# Class19: Pertussis Mini Project

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Pertussis (a.k.a. whooping cough) is a deadly lung infection caused by the bacteria B. Pertussis.

The CDC tracks Pertussis cases around the US.

https://tinyurl.com/pertussiscdc

### 1. Investigating pertussis cases by year

We can "scrape" this data using the R **datapasta** package.

> 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.

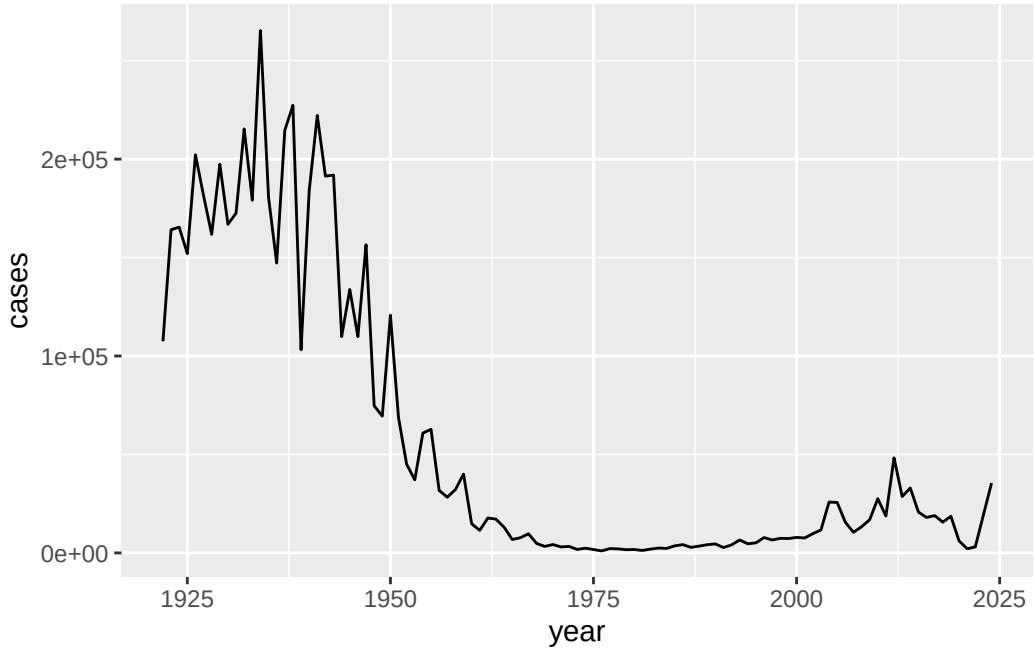
```
head(cdc)
```

```
  year  cases
1 1922 107473
2 1923 164191
3 1924 165418
4 1925 152003
5 1926 202210
6 1927 181411
```

```
library(ggplot2)
```

```
Warning: package 'ggplot2' was built under R version 4.3.3
```

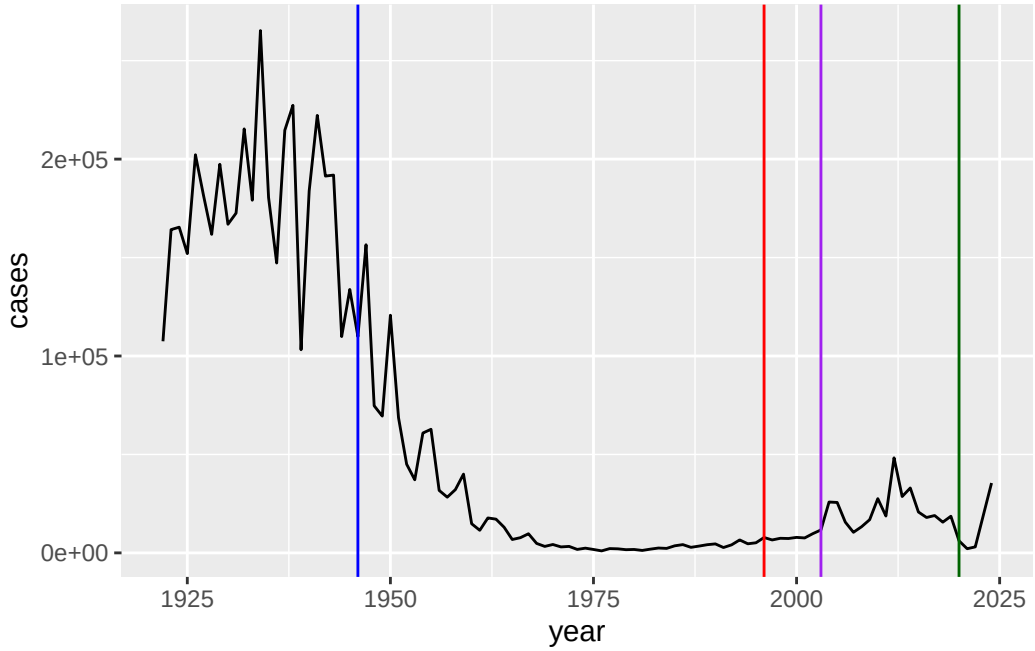
```
ggplot(cdc) +
  aes(year, cases) +
  geom_line()
```




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

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?

```
ggplot(cdc) +
  aes(year, cases) +
  geom_line() +
  geom_vline(xintercept = 1946, col="blue") +
  geom_vline(xintercept = 1996, col="red") +
  geom_vline(xintercept = 2020, col="darkgreen") +
  geom_vline(xintercept = 2003, col="purple")
```




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

There were high case numbers before the first wP (whole cell) vaccine roll out in 1946, then a rapid decline in case numbers until 2004 when we have our first large-scale outbreaks of pertussis again. There is a notable COVID related dip and recent rapid rise. After the introduction of the aP we see an increase that could be due to a decrease in immunity to Pertussis (booster shots introduced in 2007).

Q. What is different about the immune response to infection if you had an older wP vaccine vs the newer aP vaccine?

We don't yet know the answers to this question.

##3. Exploring Computational Models of Immunity - Pertussis Boost (CMI-PB) Data

The CMI-PB project aims to address this key question: what is the difference between wP and aP individuals?

We can get all the data from this ongoing project via JSON API calls.

For this we will use the **jsonlite** package.

```
library(jsonlite)
```

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

```
subject <- read_json("https://www.cmi-pb.org/api/v5_1/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
```

Q. How many individual "subjects" are in this data set?

```
nrow(subject)
```

```
[1] 172
```

Q4. How many aP and wP infancy vaccinated (primed) subjects are in the dataset?

