lab15

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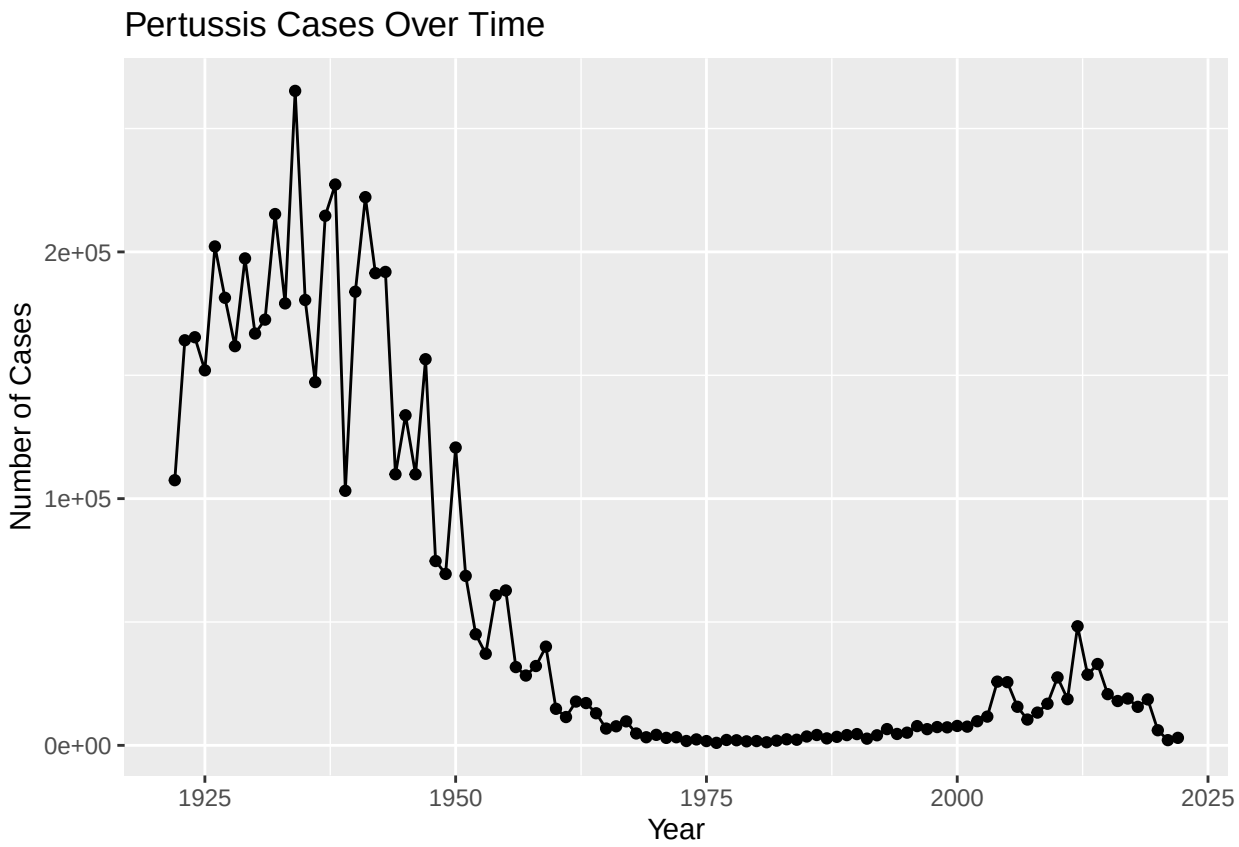
Q1. With the help of the R "addin" package datapasta assign the CDC pertussis case number data to a data framtibble::tribble(e called cdc and use ggplot to make a plot of cases numbers over time.

```
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,
                                     2019,2020,2021,2022),
  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,
                                     2116,3044)
)
```

```
colnames(cdc) <- c("Year", "Cases")
```

```
ggplot(cdc) +
  aes(x = Year, y = Cases) +
  geom_point(color = "black") +
  geom_line(color = "black") +
  labs(
    title = "Pertussis Cases Over Time",
    x = "Year",
    y = "Number of Cases"
  )
```



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?

