Class18: Pertussis and the CMI-PB project

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1. Investigating pertussis cases by year

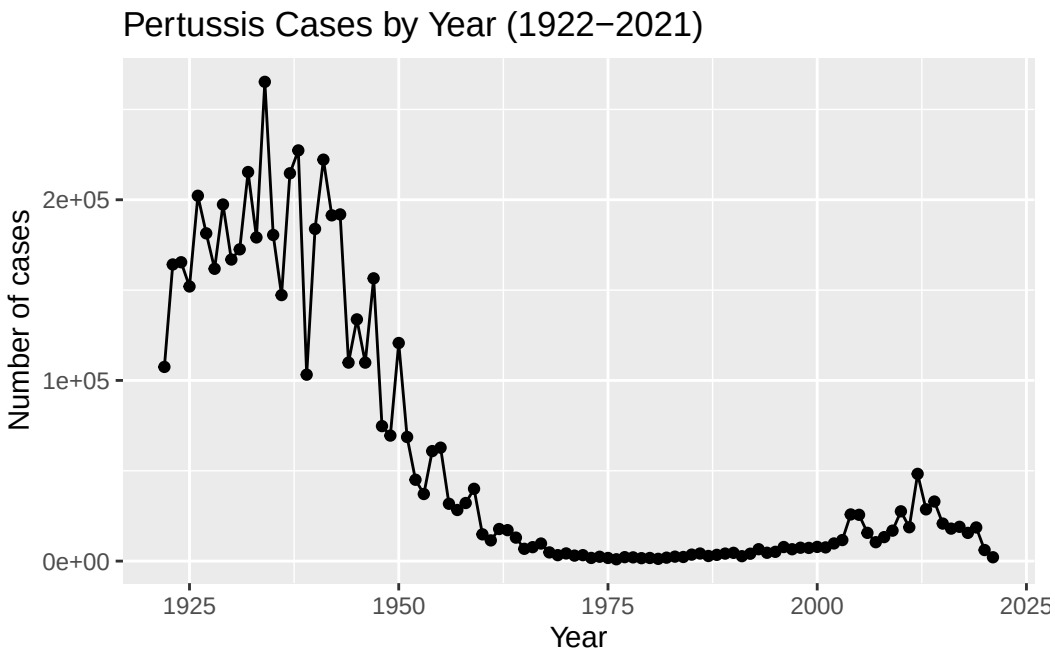
```
#install.packages("datapasta")

library(datapasta)
library(ggplot2)

cdc <- data.frame(
  Year = c(1922, 1923, 1924, 1925, 1926, 1927, 1928, 1929, 1930, 1931, 1932, 1933,
           1934, 1935, 1936, 1937, 1938, 1939, 1940, 1941, 1942, 1943, 1944, 1945,
           1946, 1947, 1948, 1949, 1950, 1951, 1952, 1953, 1954, 1955, 1956, 1957,
           1958, 1959, 1960, 1961, 1962, 1963, 1964, 1965, 1966, 1967, 1968, 1969,
           1970, 1971, 1972, 1973, 1974, 1975, 1976, 1977, 1978, 1979, 1980, 1981,
           1982, 1983, 1984, 1985, 1986, 1987, 1988, 1989, 1990, 1991, 1992, 1993,
           1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005,
           2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017,
           2018, 2019, 2020, 2021),
  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)
)


ggplot(cdc, aes(x = Year, y = Cases)) +
```

```
  geom_point() +
  geom_line() +
  labs(title = "Pertussis Cases by Year (1922-2021)",
       x = "Year",
       y = "Number of cases")
```