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

```
aP wP
87 85
```

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

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

```
Female   Male
   112     60
```

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                                     32   12
  Black or African American                  2    3
  More Than One Race                        15    4
  Native Hawaiian or Other Pacific Islander  1    1
  Unknown or Not Reported                   14    7
  White                                     48   32
```

This is not representative of the US population but it is the biggest data set of its type so lets see what we can learn…

```
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
```

```
today()
```

```
[1] "2025-03-09"
```

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

```
Time difference of 9199 days
```

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

```
[1] 25.18549
```

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?

```
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. 
     22      26      27      27      28      34 
```

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

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
     22      32      34      36      39      57 
```

They appear to be significantly different, with a median age difference of about seven years. In retrospect looking at the number seven may not seem significant, but seven years can be a significant amoutn of time.

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

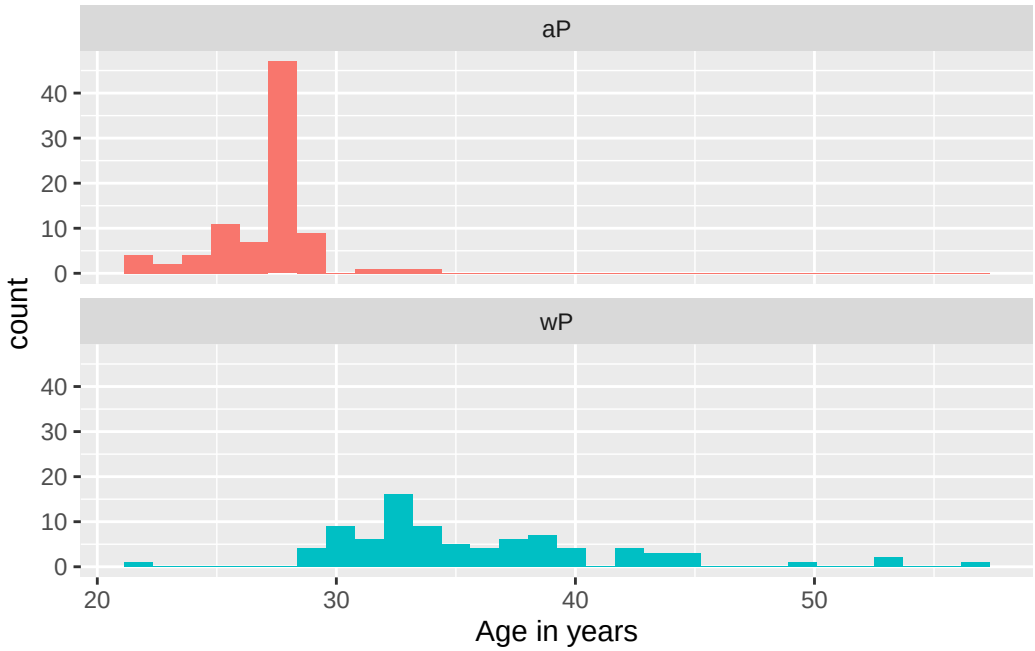
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
[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`.
```




Yes there is a significant difference between these groups. There is a visually apparent difference between wP and aP age groups. aP is predominately younger than 30 while wP is more evenly spread past 30.

```
specimen <- read_json("https://www.cmi-pb.org/api/v5_1/specimen", simplifyVector = T)
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
```

```
ab_data <- read_json("https://www.cmi-pb.org/api/v5_1/plasma_ab_titer", simplifyVector = T)
head(ab_data)
```

```
  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
```

I now have 3 tables of data from CMI-PB: `subject`, `specimen`, and `ab_data`. How can we join these tables so we can have all of the information we need to work with?

We can use the `inner_join()` function from the **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:

```
library(dplyr)

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        age specimen_id
1    1986-01-01    2016-09-12 2020_dataset 14312 days           1
2    1986-01-01    2016-09-12 2020_dataset 14312 days           2
3    1986-01-01    2016-09-12 2020_dataset 14312 days           3
4    1986-01-01    2016-09-12 2020_dataset 14312 days           4
5    1986-01-01    2016-09-12 2020_dataset 14312 days           5
6    1986-01-01    2016-09-12 2020_dataset 14312 days           6
  actual_day_relative_to_boost planned_day_relative_to_boost specimen_type
1                           -3                             0         Blood
2                            1                             1         Blood
3                            3                             3         Blood
4                            7                             7         Blood
5                           11                            14         Blood
6                           32                            30         Blood
  visit
1     1
2     2
3     3
4     4
5     5
6     6
```

```
dim(subject)
```

```
[1] 172   9
```

```
dim(specimen)
```

```
[1] 1503    6
```

```
dim(meta)
```

```
[1] 1503   14
```

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(meta, ab_data)
```

```
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        age specimen_id
1    1986-01-01    2016-09-12 2020_dataset 14312 days           1
2    1986-01-01    2016-09-12 2020_dataset 14312 days           1
3    1986-01-01    2016-09-12 2020_dataset 14312 days           1
4    1986-01-01    2016-09-12 2020_dataset 14312 days           1
5    1986-01-01    2016-09-12 2020_dataset 14312 days           1
6    1986-01-01    2016-09-12 2020_dataset 14312 days           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
```

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  7265 11993 12000 12000 12000
```

```
table(abdata$antigen)
```

```
    ACT   BETV1      DT   FELD1     FHA  FIM2/3   LOLP1     LOS Measles     OVA
   1970    1970    6318    1970    6712    6318    1970    1970    1970    6318
    PD1     PRN      PT     PTM   Total      TT
   1970    6712    6712    1970     788    6318
```

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)
```