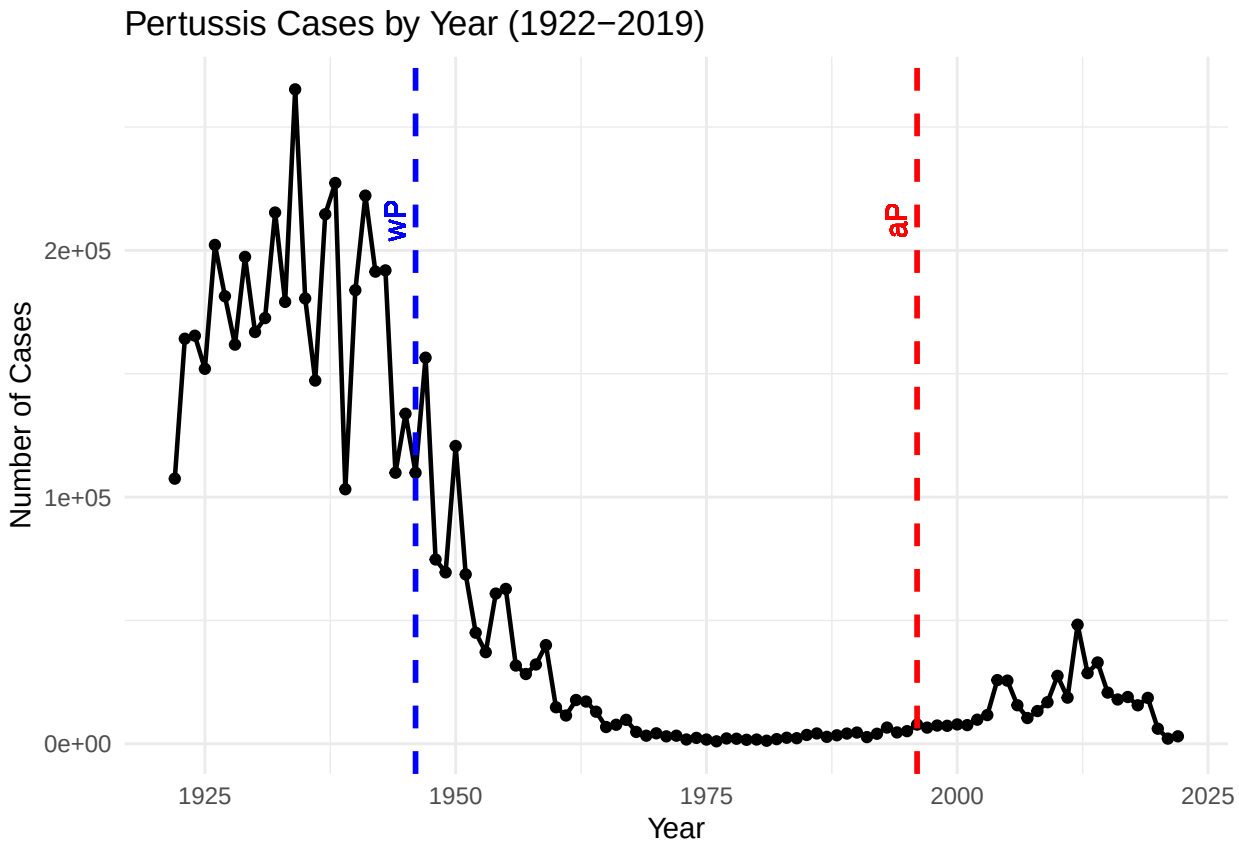
```
ggplot(cdc) +
  aes(x = Year, y = Cases) +
  geom_point(color = "black", size = 1.5) +
  geom_line(color = "black", size = 0.8) +
  geom_vline(xintercept = 1946, linetype = "dashed", color = "blue", size = 1) +
  geom_text(aes(x = 1946, y = max(Cases) * 0.8, label = "wP"),
            color = "blue", angle = 90, vjust = -0.5) +
  geom_vline(xintercept = 1996, linetype = "dashed", color = "red", size = 1) +
  geom_text(aes(x = 1996, y = max(Cases) * 0.8, label = "aP"),
            color = "red", angle = 90, vjust = -0.5) +
  labs(
    title = "Pertussis Cases by Year (1922-2019)",
```

```
    x = "Year",
    y = "Number of Cases"
  ) +
  theme_minimal()
```

```
## Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use `linewidth` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```
## Warning in geom_text(aes(x = 1946, y = max(Cases) * 0.8, label = "wP"), : All aesthetics have length
## 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) * 0.8, label = "aP"), : All aesthetics have length
## i Please consider using `annotate()` or provide this layer with data containing
##   a single row.
```



The introduction of wP vaccine in 1946 shows a significant decline in pertussis cases on the graph.

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

The introduction of aP vaccines in 1996 shoulds a slight increase in cases of pertussis.

```
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
## 87 85
```

There are 87 aP infancy vaccinated subjects and 85 wP infancy vaccinated subjects in the dataset.

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

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

```
##
## Female   Male
##    112     60
```

There are 112 females and 60 males in the dataset.

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
```

```
library(lubridate)
```

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

```
## The following objects are masked from 'package:base':
##
##     date, intersect, setdiff, union
```

```
today()
```

```
## [1] "2024-11-25"
```

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

```
## Time difference of 9095 days
```

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

```
## [1] 24.90075
```

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)
```

```
subject$age_years <- time_length(subject$age, "years")
```

```
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")
wp <- subject %>% filter(infancy_vac == "wP")
```

```
round(summary(ap$age_years))
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##      22      26      27      27      28      34
```

```
round(summary(wp$age_years))
```

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

```
t.test(ap$age_years, wp$age_years)
```

```
##
##  Welch Two Sample t-test
##
## data:  ap$age_years and wp$age_years
## t = -12.918, df = 104.03, p-value < 2.2e-16
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -10.094058  -7.407351
## sample estimates:
## mean of x mean of y
##  26.79610  35.54681
```

Since the p-value is less than 0.05, the difference in average ages between the two groups is statistically significant.

