Class 17: Investigating Pertussis

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Pertussis, or whooping cough, is a highly contagious lung infection caused by a bacteria *B. pertussis*.

The CDC tracks reported cases in the U.S. since the 1920s.

#Investigating Pertussis Cases by Year

```
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),
         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)
       )
```

```
library(ggplot2)
```

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.

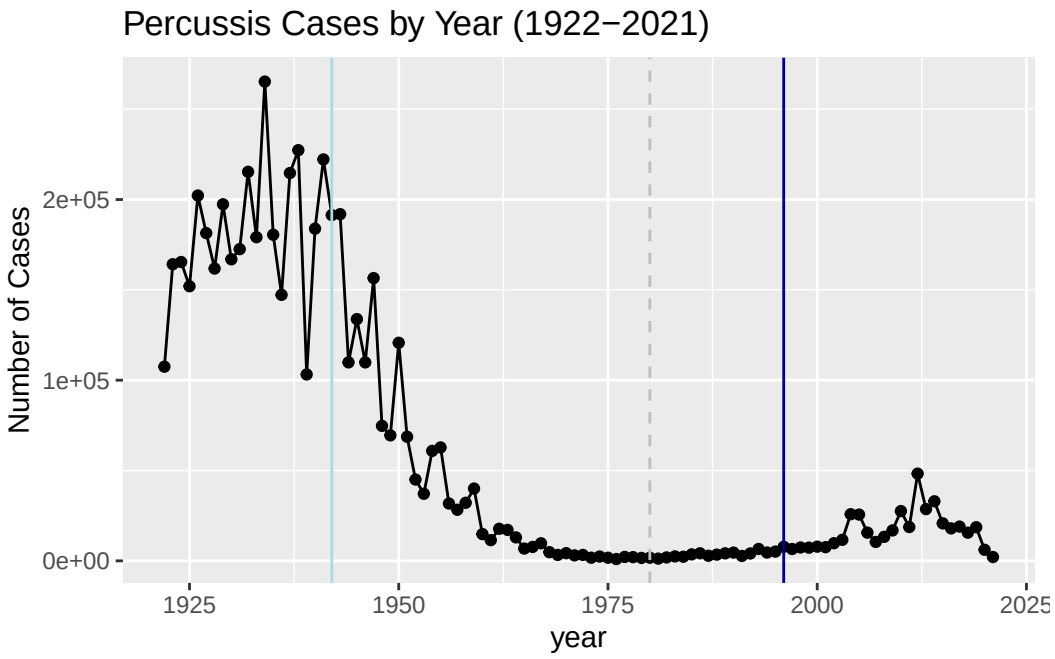
```
baseplot <- ggplot(cdc) +
  aes(Year, Cases) +
  geom_point() +
  geom_line() +
  labs(title = "Percussis Cases by Year (1922-2021)") +
  xlab("year") +
  ylab("Number of Cases")
baseplot
```

#The Tale of Two Vaccines

> 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?

The first big "whole-cell" pertussis vaccine program started in 1942.

```
baseplot +
  geom_vline(xintercept = 1942, color = "light blue") +
  geom_vline(xintercept = 1980, color = "grey", linetype = 2) +
  geom_vline(xintercept = 1996, color = "dark blue")
```



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 aP vaccine, we see a very gradual uptick in the amount of cases. There is a couple of possible explanations for this trend: some people could still be vaccine hesitant, *the new vaccine could be less effective long-term*, or the bacteria could be evolving.

Exploring CMI-PB Data

Something big is happening with pertussis cases and big outbreaks are once again a major public health concern! BUGGER

One of the main hypothesis for the increasing case numbers is waning vaccine efficiency with the newer aP vaccine.

Enter the CMI-PB project, which is studying this problem on large scale. Let's see what data they have.

Their data is available in JSON format ("key:value" pair style) We will use the "jsonlight" package to read their data.

```
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?

```
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$race, subject$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
```

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

head(specimen, 6)
```

```
  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
```

I want to "merge" the `subject` and `specimen` tables together. I will use the **dplyr** package for this.