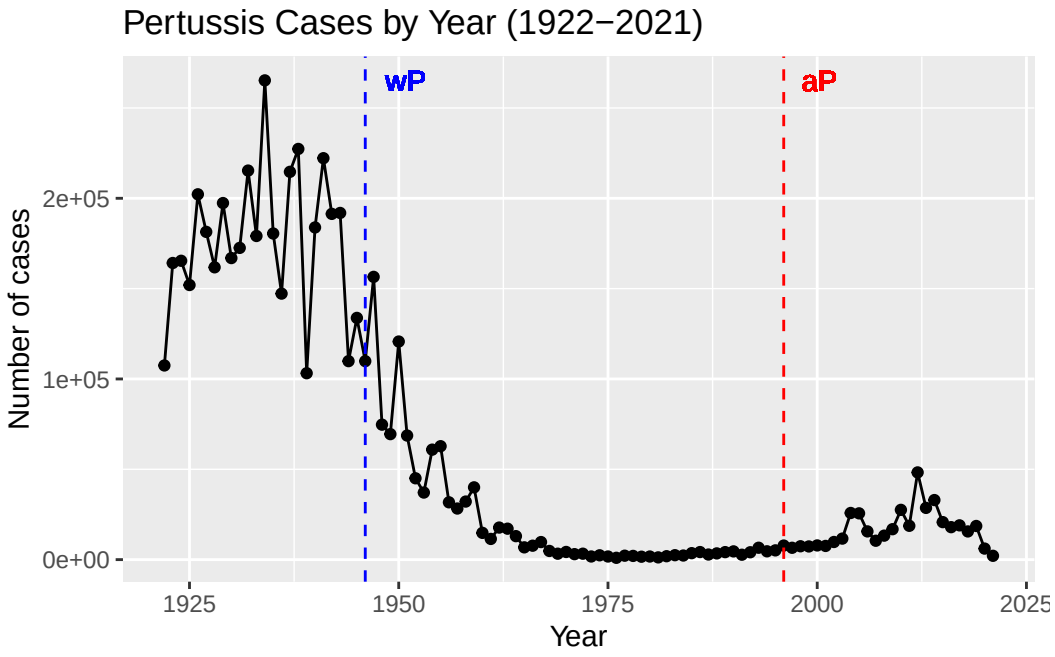
2. A tale of two vaccines (wP & aP)

```
ggplot(cdc, aes(x = Year, y = Cases)) +
  geom_point() +
  geom_line() +
  geom_vline(xintercept = 1946, linetype = "dashed", color = "blue") +
  geom_vline(xintercept = 1996, linetype = "dashed", color = "red") +
  geom_text(aes(x = 1946, y = max(Cases), label = "wP"), color = "blue", hjust = -0.5) +
  geom_text(aes(x = 1996, y = max(Cases), label = "aP"), color = "red", hjust = -0.5) +
  labs(title = "Pertussis Cases by Year (1922-2021)",
       x = "Year",
       y = "Number of cases")
```

```
Warning in geom_text(aes(x = 1946, y = max(Cases), label = "wP"), color = "blue", : All aestl
```

```
i Please consider using `annotate()` or provide this layer with data containing
  a single row.

Warning in geom_text(aes(x = 1996, y = max(Cases), label = "aP"), color = "red", : All aesthe
i Please consider using `annotate()` or provide this layer with data containing
  a single row.
```



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

According to CDC data, pertussis cases are on the rise again. For instance, in 2012, the CDC reported 48,277 cases of pertussis in the U.S., the highest number since 1955, when 62,786 cases were reported. Experts in the field suggest several possible reasons for this resurgence, including: (1) the increased sensitivity of PCR-based testing, (2) vaccine hesitancy, (3) bacterial evolution allowing escape from vaccine-induced immunity, and (4) diminishing immunity in adolescents who were initially vaccinated with the newer aP vaccine compared to those who received the older wP vaccine.

3. Exploring CMI-PB data

```
# Allows us to read, write and process JSON 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 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
```

```
#install.packages("lubridate")
library(lubridate)
```

```
Attaching package: 'lubridate'

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

    date, intersect, setdiff, union
```

```
today()
```

```
[1] "2024-06-02"
```

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

```
Time difference of 8919 days
```