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

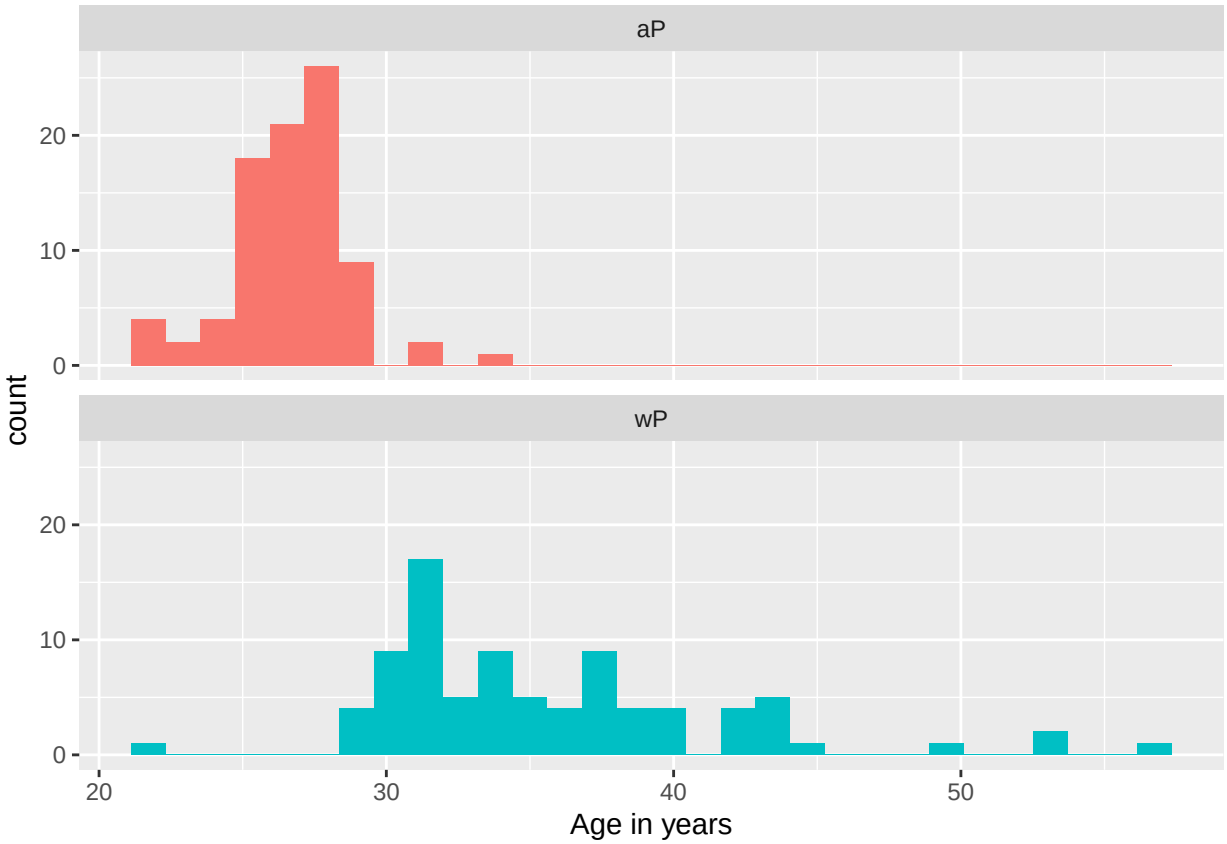
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
## [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`.
```




```
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 <- left_join(specimen, subject)
```

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

```
dim(meta)
```

```
## [1] 1503   15
```

```
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 age_years
## 1 14208 days  38.89938
## 2 14208 days  38.89938
## 3 14208 days  38.89938
## 4 14208 days  38.89938
## 5 14208 days  38.89938
## 6 14208 days  38.89938
```

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, by = "specimen_id")
dim(abdata)
```

```
## [1] 52576    22
```

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  5389 10117 10124 10124 10124
```

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         5670
```

```
most_recent_dataset <- tail(sort(unique(abdata$dataset)), 1)
recent_data_rows <- nrow(abdata[abdata$dataset == most_recent_dataset, ])
cat("Most recent dataset:", most_recent_dataset, "\n")
```

```
## Most recent dataset: 2023_dataset
```

```
cat("Number of rows in the most recent dataset:", recent_data_rows, "\n")
```

```
## Number of rows in the most recent dataset: 5670
```

The most recent dataset might have the smallest number of rows.

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

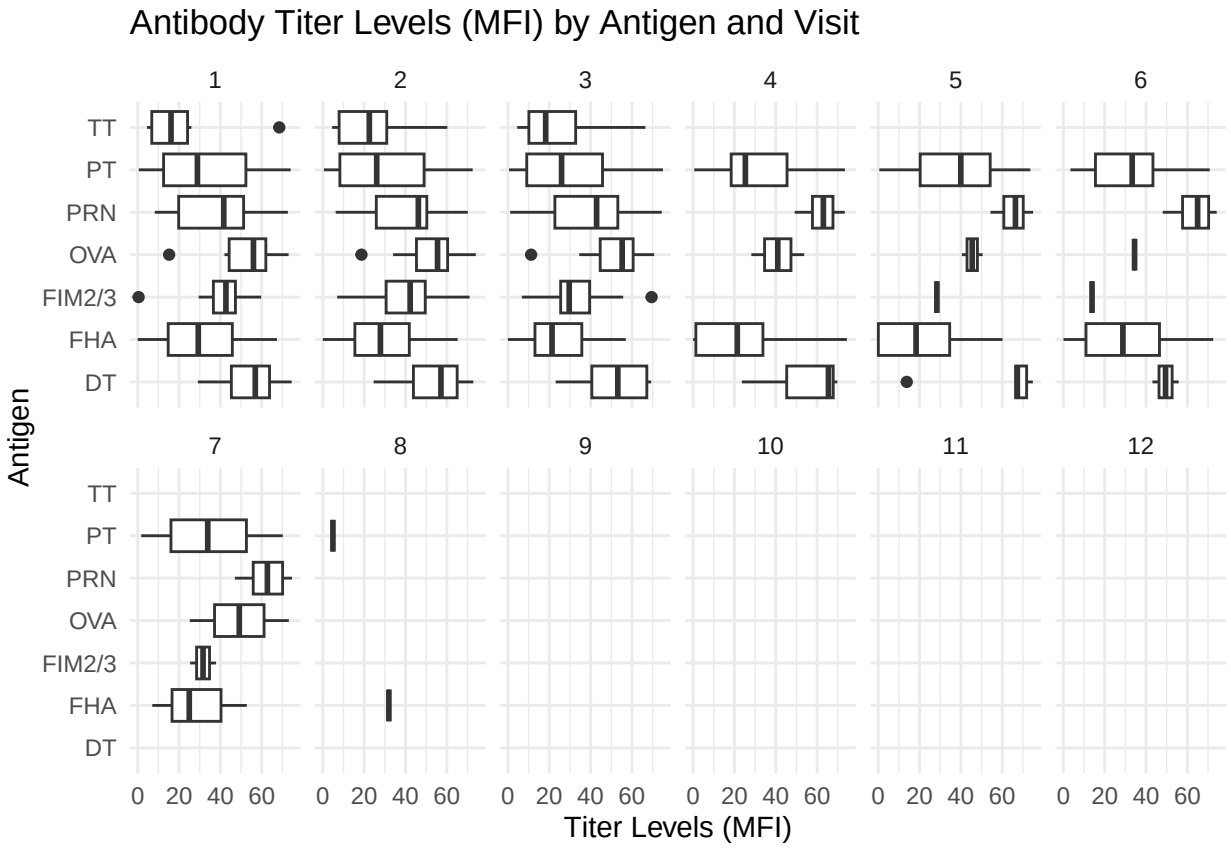
```
##   specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
## 1           1     IgG                TRUE      PT   68.56614       3.736992
## 2           1     IgG                TRUE     PRN  332.12718       2.602350
## 3           1     IgG                TRUE     FHA 1887.12263      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 age_years
## 1 14208 days  38.89938
## 2 14208 days  38.89938
## 3 14208 days  38.89938
## 4 15304 days  41.90007
## 5 15304 days  41.90007
## 6 15304 days  41.90007
```

