class_19

Jimmi Nguyen

1. Investigating pertussis cases by year

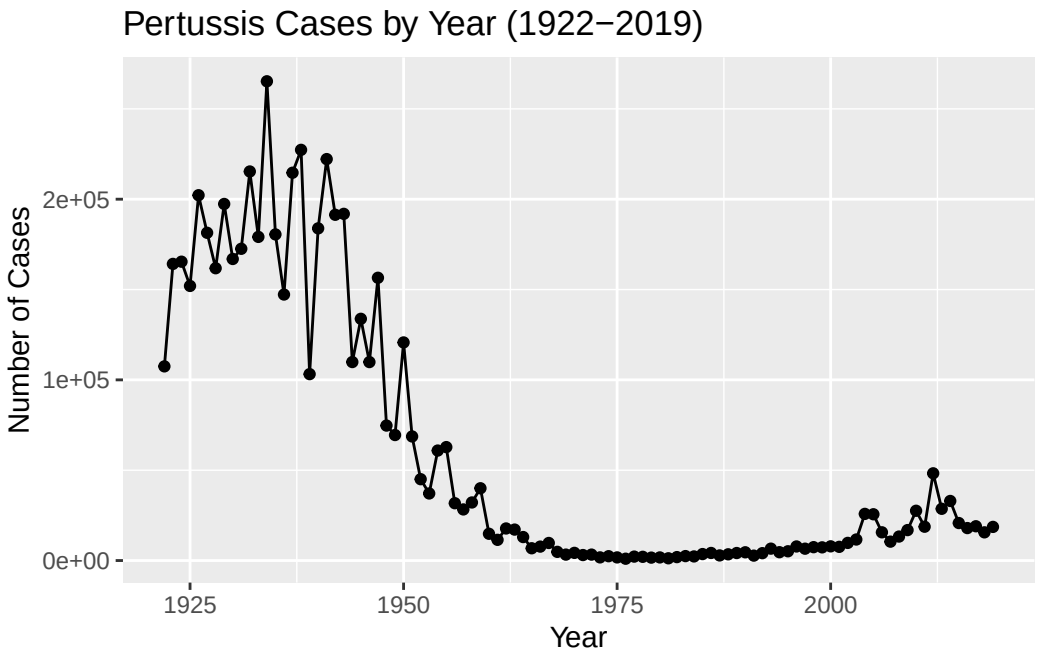
Q1. With the help of the R "addin" package datapasta assign the CDC pertussis case number data to a data frame called cdc and use ggplot to make a plot of cases numbers over time.

```
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)
)
```

```
library(ggplot2)

plot = ggplot(cdc) +
  aes(x = Year, y = No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  labs(title = "Pertussis Cases by Year (1922-2019)", x="Year",y="Number of Cases")
plot
```

