Class_19:Investigating_Pertussis_Resurgence

Investigating Pertussis Resurgence

Investigating pertussis cases by year

The CDC tracks cases of Pertussis in the US. We can get their data via web-scrapping.

```
cdc
```

```
   Year  Cases
1  1922 107473
2  1923 164191
3  1924 165418
4  1925 152003
5  1926 202210
6  1927 181411
7  1928 161799
8  1929 197371
9  1930 166914
10 1931 172559
11 1932 215343
12 1933 179135
13 1934 265269
14 1935 180518
15 1936 147237
16 1937 214652
17 1938 227319
18 1939 103188
19 1940 183866
20 1941 222202
21 1942 191383
22 1943 191890
```

```
23 1944 109873
24 1945 133792
25 1946 109860
26 1947 156517
27 1948  74715
28 1949  69479
29 1950 120718
30 1951  68687
31 1952  45030
32 1953  37129
33 1954  60886
34 1955  62786
35 1956  31732
36 1957  28295
37 1958  32148
38 1959  40005
39 1960  14809
40 1961  11468
41 1962  17749
42 1963  17135
43 1964  13005
44 1965   6799
45 1966   7717
46 1967   9718
47 1968   4810
48 1969   3285
49 1970   4249
50 1971   3036
51 1972   3287
52 1973   1759
53 1974   2402
54 1975   1738
55 1976   1010
56 1977   2177
57 1978   2063
58 1979   1623
59 1980   1730
60 1981   1248
61 1982   1895
62 1983   2463
63 1984   2276
64 1985   3589
65 1986   4195
```

```
66 1987   2823
67 1988   3450
68 1989   4157
69 1990   4570
70 1991   2719
71 1992   4083
72 1993   6586
73 1994   4617
74 1995   5137
75 1996   7796
76 1997   6564
77 1998   7405
78 1999   7298
79 2000   7867
80 2001   7580
81 2002   9771
82 2003  11647
83 2004  25827
84 2005  25616
85 2006  15632
86 2007  10454
87 2008  13278
88 2009  16858
89 2010  27550
90 2011  18719
91 2012  48277
92 2013  28639
93 2014  32971
94 2015  20762
95 2016  17972
96 2017  18975
97 2018  15609
98 2019  18617
```

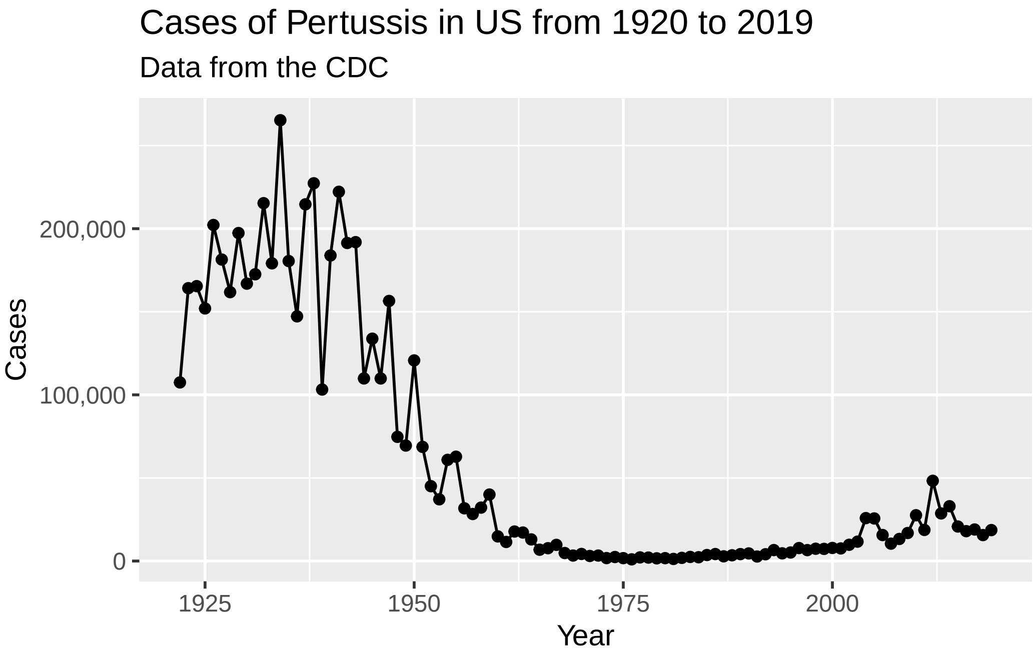
Make a plot with a trendline

```r
library("ggplot2")
library(scales)
```

> 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.