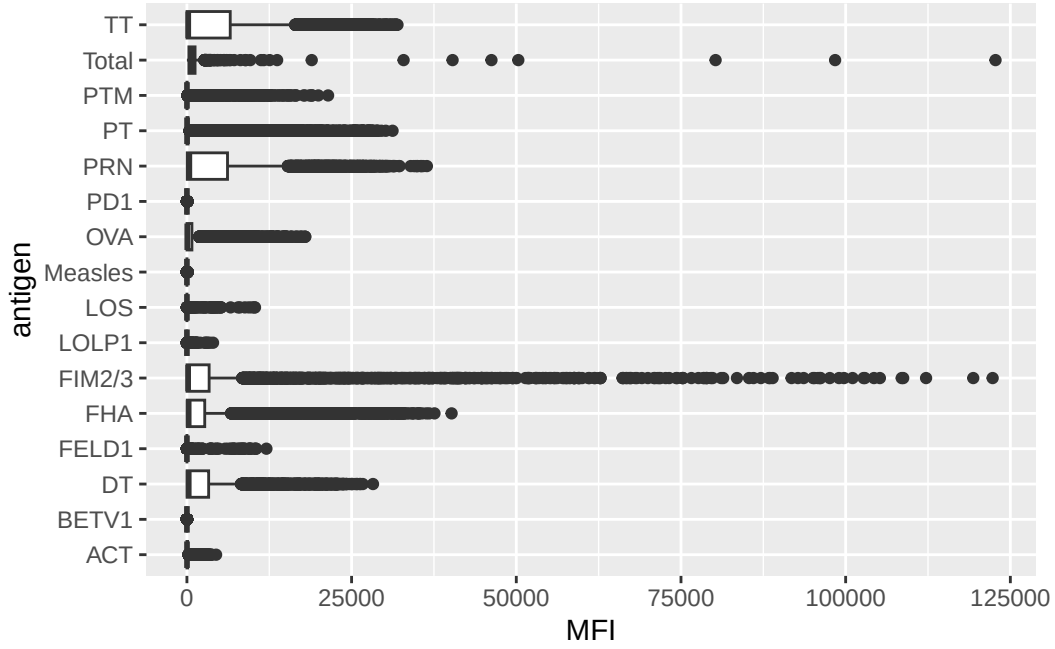
```
2020_dataset 2021_dataset 2022_dataset 2023_dataset
       31520         8085         7301        15050
```

These are data sets per year, the most recent data set has a higher number of rows than the previous year.

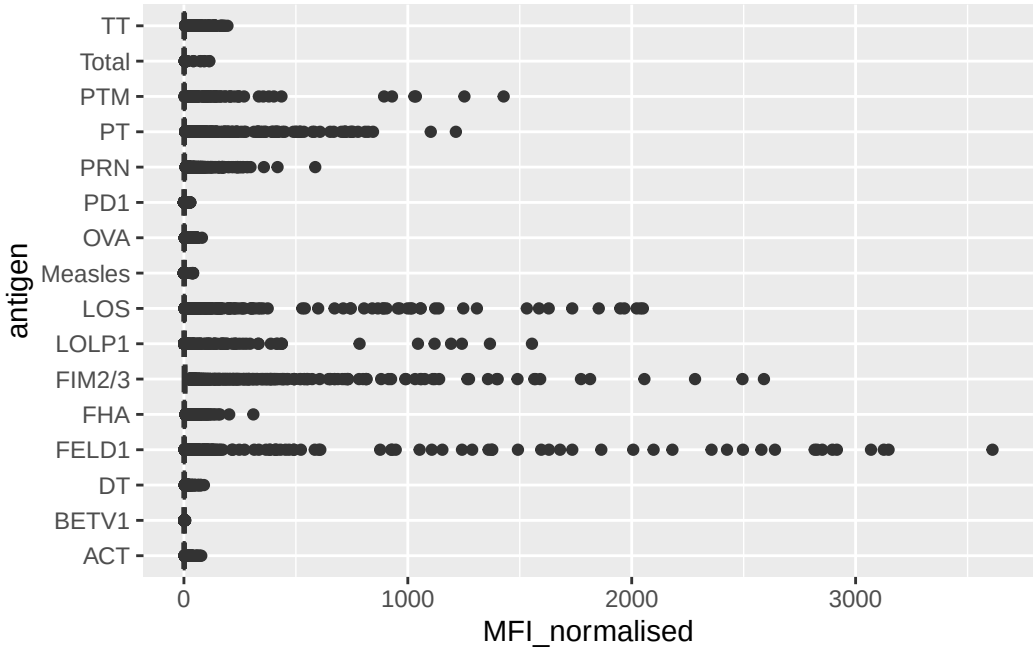
Visualizing our data:

```
ggplot(abdata) +
  aes(MFI, antigen) +
  geom_boxplot()
```

```
Warning: Removed 1 row containing non-finite outside the scale range
(`stat_boxplot()`).
```




```
ggplot(abdata) +
  aes(MFI_normalised, antigen) +
  geom_boxplot()
```

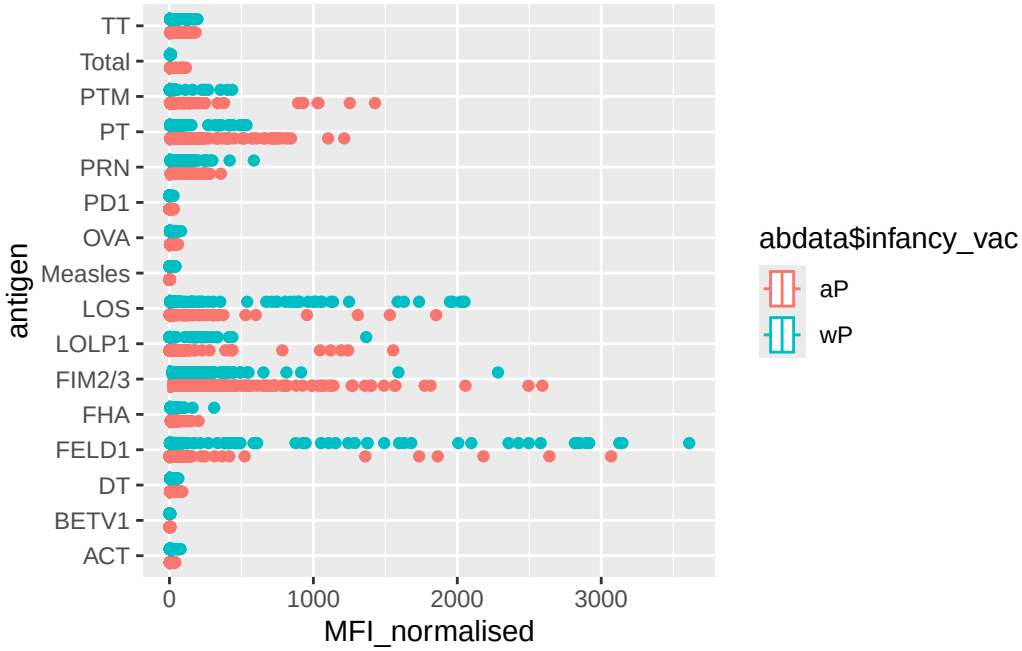



Antigens like FIM2/3, PT, FELD1 have quite a large range of values. Others like measles dont show much activity.

