Class 18: Pertussis mini-project

Georgina Canto-Encalada

Pertusssis case numbers are tracked by the CDC and made available here: https://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html

> 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

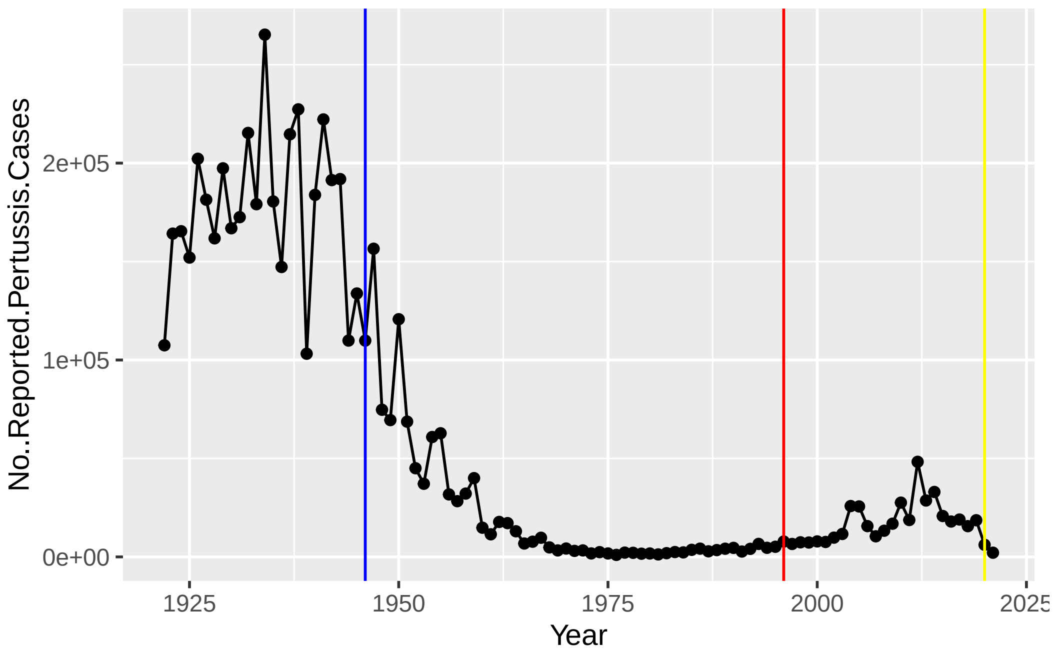
We will use the datapasta pacjage to "scrape" thus data.

```
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)
       )
```

> 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?

Using ggplot to make a plot of cases numbers over time.

```
library(ggplot2)
ggplot(cdc, aes(x=Year,y=No..Reported.Pertussis.Cases)) + geom_point() +geom_line() + geom
```



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

ANS: The number of people rejecting the vaccination increased

Read data from the CMI-PB project

It returns JSON data that we can read into R with the help of a package called **jsonlite**

```
library(jsonlite)
```

```
Warning: package 'jsonlite' was built under R version 4.3.3
```

```
subject <- read_json("https://www.cmi-pb.org/api/subject", simplifyVector = TRUE)
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 many aP and wP infancy vaccinated subjects are in the dataset?

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

```

aP wP 
60 58 
```

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

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

```

Female   Male 
    79     39 
```

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

```
table(subject$race, subject$biological_sex)
```

```
                                           
                                            Female Male
  American Indian/Alaska Native                  0    1
  Asian                                         21   11
  Black or African American                      2    0
  More Than One Race                             9    2
  Native Hawaiian or Other Pacific Islander      1    1
  Unknown or Not Reported                       11    4
  White                                         35   20
```

```r
specimen<-read_json("http://cmi-pb.org/api/specimen",simplifyVector = TRUE)
head(specimen)
```

```
  specimen_id subject_id actual_day_relative_to_boost
1           1          1                           -3
2           2          1                            1
3           3          1                            3
4           4          1                            7
5           5          1                           11
6           6          1                           32
  planned_day_relative_to_boost specimen_type visit
1                             0         Blood     1
2                             1         Blood     2
3                             3         Blood     3
4                             7         Blood     4
5                            14         Blood     5
6                            30         Blood     6
```

> 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
library(lubridate)
```

```
Warning: package 'lubridate' was built under R version 4.3.3


Attaching package: 'lubridate'

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

    date, intersect, setdiff, union
```

```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
```