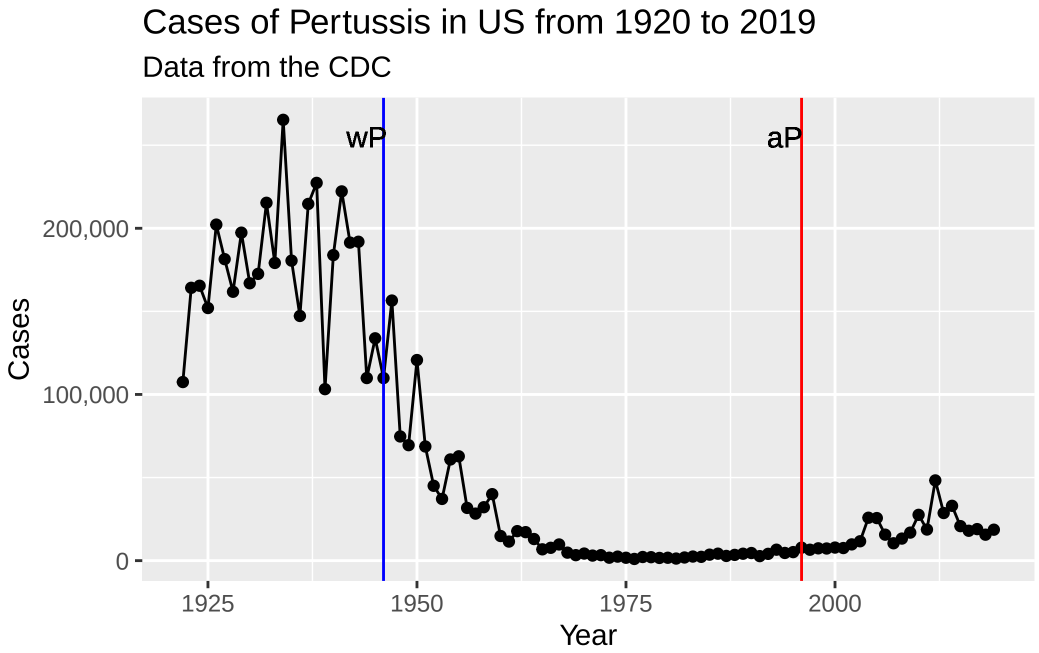
```
baseplot <- ggplot(cdc, aes(Year, Cases))+
  geom_point()+
  geom_line()+
  labs(title="Cases of Pertussis in US from 1920 to 2019", subtitle="Data from the CDC")+
  scale_y_continuous(labels = label_comma())
```

```
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?

```
baseplot+
  geom_vline(xintercept=1946, col="blue")+
  geom_vline(xintercept=1996, col="red")+
  geom_text(aes(x=1944,y=255000,label="wP"))+
  geom_text(aes(x=1994, y=255000, label="aP"))
```



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

When the wP vaccine was introduced, case numbers went down since people were getting the vaccine and it was very effective. When the aP vaccine was introduced, the number of cases remained low for a while but began to rise and is on an upward trend. This could be due to a few different reasons such as people being more skeptical to get vaccines due to misinformation or that the aP vaccine isn't as effective as the wP vaccine over a long period of time since cases were low for a while and then began to spike. It could also be that the bacteria evolved and gained some immunity to the vaccine which would explain the later spike in cases.

The CMI-PB Project

The CMI=PB project is collecting data on aP and wP individuals and their immune response to infection and/or booster shots.

CMI-PB returns data from it's API and JSON format (like most APIs). We will use the jsonlite package to get data from this API.