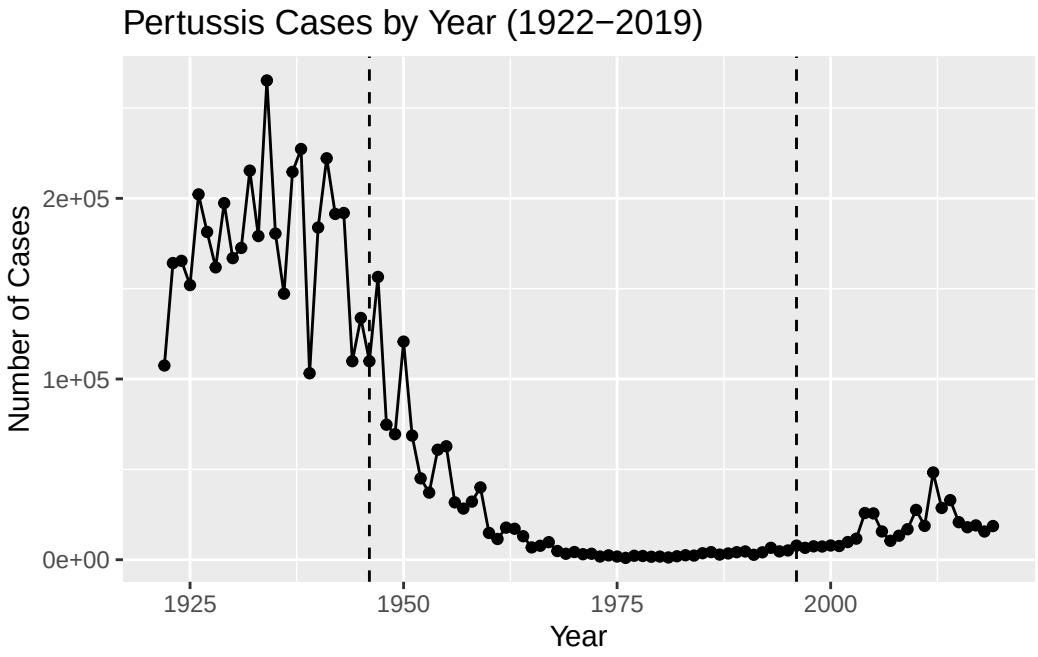


2. A tale of two vaccines (wP & aP)

Let's return to our CDC data plot and examine what happened after the switch to the acellular pertussis (aP) vaccination program

> 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?

```
plot + geom_vline(xintercept = c(1946,1996), linetype=2)
```



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

There was a slight increase in number of pertussis cases after the aP vaccine was introduced. An explanation for the trend would be resistance through bacterial evolution.

3. Exploring CMI-PB data

The CMI-PB API (like most APIs) sends responses in JSON format. Briefly, JSON data is formatted as a series of key-value pairs, where a particular word ("key") is associated with a particular value. An example of the JSON format for Ab titer data is shown below:

```
{
"specimen_id":1,
 "isotype":"IgG",
 "is_antigen_specific":true,
 "antigen":"PT",
 "ab_titer":68.5661390514946,
 "unit":"IU/ML",
 "lower_limit_of_detection":0.53
 }
```


To read these types of files into R we will use the read_json() function from the jsonlite package. Note that if you want to do more advanced querys of APIs directly from R you will likely want to explore the more full featured rjson package. The big advantage of using jsonlite for our current purposes is that it can simplify JSON key-value pair arrays into R data frames without much additional effort on our part.

```
# Allows us to read, write and process JSON data
library(jsonlite)
```

Let's now read the main subject database table from the CMI-PB API. You can find out more about the content and format of this and other tables here: https://www.cmi-pb.org/blog/understand-data/

```
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 may 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$biological_sex,subject$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
```

Two of the columns of subject contain dates in the Year-Month-Day format. Recall from our last mini-project that dates and times can be annoying to work with at the best of times. However, in R we have the excellent lubridate package, which can make life allot easier. Here is a quick example to get you started:

```
library(lubridate)
```

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

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

    date, intersect, setdiff, union
```

What is today's date (at the time I am writing this obviously)

```
today()
```

```
[1] "2023-03-18"
```

How many days have passed since new year 2000

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

```
Time difference of 8477 days
```

What is this in years?

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

```
[1] 23.20876
```

Note that here we are using the ymd() function to tell lubridate the format of our particular date and then the time_length() function to convert days to years.

> 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)
```

i.

```
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      36      40      55
```

wP Average is 36 years old.

ii.

```
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
```

aP Average age is 26 years old

iii.

They significantly differ by a 10 years difference.

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

```
subject$age_vac <- ymd(subject$date_of_boost)-ymd(subject$year_of_birth)

round( summary( time_length( subject$age_vac, "years" ) ) )
```

```
 Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
   19      20      23      26      29      51
```