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

The average age of aP individuals

```
# aP group
ap <- subject %>% filter(infancy_vac == "aP")
round( summary( time_length( ap$age, "years" ) ) )
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
     21      26      26      26      27      30 
```

The average age of wP individuals

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

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
     28      31      36      37      39      56 
```

are they significantly different?

ANS: Yes

```
# Statistical test to compare average ages
# Assuming normal distribution and equal variances
t_test_result <- t.test(age ~ infancy_vac, data = subject)

# Print the t-test result
print(t_test_result)
```

```

    Welch Two Sample t-test

data:  age by infancy_vac
```

```
t = -12.436 days, df = 65.411, p-value < 2.2e-16
alternative hypothesis: true difference in means between group aP and group wP is not equal
95 percent confidence interval:
 -4364.767 days -3156.997 days
sample estimates:
Time differences in days
mean in group aP mean in group wP
        9606.567        13367.448
```

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

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

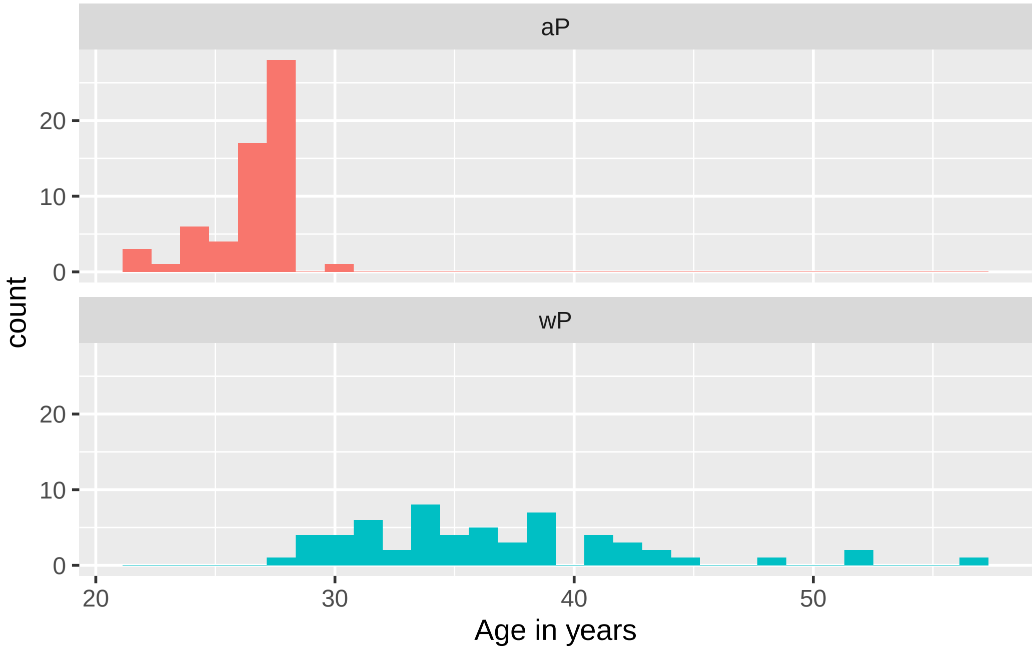
```
[1] 30.69678 51.07461 33.77413 28.65982 25.65914 28.77481
```

Q9. With the help of a faceted boxplot or histogram (see below), do you think these two groups are significantly different?

Yes, they are 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`.
```



Joining multiple tables

Let's join subject and specimen list based on subject_id. We will use the `inner_join()` from dplyr package

> 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)`
```

```
head(meta)
```

```
  specimen_id subject_id actual_day_relative_to_boost
1           1          1                           -3
2           2          1                            1
3           3          1                            3
4           4          1                            7
```

```
5           5          1                           11
6           6          1                           32
  planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
1                             0         Blood     1          wP         Female
2                             1         Blood     2          wP         Female
3                             3         Blood     3          wP         Female
4                             7         Blood     4          wP         Female
5                            14         Blood     5          wP         Female
6                            30         Blood     6          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 13953 days
2 13953 days
3 13953 days
4 13953 days
5 13953 days
6 13953 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.

