Class 19: Mini Project: Investigating Pertussis Resurgence

AUTHOR
Emily Hickey (A15575724)

Investigating pertussis cases by year

> 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.

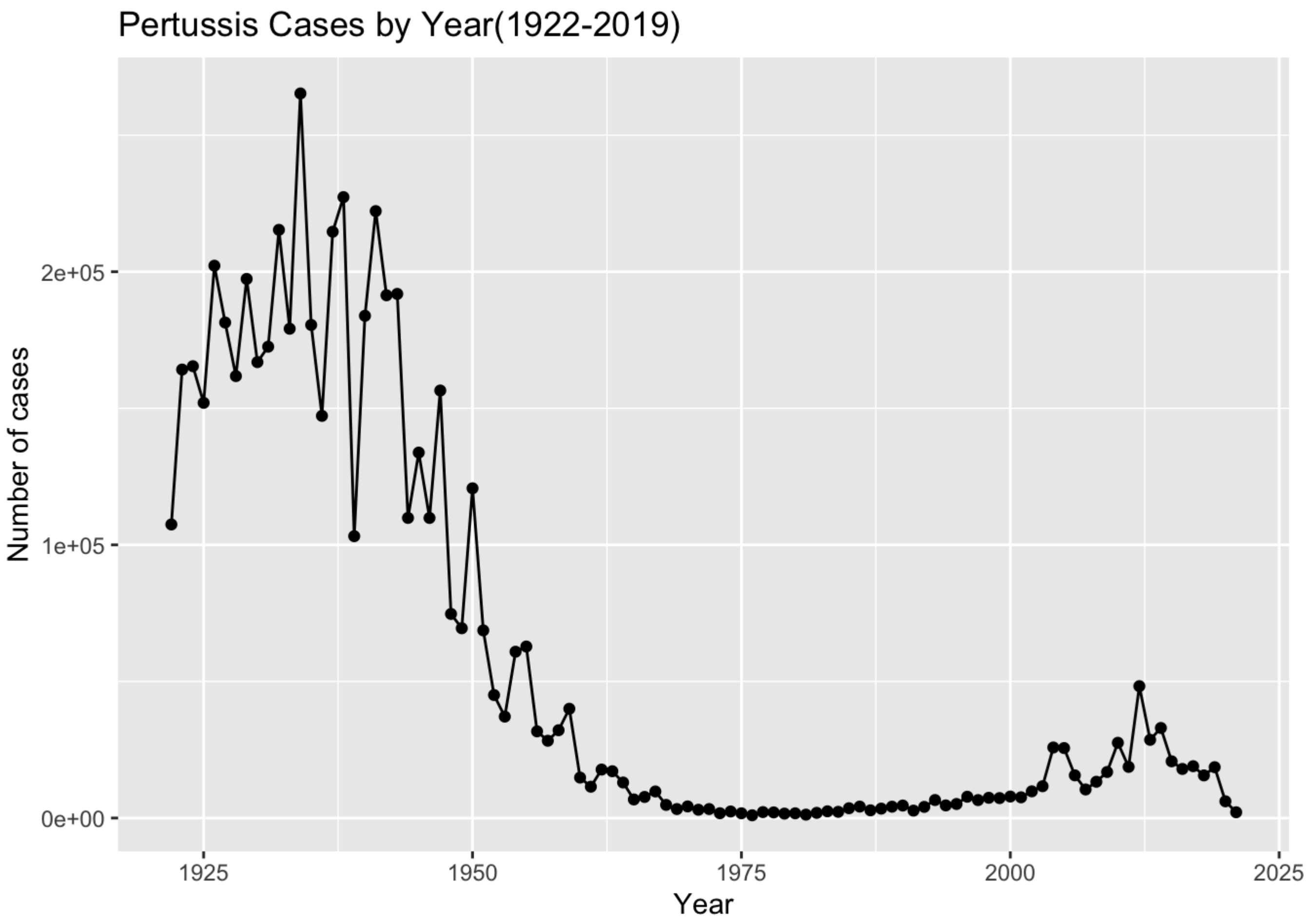
```
library(datapasta)
```

```
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,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)
)
```

```
library(ggplot2)
```

```
ggplot(cdc) +
  aes(cdc$Year, cdc$No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  labs(title= "Pertussis Cases by Year(1922-2019)", x="Year", y="Number of cases")
```

```
Warning: Use of `cdc$Year` is discouraged.
ℹ Use `Year` instead.

Warning: Use of `cdc$No..Reported.Pertussis.Cases` is discouraged.
ℹ Use `No..Reported.Pertussis.Cases` instead.

Warning: Use of `cdc$Year` is discouraged.
ℹ Use `Year` instead.

Warning: Use of `cdc$No..Reported.Pertussis.Cases` is discouraged.
ℹ Use `No..Reported.Pertussis.Cases` instead.
```

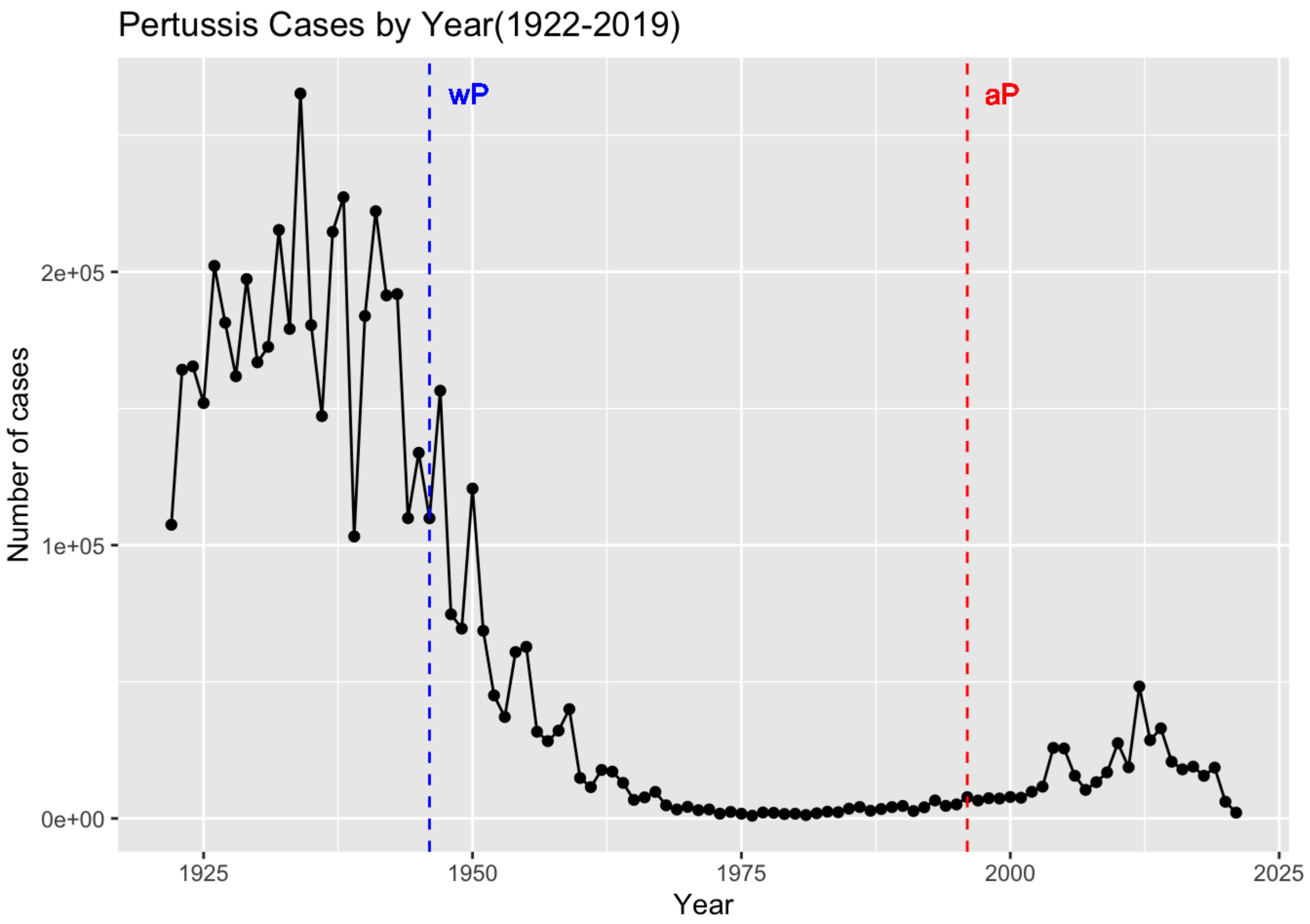


A tale of two vaccines (wP & aP)

