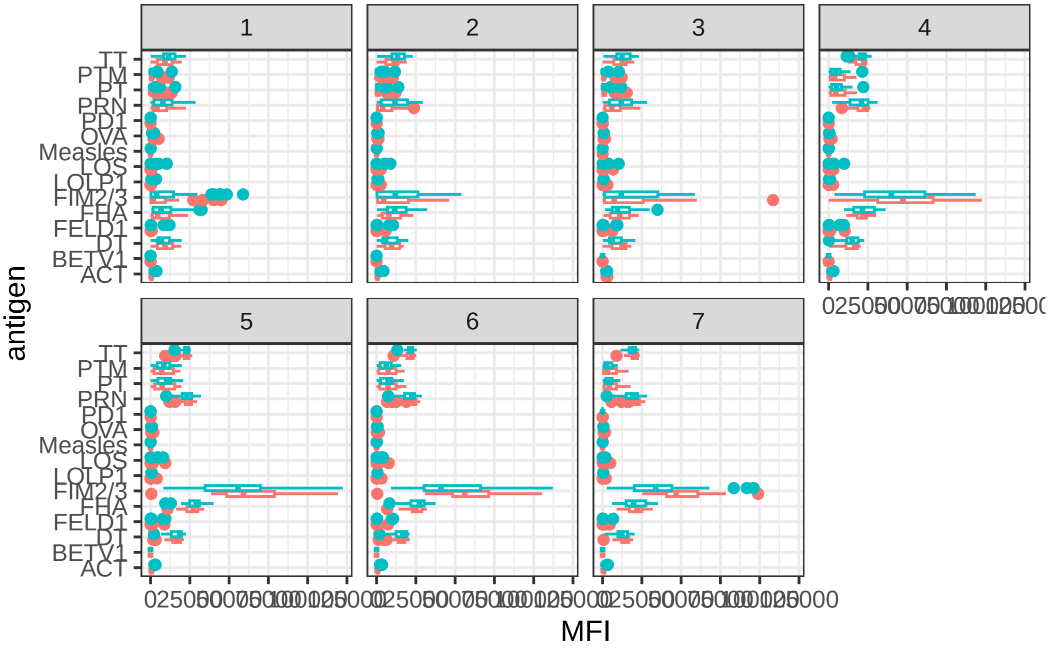
```r
ggplot(ig1) +
  aes(MFI, antigen) +
  geom_boxplot() +
  facet_wrap(vars(visit), nrow=2)
```



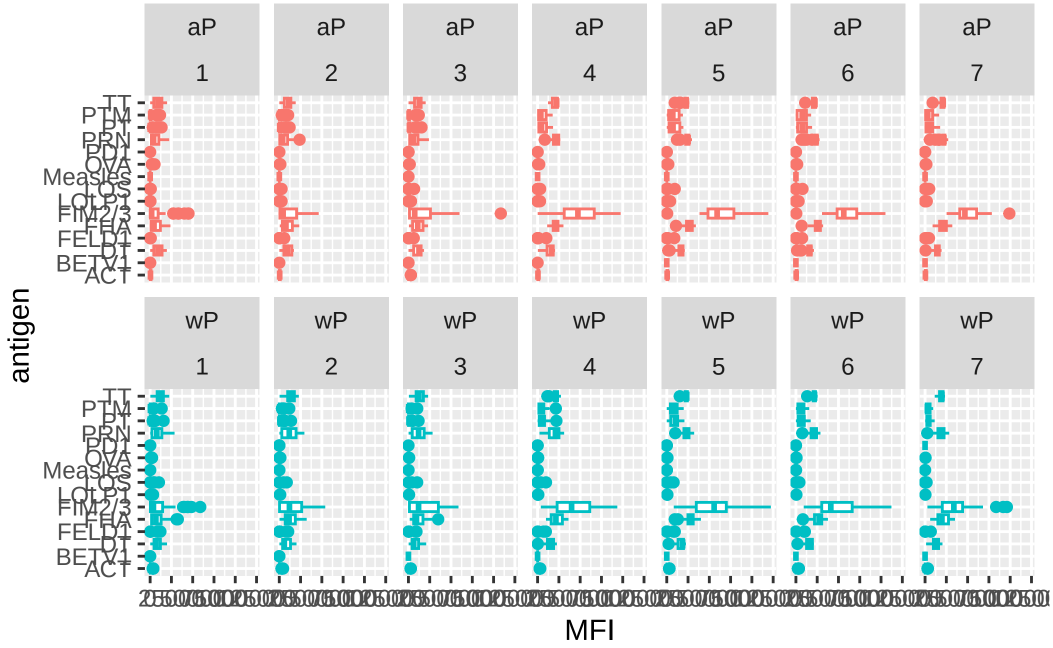
Q14. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

Q14A. FIM2/3, PRN, and FHA show differences in the level of IgG1 antibody titers recognizing them over time.