Another inner_join to add all the meta data onto these measurements

```r
ab_titer <- read_json("http://cmi-pb.org/api/v4/plasma_ab_titer",simplifyVector = TRUE)
abdata <- inner_join(ab_titer,meta)
```

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

```r
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 13953 days
2 13953 days
3 13953 days
4 13953 days
5 13953 days
6 13953 days
```

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

How many Ab isotyopes are there?

```r
table(abdata$isotype)
```

```

 IgE  IgG IgG1 IgG2 IgG3 IgG4 
6698 3233 7961 7961 7961 7961 
```

> Q12. What are the different $dataset values in abdata and what do you notice about the number of rows for the most "recent" dataset?

ANS: More samples were collected during 2020 than in 2022. It has sense since it was the COVID outbreak.

```
table(abdata$dataset)
```

```
2020_dataset 2021_dataset 2022_dataset 
       31520         8085         2170 
```

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

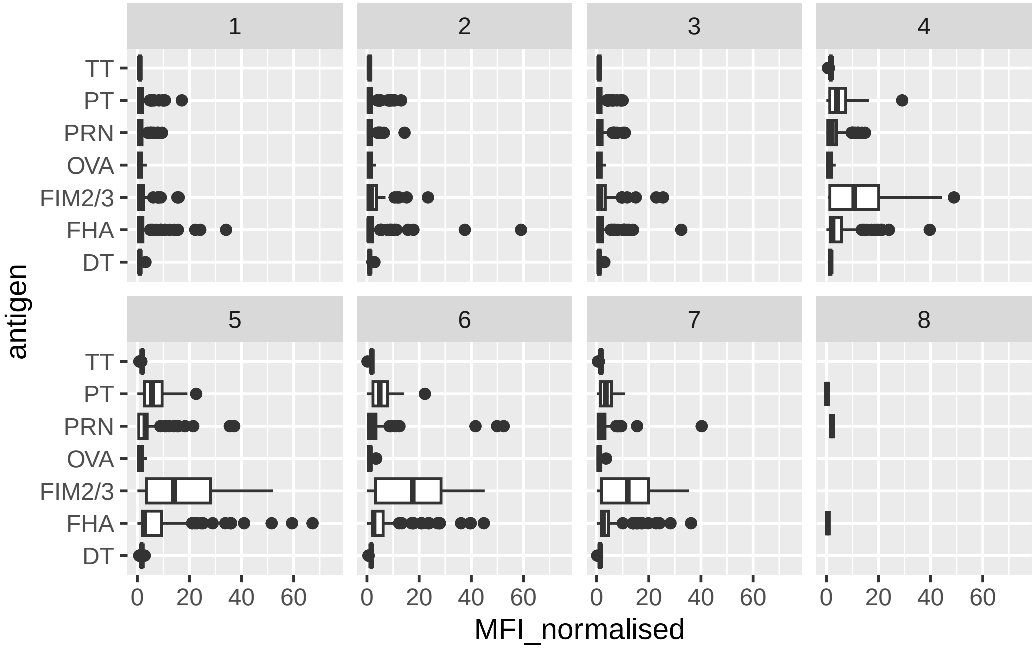
```
igg <- abdata %>% filter(isotype == "IgG")
head(igg)
```

```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgG                TRUE      PT   68.56614       3.736992
2           1     IgG                TRUE     PRN  332.12718       2.602350
3           1     IgG                TRUE     FHA 1887.12263      34.050956
4          19     IgG                TRUE      PT   20.11607       1.096366
5          19     IgG                TRUE     PRN  976.67419       7.652635
6          19     IgG                TRUE     FHA   60.76626       1.096457
   unit lower_limit_of_detection subject_id actual_day_relative_to_boost
1 IU/ML                 0.530000          1                           -3
2 IU/ML                 6.205949          1                           -3
3 IU/ML                 4.679535          1                           -3
4 IU/ML                 0.530000          3                           -3
5 IU/ML                 6.205949          3                           -3
6 IU/ML                 4.679535          3                           -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                Unknown White    1983-01-01    2016-10-10 2020_dataset
```

```
5                 Unknown White    1983-01-01    2016-10-10 2020_dataset
6                 Unknown White    1983-01-01    2016-10-10 2020_dataset
         age
1 13953 days
2 13953 days
3 13953 days
4 15049 days
5 15049 days
6 15049 days
```

```
ggplot(igg) +
  aes(MFI_normalised,antigen) +
  xlim(0,75) +
  geom_boxplot() +
  facet_wrap(vars(visit), nrow=2)
```

```
Warning: Removed 5 rows containing non-finite values (`stat_boxplot()`).
```