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

```
[1] 24.41889
```

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

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

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

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
     28      32      36      37      39      56
```

The average age for wP individuals is 37 years, and for aP is 27 years.

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

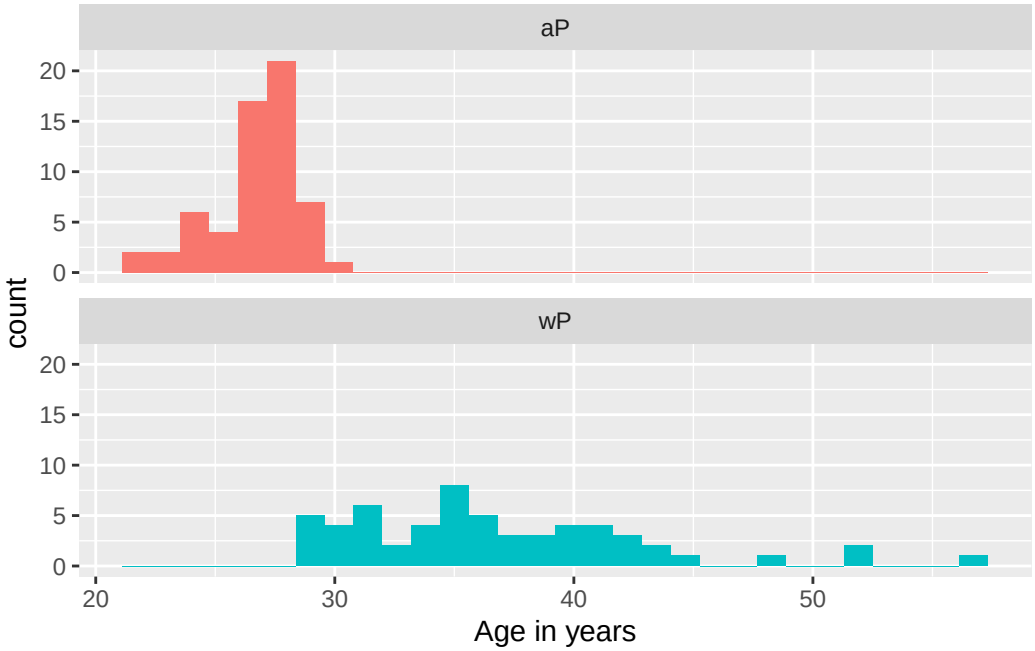
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

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

Q9. With the help of a faceted boxplot or histogram (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) +
  xlab("Age in years")
```

```
`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] 6.813505e-19
```

From the plot and the p-value, it is obvious that the two groups are significantly different.

Joining multiple tables

```
specimen <- read_json("https://www.cmi-pb.org/api/specimen", simplifyVector = TRUE)
titer <- read_json("https://www.cmi-pb.org/api/plasma_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] 939  14
```

```
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 14032 days
2 14032 days
3 14032 days
4 14032 days
5 14032 days
6 14032 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] 46906    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 4255 8983 8990 8990 8990
```

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

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

```
2020_dataset 2021_dataset 2022_dataset
       31520         8085         7301
```

The most recent one has least number of columns.

4. Examine IgG Ab titer levels

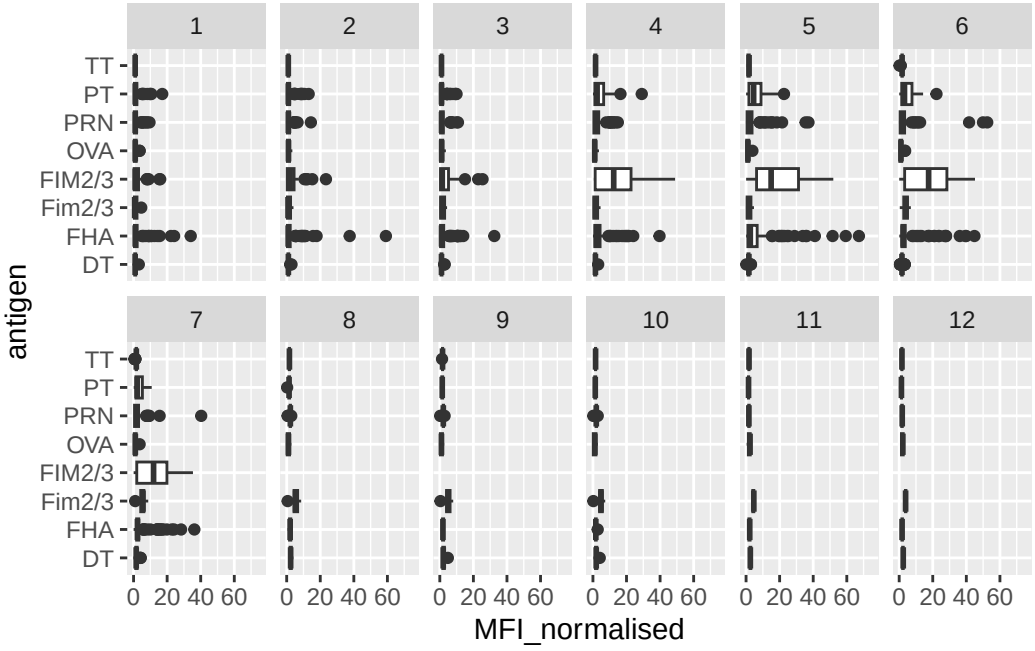
```
igg <- abdata %>% filter(isotype == "IgG")
head(igg)
```

```
  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 14032 days
2 14032 days
3 14032 days
4 15128 days
5 15128 days
6 15128 days
```

