Class 19: Mini Project: Investigating Pertussis Resurgence

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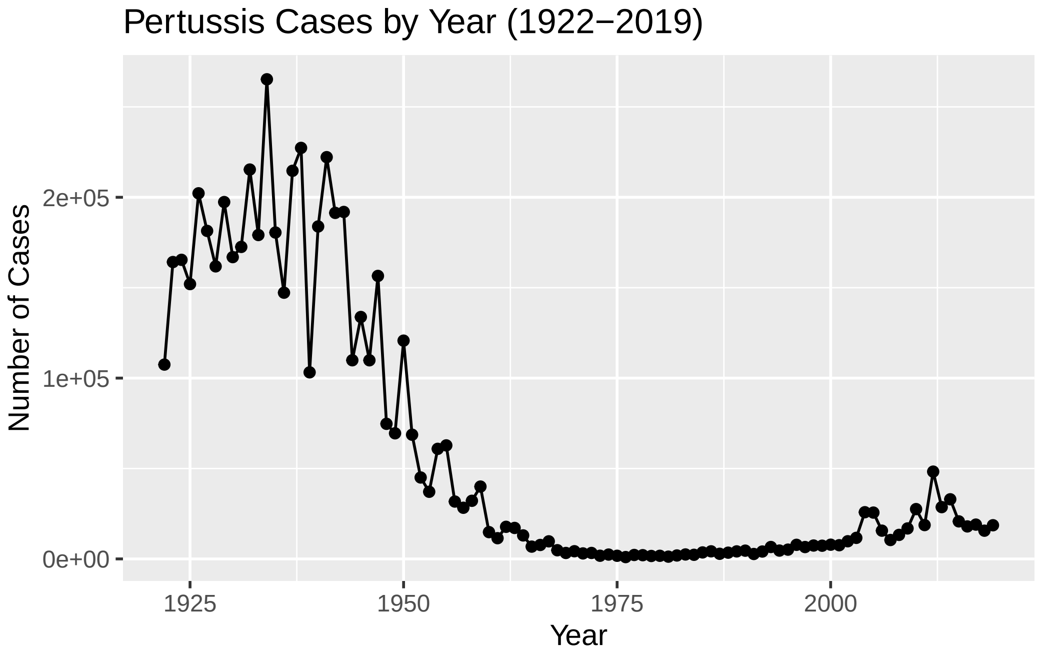
Q1. With the help of the R "addin" package datapasta assign the CDC pertussis case number data to a data frame called cdc and use ggplot to make a plot of cases numbers over time.

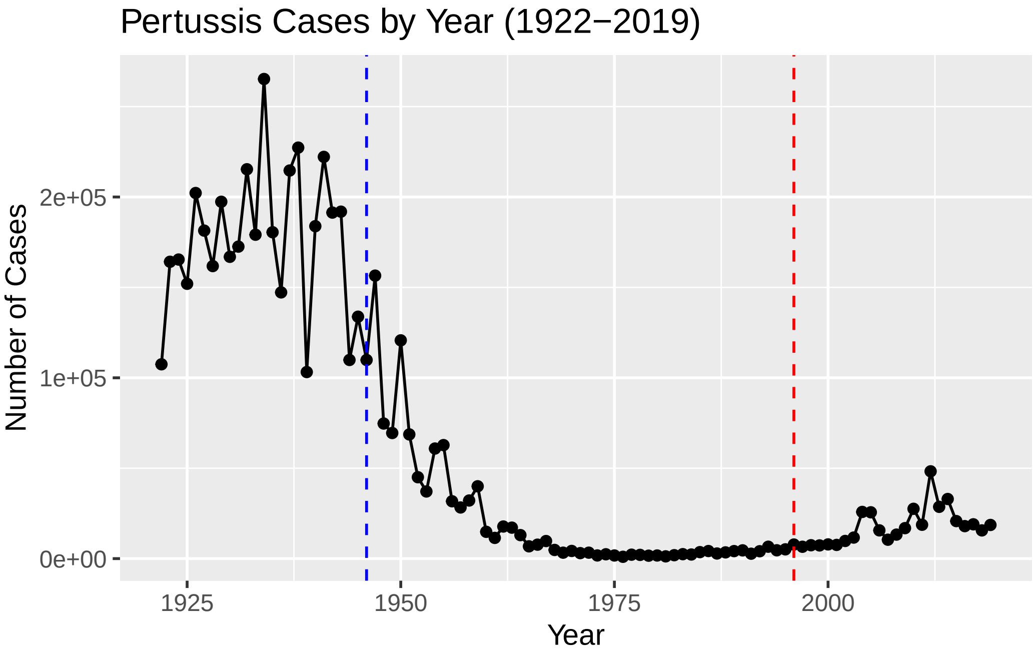
```
library(datapasta)
cdc <- data.frame(
                                 Year = c(1922L,1923L,1924L,1925L,
                                          1926L,1927L,1928L,1929L,1930L,1931L,
                                          1932L,1933L,1934L,1935L,1936L,
                                          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),
         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)
ggplot(cdc) +
  aes(Year, Cases) +
  geom_point() +
  geom_line() +
  labs(x = "Year", y = "Number of Cases" , title = "Pertussis Cases by Year (1922-2019)")
```