> 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?

```r
ggplot(cdc) +
  aes(cdc$Year, cdc$No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  geom_vline(xintercept=1946, linetype="dashed", color="blue") +
  geom_vline(xintercept=1996, linetype="dashed", color="red") +
  geom_text(aes(label="wP"), x=1946, y=max(cdc$No..Reported.Pertussis.Cases), hjust=-0.5,
  geom_text(aes(label="aP"), x=1996, y=max(cdc$No..Reported.Pertussis.Cases), hjust=-0.5,
  labs(title= "Pertussis Cases by Year(1922-2019)", x="Year", y="Number of cases")
```

```
Warning: Use of `cdc$Year` is discouraged.
ℹ Use `Year` instead.

Warning: Use of `cdc$No..Reported.Pertussis.Cases` is discouraged.
ℹ Use `No..Reported.Pertussis.Cases` instead.

Warning: Use of `cdc$Year` is discouraged.
ℹ Use `Year` instead.

Warning: Use of `cdc$No..Reported.Pertussis.Cases` is discouraged.
ℹ Use `No..Reported.Pertussis.Cases` instead.
```



I notice that cases dropped after the wP vaccine was given but rose again after the aP vaccine.

> 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, there was a spike in pertussis cases before cases began to drop again. Possible explanations for this could be: better/more sensitive PCR tests, people being hesitant to the new vaccine, bacterial evolution, and waning of immunity for those who were given the newer aP vaccine as infants.

Exploring CMI-PB 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 
```

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

```
         American Indian/Alaska Native Asian Black or African American
  Female                             0    21                         2
  Male                               1    11                         0

         More Than One Race Native Hawaiian or Other Pacific Islander
  Female                  9                                         1
  Male                    2                                         1

         Unknown or Not Reported White
  Female                      11    35
  Male                         4    20
```

Working with dates

```
library(lubridate)
```

```
Attaching package: 'lubridate'
```

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

    date, intersect, setdiff, union
```

```
today()
```

```
[1] "2023-12-05"
```

How many days have passed since new year 2000?

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

```
Time difference of 8739 days
```

What is this in years?

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

```
[1] 23.92608
```

> 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)
subject$age
```

```
Time differences in days
  [1] 13852 20427 14948 13122 12026 13122 15678 14217 10200 15313 13852 15313
 [13]  9834 11295 12756 13487 16044  9834 10930 15678 14948 14217 12026 11661
 [25] 13122 14948  9834 15313  9834 13122 12756  9834 12391 14948 12026  9834
 [37]  9469  9834 14217 10930 14217  9834  9469  9469  9834  9469 10200  9469
 [49]  9834  9834  9834  9469  9469  9834  9834  9834 10200  9834  9834  9834
 [61] 13487 11295 10565 11295 12391 17505 18966 18966 12391  9469  9469 12026
 [73] 10565 10565  9469  9469 13122 11295 13487 11661 11295  9469  9104  9834
 [85]  8739  9469  8739  8739  9834  9104  9469  8739 10200  9104  9469  8739
 [97] 13852 11295  9104  8373  7643  7643 10930 12756 10930 10200  9469 10565
[109] 12756  9834 10200 10200 10200 12391  8008  8739 10930  9469
```

```
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. 
     21      26      26      26      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      31      35      36      39      56 
```

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

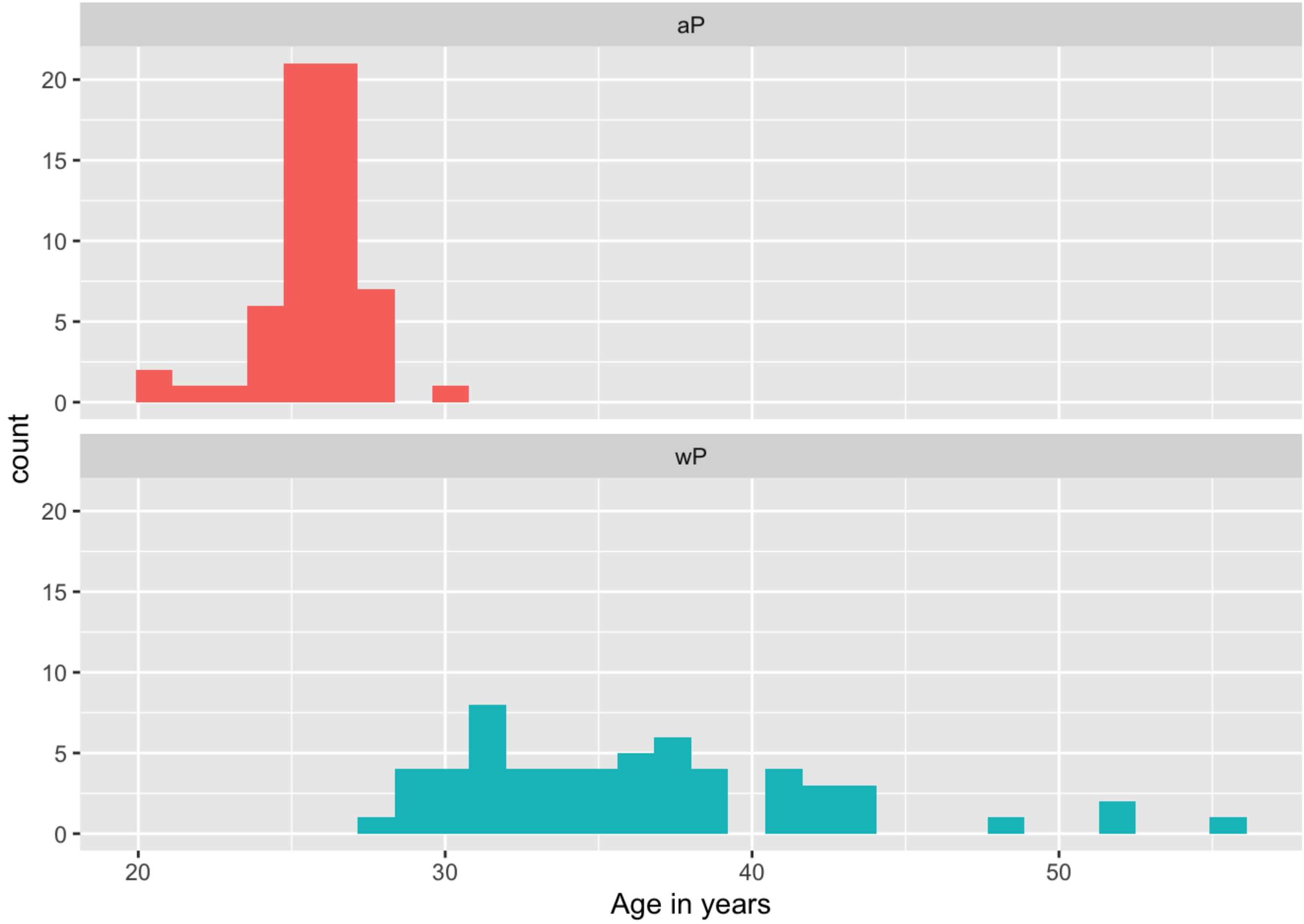
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
[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, these two groups are significantly different

Joining multiple tables