```r
library(jsonlite)

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

```r
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?

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

```

aP wP
47 49
```

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

```r
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…)?

```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
```

```
library(lubridate)
```

```

Attaching package: 'lubridate'

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

    date, intersect, setdiff, union
```

```
today()
```

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

> 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?

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

Now find the average age of all individuals:

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

```
[1] 31.05079
```

Now use splyr to subset to wP or aP subjects

(i) average age of wP individuals

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

```
[1] 36.36006
```

(ii) average age of aP individuals

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

```
[1] 25.5156
```

(iii) are they significantly different?

```r
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:
  9.044045 12.644857
sample estimates:
mean of x mean of y 
 36.36006  25.51560 
```

T-test tells us that the data is significantly different.

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

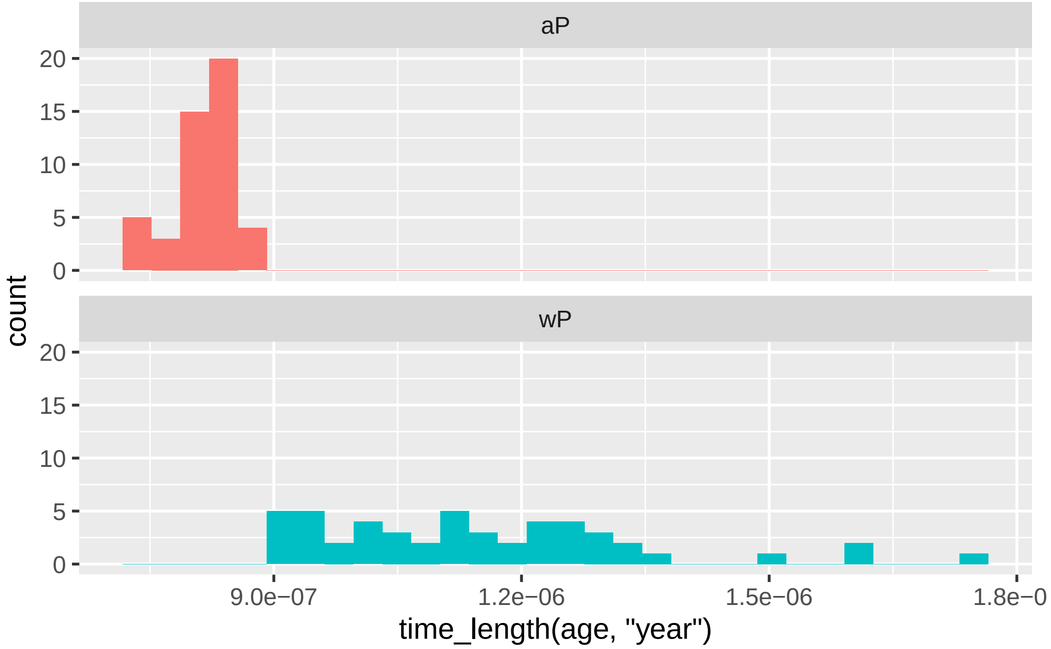
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

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

Q9. With the help of a faceted boxplot (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`.
```



Yes they are significantly different, if they were similar their graphs would look much more similar.

Joining multiple tables

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

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

titer <- read_json("https://www.cmi-pb.org/api/ab_titer", simplifyVector = T)
```

```
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
```

```
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
```

To know whether a given specimen_id comes from an aP or wP individual we need to link (a.k.a. "join" or merge) our specimen and subject data frames. The excellent dplyr package (that we have used previously) has a family of join() functions that can help us with this common task:

> 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 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.

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

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

```
dim(abdata)
```

```
[1] 32675    21
```

```
head(abdata,4)
```

```
  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
   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
```

```
  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
               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
       age
1 37.19644
2 37.19644
3 37.19644
4 37.19644
```

> 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?

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

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

The number of specimens for 8 visits is significantly lower than the other number of visits. The reason visit 8 is so small is because the project is still ongoing so the data hasn't fully been collected for visit 8 yet.