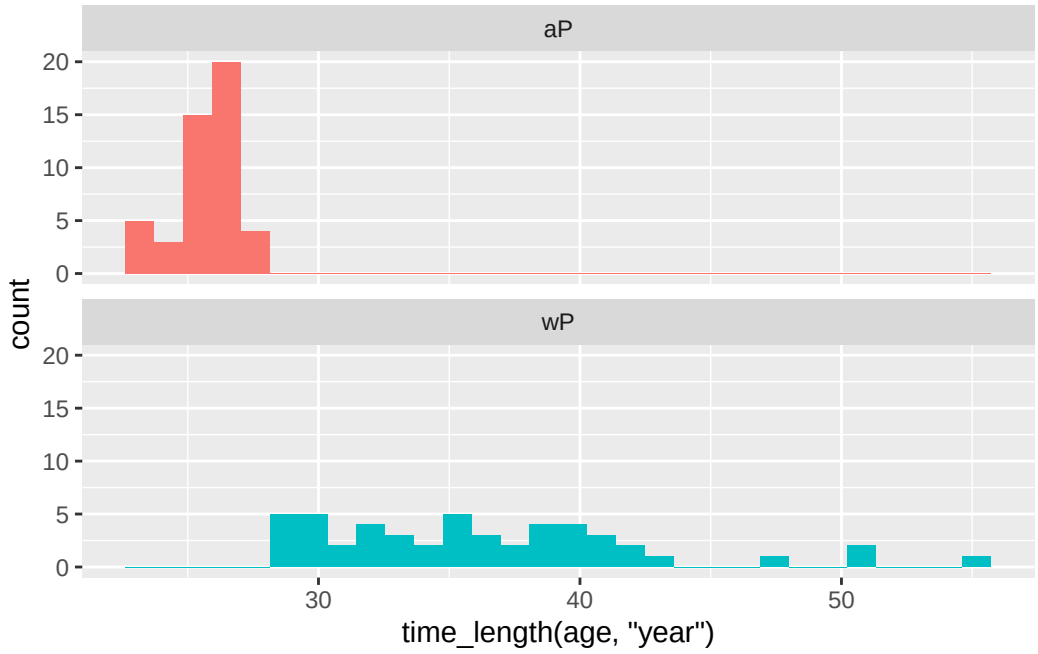
```
head(time_length(subject$age_vac, "year"))
```

```
[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?

```
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`.
```




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

x$p.value
```

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

these two groups are significantly different with a p-value of $1.3160451 \times 10^{-16}$

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)
```

> 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  15
```

```
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    age_vac
1 13590 days 11212 days
2 13590 days 11212 days
3 13590 days 11212 days
4 13590 days 11212 days
5 13590 days 11212 days
6 13590 days 11212 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.

```
abdata <- inner_join(titer, meta)
```

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

```
dim(abdata)
```

```
[1] 32675    22
```

11. 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?

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

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

They have the least amount of specimens.

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.

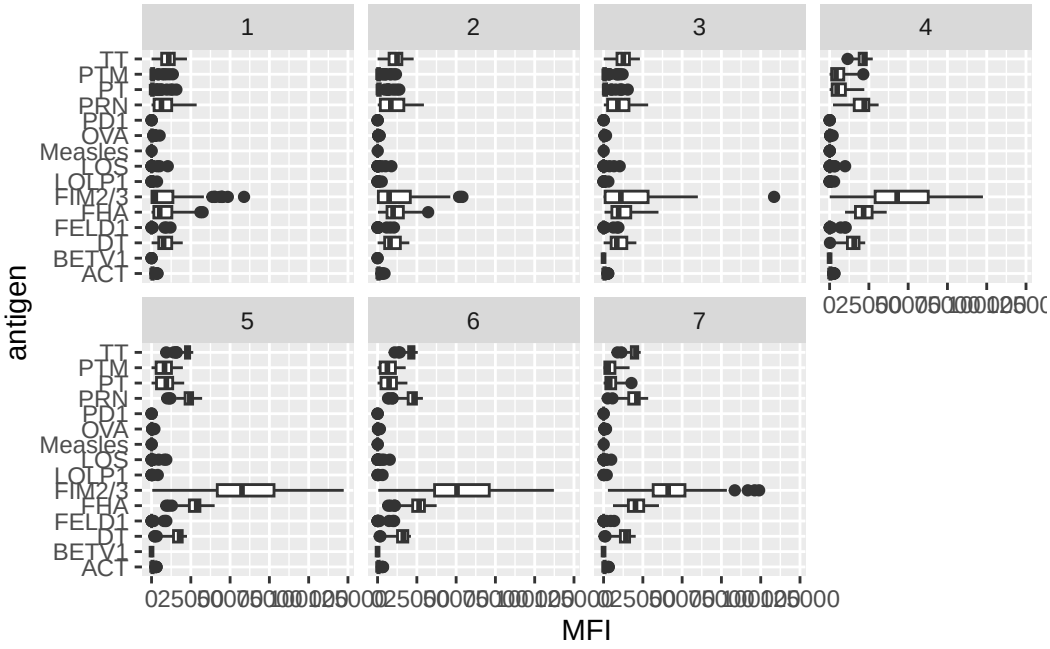
```
ig1 <- abdata %>% filter(isotype == "IgG1", visit!=8)
head(ig1)
```

```
  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    age_vac
1 13590 days 11212 days
2 13590 days 11212 days
3 13590 days 11212 days
4 13590 days 11212 days
5 13590 days 11212 days
6 13590 days 11212 days
```

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)
```