```
# 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/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 13852 days
2 13852 days
3 13852 days
4 13852 days
5 13852 days
6 13852 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.

```r
abdata <- inner_join(titer, meta)
```

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

```r
by=join_by(specimen_id)
dim(abdata)
```

```
[1] 41810    21
```

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

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

```

 IgE  IgG IgG1 IgG2 IgG3 IgG4 
6698 3240 7968 7968 7968 7968 
```

> 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         2205 
```

The number of rows for the most “recent” data set are less than the later data sets.

Examine IgG Ab titer levels

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

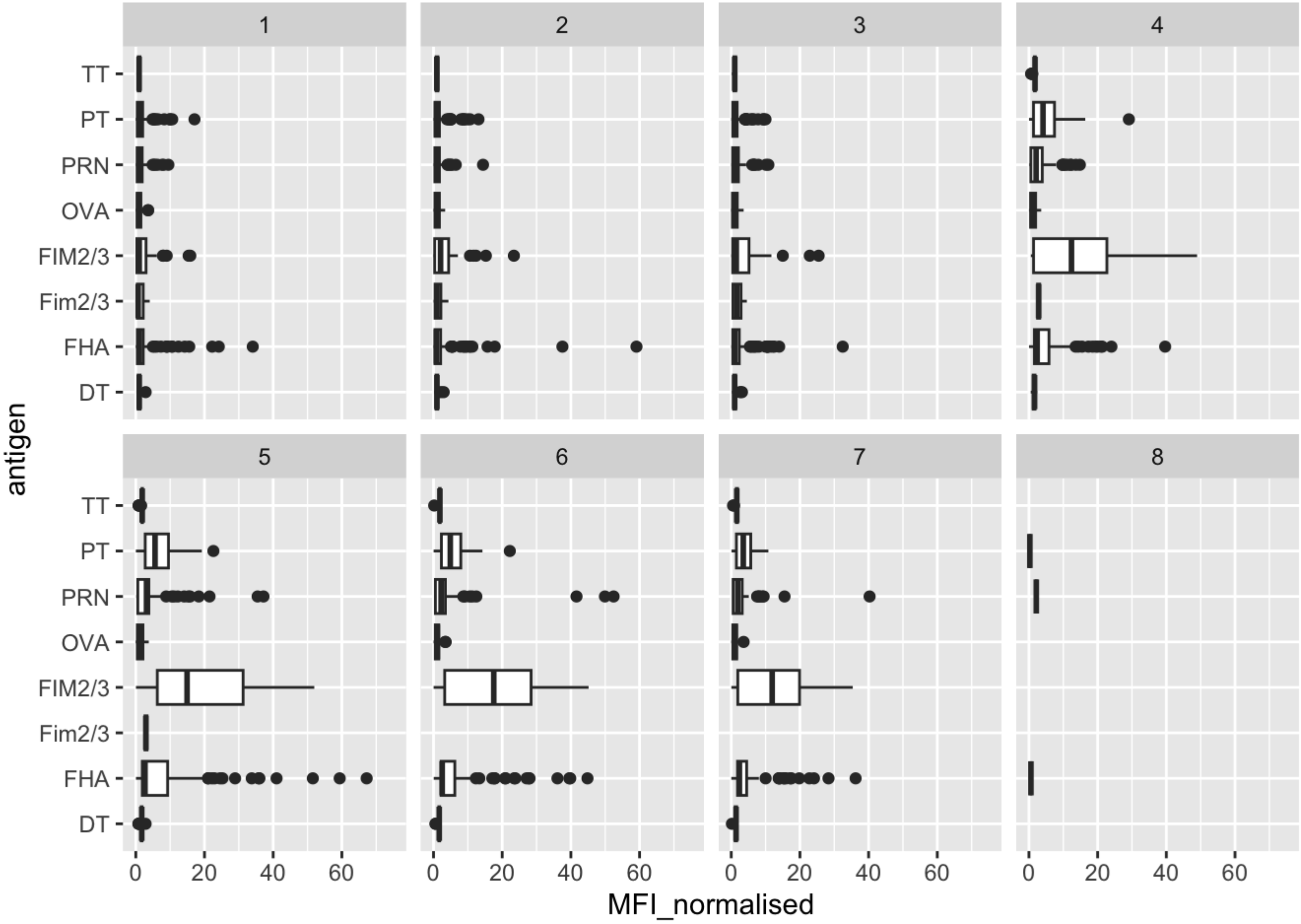
```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgG                TRUE      PT   68.56614       3.736992
2           1     IgG                TRUE     PRN  332.12718       2.602350
3           1     IgG                TRUE     FHA 1887.12263      34.050956
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 13852 days
2 13852 days
```