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

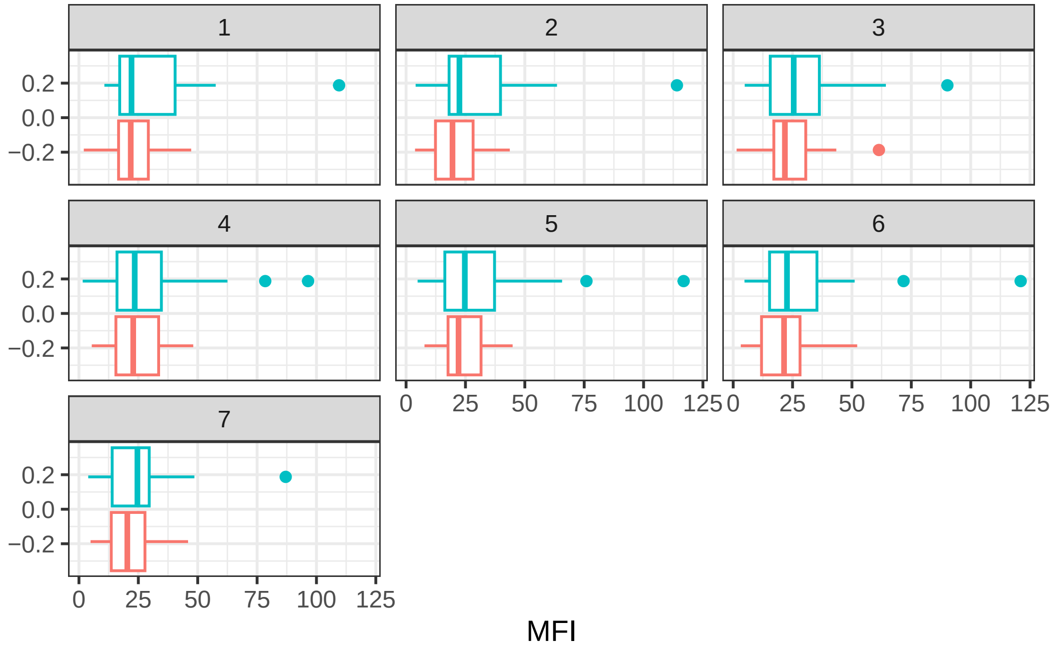


```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

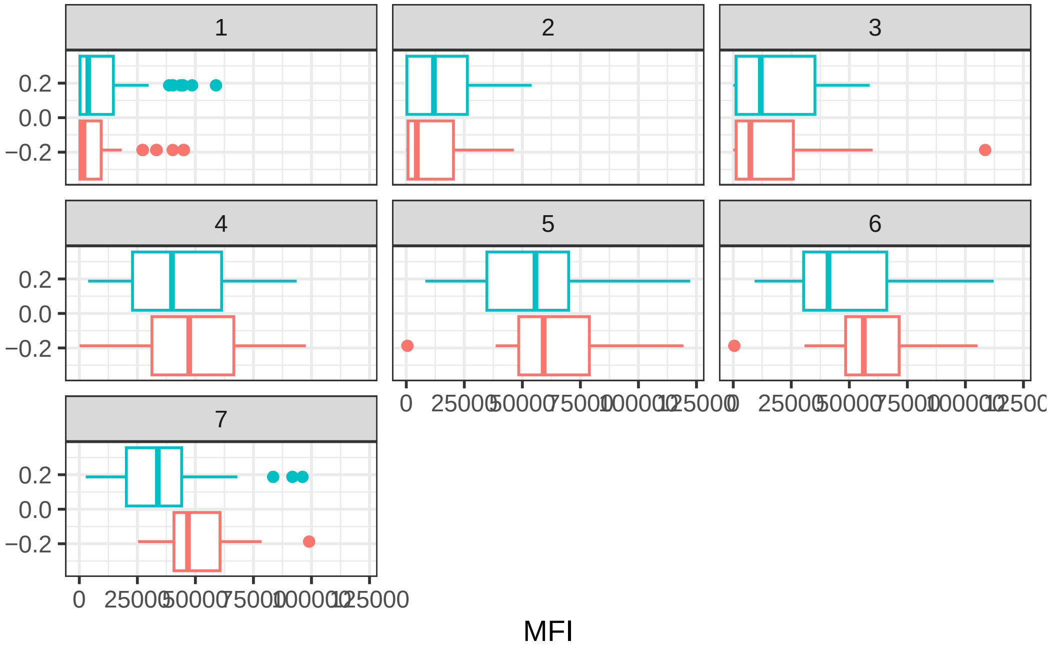


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("Measles", that is not in our vaccines) and a clear antigen of interest ("FIM2/3", extra-cellular fimbriae proteins from B. pertussis that participate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

```
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?