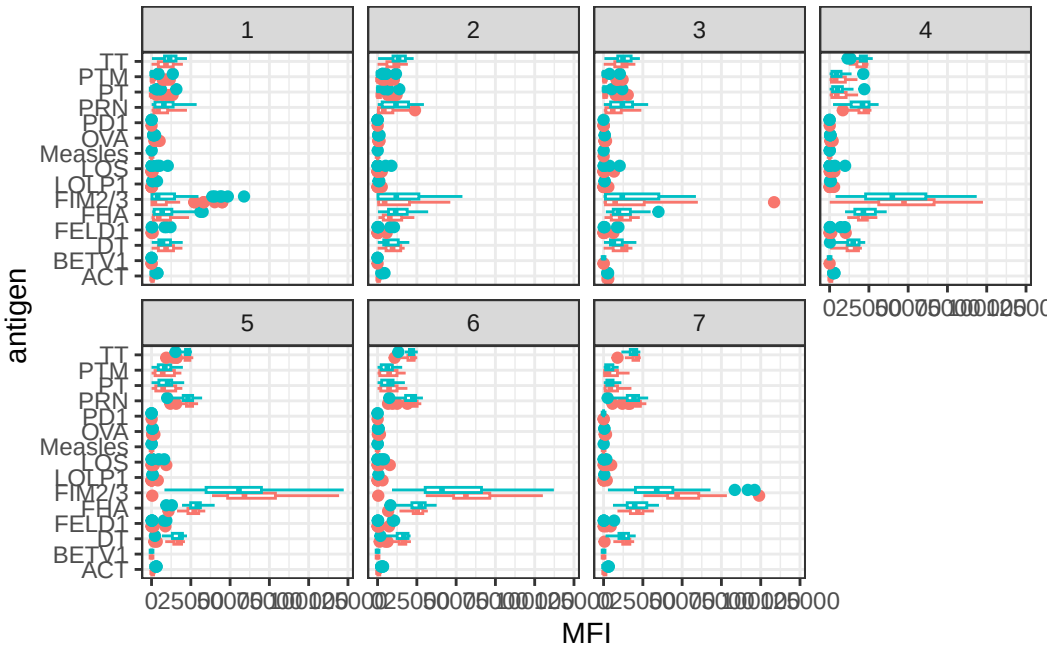
Q14. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

FIM2/3 show differences over time

We can attempt to examine differences between wP and aP here by setting color and/or facet

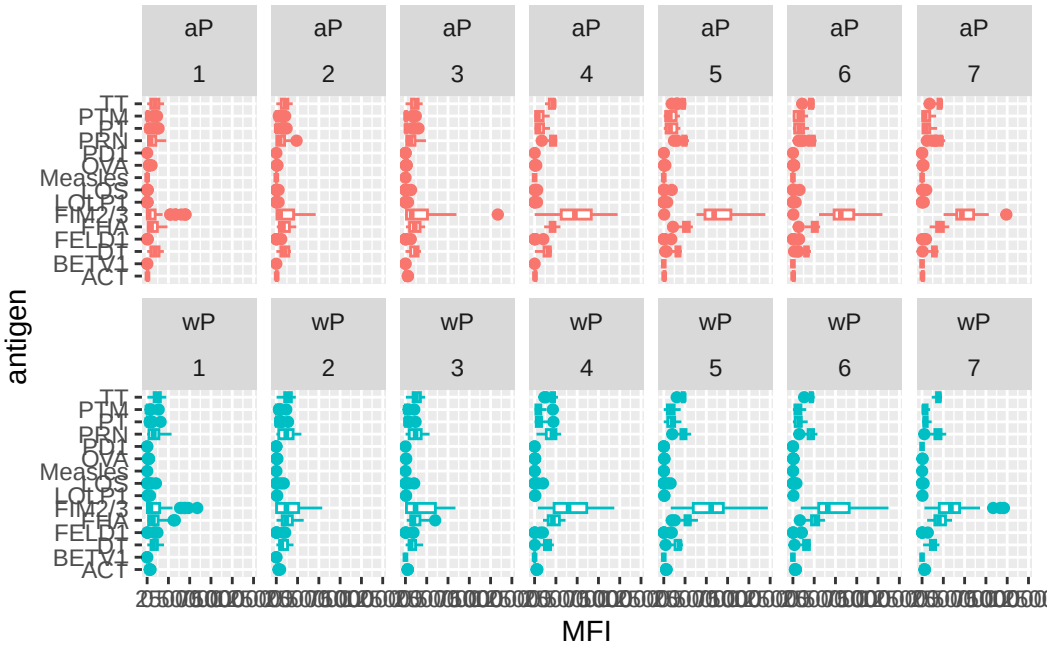
values of the plot to include infancy_vac status (see below). However these plots tend to be rather busy and thus hard to interpret easily.