> 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?

```r
library(ggplot2)
ggplot(cdc) +
  aes(Year, Cases) +
  geom_point() +
  geom_line() +
  labs(x = "Year", y = "Number of Cases" , title = "Pertussis Cases by Year (1922-2019)")
  geom_vline( xintercept = 1946, color = "blue", linetype = 2) +
  geom_vline( xintercept = 1996, color = "red" , linetype = 2)
```



I notice a drastic reduction in number of cases after the 1946 vaccine and a rise in cases after the 1996 vaccine. The increase could be because of anti-vaxers or that the original vaccine wore off after a certain amount of time.

> 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 1996 vaccine there was an increase in number of cases. This can be due to anti-vaxers, more sensitive testing, bacterial evolution, and possibly the waning of immunity earlier from the newer vaccine.

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

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

```

aP wP 
47 49 
```

47 aP and 49wP.

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

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

```

Female   Male 
    66     30 
```

66 Female and 30 Male.

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

```r
table(subject$ethnicity, subject$biological_sex)
```

```
                         Female Male
  Hispanic or Latino         18    5
  Not Hispanic or Latino     47   22
  Unknown                     1    3
```

For females there are 18 Hispanic or Latino, 47 non Hispanic or Latino, and 1 Unknown. For males there are 5 Hispanic or Latino, 22 non Hispanic or Latino, and 3 Unknown.

```r
library(lubridate)
```

```
Loading required package: timechange


Attaching package: 'lubridate'


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

    date, intersect, setdiff, union
```

```r
today()
```

```
[1] "2022-11-29"
```

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

```
Time difference of 8368 days
```

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

```
[1] 22.91034
```

> 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?

```
# Use todays date to calculate age in days
subject$age <- today() - ymd(subject$year_of_birth)

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
```

```
ap <- subject %>% filter(infancy_vac == "aP")

round( summary( time_length( ap$age, "years" ) ) )
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
     23      25      26      25      26      27 
```

```
# wP
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 
```

```
t.test(wp$age, ap$age,)
```

```

    Welch Two Sample t-test

data:  wp$age and ap$age
t = 12.092 days, df = 51.082, p-value < 2.2e-16
```

```
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 3303.337 days 4618.534 days
sample estimates:
Time differences in days
mean of x mean of y
13175.510  9214.574
```

Average ap age is 25. Average wp age is 36. They are statistically significant with a p-value of 2.2e-16.