> 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:

```
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
```

```
meta <- inner_join(subject, specimen)
```

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

```
head(meta)
```

```
  subject_id infancy_vac biological_sex              ethnicity  race
1          1          wP         Female Not Hispanic or Latino White
2          1          wP         Female Not Hispanic or Latino White
3          1          wP         Female Not Hispanic or Latino White
4          1          wP         Female Not Hispanic or Latino White
5          1          wP         Female Not Hispanic or Latino White
6          1          wP         Female Not Hispanic or Latino White
  year_of_birth date_of_boost      dataset specimen_id
1    1986-01-01    2016-09-12 2020_dataset           1
2    1986-01-01    2016-09-12 2020_dataset           2
3    1986-01-01    2016-09-12 2020_dataset           3
4    1986-01-01    2016-09-12 2020_dataset           4
5    1986-01-01    2016-09-12 2020_dataset           5
6    1986-01-01    2016-09-12 2020_dataset           6
  actual_day_relative_to_boost planned_day_relative_to_boost specimen_type
1                           -3                             0         Blood
2                          736                           736         Blood
3                            1                             1         Blood
4                            3                             3         Blood
5                            7                             7         Blood
6                           11                            14         Blood
  visit
1     1
2    10
3     2
4     3
5     4
6     5
```

```
ncol(specimen)
```

```
[1] 6
```

```
ncol(subject)
```

```
[1] 8
```

```
ncol(meta)
```

```
[1] 13
```

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.

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

```
  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
```

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

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

```
head(abdata)
```

```
  subject_id infancy_vac biological_sex              ethnicity  race
1          1          wP         Female Not Hispanic or Latino White
2          1          wP         Female Not Hispanic or Latino White
3          1          wP         Female Not Hispanic or Latino White
4          1          wP         Female Not Hispanic or Latino White
5          1          wP         Female Not Hispanic or Latino White
6          1          wP         Female Not Hispanic or Latino White
  year_of_birth date_of_boost      dataset specimen_id
1    1986-01-01    2016-09-12 2020_dataset           1
2    1986-01-01    2016-09-12 2020_dataset           1
3    1986-01-01    2016-09-12 2020_dataset           1
4    1986-01-01    2016-09-12 2020_dataset           1
5    1986-01-01    2016-09-12 2020_dataset           1
6    1986-01-01    2016-09-12 2020_dataset           1
  actual_day_relative_to_boost planned_day_relative_to_boost specimen_type
1                           -3                             0         Blood
2                           -3                             0         Blood
3                           -3                             0         Blood
4                           -3                             0         Blood
5                           -3                             0         Blood
6                           -3                             0         Blood
  visit isotype is_antigen_specific antigen        MFI MFI_normalised  unit
1     1     IgE               FALSE   Total 1110.21154       2.493425 UG/ML
2     1     IgE               FALSE   Total 2708.91616       2.493425 IU/ML
3     1     IgG                TRUE      PT   68.56614       3.736992 IU/ML
4     1     IgG                TRUE     PRN  332.12718       2.602350 IU/ML
5     1     IgG                TRUE     FHA 1887.12263      34.050956 IU/ML
6     1     IgE                TRUE     ACT    0.10000       1.000000 IU/ML
  lower_limit_of_detection
1                 2.096133
2                29.170000
3                 0.530000
4                 6.205949
5                 4.679535
6                 2.816431
```

```
dim(abdata)
```

```
[1] 32675    20
```

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
```

There are way less visit 8 specimens because the project is still ongoing. The 8th visits are happening now and therefore we do not have that data for all individuals yet.

Examine IgG1 Ab Titer Levels

We will use the `filter()` function from dplyr to focus on just IgG1 isotype and visits 1 to 7.

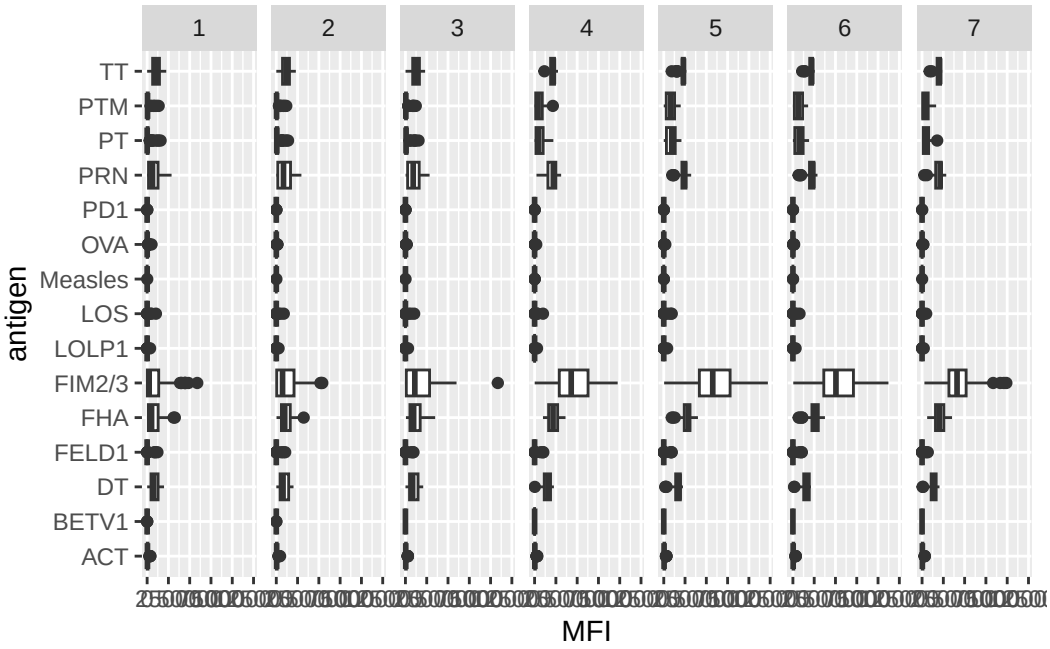
```
ig1 <- abdata %>% filter(isotype == "IgG1", visit!=8)
head(ig1)
```