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

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

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```

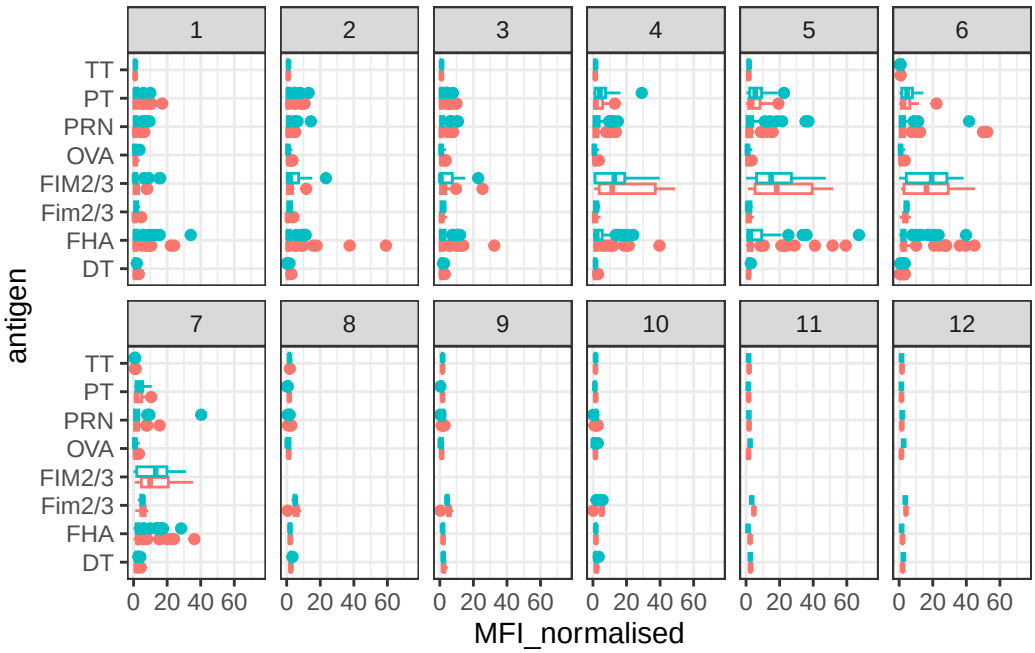


Q14. What antigens show differences in the level of IgG antibody titers recognizing them over time? Why these and not others?

TT and DT show high and consistent IgG antibody titers, likely due to regular booster vaccinations. PRN and FHA show higher variability, possibly due to differences in vaccine formulations and individual responses. Fim2/3 show lower and less variable titers, suggesting they might be less immunogenic.