```
3 13852 days
4 14948 days
5 14948 days
6 14948 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 values (`stat_boxplot()`).
```

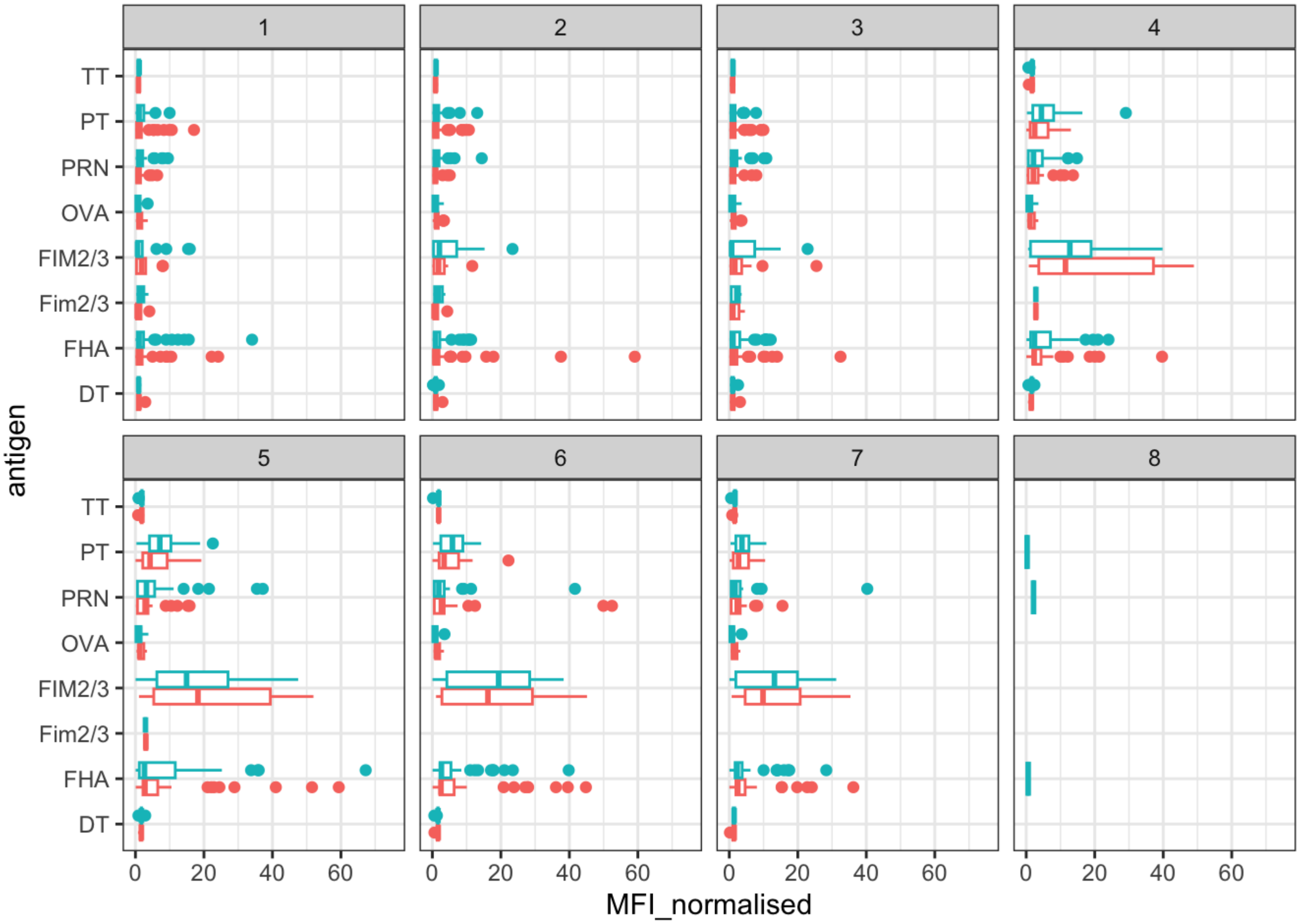


> Q14. What antigens show differences in the level of IgG antibody titers recognizing them over time? Why these and not others?

FIM2/3, PT, and FHA; website was not working, however my best guess would be that it has to do with available tests for those specific antibodies and the antibody properties such as specificity, antigenicity, affinity, and antibody isotopes.

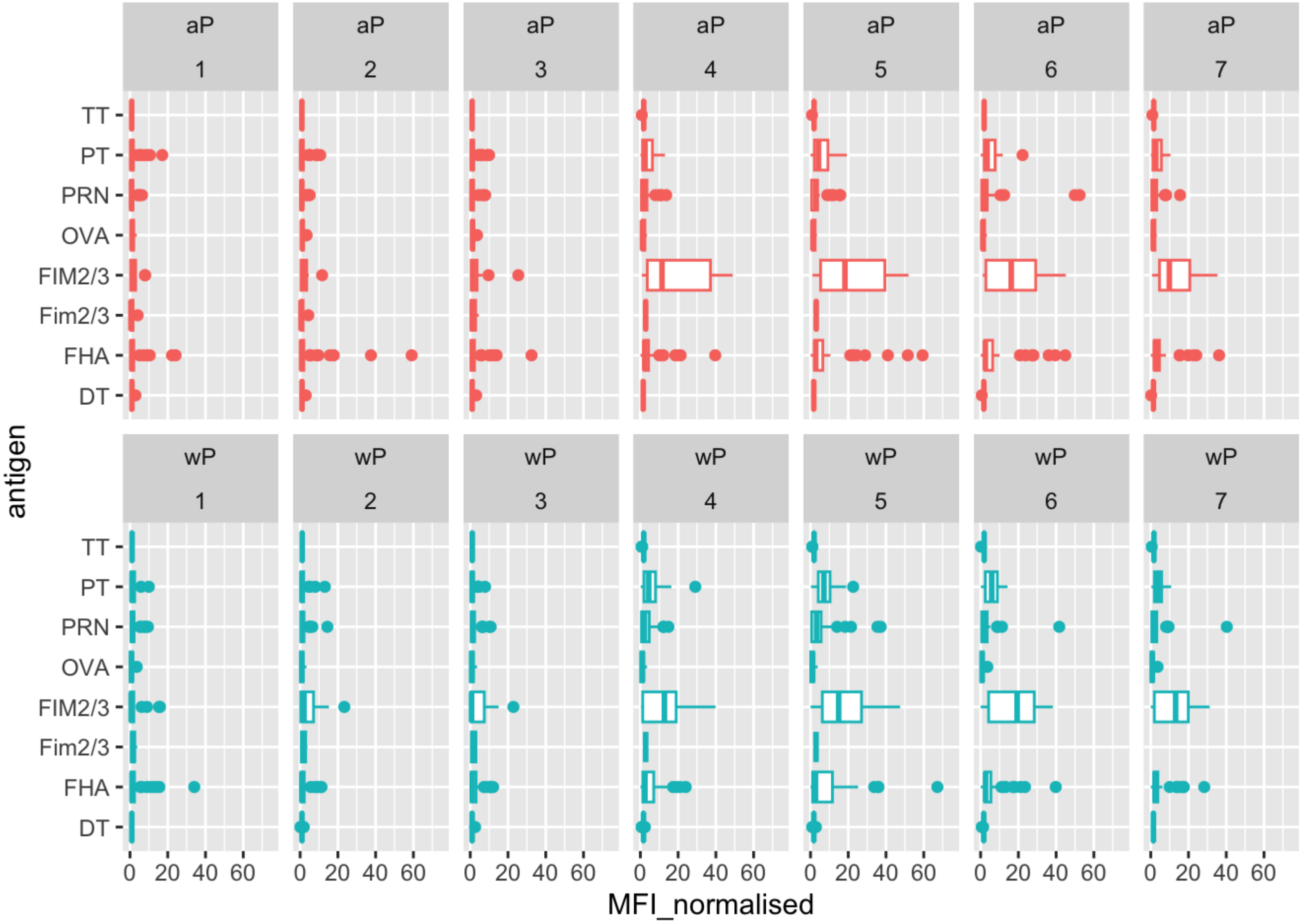
```
ggplot(igg) +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  xlim(0,75) +
  theme_bw()
```

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



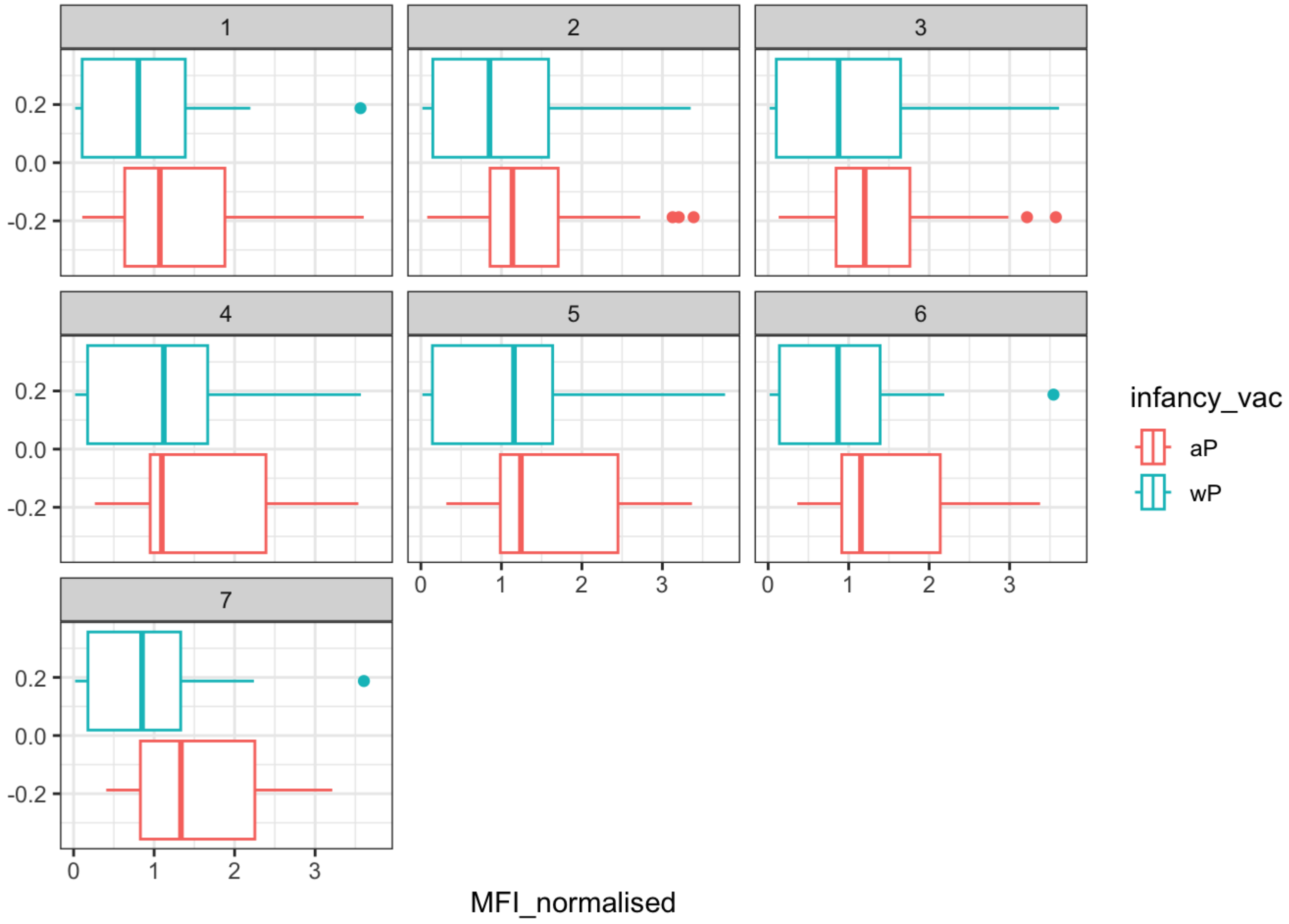
```
igg %>% filter(visit != 8) %>%
ggplot() +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  xlim(0,75) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