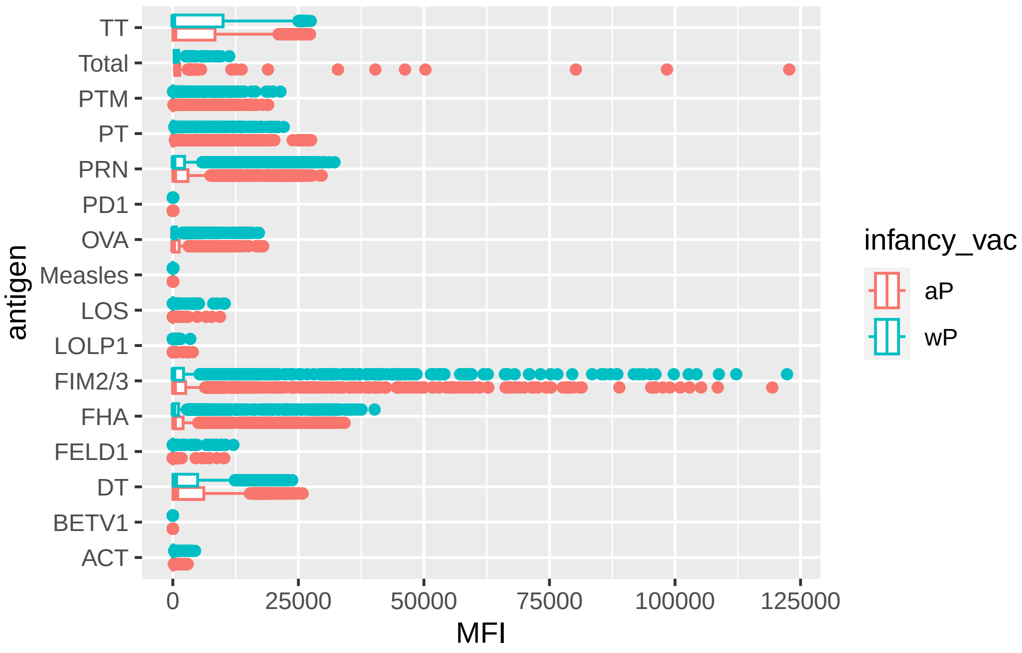


Q14. What antigens show differences in the level of IgG antibody titers recognizing them over time? Why these and not others?

ANS: FIM2/3. This antigen is associated with Bordetella pertussis infection.

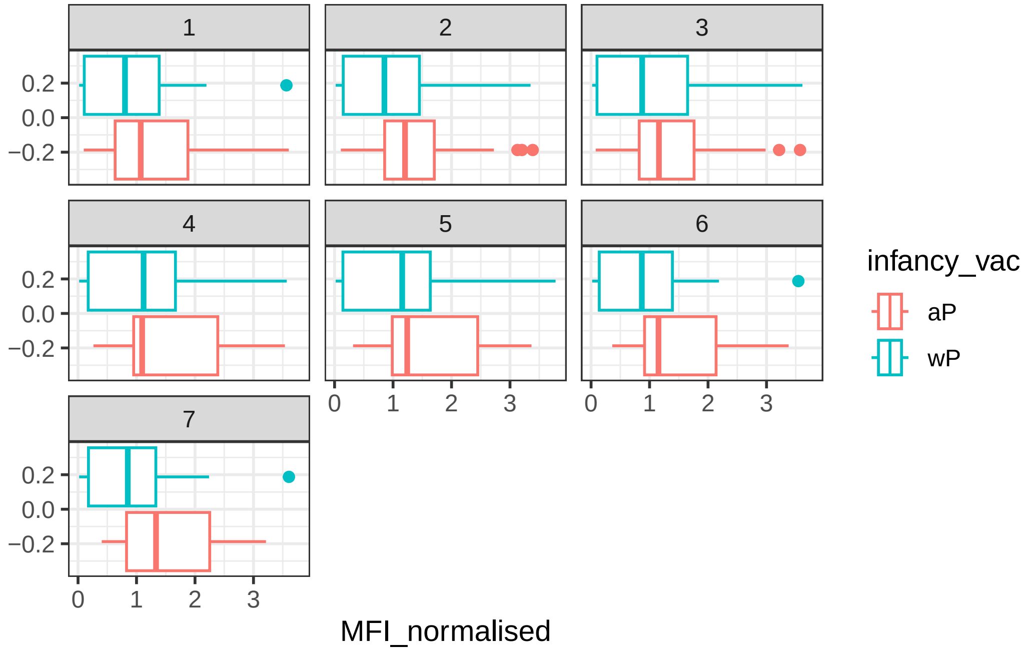
```
ggplot(abdata) +
  aes(MFI,antigen, col=infancy_vac) +
  geom_boxplot()
```

```
Warning: Removed 1 rows containing non-finite values (`stat_boxplot()`).
```