```
  subject_id infancy_vac biological_sex              ethnicity  race
1          1          wP         Female Not Hispanic or Latino White
2          1          wP         Female Not Hispanic or Latino White
3          1          wP         Female Not Hispanic or Latino White
4          1          wP         Female Not Hispanic or Latino White
5          1          wP         Female Not Hispanic or Latino White
6          1          wP         Female Not Hispanic or Latino White
  year_of_birth date_of_boost      dataset specimen_id
1    1986-01-01    2016-09-12 2020_dataset           1
2    1986-01-01    2016-09-12 2020_dataset           1
3    1986-01-01    2016-09-12 2020_dataset           1
```

```
4    1986-01-01    2016-09-12 2020_dataset           1
5    1986-01-01    2016-09-12 2020_dataset           1
6    1986-01-01    2016-09-12 2020_dataset           1
  actual_day_relative_to_boost planned_day_relative_to_boost specimen_type
1                           -3                             0         Blood
2                           -3                             0         Blood
3                           -3                             0         Blood
4                           -3                             0         Blood
5                           -3                             0         Blood
6                           -3                             0         Blood
  visit isotype is_antigen_specific antigen        MFI MFI_normalised  unit
1     1    IgG1                TRUE     ACT 274.355068      0.6928058 IU/ML
2     1    IgG1                TRUE     LOS  10.974026      2.1645083 IU/ML
3     1    IgG1                TRUE   FELD1   1.448796      0.8080941 IU/ML
4     1    IgG1                TRUE   BETV1   0.100000      1.0000000 IU/ML
5     1    IgG1                TRUE   LOLP1   0.100000      1.0000000 IU/ML
6     1    IgG1                TRUE Measles  36.277417      1.6638332 IU/ML
  lower_limit_of_detection
1                 3.848750
2                 4.357917
3                 2.699944
4                 1.734784
5                 2.550606
6                 4.438966
```

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 = 1)
```