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

```
ggplot(igg) +
  aes(x = MFI, y = antigen) +
  geom_boxplot() +
  xlim(0, 75) +
  facet_wrap(vars(visit), nrow = 2) +
  labs(
    title = "Antibody Titer Levels (MFI) by Antigen and Visit",
    x = "Titer Levels (MFI)",
```

```
    y = "Antigen"
  ) +
  theme_minimal()
```

```
## Warning: Removed 4475 rows containing non-finite outside the scale range
## (`stat_boxplot()`).
```

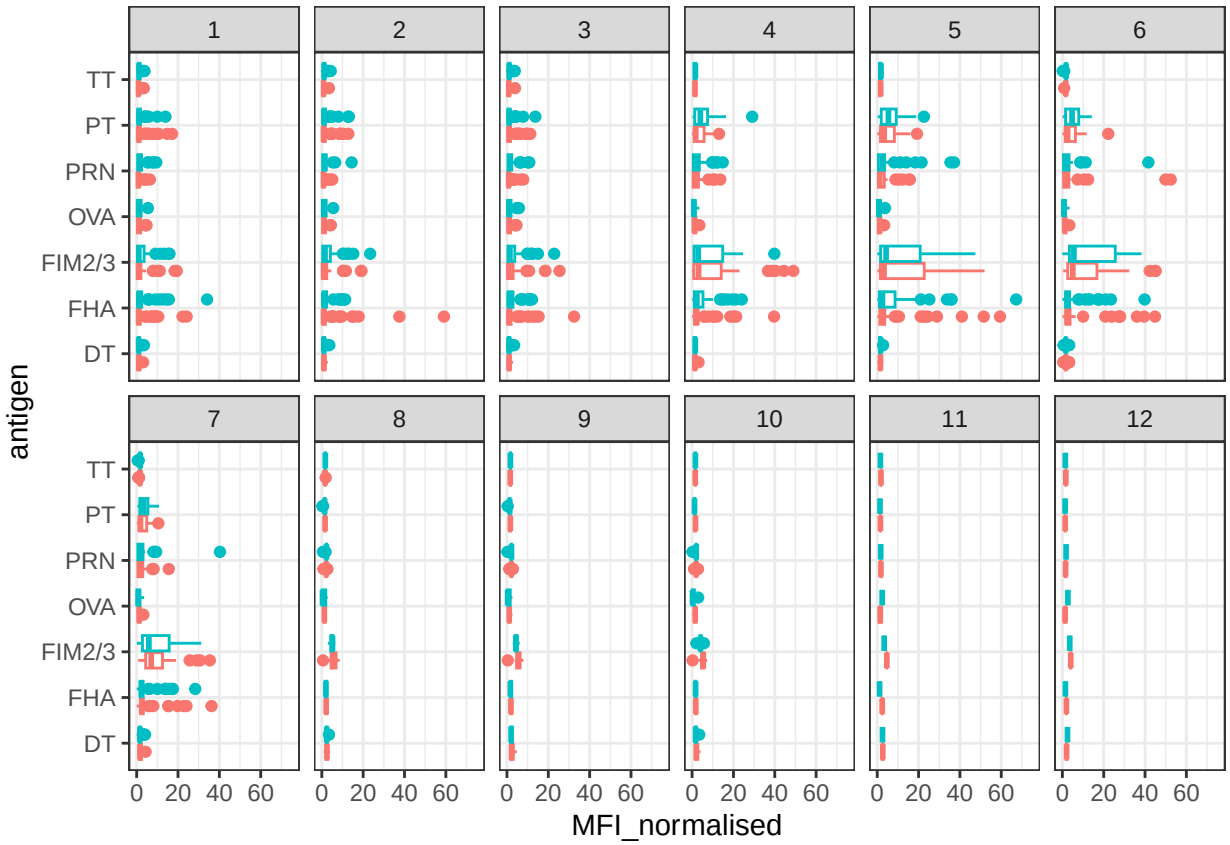


Q14. What antigens show differences in the level of IgG antibody titers recognizing them over time? Why these and not others?

Pertactin (Prn), Fimbriae (Fim2/3), Tetanus Toxoid (TT), and Diphtheria Toxoid (DT). These antigens as they are widely administered vaccines.