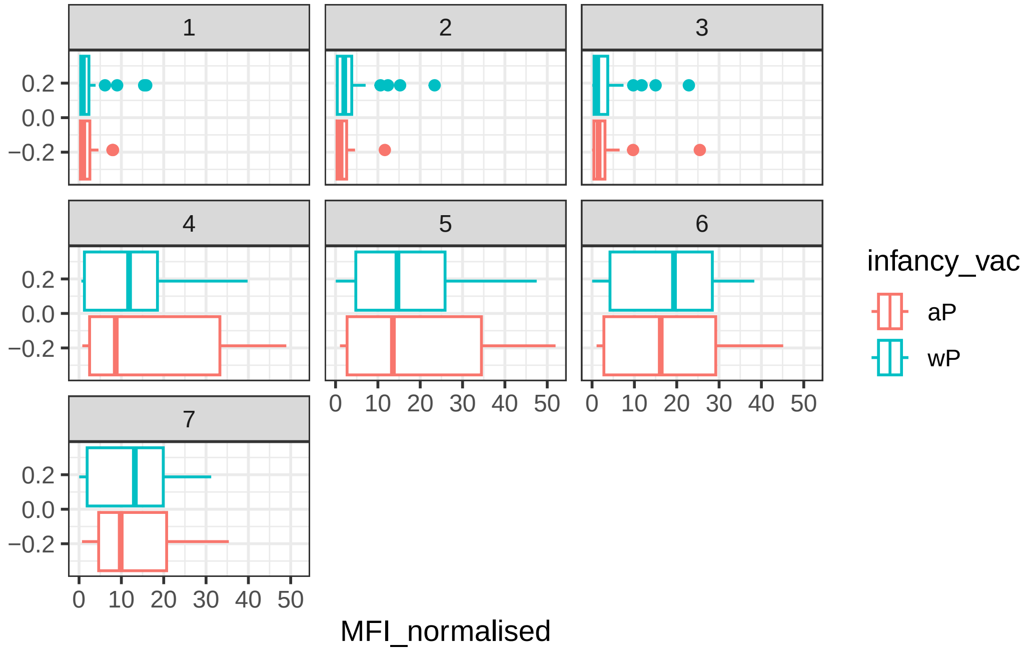


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a “control” antigen (“OVA”, that is not in our vaccines) and a clear antigen of interest (“PT”, Pertussis Toxin, one of the key virulence factors produced by the bacterium B. pertussis).

```
filter(igg, antigen=="OVA") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = T) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



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



> Q16. What do you notice about these two antigens time courses and the PT data in particular?

It seems that there is a distinction between the immune responses to different vaccine formulations across different visits. The PT antigen, which is a component of the pertussis vaccine, appears to induce a variable immune response over time, which is evident from the spread of the data points.

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

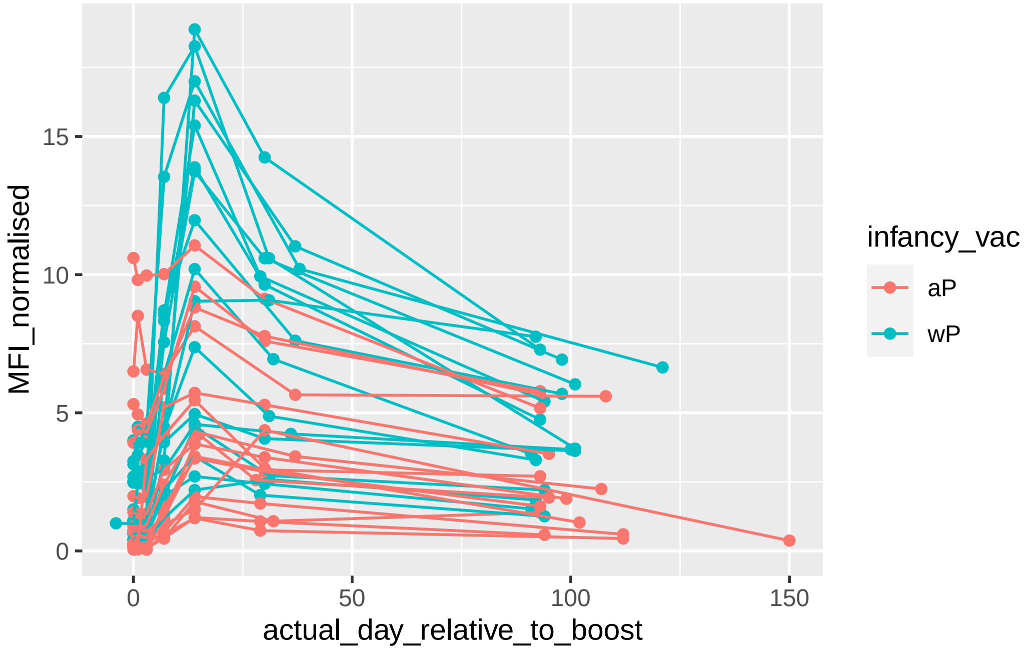
No