Q16A. FIM2/3 levels increased over time and exceeded those of measles. They also peak at visit 5 and then decrease after. WP and aP subjects both experience these trends.

Q17. Do you see any clear difference in aP vs. wP responses?

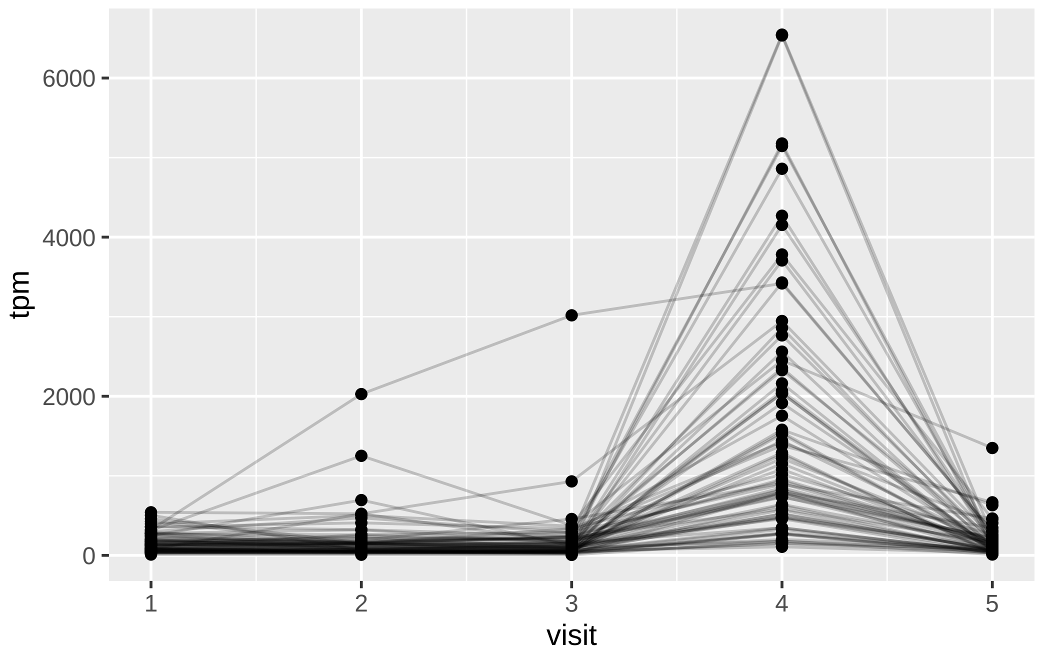
Q17A. The FIM2/3 levels of aP subjects increased more than wP subjects.

```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.
rna <- read_json(url, simplifyVector = TRUE)
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)
```



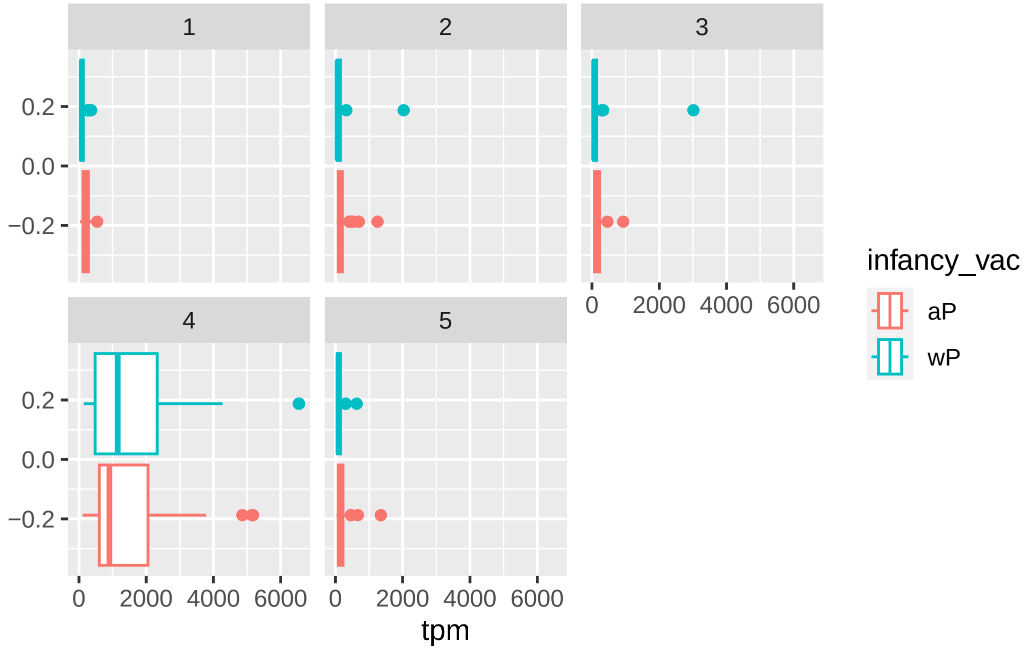
Q19.: What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

Q19A. I notice that the maximal expression of this gene occurs on visit 4 and then decreases after that.