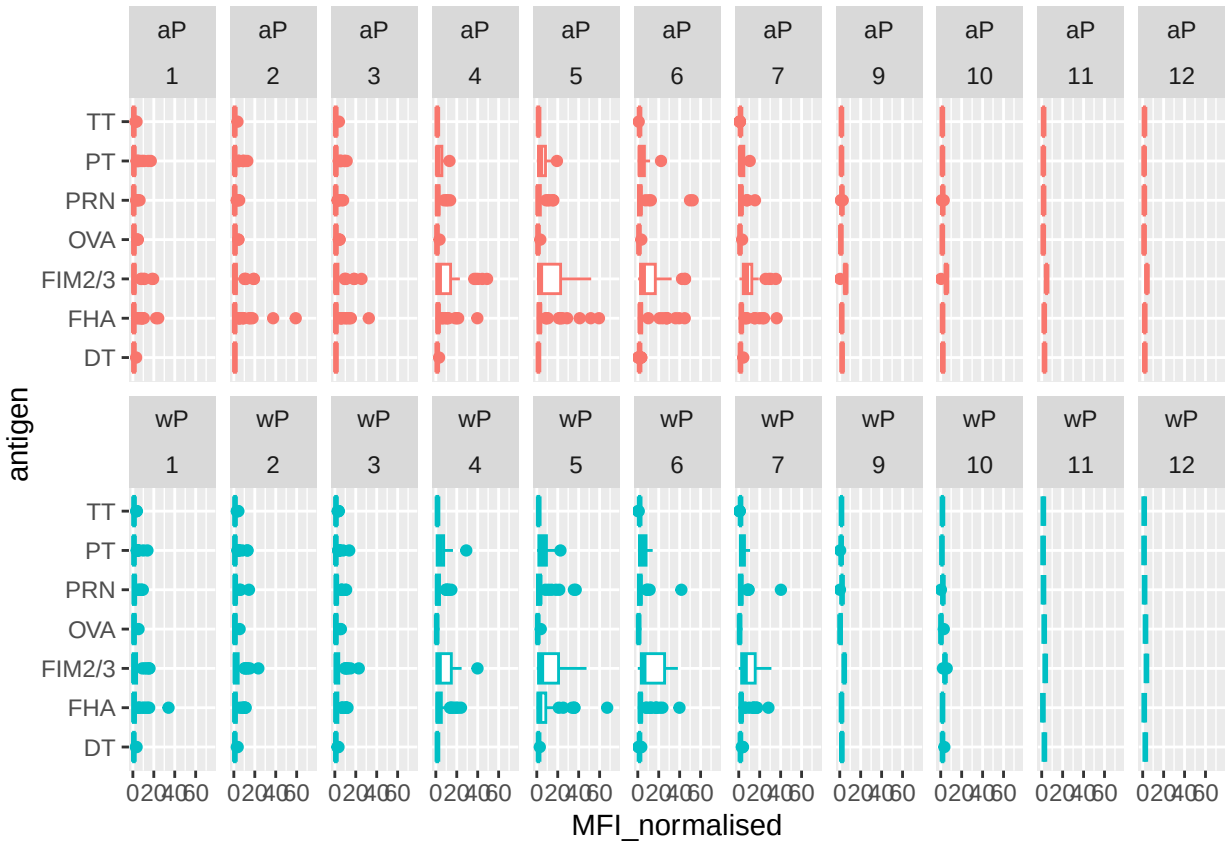
```
ggplot(igg) +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  xlim(0,75) +
  theme_bw()
```