> 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
```

```r
age_at_boost
```

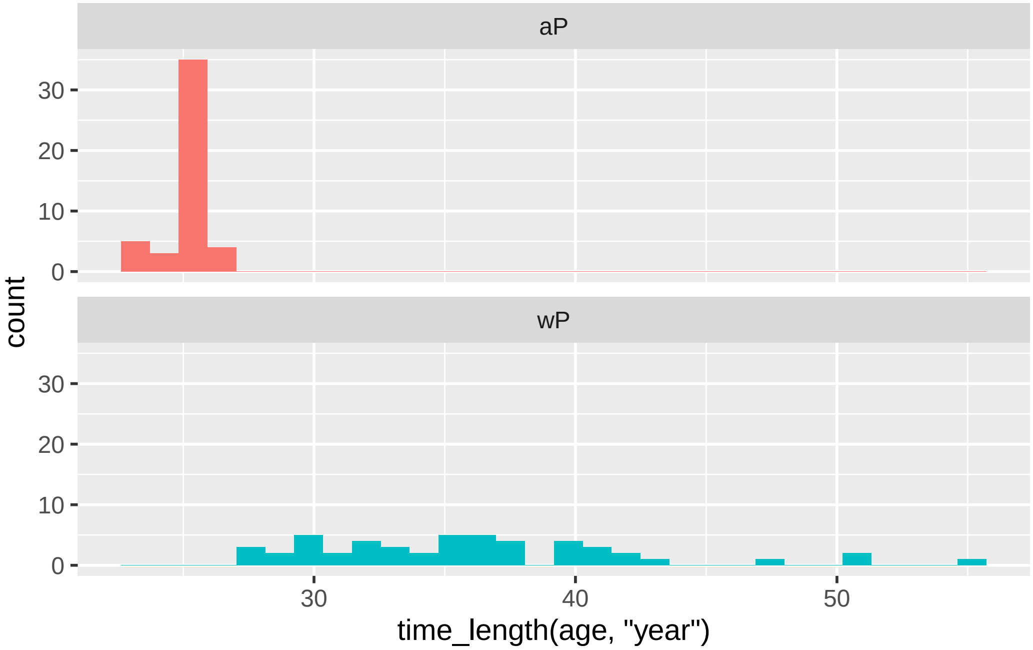
```
 [1] 30.69678 51.07461 33.77413 28.65982 25.65914 28.77481 35.84942 34.14921
 [9] 20.56400 34.56263 30.65845 34.56263 19.56194 23.61944 27.61944 29.56331
[17] 36.69815 19.65777 22.73511 32.26557 25.90007 23.90144 25.90007 28.91992
[25] 42.92129 47.07461 47.07461 29.07324 21.07324 21.07324 28.15058 24.15058
[33] 24.15058 21.14990 21.14990 31.20876 26.20671 32.20808 27.20876 26.20671
[41] 21.20739 20.26557 22.26420 19.32375 21.32238 19.32375 19.32375 22.41752
[49] 20.41889 21.41821 19.47707 23.47707 20.47639 21.47570 19.47707 35.65777
[57] 33.65914 31.65777 25.73580 24.70089 28.70089 33.73580 19.73443 34.73511
[65] 19.73443 28.73648 27.73443 19.81109 26.77344 33.81246 25.77413 19.81109
[73] 18.85010 19.81109 31.81109 22.81177 31.84942 19.84942 18.85010 18.85010
[81] 19.90691 18.85010 20.90897 19.04449 20.04381 19.90691 19.90691 19.00616
[89] 19.00616 20.04381 20.04381 20.07940 21.08145 20.07940 20.07940 20.07940
```

The age_at_boost table shows the age at which all individuals were boosted.

> 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`.
```



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

x$p.value
```

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

The two groups are most definitely statistically significant with a p-value of 1.31e-16.