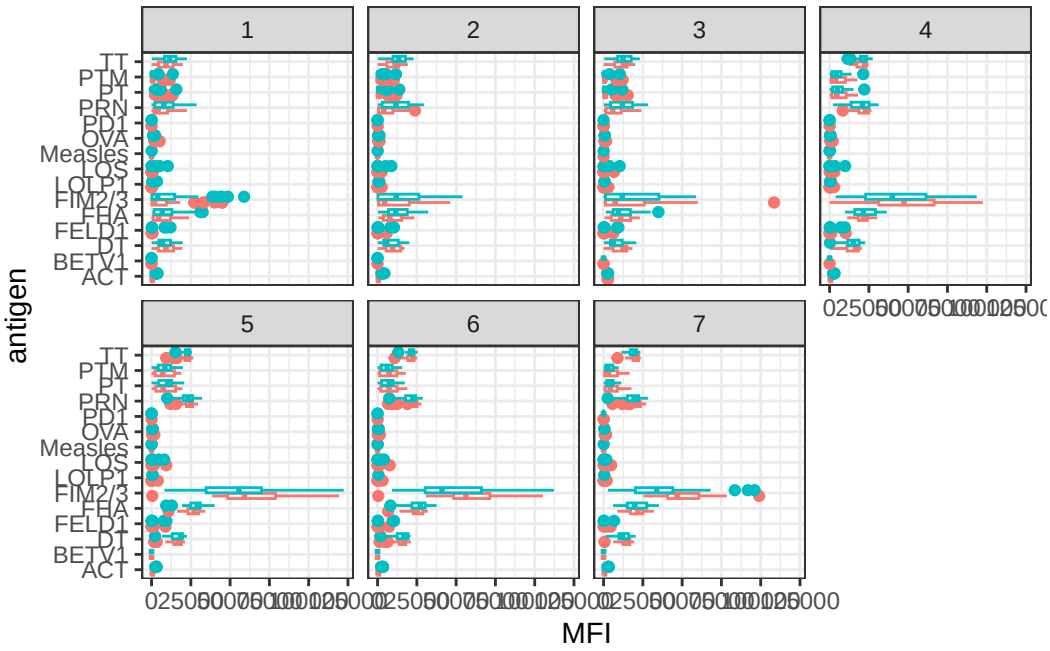


Q14. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

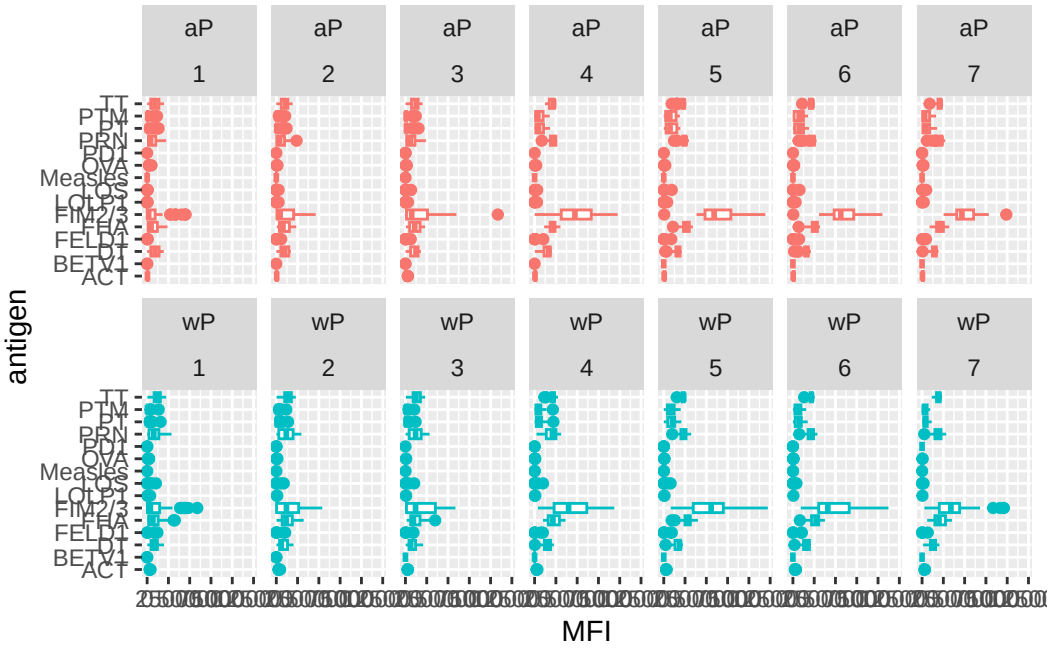
FIM2/3 (Fimbrial Protein) clearly changes. This is a protein involved in making bacteria pilus and is involved in cell adhesion FHA (Filamentous hemagglutinin) is secreted onto the cell surface of bacteria. DT (Diphtheria Toxin) PT, PTM, PRN

All the above antigens are involved in extracellular function that should rise and change when exposed to a vaccine.