Q. Are there differences at this whole-data set level between aP and wP?

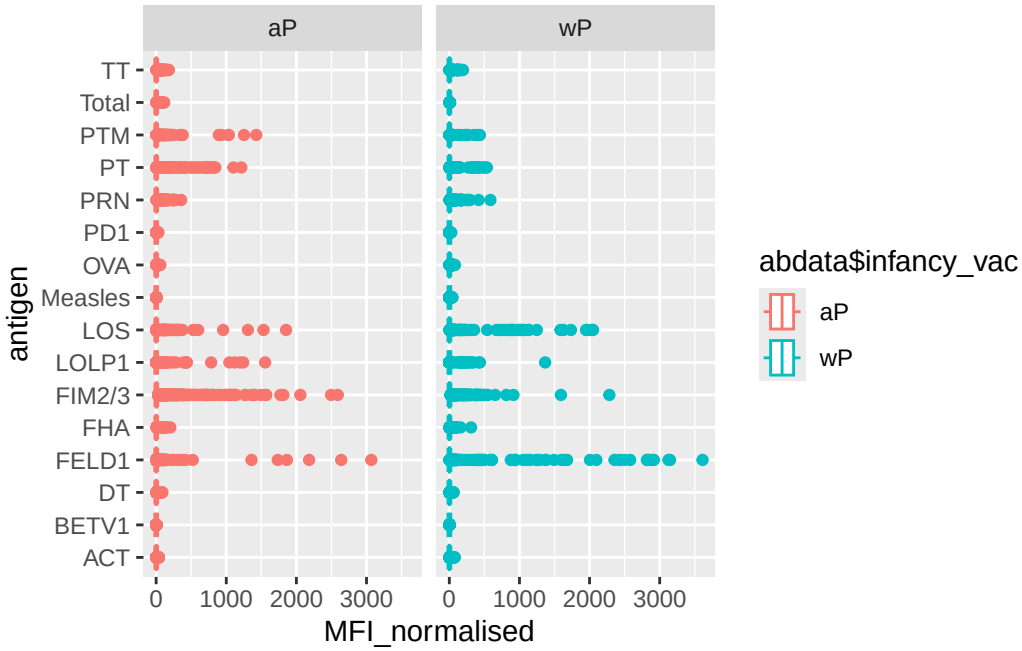
```
ggplot(abdata) +
  aes(MFI_normalised, antigen, col=abdata$infancy_vac) +
  geom_boxplot()
```

```
Warning: Use of `abdata$infancy_vac` is discouraged.
i Use `infancy_vac` instead.
```




```
ggplot(abdata) +
  aes(MFI_normalised, antigen, col=abdata$infancy_vac) +
  geom_boxplot() +
  facet_wrap(~infancy_vac)
```

```
Warning: Use of `abdata$infancy_vac` is discouraged.
i Use `infancy_vac` instead.
```




## 4. Examine IgG Ab titer levels

For this I need to select out just isotype IgG rather than all of them (like above)

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

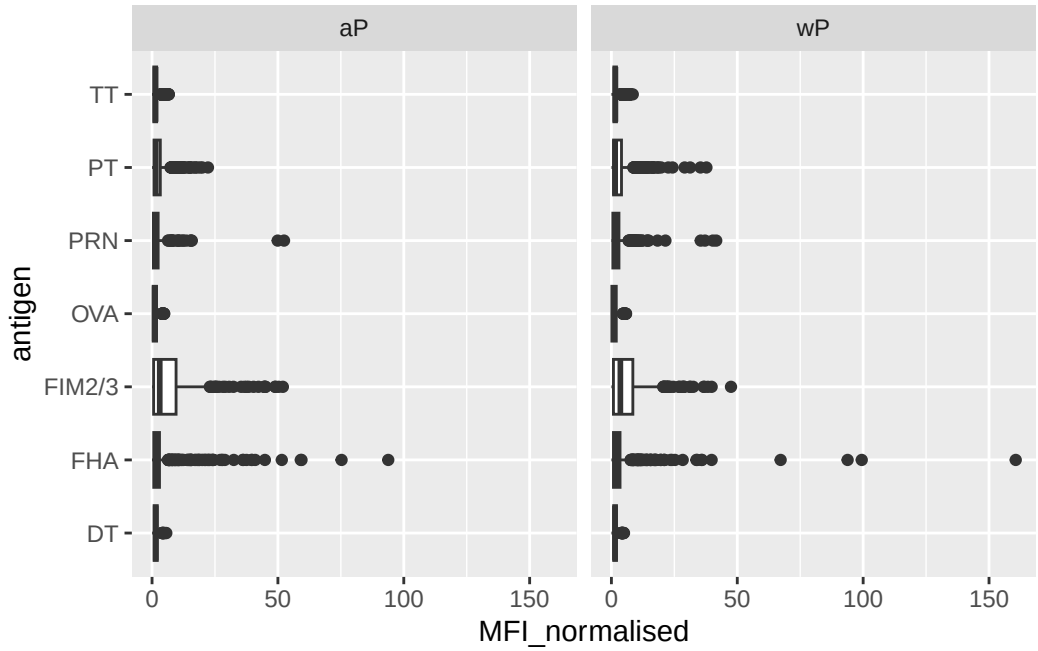
```
igg <- abdata |>
  filter(isotype == "IgG")
head(igg)
```

```
  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        age specimen_id
1    1986-01-01    2016-09-12 2020_dataset 14312 days           1
2    1986-01-01    2016-09-12 2020_dataset 14312 days           1
3    1986-01-01    2016-09-12 2020_dataset 14312 days           1
```

```
4    1986-01-01    2016-09-12 2020_dataset 14312 days           2
5    1986-01-01    2016-09-12 2020_dataset 14312 days           2
6    1986-01-01    2016-09-12 2020_dataset 14312 days           2
  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                            1                              1         Blood
5                            1                              1         Blood
6                            1                              1         Blood
  visit isotype is_antigen_specific antigen        MFI MFI_normalised  unit
1     1     IgG                TRUE      PT   68.56614       3.736992 IU/ML
2     1     IgG                TRUE     PRN  332.12718       2.602350 IU/ML
3     1     IgG                TRUE     FHA 1887.12263      34.050956 IU/ML
4     2     IgG                TRUE      PT   41.38442       2.255534 IU/ML
5     2     IgG                TRUE     PRN  174.89761       1.370393 IU/ML
6     2     IgG                TRUE     FHA  246.00957       4.438960 IU/ML
  lower_limit_of_detection
1                 0.530000
2                 6.205949
3                 4.679535
4                 0.530000
5                 6.205949
6                 4.679535
```