```
#pt_data<-filter(abdata,antigen=="PT",isotype=="IgG", dataset == "2020_dataset")
pt_data<-filter(abdata,antigen=="PT",isotype=="IgG",dataset=="2021_dataset")
```

```
table(pt_data$dataset)
```

```
2021_dataset
         231
```

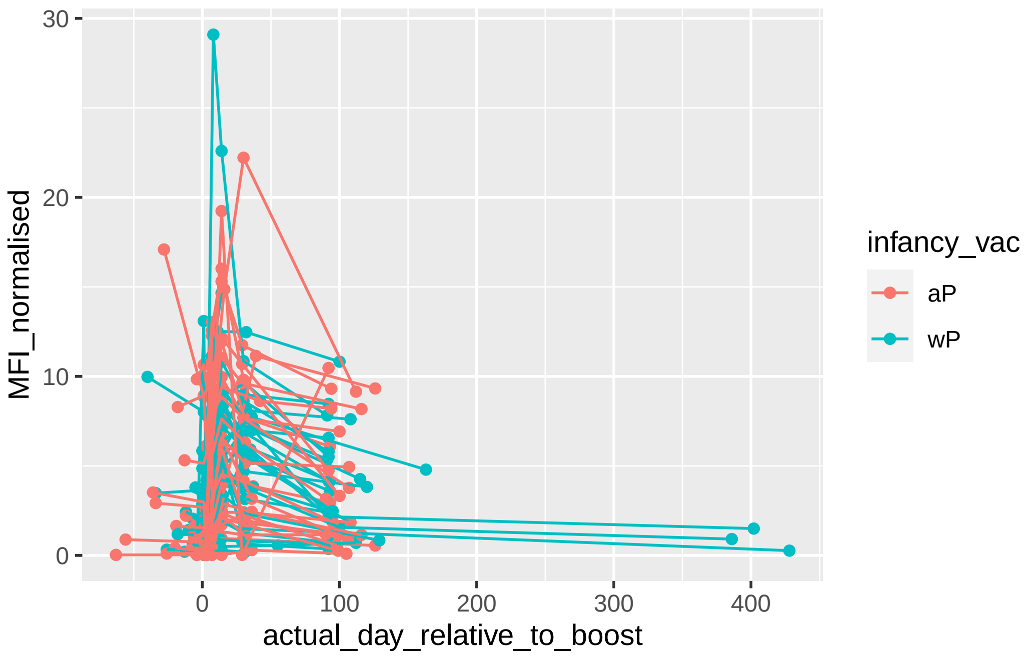
```
ggplot(pt_data) +
  aes(x=actual_day_relative_to_boost, y=MFI_normalised, col=infancy_vac,
      group=subject_id) +
  geom_point() +
  geom_line()
```



Q18. Does this trend look similar for the 2020 dataset?

No

```
pt_data<-filter(abdata,antigen=="PT",isotype=="IgG", dataset == "2020_dataset")
ggplot(pt_data) +
  aes(x=actual_day_relative_to_boost, y=MFI_normalised, col=infancy_vac,
      group=subject_id) +
  geom_point() +
  geom_line()
```


30
20
10
0
MFI_normalised
0
100
200
300
400
actual_day_relative_to_boost
infancy_vac
aP
wP