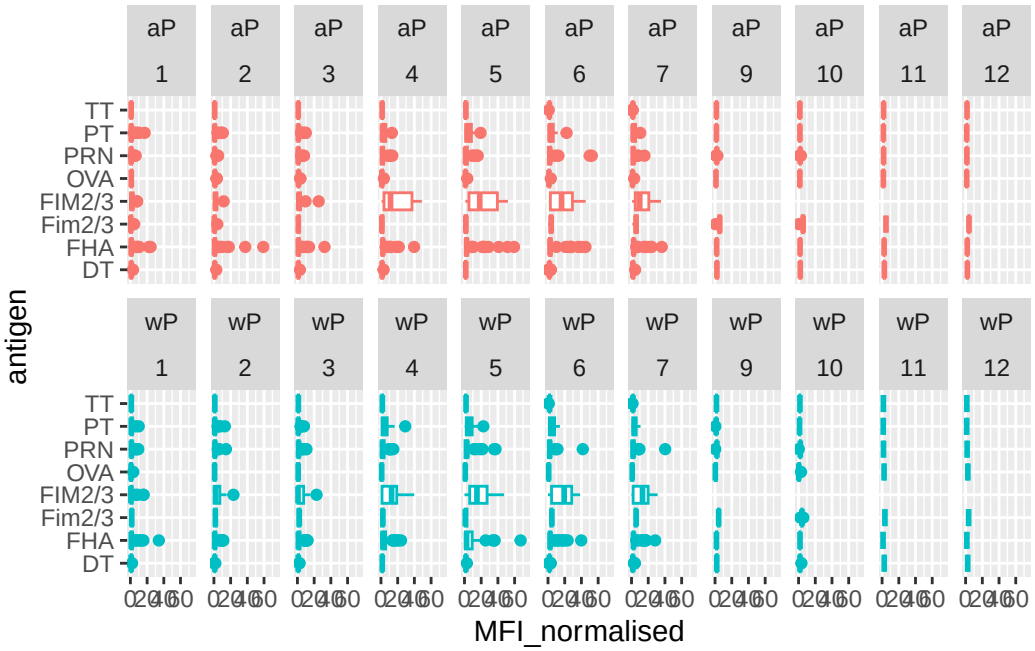
```
ggplot(igg) +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  xlim(0,75) +
  theme_bw()
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```



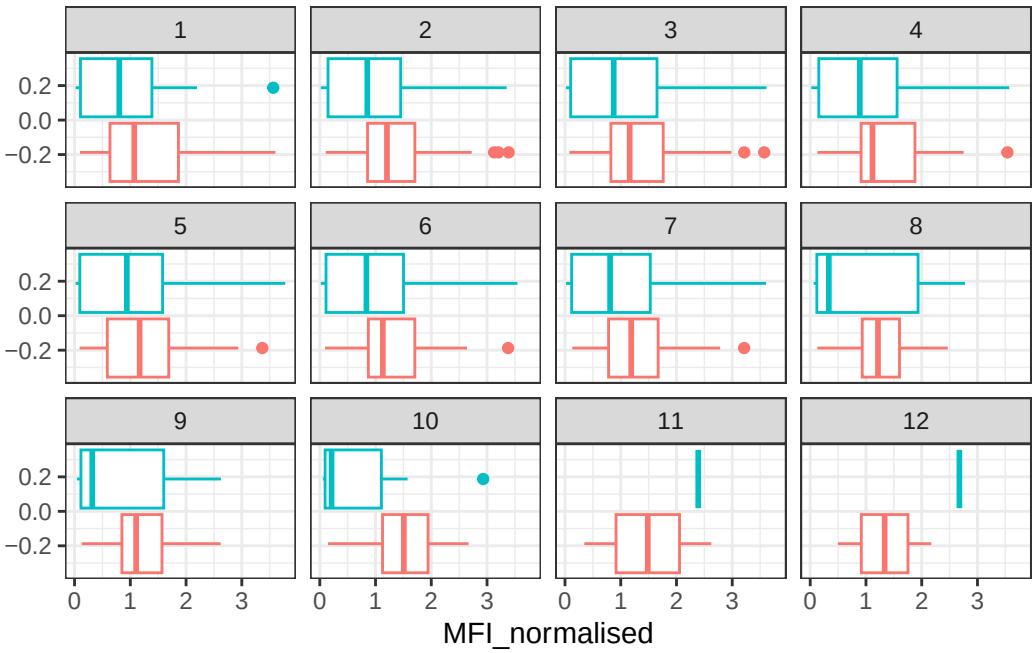

```
igg %>% filter(visit != 8) %>%
ggplot() +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  xlim(0,75) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```

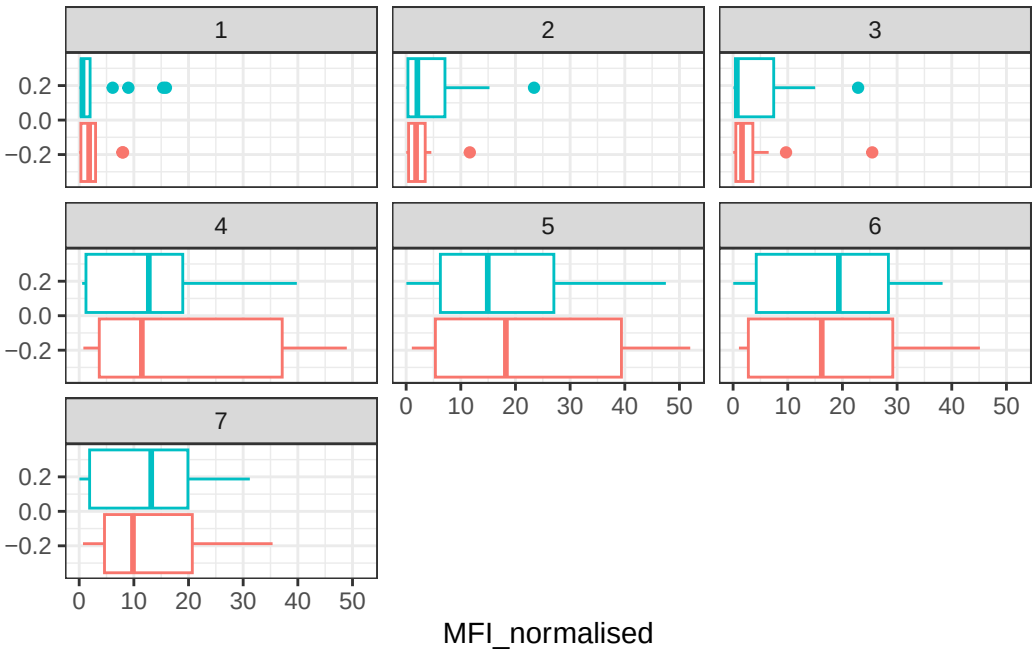


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each.