```
# 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, 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 13481 days
2 13481 days
3 13481 days
4 13481 days
5 13481 days
6 13481 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, 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 
```

6698 IgE, 1413 IgG, 6141 IgG1, 6141 IgG2, 6141 IgG3, and 6141 IgG4.

> 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 
```

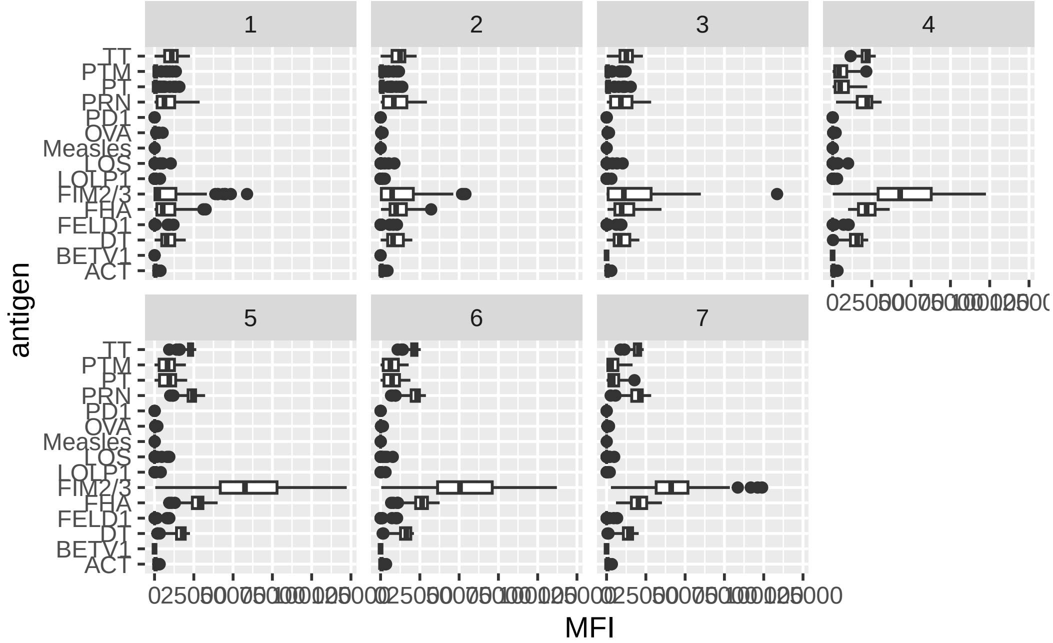
Visit 8 specimens are significantly lower than 1-7.

```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 13481 days
2 13481 days
3 13481 days
4 13481 days
5 13481 days
6 13481 days
```

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

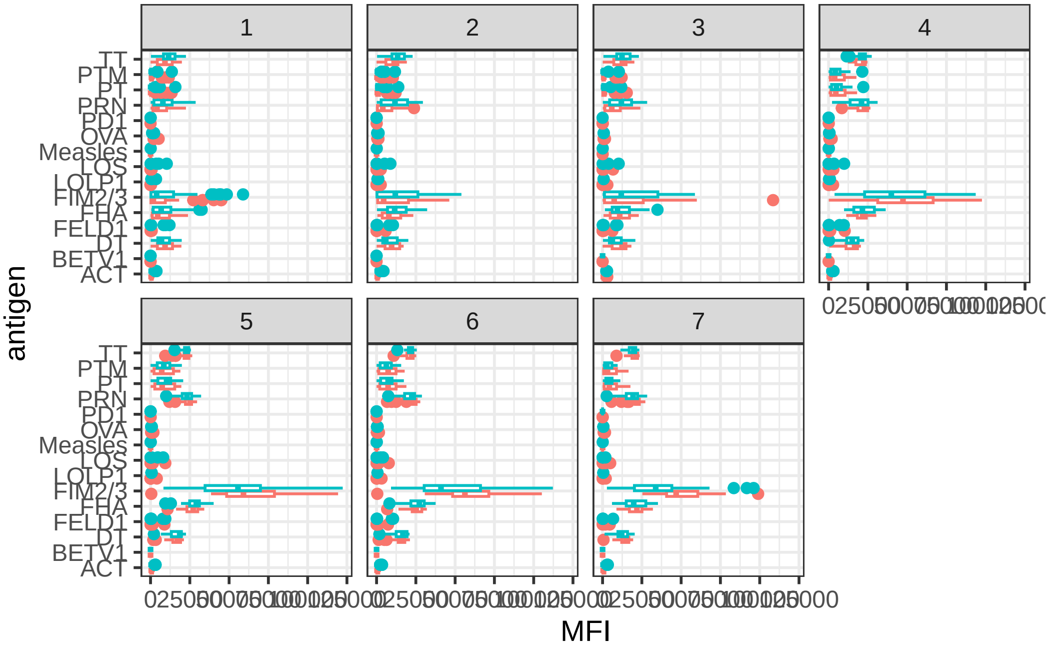
```
ggplot(ig1) +
  aes(MFI, antigen) +
  geom_boxplot() +
  facet_wrap(vars(visit), nrow=2)
```