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



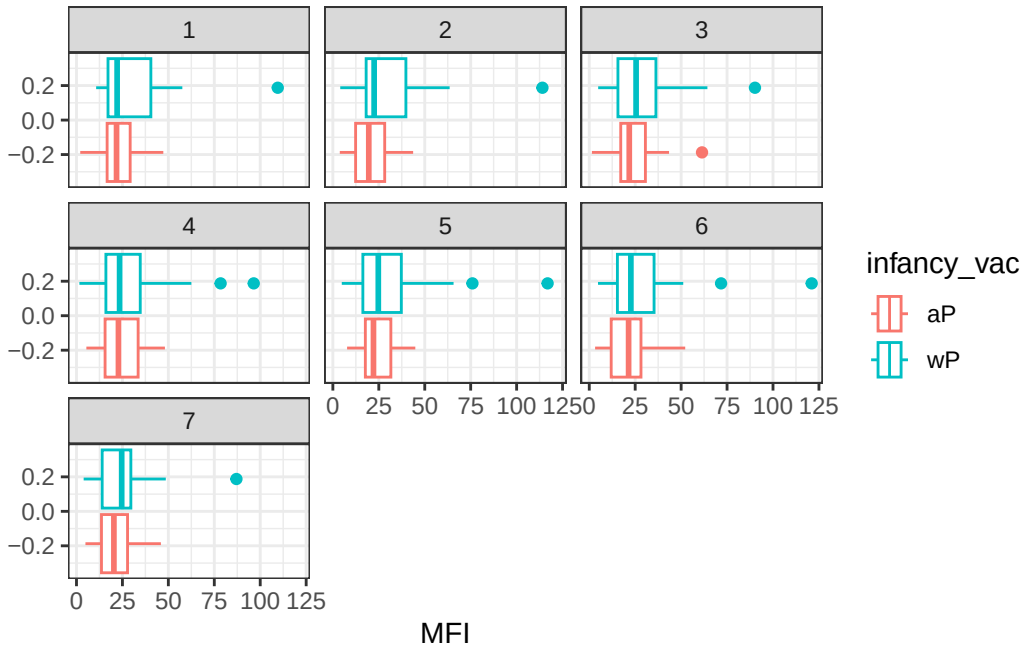
Another version of this plot adding `infancy_vac` to the faceting:

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



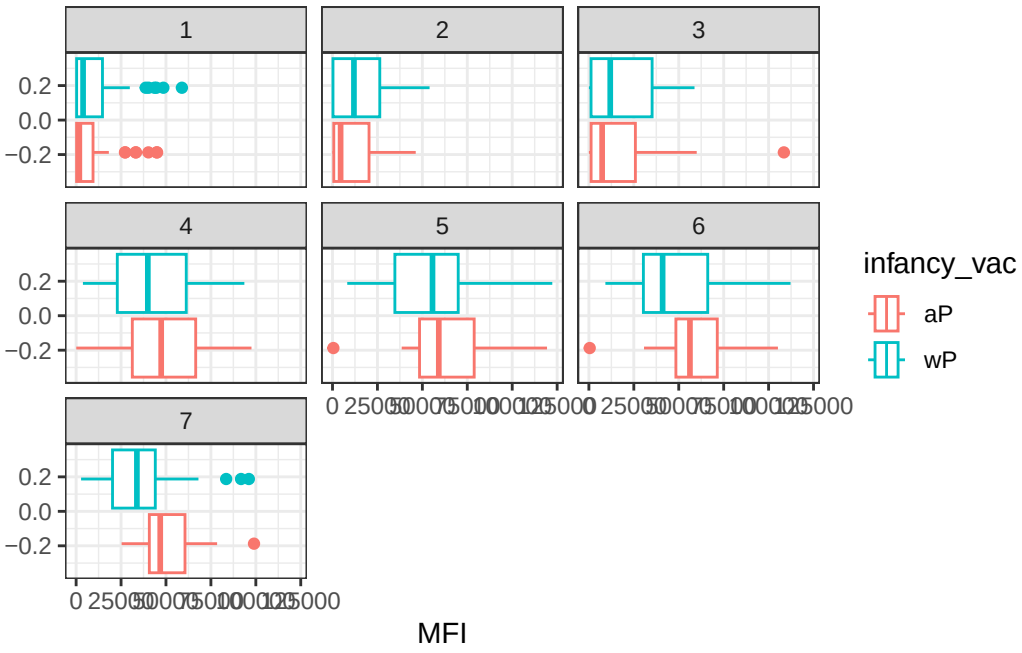
Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("Measles", that is not in our vaccines) and a clear antigen of interest ("FIM2/3", extra-cellular fimbriae proteins from B. pertussis that participate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