Examine IgG1 Ab titer levels

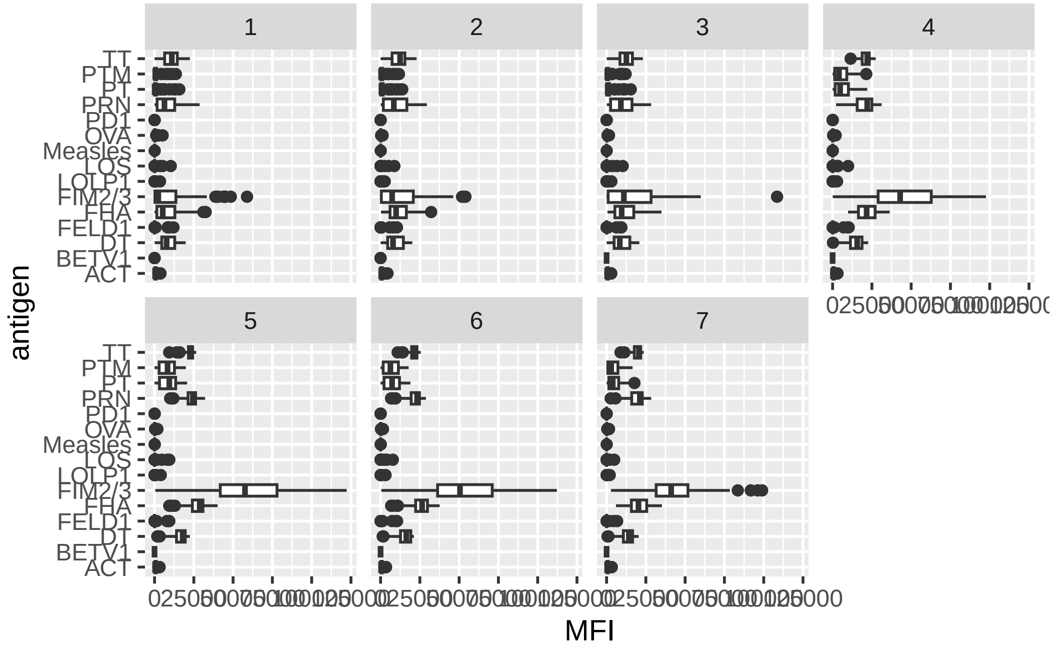
Exclude visit 8 from the analysis

```
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:

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

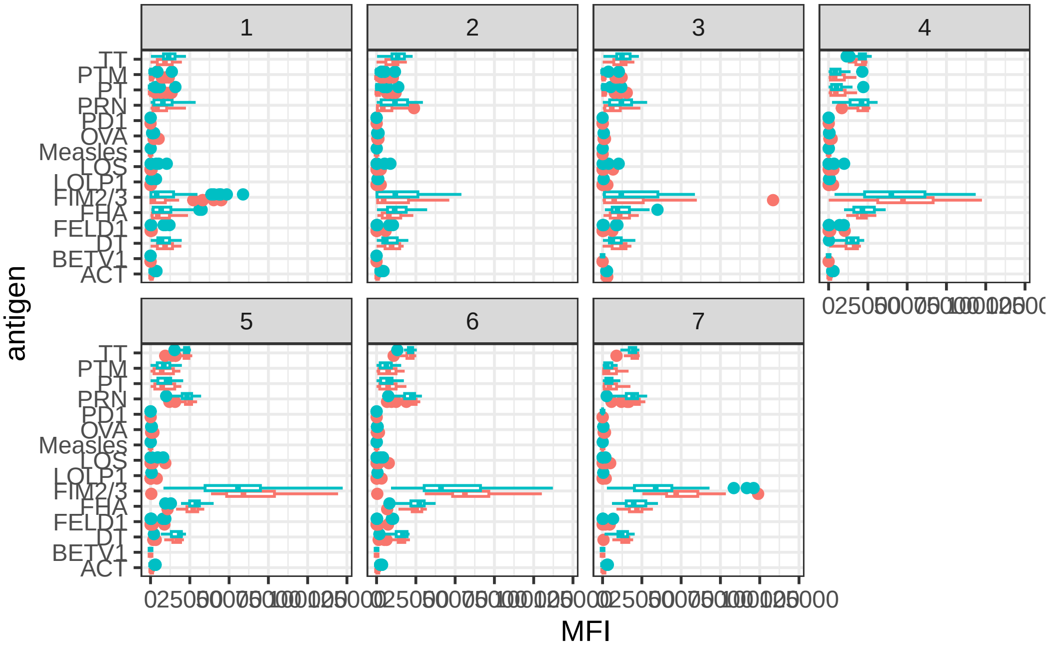


> Q14. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

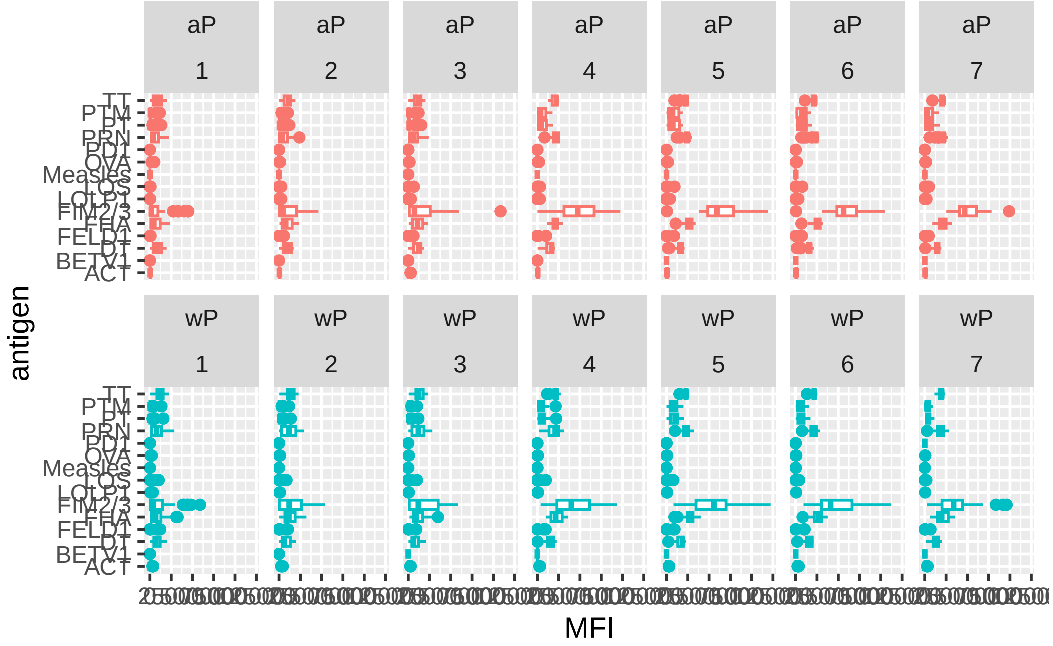