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

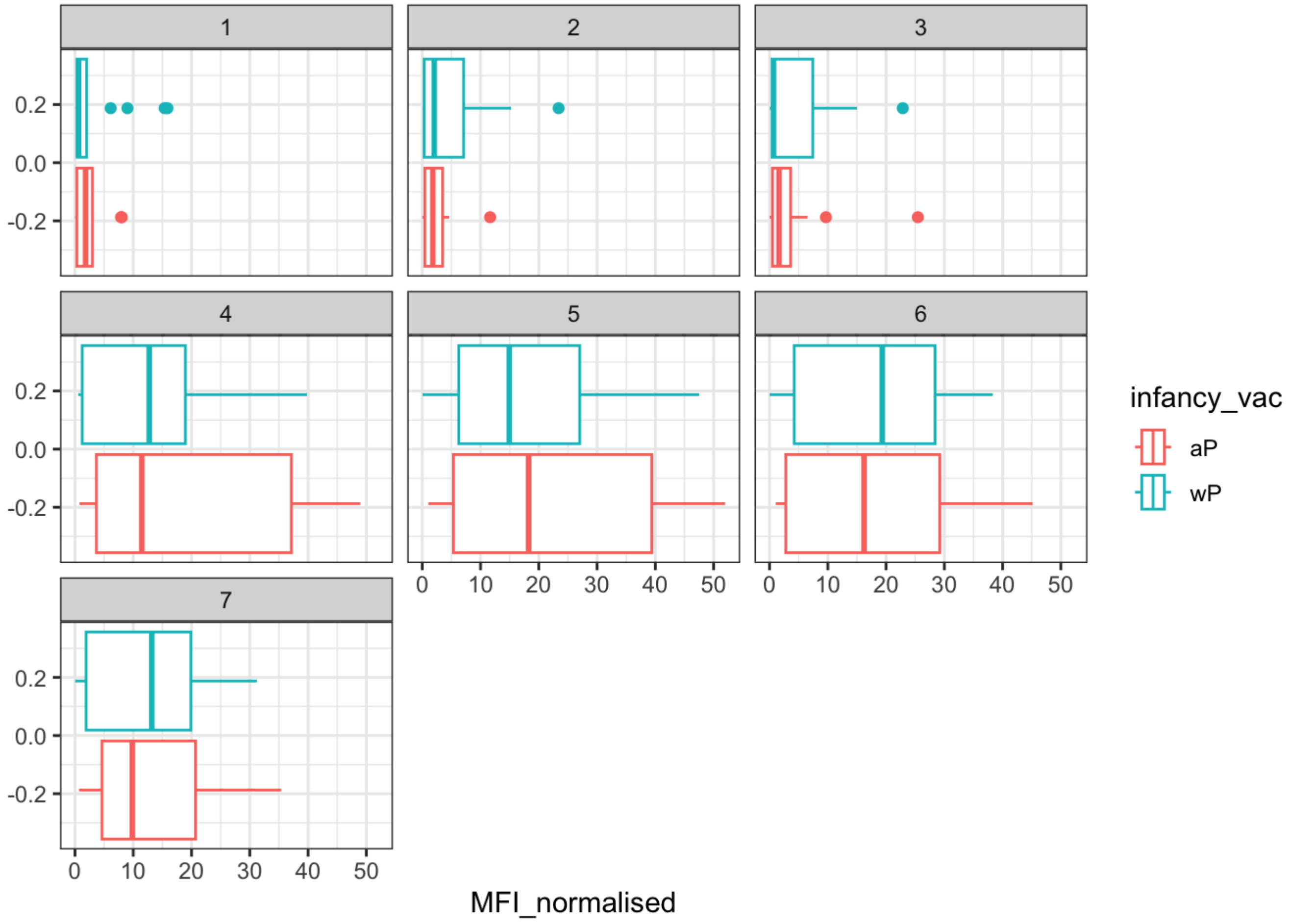


> Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("OVA", that is not in our vaccines) and a clear antigen of interest ("PT", Pertussis Toxin, one of the key virulence factors produced by the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