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




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



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

PT level increased over time and is significantly higher than OVA levels.

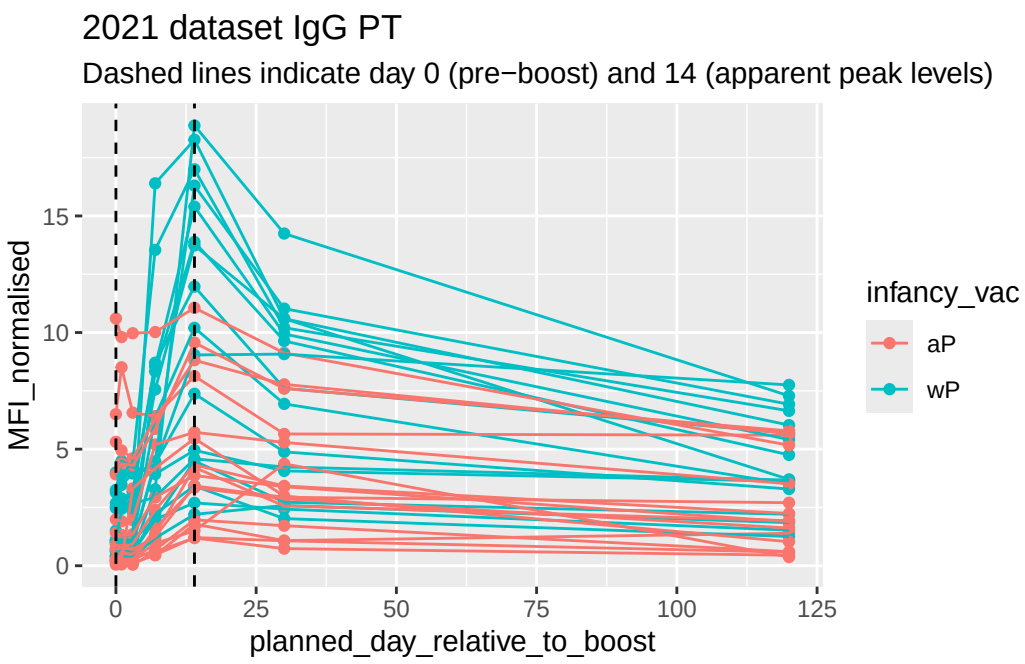
Q17. Do you see any clear difference in aP vs. wP responses?

aP appeared to respond slower than wP did.

```
abdata.21 <- abdata %>% filter(dataset == "2021_dataset")

abdata.21 %>%
  filter(isotype == "IgG",  antigen == "PT") %>%
  ggplot() +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
        col=infancy_vac,
        group=subject_id) +
    geom_point() +
    geom_line() +
    geom_vline(xintercept=0, linetype="dashed") +
    geom_vline(xintercept=14, linetype="dashed") +
  labs(title="2021 dataset IgG PT",
```

```
      subtitle = "Dashed lines indicate day 0 (pre-boost) and 14 (apparent peak levels)")
```