FIM 2/3 (fimbrial protein), FHA (filamentous hemagglutinin), and PRN (pertactin autotransporter). These are rising because they're part of the aP boost vaccine and the immune system is recognizing them. DT also increases as it is one of the antibodies respondign to the bacteria.

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.

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

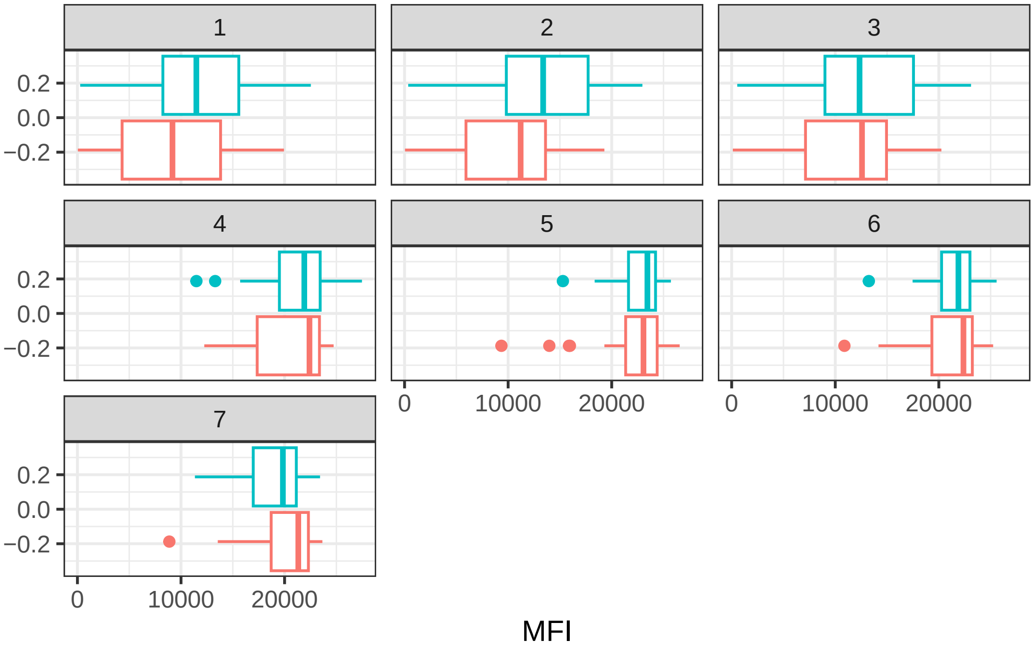