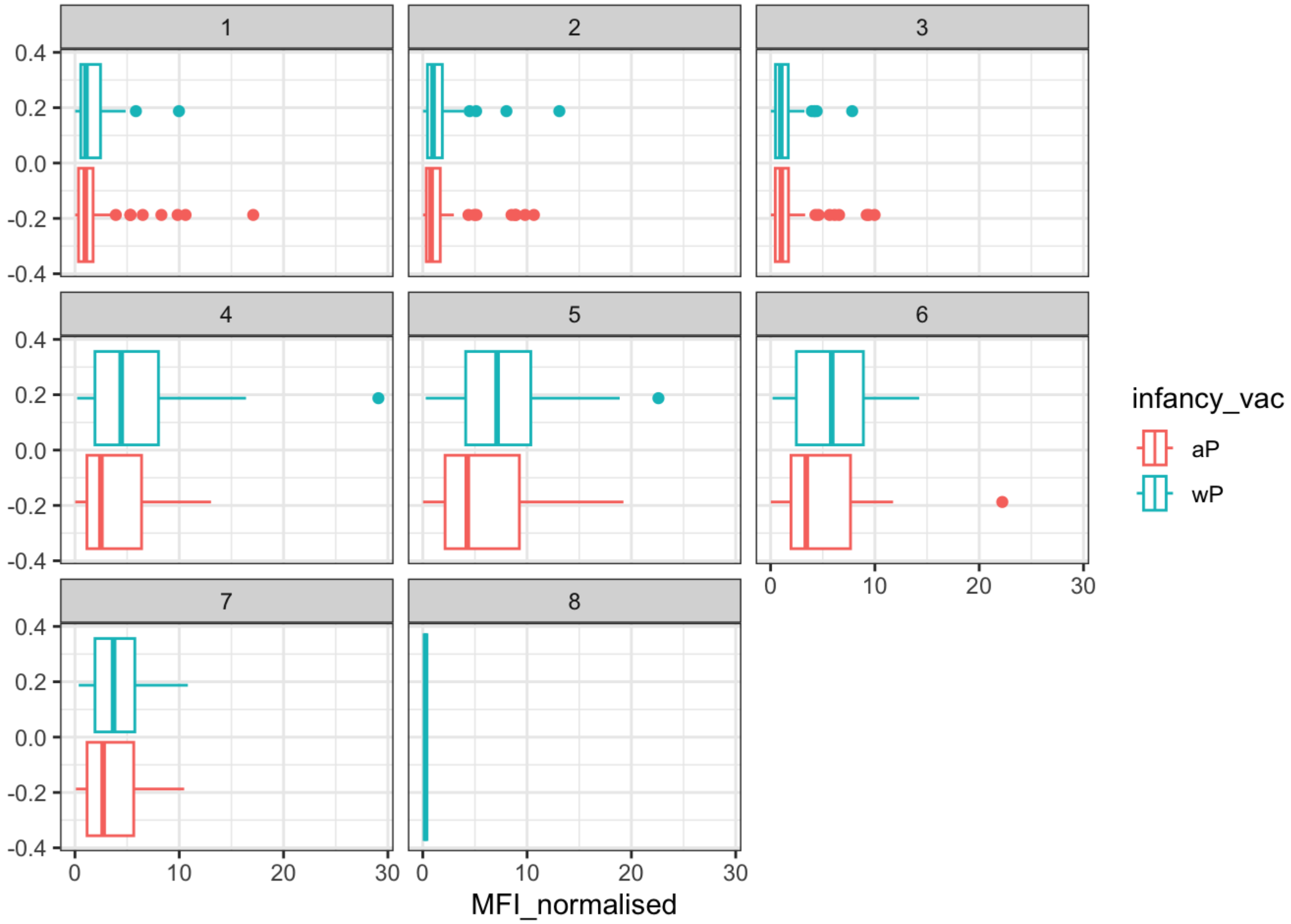
```
filter(igg, antigen=="OVA") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = TRUE) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



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



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



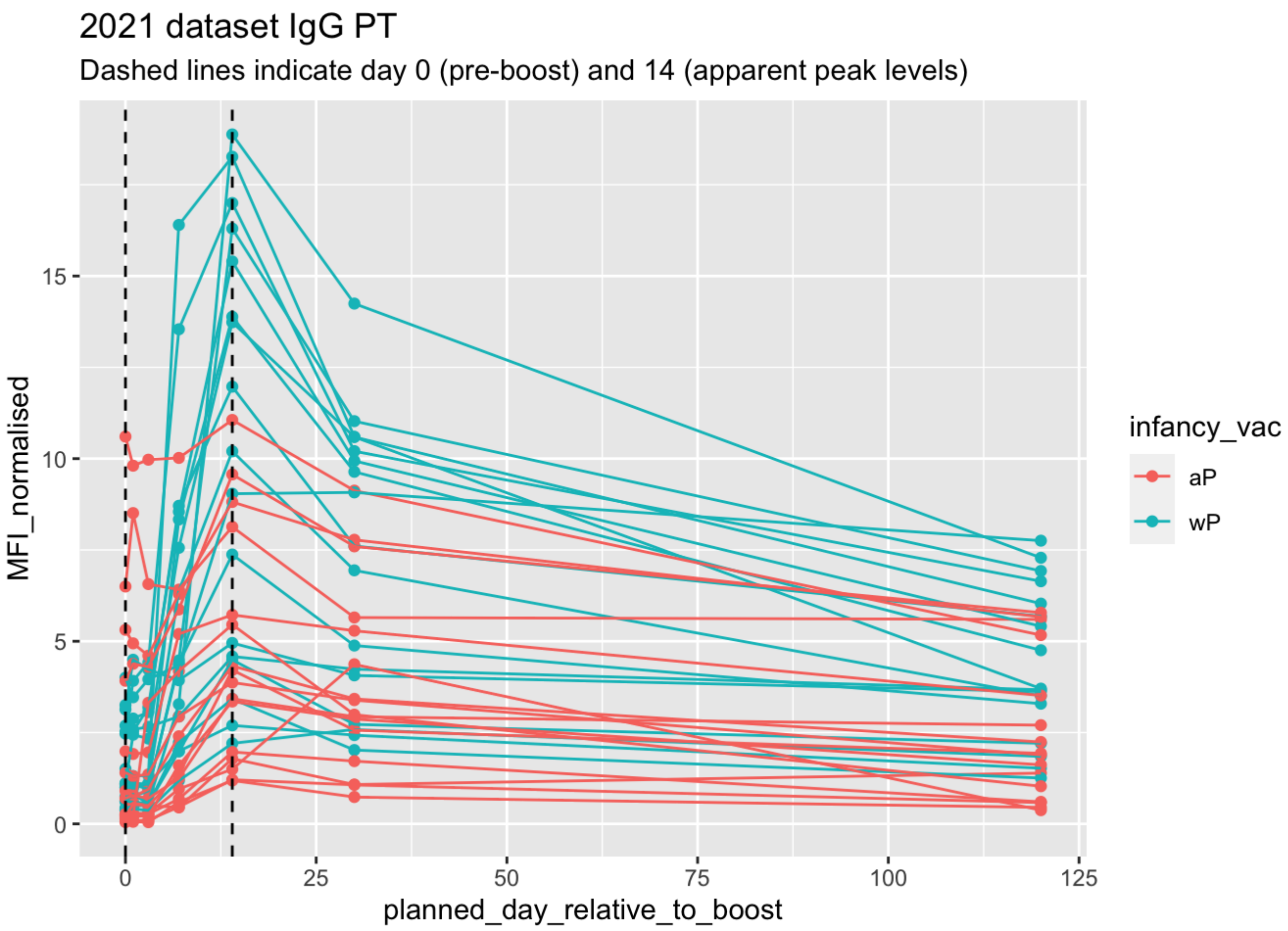
> Q16. What do you notice about these two antigens time courses and the PT data in particular?

PT antigen levels rise over time, significantly more than “OVA” and “FIM2/3”,and peak around visit 5/6, then decrease. This happens to both aP and wP subjects.

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

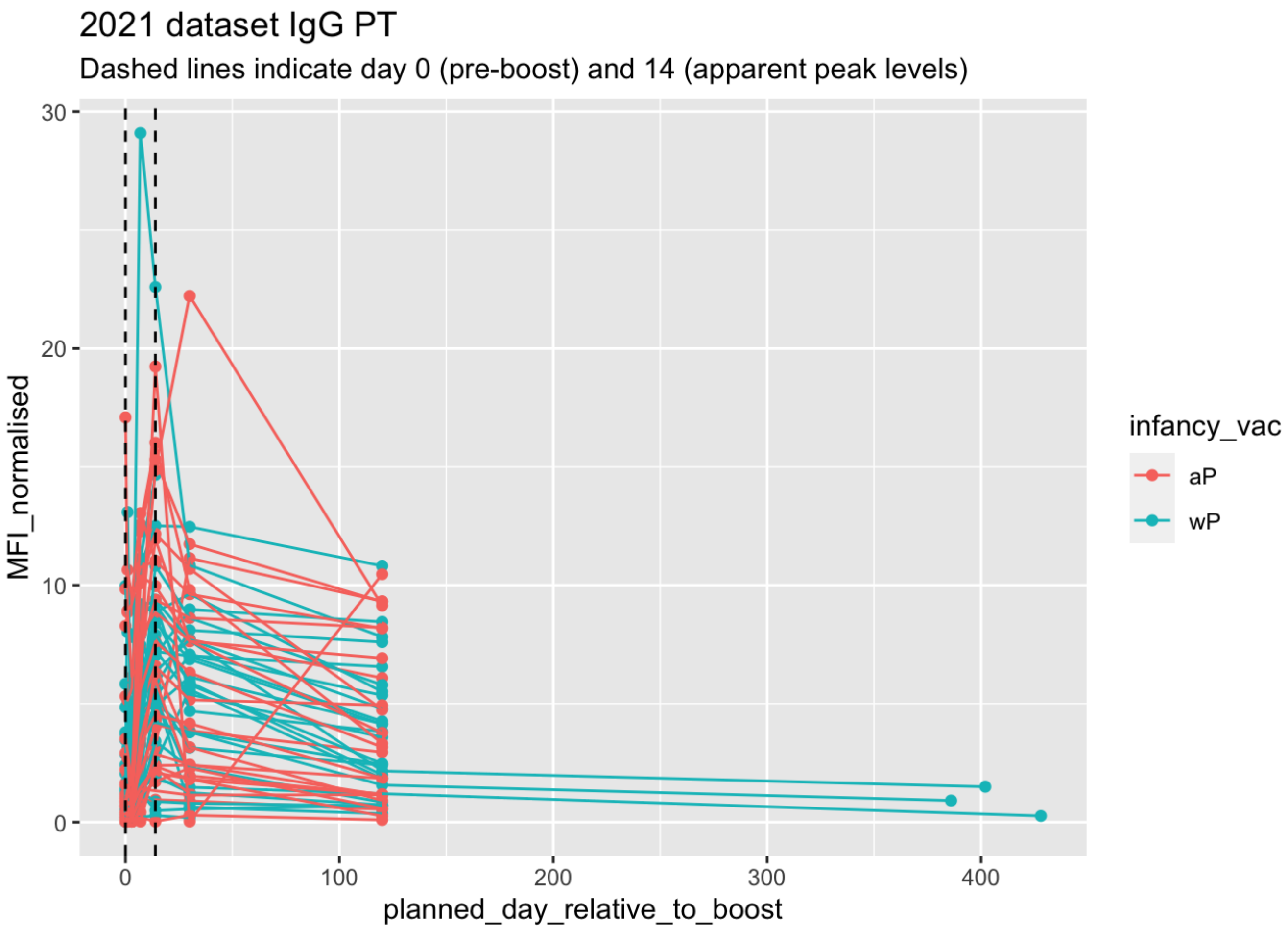
wP response is higher than aP.