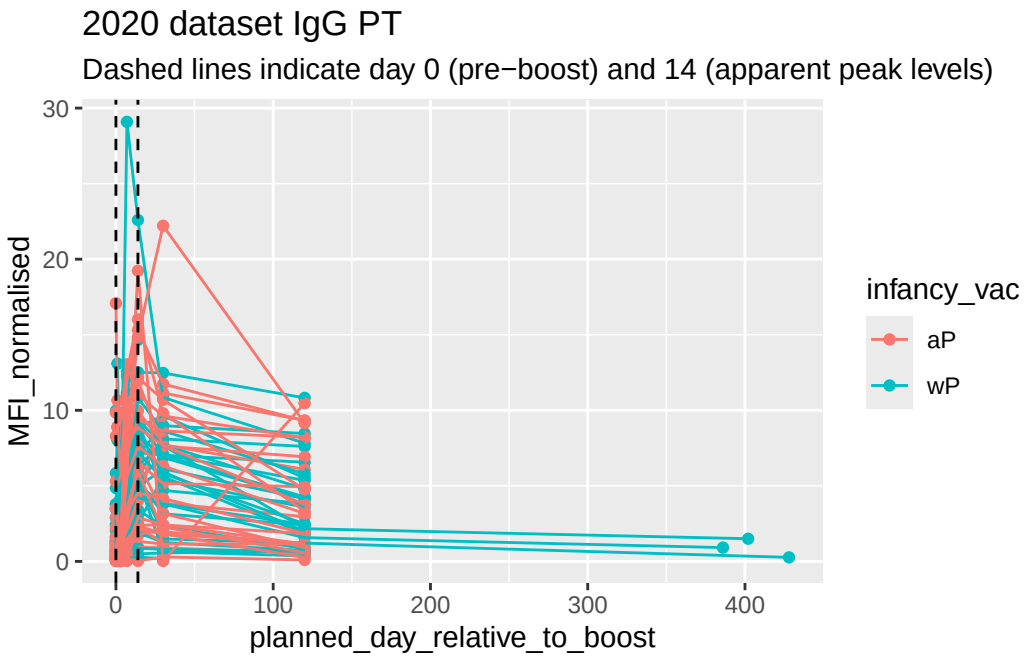


Q18. Does this trend look similar for the 2020 dataset?

Yes, this trend look similar for the 2020 dataset.

```
abdata.20 <- abdata %>% filter(dataset == "2020_dataset")

abdata.20 %>%
  filter(isotype == "IgG",  antigen == "PT") %>%
  ggplot() +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
        col=infancy_vac,
        group=subject_id) +
    geom_point() +
    geom_line() +
    geom_vline(xintercept=0, linetype="dashed") +
    geom_vline(xintercept=14, linetype="dashed") +
  labs(title="2020 dataset IgG PT",
       subtitle = "Dashed lines indicate day 0 (pre-boost) and 14 (apparent peak levels)")
```



5. Obtaining CMI-PB RNASeq data

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

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

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

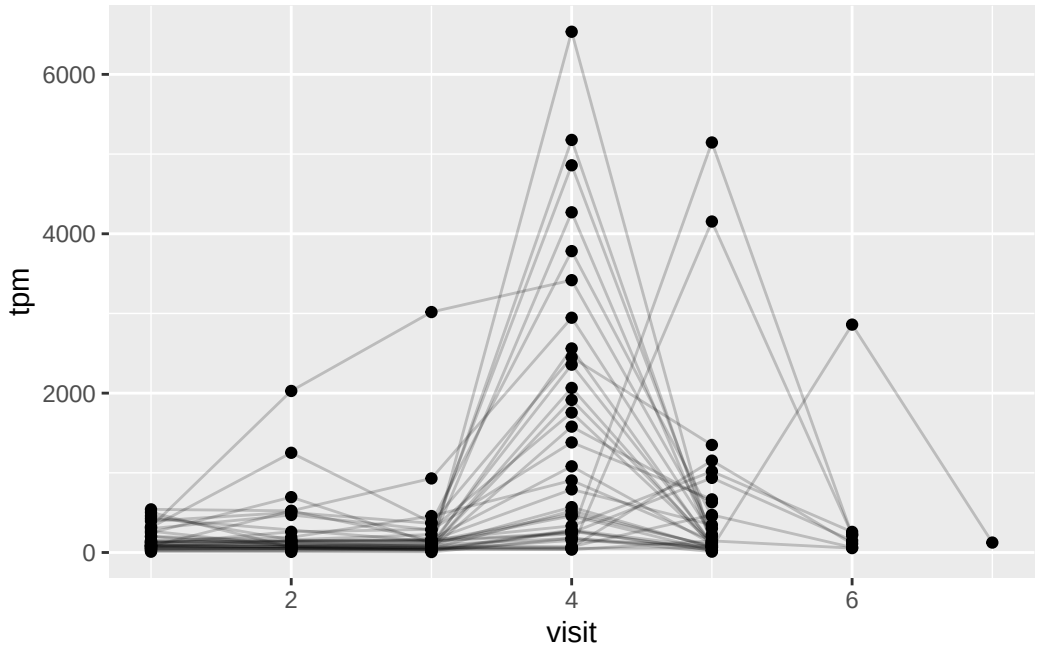
```
Joining with `by = join_by(subject_id)`
```

```
ssrna <- inner_join(rna, meta)
```

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

Q19. Make a plot of the time course of gene expression for IGHG1 gene (i.e. a plot of visit vs. tpm).