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



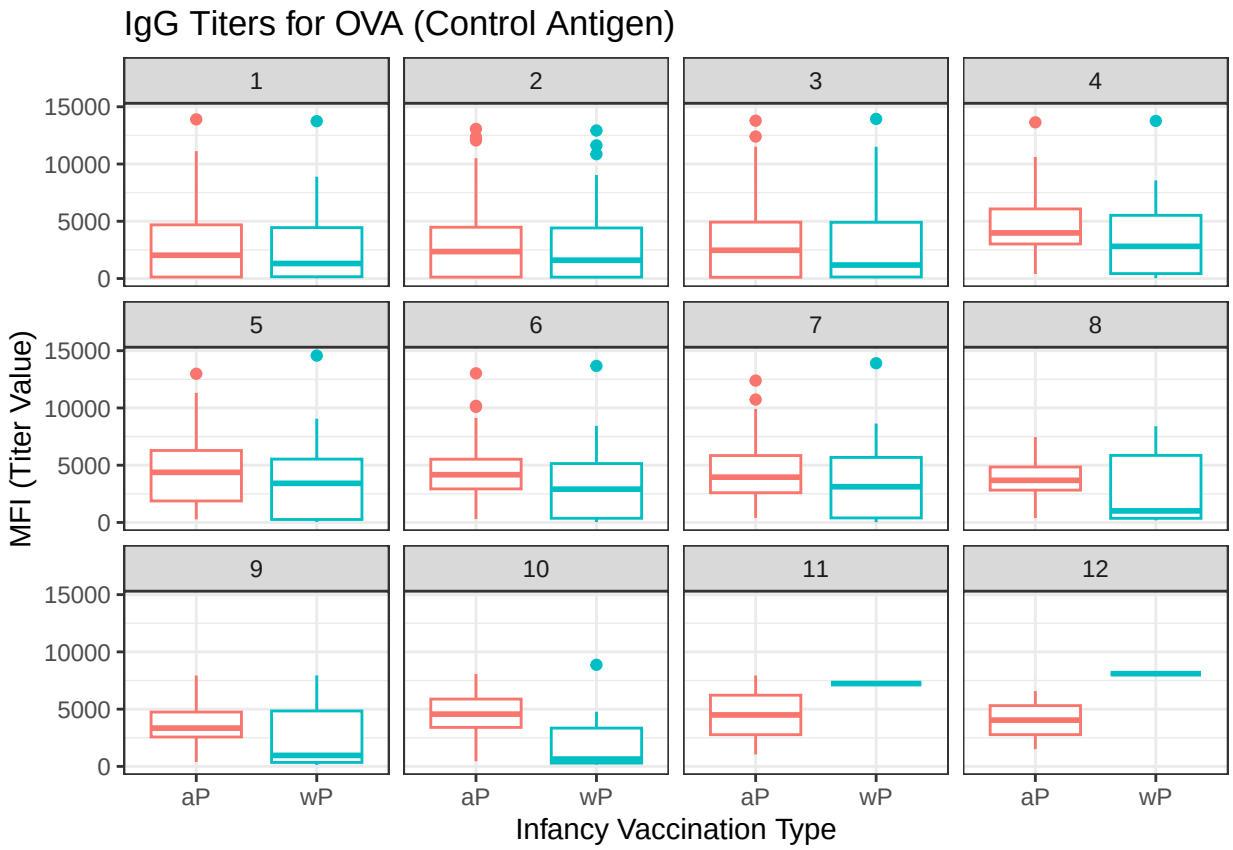

```
igg %>% filter(visit != 8) %>%
ggplot() +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  xlim(0,75) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

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

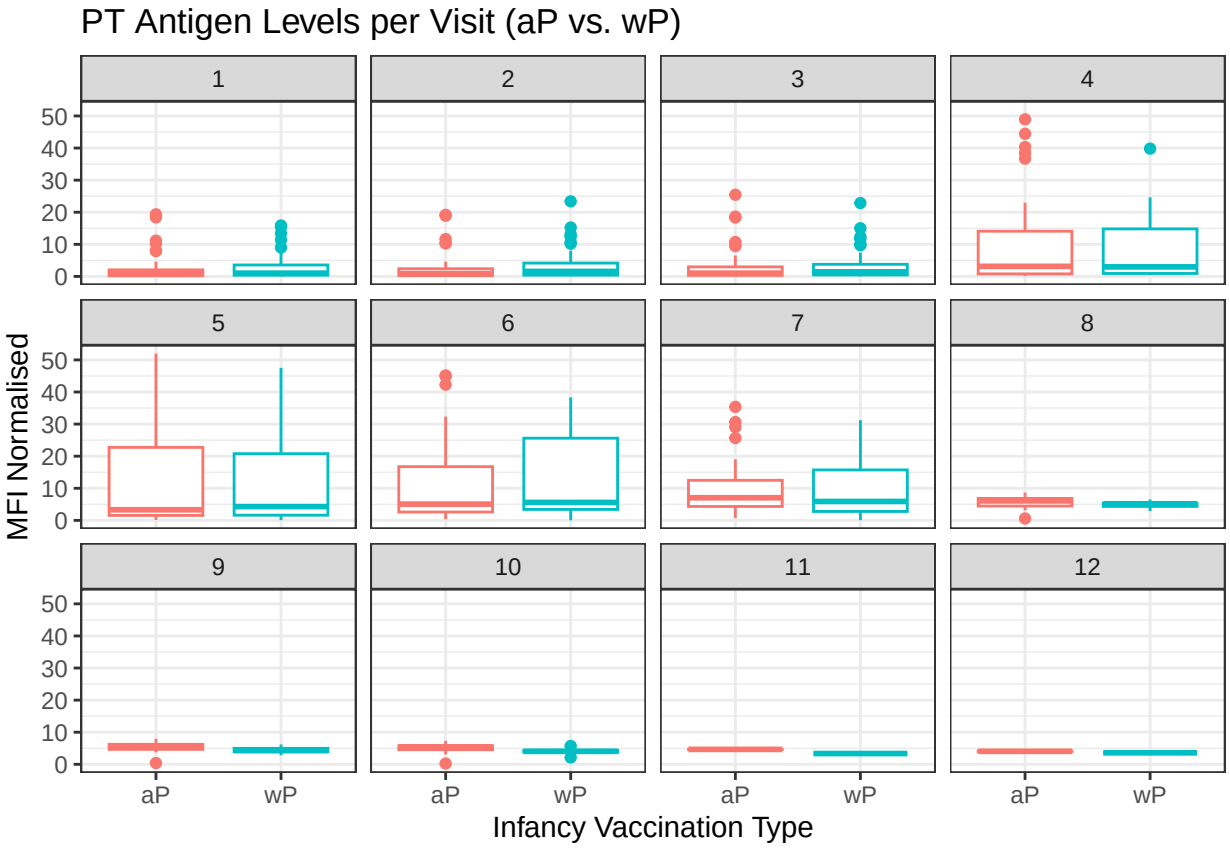


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("OVA", that is not in our vaccines) and a clear antigen of interest ("PT", Pertussis Toxin, one of the key virulence factors produced by the bacterium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

```
filter(igg, antigen == "OVA") %>%
  ggplot() +
  aes(x = infancy_vac, y = MFI, col = infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  labs(
    title = "IgG Titers for OVA (Control Antigen)",
    x = "Infancy Vaccination Type",
    y = "MFI (Titer Value)"
  ) +
  theme_bw()
```