```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

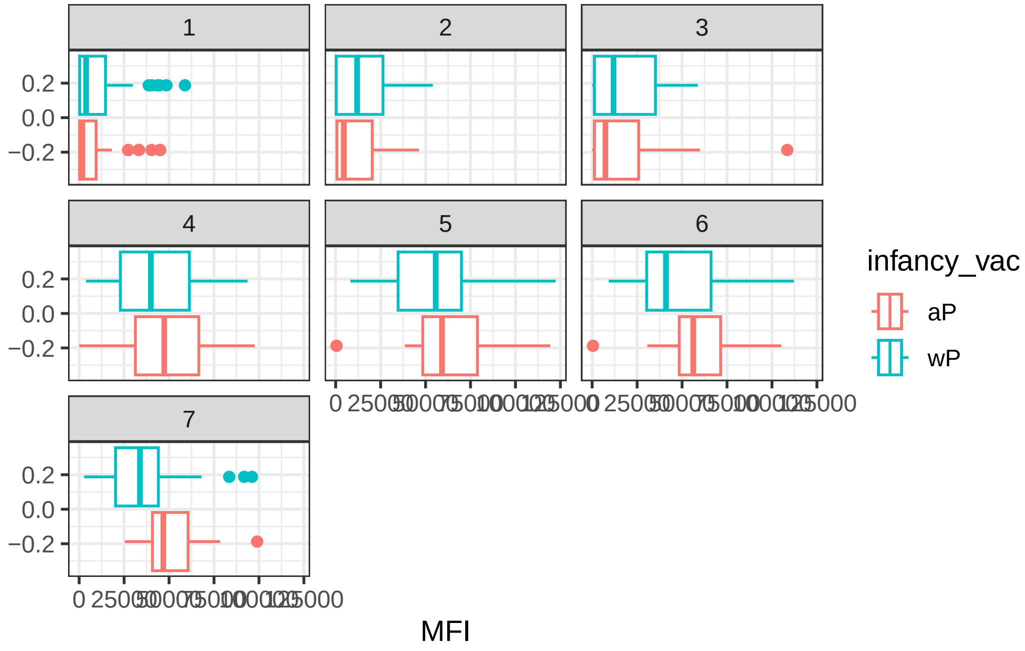


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("Measles", that is not in our vaccines) and a clear antigen of interest ("FIM2/3", extra-cellular fimbriae proteins from B. pertussis that participate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