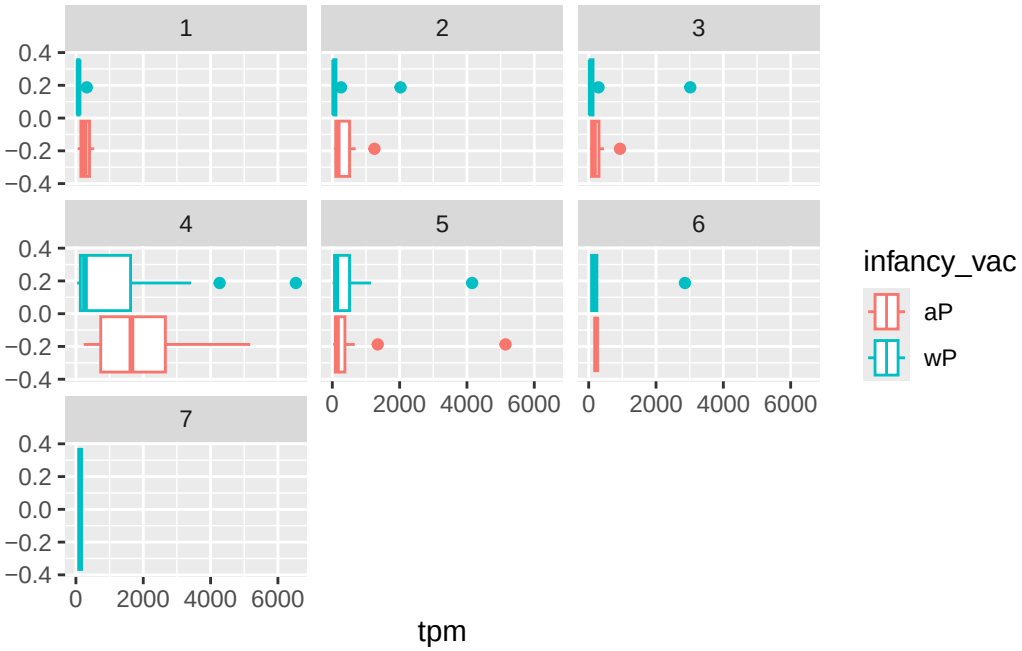
```
ggplot(ssrna) +
  aes(visit, tpm, group=subject_id) +
  geom_point() +
  geom_line(alpha=0.2)
```



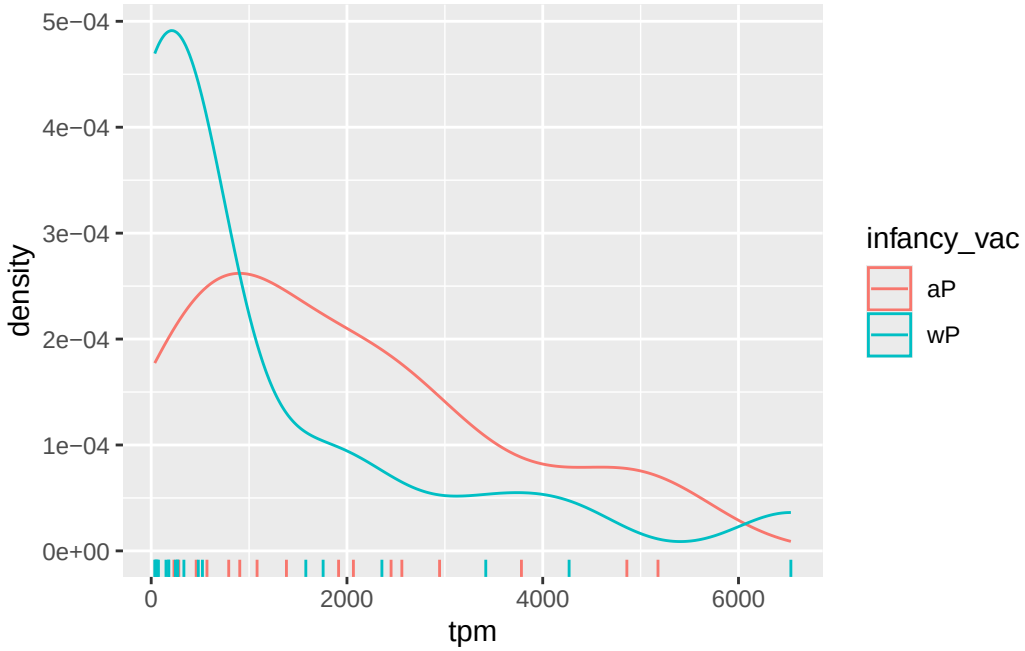
Q20.: What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

The expression of this gene is at its max level at visit 4.

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




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



6. Working with larger datasets [OPTIONAL]