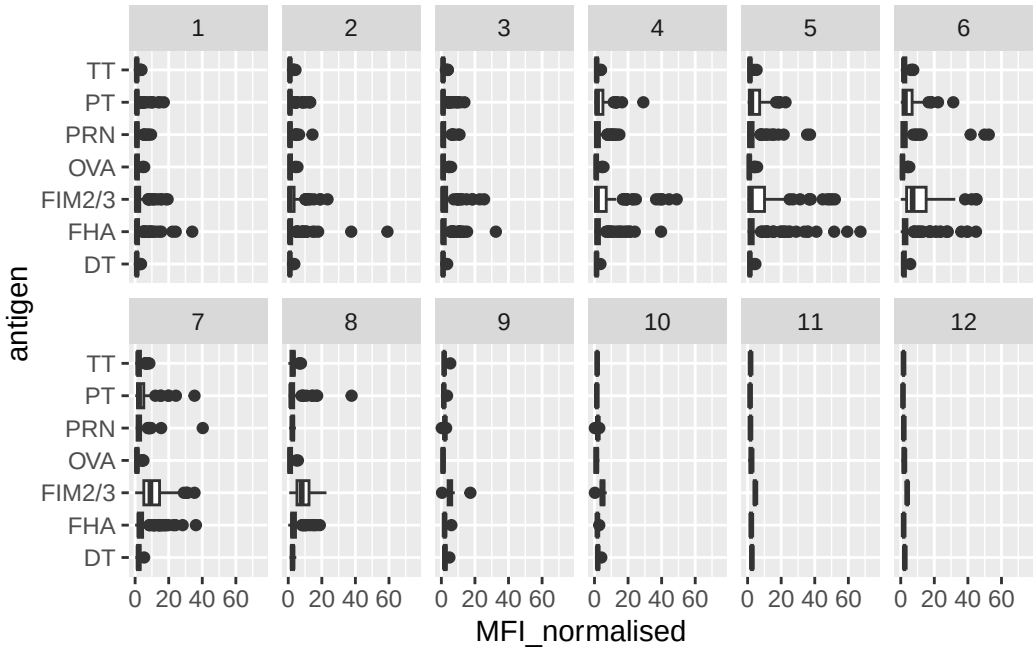
An overview boxplot:

```
ggplot(igg) +
  aes(MFI_normalised, antigen) +
  geom_boxplot() +
  facet_wrap(~infancy_vac)
```




```
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()`).
```

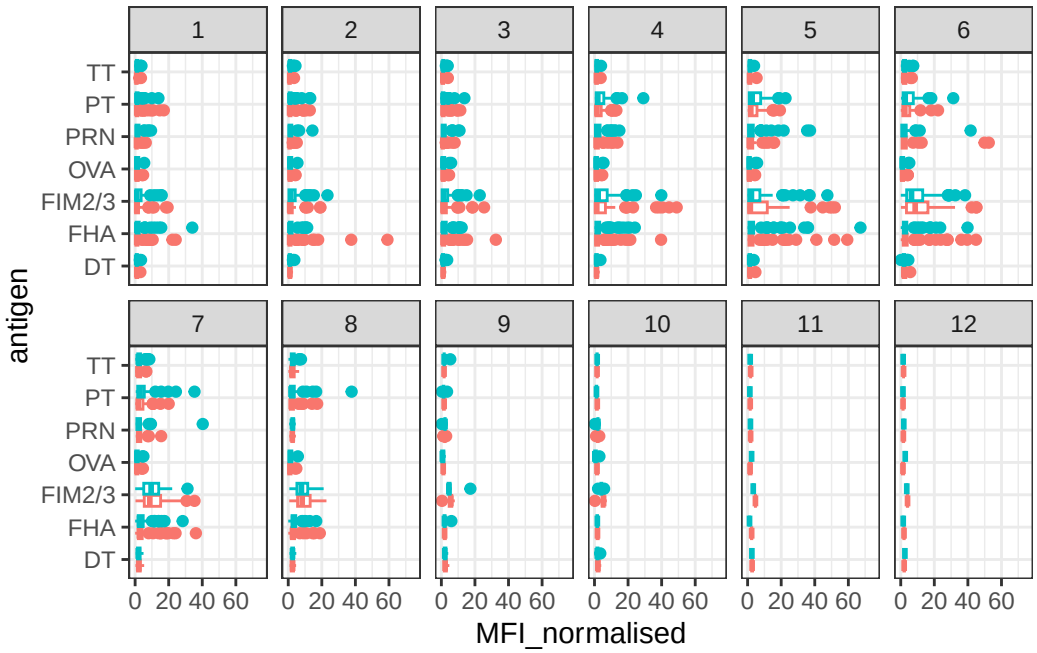



Q14. What antigens show differences in the level of IgG antibody titers recognizing them over time? Why these and not others?

Antigens TT, PRN, FHA, and FIM2/3 all show differences over time. All others show a somewhat consistent recogniction level over time but these 4 show an increase at around day 7 to 14 after the booster. I think these are recognized at later because of their binding/inhibatory functions.