```r
filter(igg, antigen == "FIM2/3") %>%
  ggplot() +
  aes(x = infancy_vac, y = MFI_normalised, col = infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  labs(
    title = "PT Antigen Levels per Visit (aP vs. wP)",
    x = "Infancy Vaccination Type",
    y = "MFI Normalised"
  ) +
  theme_bw()
```



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

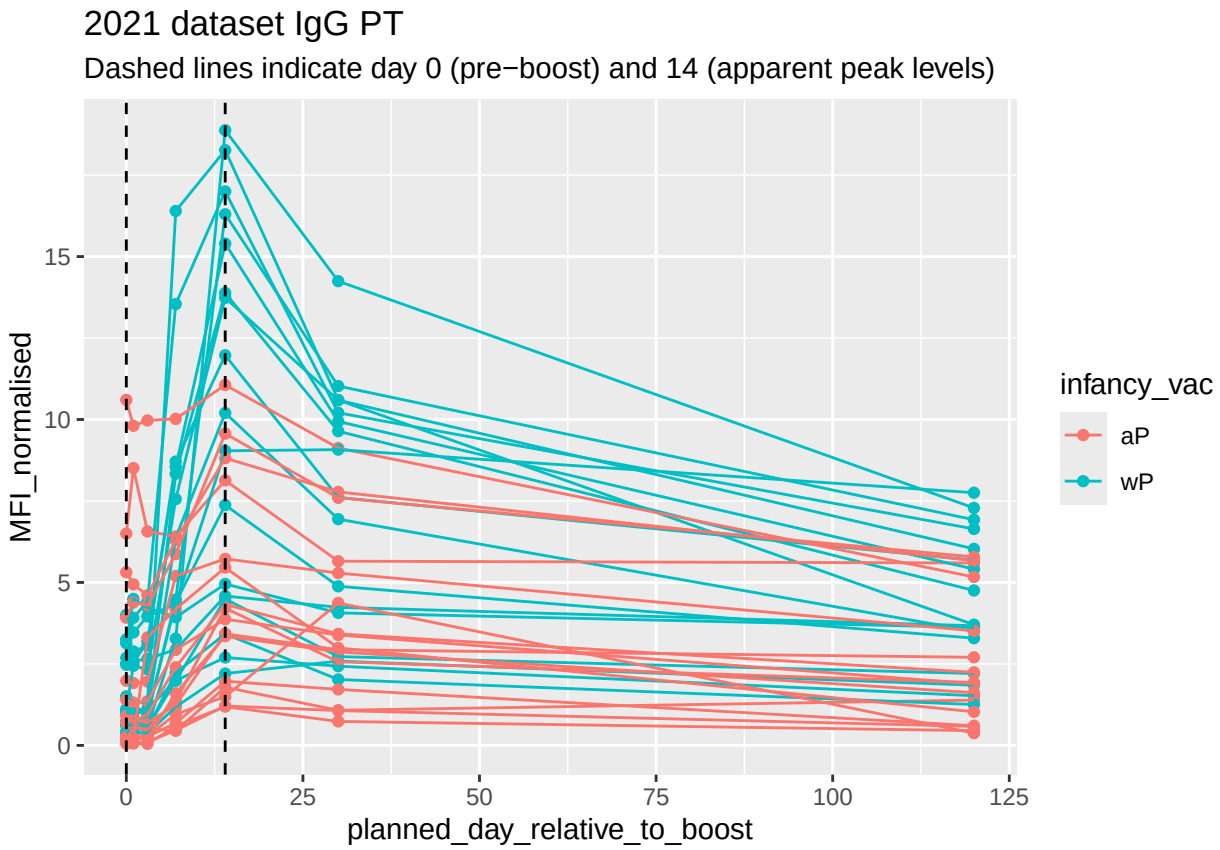
PT levels clearly rise over time and far exceed those of OVA. They also appear to peak at visit 5 and then decline. This trend appears similar for wP and aP subjects

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

For OVA antigen, there is no significant differences in MFI levels between aP and wP. For PT antigen, The aP group consistently shows higher IgG titers compared to the wP group.

```
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?

The 2021 dataset shows a more significant peak for the wP group compared to the aP group.