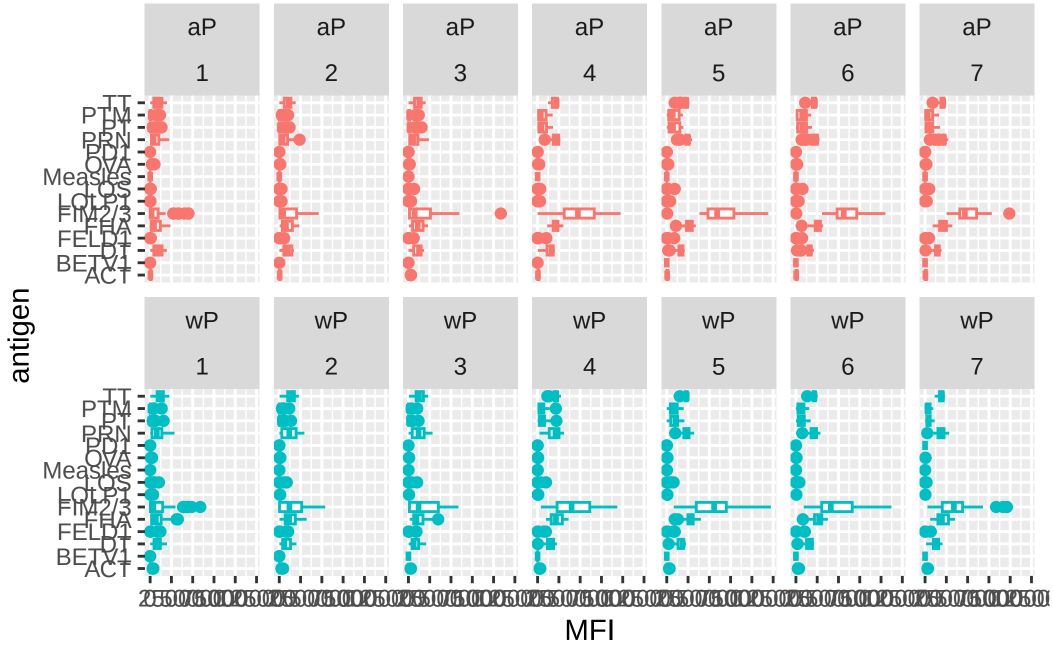
Q14. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

I believe it is FIM2/3 since it has the largest range, IQR, and median MFI.