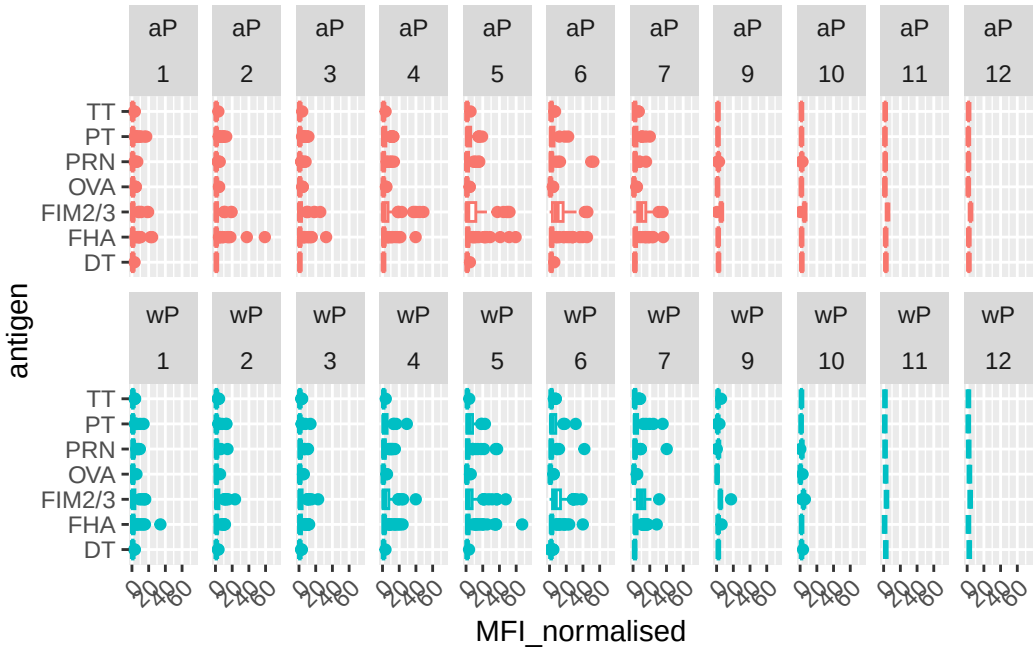
```
ggplot(igg) +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  xlim(0,75) +
  theme_bw()
```

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



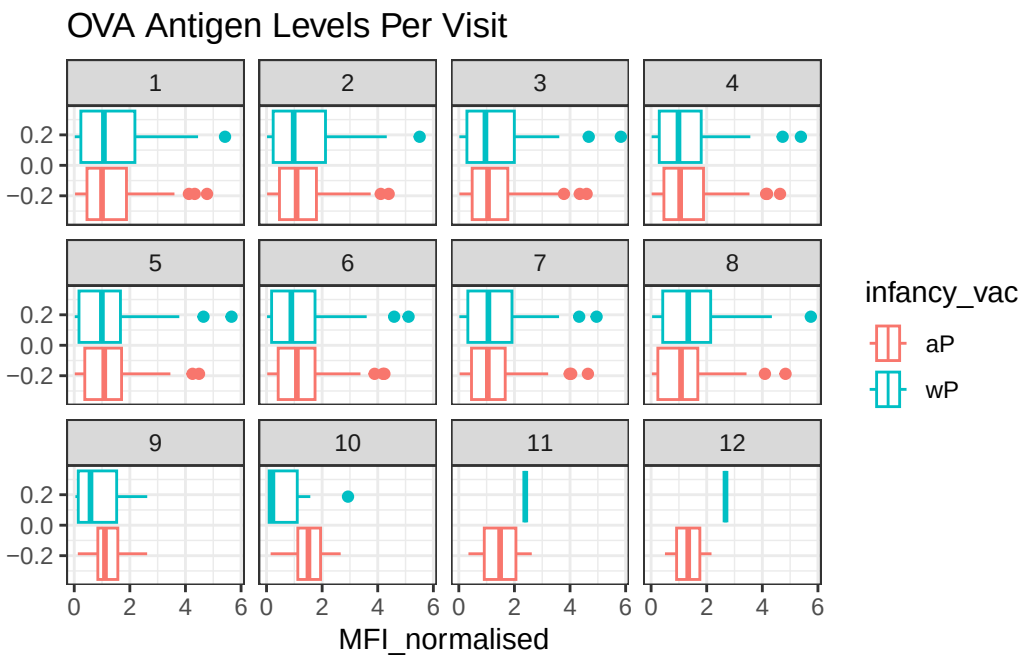

```
igg %>% filter(visit != 8) %>%
ggplot() +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  xlim(0,75) +
  facet_wrap(vars(infancy_vac, visit), nrow=2) +
  theme(axis.text.x = element_text(angle = 45, hjust=1))
```

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

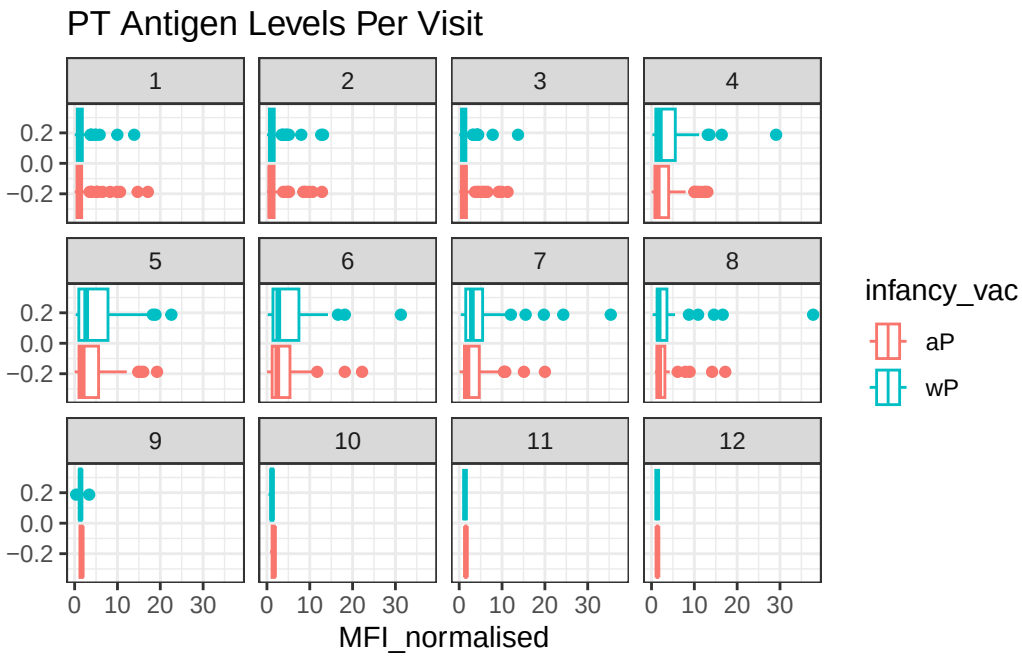



Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("OVA", that is not in our vaccines) and a clear antigen of interest ("PT", Pertussis Toxin, one of the key virulence factors produced by the bacterium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

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




```
filter(igg, antigen=="PT") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = T) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  labs(title = "PT Antigen Levels Per Visit")
```




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

OVA antigen levels are higher on average than PT and over the course of time stay higher.