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

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(x = visit, y = tpm, group = subject_id, color = infancy_vac) +
  geom_point() +
  geom_line(alpha = 0.2) +
  labs(
    title = "Time Course of IGHG1 Gene Expression (TPM)",
    x = "Visit",
    y = "TPM Expression Level",
    color = "Infancy Vaccination"
  ) +
  theme_minimal()
```

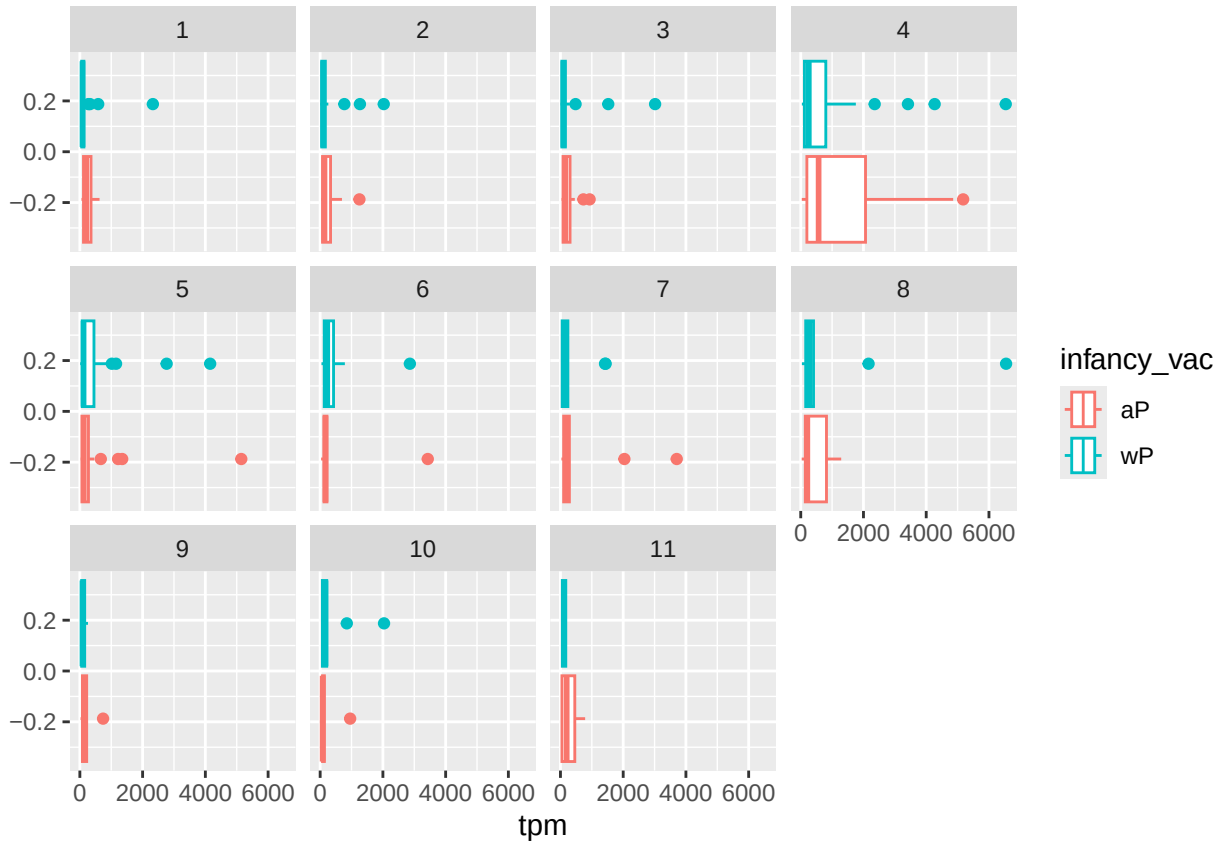
Q20. What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

The IGHG1 gene expression (TPM) peaks sharply at visit 4.

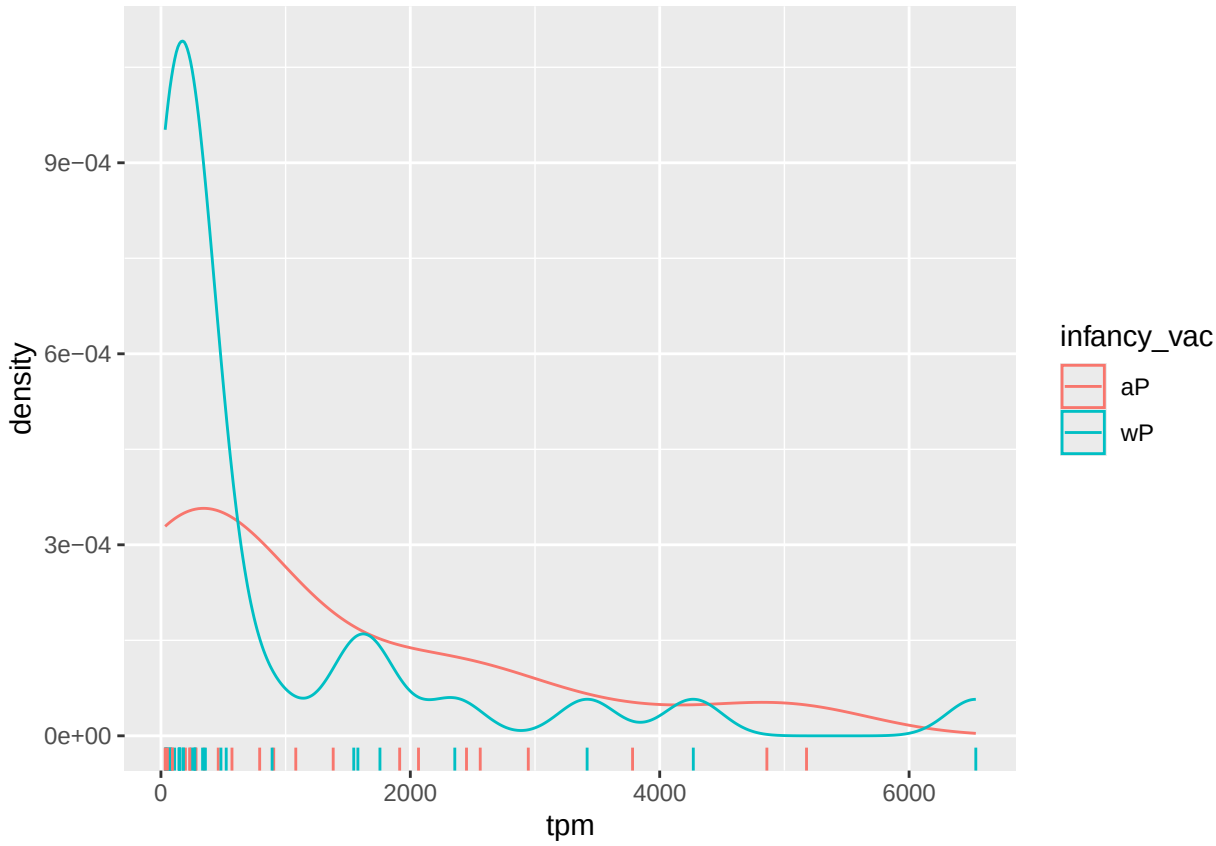
Q21. Does this pattern in time match the trend of antibody titer data? If not, why not?

Yes, this pattern closely matches the antibody titer trends as both gene expression and antibody titers peak after boosting, indicating a coordinated immune response

```
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