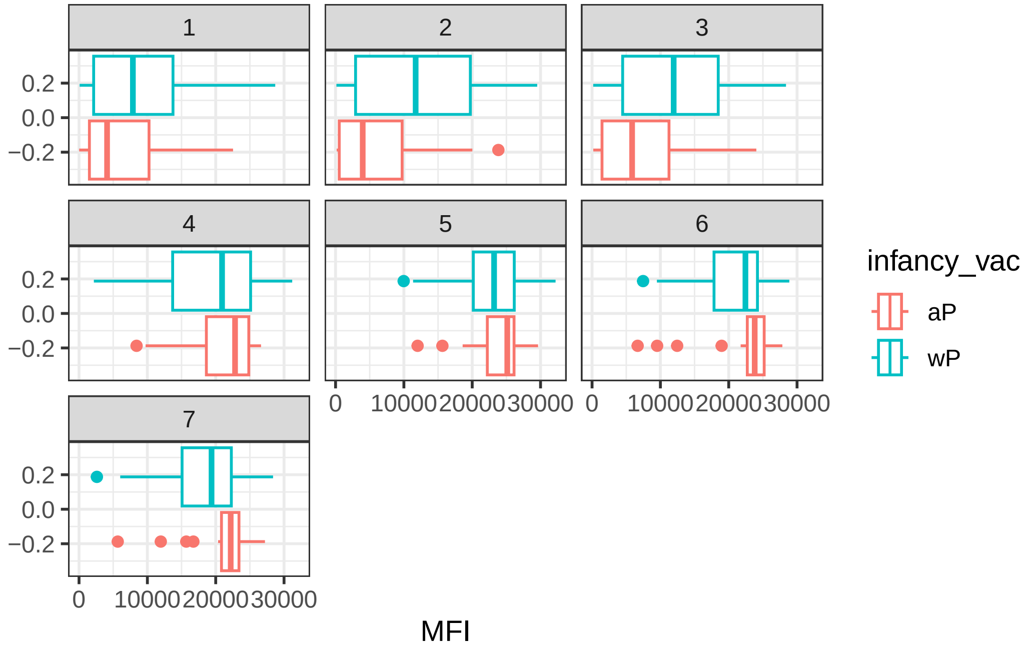
```
filter(ig1, antigen=="TT") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = F) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



```
filter(ig1, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = T) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



```
filter(ig1, antigen=="PRN") %>%
  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?

The TT antigen doesn't increase much over the time course and stays pretty consistent the whole time. For the FIM2/3 data, the boxplot is increasing over the time course and ends at a much higher MFI than the TT. The FIM2/3 peaks around visit 5 and 6 and then starts to decline a bit.

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

Looking at FIM2/3 and PRN which we saw earlier is involved with the vaccine, the aP response seems to be greater than the wP responses by visit 3 or 4 and then continues to increase at a greater rate than wP and then both the aP and wP vaccine begin to decline around visit 6 or 7.

Obtaining CMI-PB RNASeq data

The link 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)
```

```
head(rna)
```

```
  versioned_ensembl_gene_id specimen_id raw_count      tpm
1         ENSG00000211896.7         344     18613  929.640
2         ENSG00000211896.7         243      2011  112.584
3         ENSG00000211896.7         261      2161  124.759
4         ENSG00000211896.7         282      2428  138.292
5         ENSG00000211896.7         345     51963 2946.136
6         ENSG00000211896.7         244     49652 2356.749
```

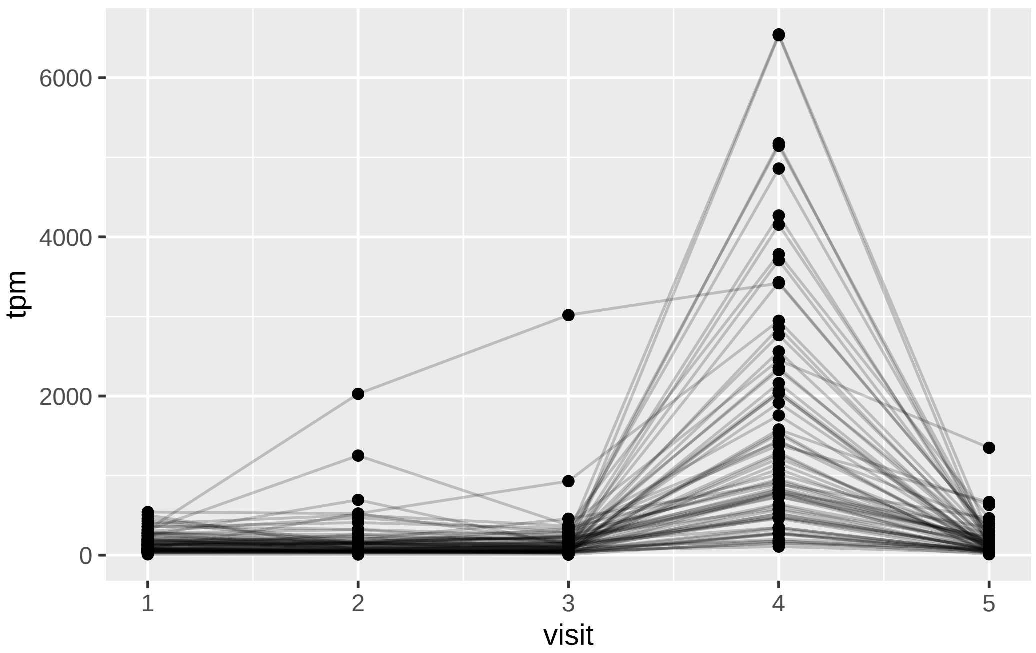
To facilitate further analysis we need to “join” the rna expression data with our metadata meta, which is itself a join of sample and specimen data. This will allow us to look at this genes TPM expression values over aP/wP status and at different visits (i.e. times):