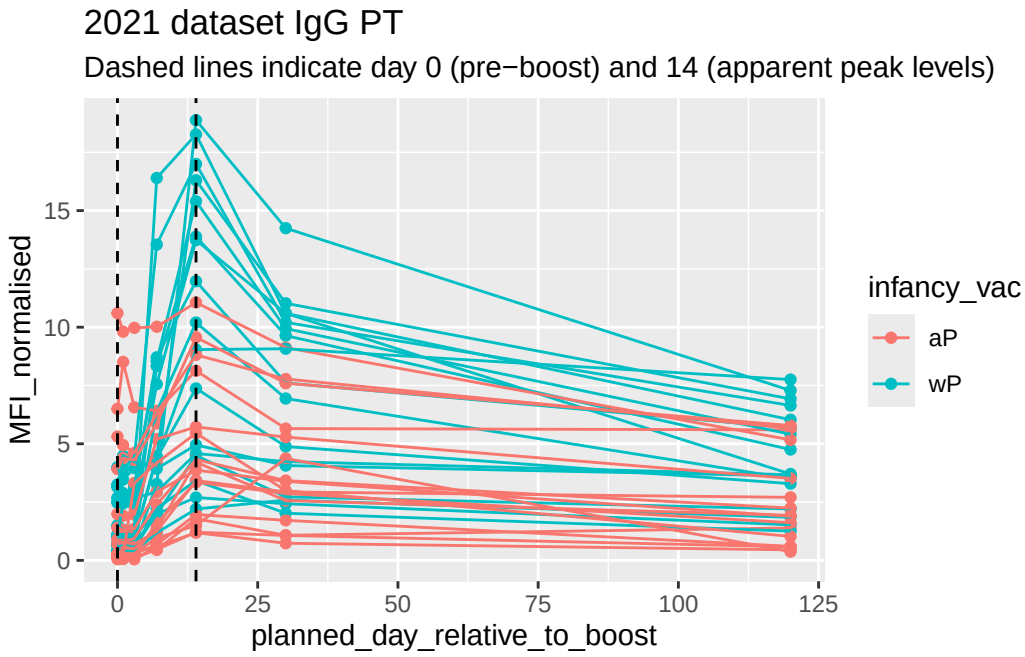
Q17. Do you see any clear difference in aP vs. wP responses?

For the OVA antigen there is a clear difference for aP compared to wP. Longer after the booster there is still binding.

Digging in further to look at the time course for IgG isotype PT antigen levels across aP and wP individuals:

```
## Filter to include 2021 data only
abdata.21 <- abdata |>
  filter(dataset == "2021_dataset")

##Filter to look at IgG PT data only
pt.igg <- abdata.21 |>
  filter(isotype == "IgG",  antigen == "PT")

ggplot(pt.igg) +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
```

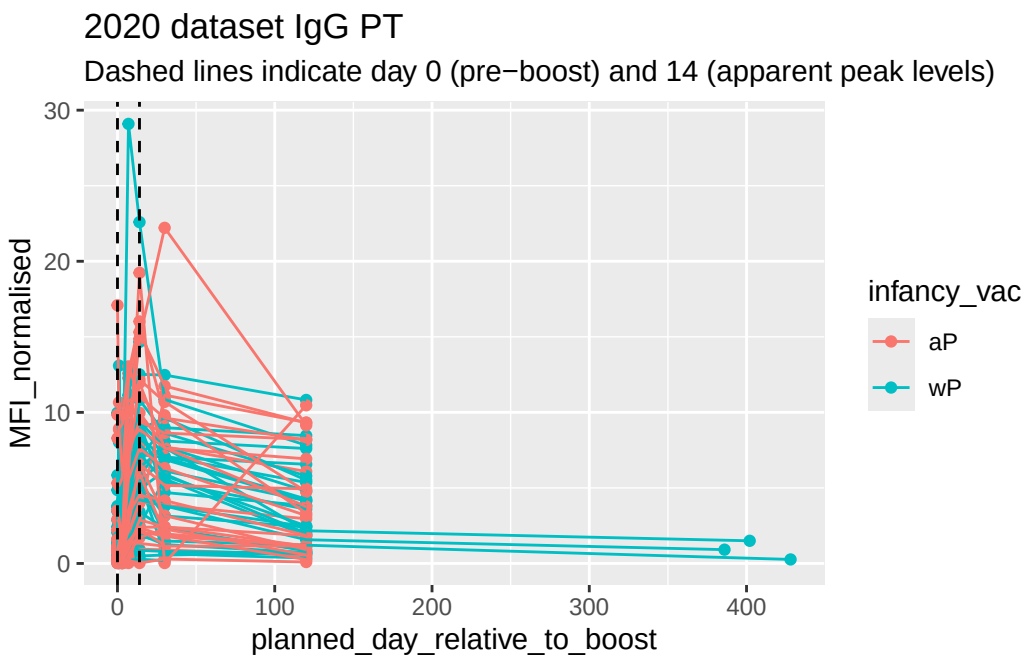
```
        col=infancy_vac,
        group=subject_id) +
    geom_point() +
    geom_line() +
    geom_vline(xintercept=0, linetype="dashed") +
    geom_vline(xintercept=14, linetype="dashed") +
  labs(title="2021 dataset IgG PT",
       subtitle = "Dashed lines indicate day 0 (pre-boost) and 14 (apparent peak levels)")
```




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

```
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)")
```




I think the general trend compared to 2020 is similar, with a general decreased in MFI over time.