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

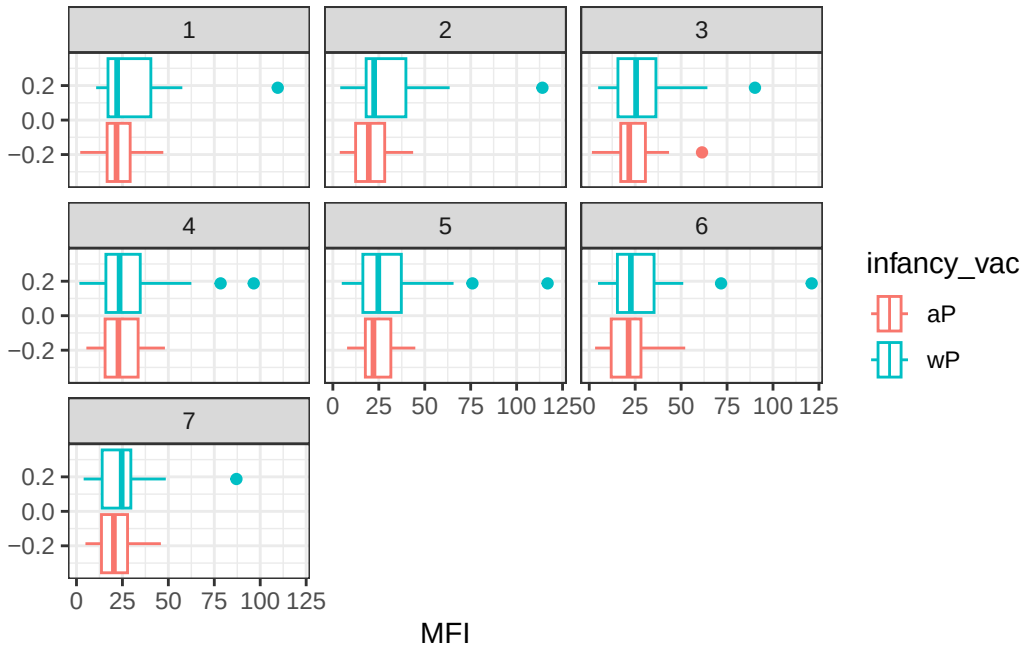



```
ggplot(ig1) +
  aes(MFI, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

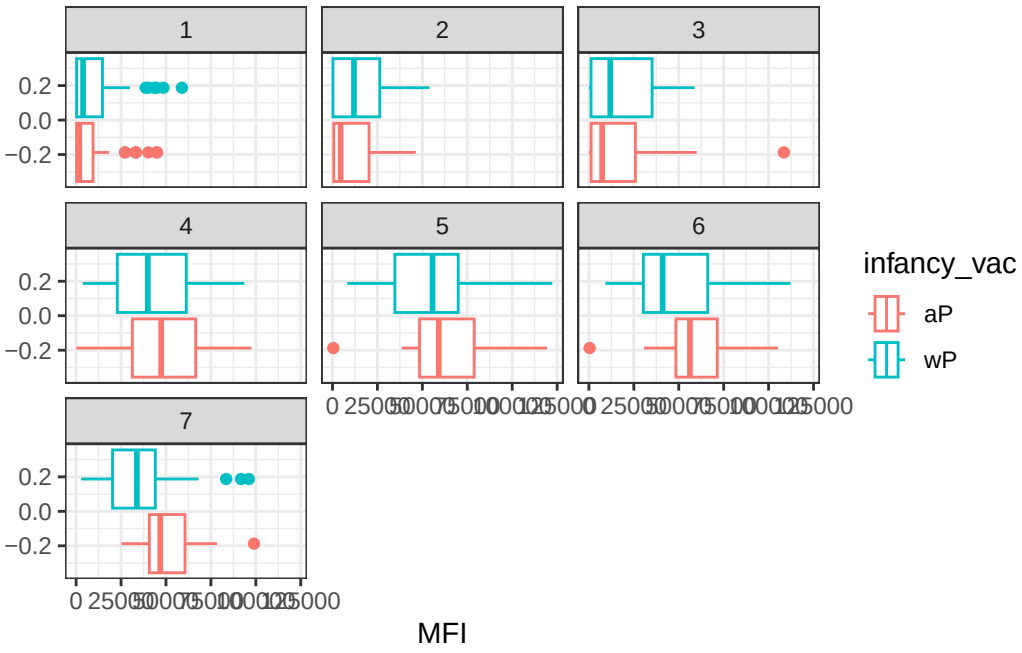


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("Measles", that is not in our vaccines) and a clear antigen of interest ("FIM2/3", extra-cellular fimbriae proteins from B. pertussis that participate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

The median MFI for the measles antigen is consistent throughout time, regardless of the type of vaccine the person was exposed to. This is expected as the measles antigen is a control. However, the FIM2/3 vaccine appears to be a lot more variable across the visit days as well as when comparing which vaccines the people had been exposed to.

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

There is possibly a clear difference in aP vs wP response for day 7 visit for the FIM2/3 but more statistical analysis is needed to know for sure.