```
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?

```
abdata.21 <- abdata %>% filter(dataset == "2020_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)"
```



No, it does not look similar.

Obtaining CMI-PB RNASeq data

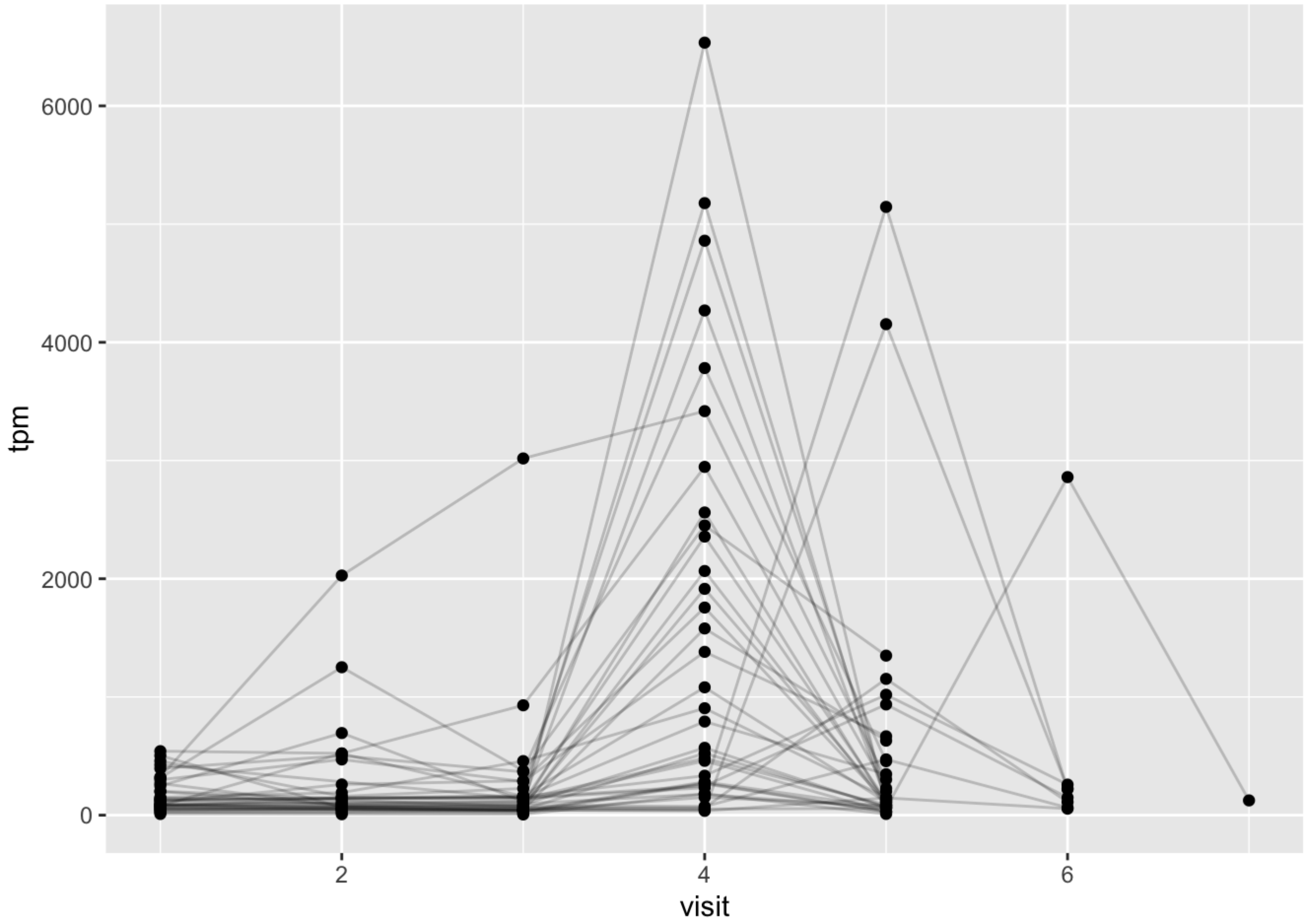
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896