## 5. Obtaining CMI-PB RNASeq data

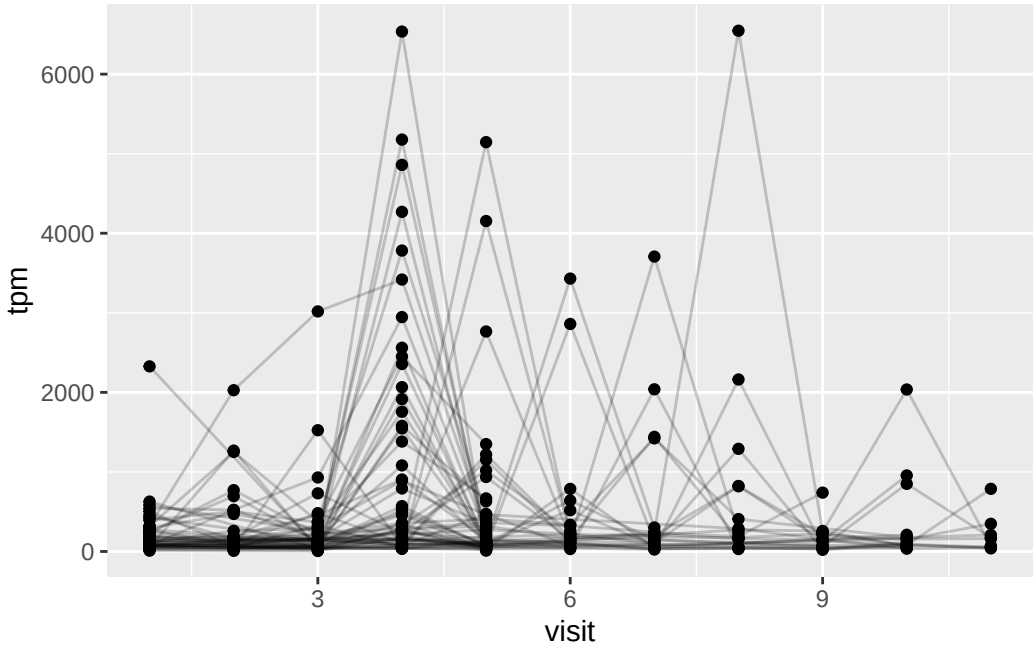
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.7"

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

```
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)
```



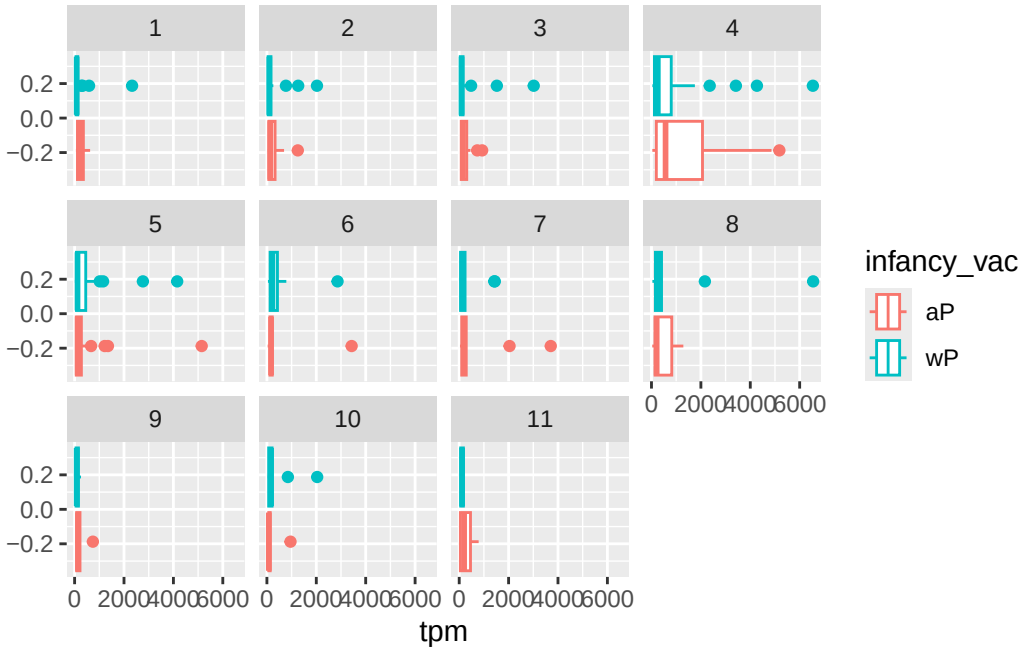

Q20.: What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

It appears its maximum level is at visit 4, but it is also expressed at visit 8.

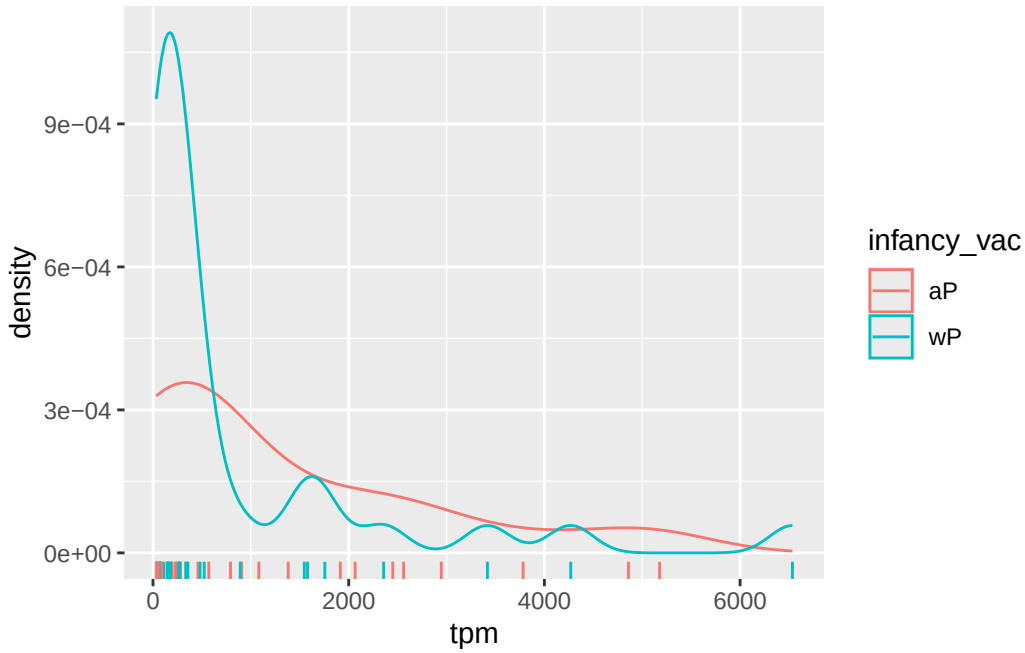
Q21. Does this pattern in time match the trend of antibody titer data? If not, why not?

It does seem to match. Day 4 and 8 appear to have high MFI values, but other days also show levels of similar expression.

```
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()
```


density
9e−04
6e−04
3e−04
0e+00
0
2000
4000
6000
tpm
infancy_vac
aP
wP