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

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

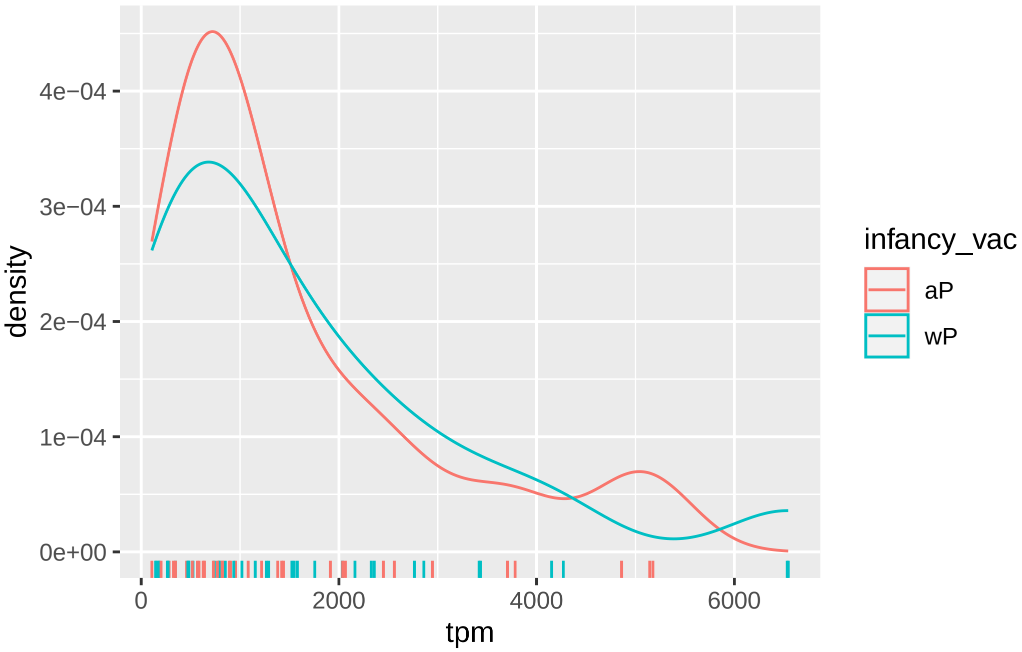
> Q18. Make a plot of the time course of gene expression for IGHG1 gene (i.e. a plot of visit vs. tpm).

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



Focus in on visit 4 and facet by aP/wP subjects

```
ssrna %>%
  filter(visit==4) %>%
  ggplot() +
    aes(tpm, col=infancy_vac) + geom_density() +
    geom_rug()
```



> Q19.: 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 reaches its maximum level at visit 4 and then immediately drops back down to 0 by visit 5.

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

This pattern in time does not match the trend of antibody titer data because this pattern has the maximum level being reached at visit 4 whereas the trend of antibody titer data seems to reach the maximum level around visit 5 and 6. Also, this pattern has the levels going from 0 visit 3 to max at visit 4 and then back to 0 by visit 5. With the antibody titer data, the change among visits was much more gradual.