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

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



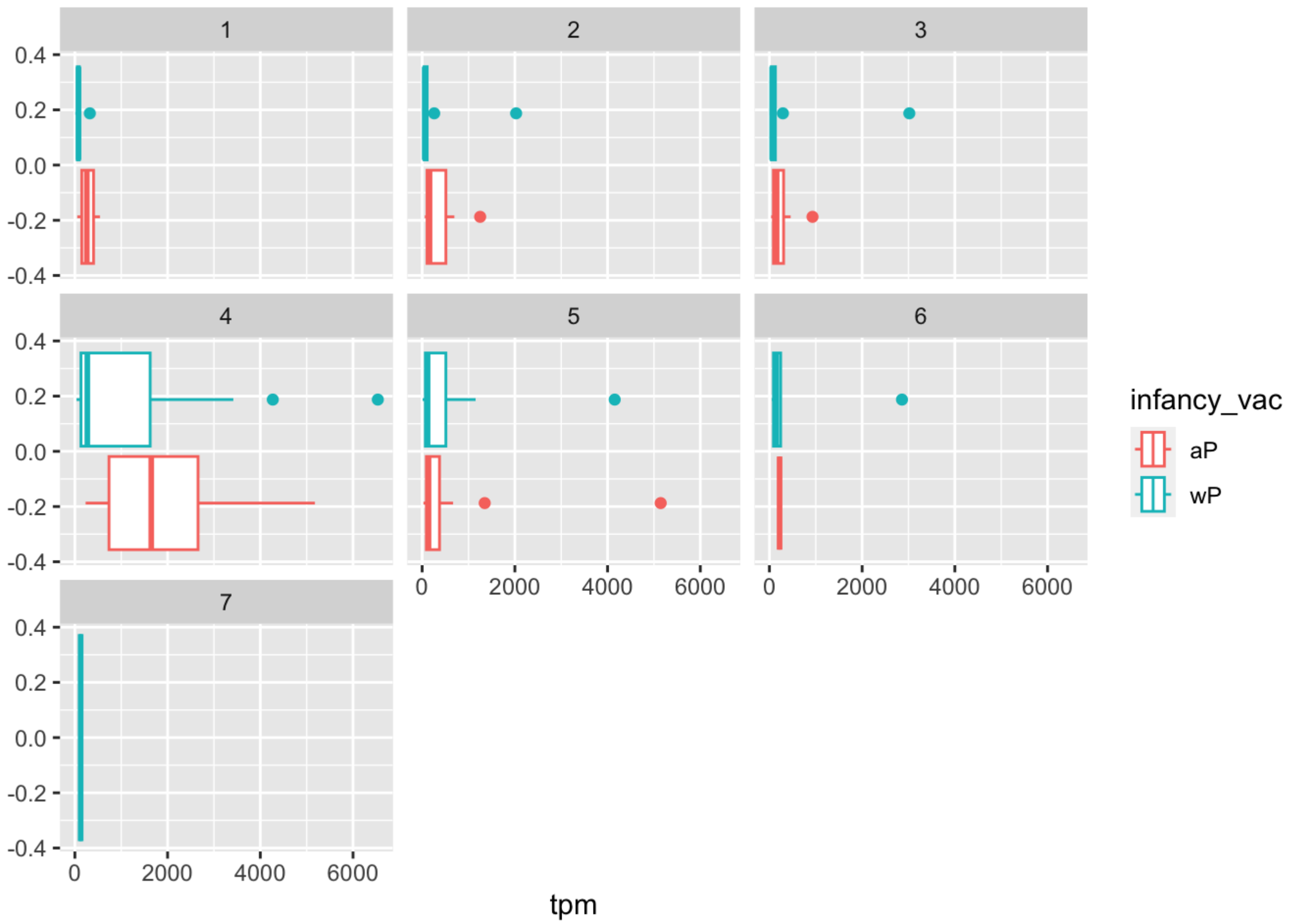
> Q20.: What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

It is at its maximum level at visit 4.

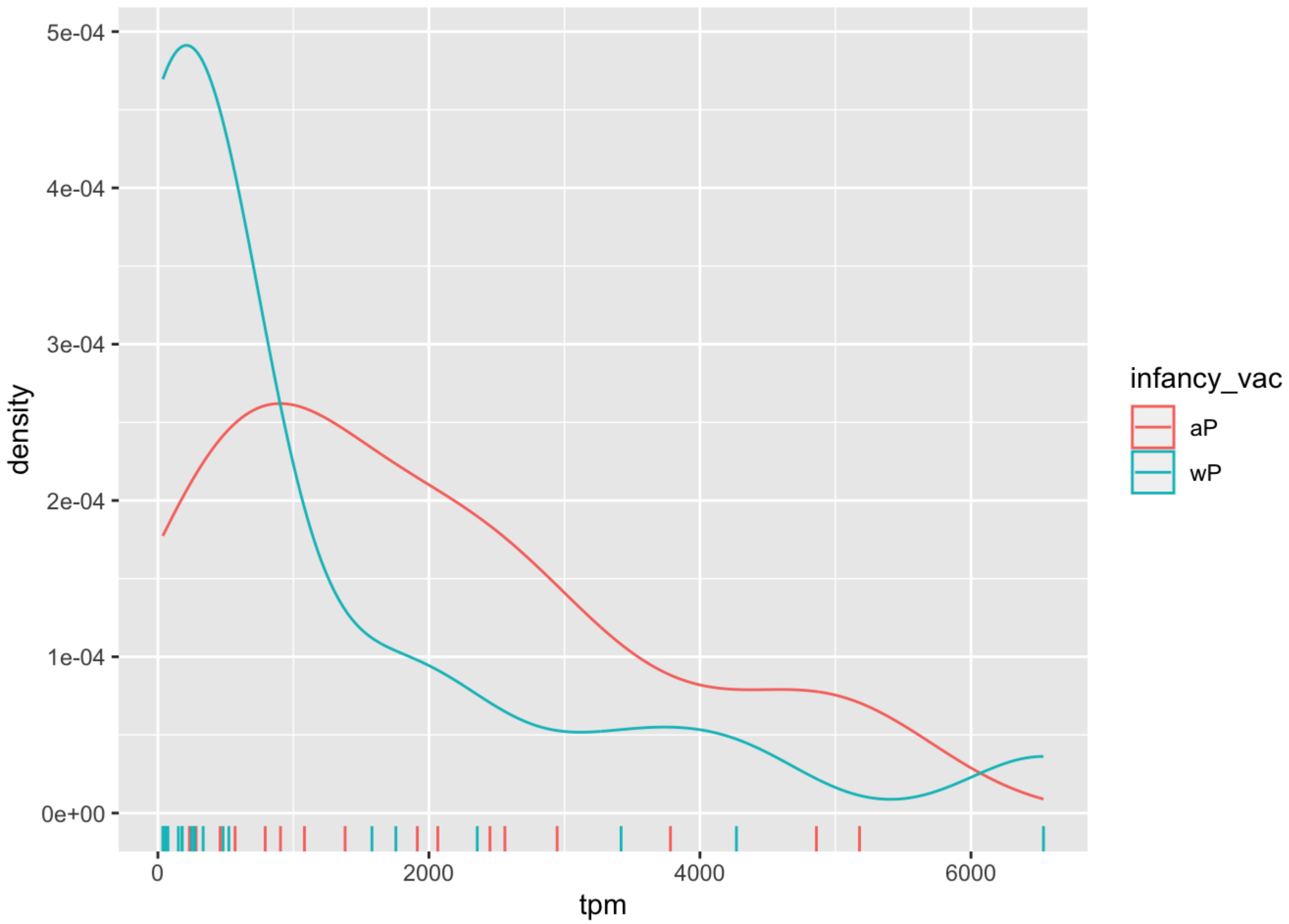
> Q21. Does this pattern in time match the trend of antibody titer data? If not, why not?

No. The trend of antibody titer data reached a peak at visit 5, while here is peaks 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()
```


5e-04
4e-04
3e-04
2e-04
1e-04
0e+00
density
0
2000
4000
6000
tpm
infancy_vac
aP
wP