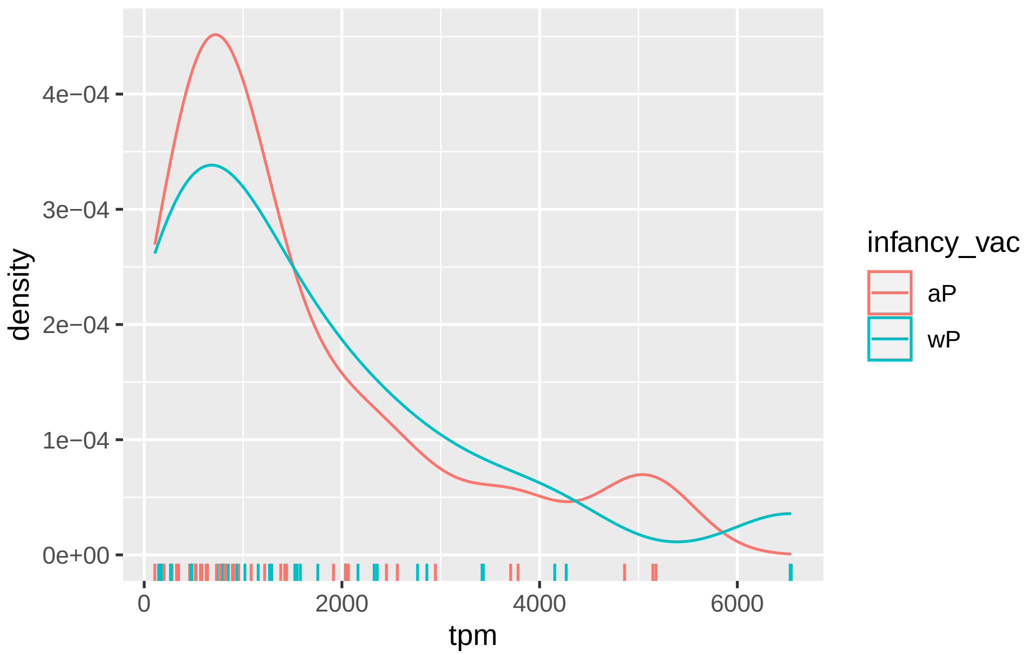
Q20. Does this pattern in time match the trend of antibody titer data? If not, why not?

Q20A. The pattern in time does match the trend of antibody titer data because the peak of expression levels occured on visit 5 for the antibody titer data. This means that the antibodies from the gene expression were remaining after gene expression was highest.

```
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()
```



```r
sessionInfo()
```

```
R version 4.2.2 (2022-10-31 ucrt)
Platform: x86_64-w64-mingw32/x64 (64-bit)
Running under: Windows 10 x64 (build 19044)

Matrix products: default

locale:
[1] LC_COLLATE=English_United States.utf8
[2] LC_CTYPE=English_United States.utf8
[3] LC_MONETARY=English_United States.utf8
[4] LC_NUMERIC=C
[5] LC_TIME=English_United States.utf8

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
[1] dplyr_1.1.0     lubridate_1.9.2 jsonlite_1.8.4  ggplot2_3.4.1
[5] datapasta_3.1.0
```

```
loaded via a namespace (and not attached):
 [1] rstudioapi_0.14  knitr_1.42       magrittr_2.0.3   tidyselect_1.2.0
 [5] munsell_0.5.0    timechange_0.2.0 colorspace_2.1-0 R6_2.5.1
 [9] rlang_1.0.6      fastmap_1.1.1    fansi_1.0.4      tools_4.2.2
[13] grid_4.2.2       gtable_0.3.1     xfun_0.37        utf8_1.2.3
[17] cli_3.6.0        withr_2.5.0      htmltools_0.5.4  yaml_2.3.7
[21] digest_0.6.31    tibble_3.1.8     lifecycle_1.0.3  farver_2.1.1
[25] vctrs_0.5.2      glue_1.6.2       evaluate_0.20    rmarkdown_2.20
[29] labeling_0.4.2   compiler_4.2.2   pillar_1.8.1     generics_0.1.3
[33] scales_1.2.1     pkgconfig_2.0.3
```