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



the same for antigen=="FIM2/3"

```
filter(ig1, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = T) +
  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 levels rise over time and far exceed Measles.

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

ap is generally higher in measles while there is no differences in FIM2/3 with a slight shift toward wp.

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.

For example we can obtain RNA-Seq results for a specific ENSEMBLE gene identifier or multiple identifiers combined with the & character:

```
# For example use the following URL
https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.7
```

The link above is for the key gene involved in expressing any IgG1 antibody, namely the IGHG1 gene. Let's read available RNA-Seq data for this gene into R and investigate the time course of it's gene expression values.

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

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

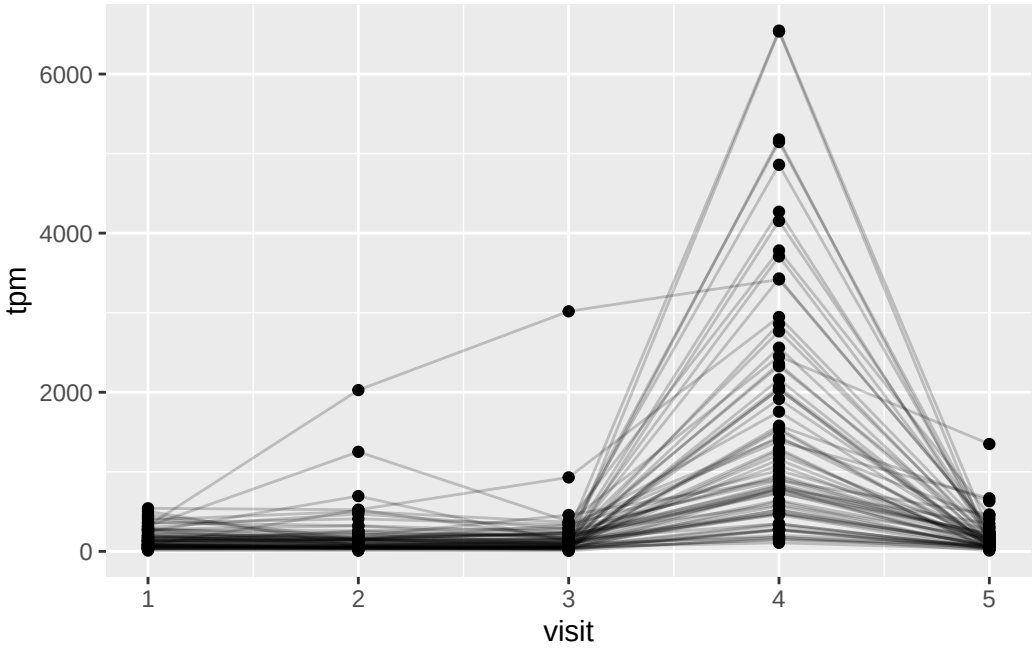
To facilitate further analysis we need to "join" the rna expression data with our metadata meta, which is itself a join of sample and specimen data. This will allow us to look at this genes TPM expression values over aP/wP status and at different visits (i.e. times):

```
#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)?