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

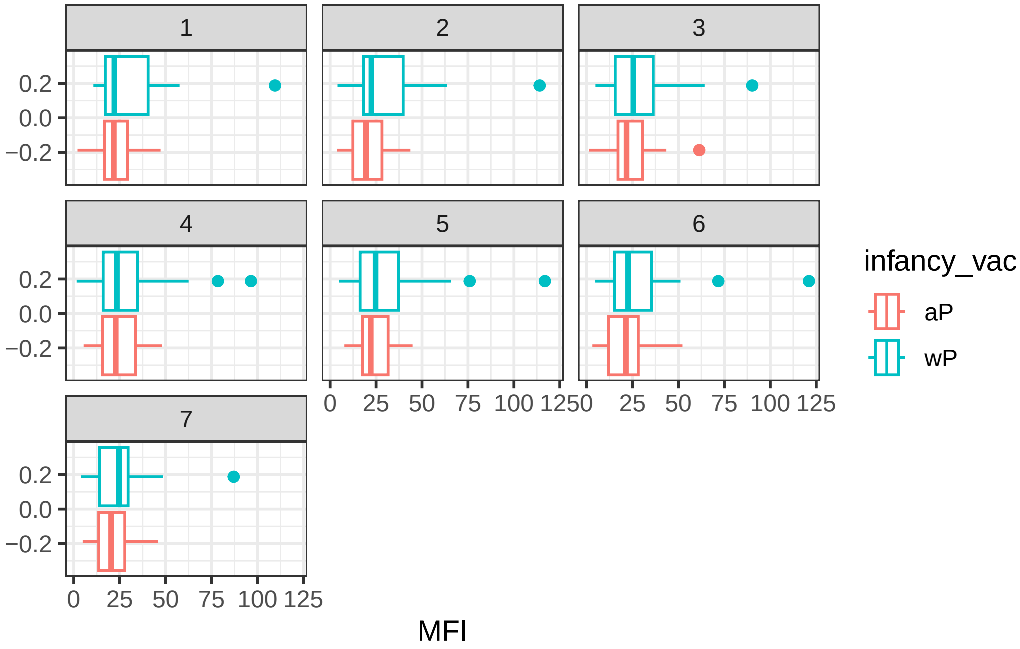


```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

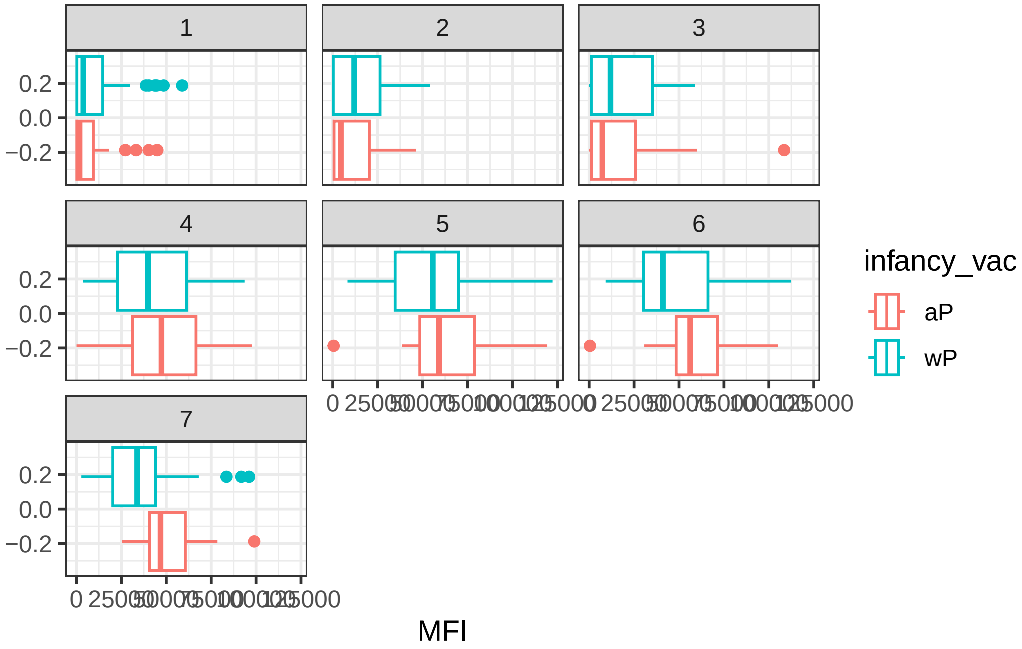


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a “control” antigen (“Measles”, that is not in our vaccines) and a clear antigen of interest (“FIM2/3”, extra-cellular fimbriae proteins from B. pertussis that participate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

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



```
filter(ig1, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI, col=infancy_vac) +
  geom_boxplot(show.legend = TRUE) +
  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 clearly rise over time and far exceed those of Measles. They also appear to peak at visit 5 and then decline. This trend appears similar for for wP and aP subjects. (from the hint on lab guide)

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

I do not see a clear difference between the two.