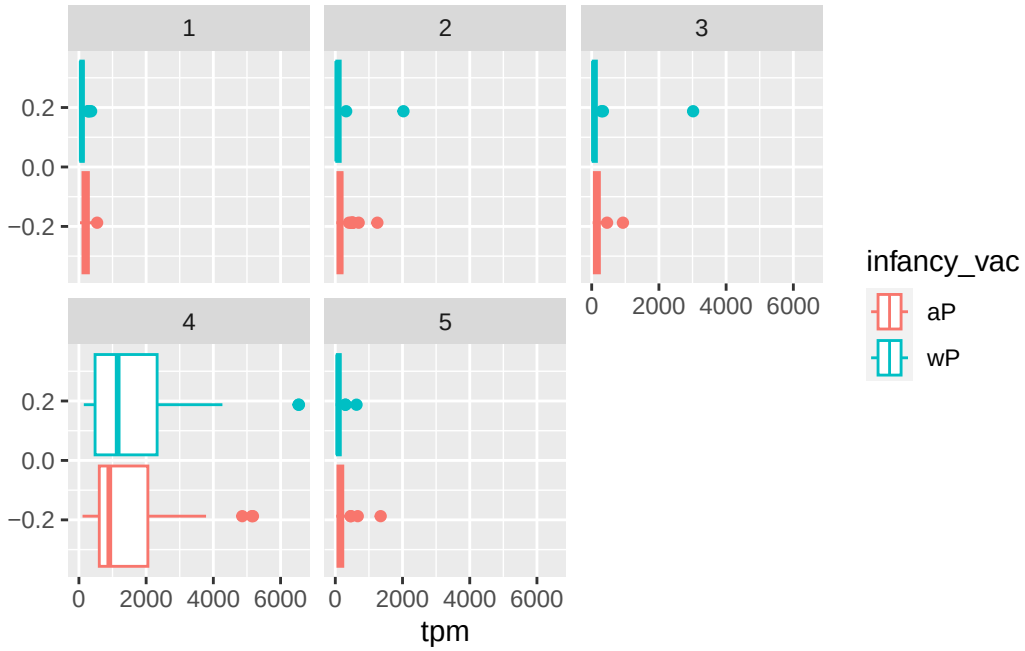
It has a maximum at 6500 spiking at visit 4 and subsequently falling dramatically by visit 5

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

it does not match because Cells make antibodies

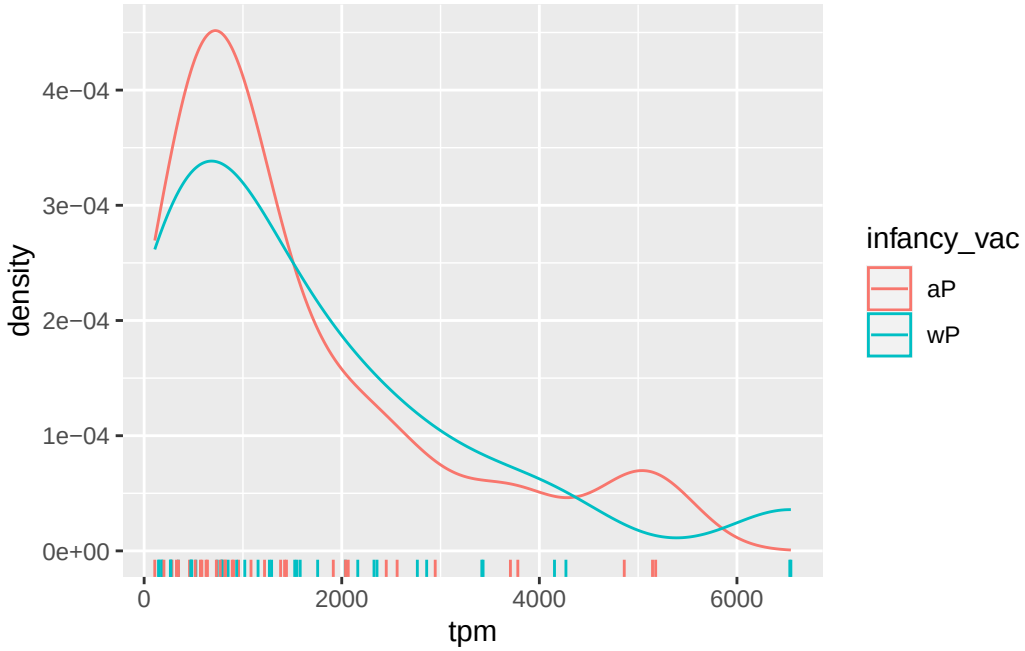
We can dig deeper and color and/or facet by infancy_vac status:

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



There is 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()
```




```
sessionInfo()
```

```
R version 4.2.2 (2022-10-31 ucrt)
Platform: x86_64-w64-mingw32/x64 (64-bit)
Running under: Windows 10 x64 (build 22621)

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

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
```