Pertussis Project

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Investigating Pertussis Resurgence

#Pertussis (more commonly known as whooping cough) is a highly contagious respiratory disease caused by the bacterium Bordetella pertussis. People of all ages can be infected leading to violent coughing fits followed by a high-pitched intake of breath that sounds like "whoop". Infants and toddlers have the highest risk for severe complications and death. Recent estimates from the WHO suggest that ~16 million cases and 200,000 infant deaths are due to pertussis annually 1.

1. Investigating pertussis cases by year

```
library(datapasta)
```

```
## Warning: package 'datapasta' was built under R version 4.1.3
```

```
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),
         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)
       )
```

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.

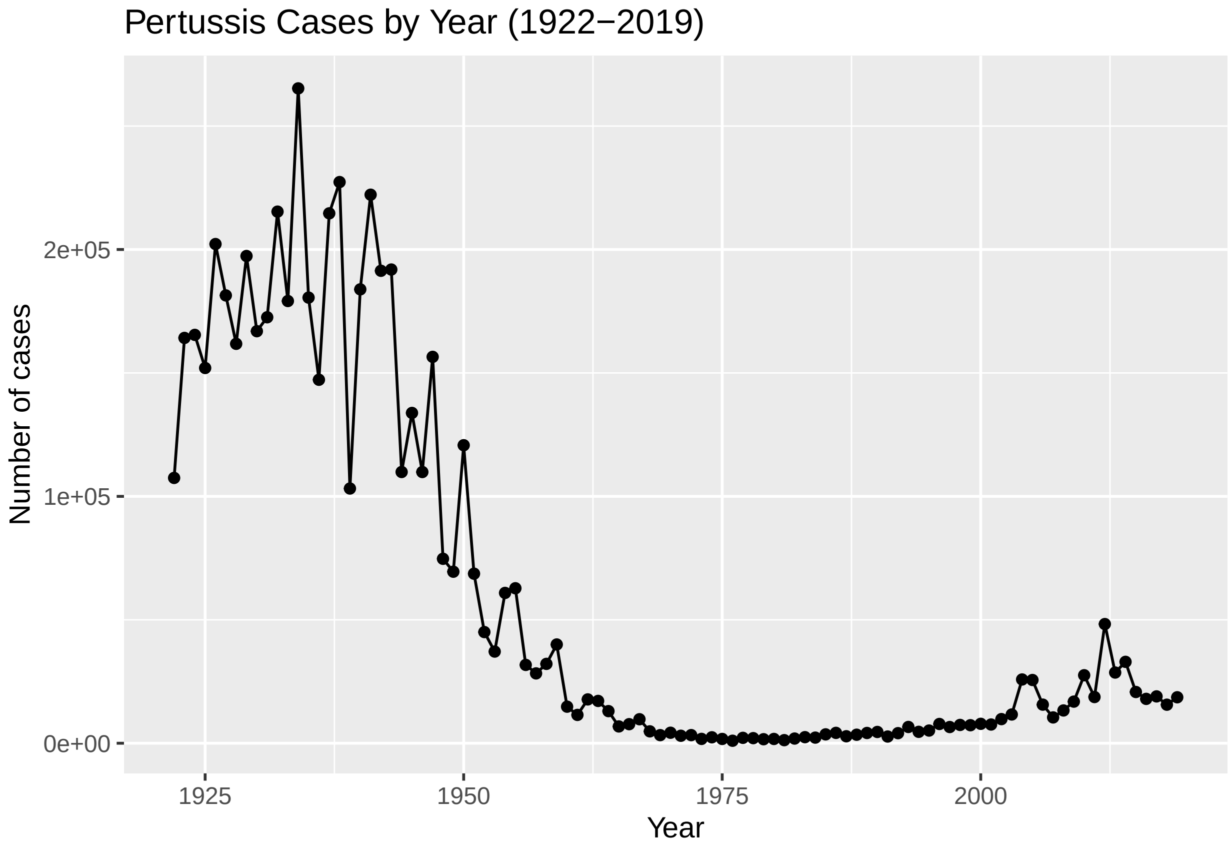
```
library(ggplot2)
ggplot(cdc) +
  aes(x = cdc$Year, y= cdc$No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  labs(x = "Year", y= "Number of cases", title= "Pertussis Cases by Year (1922-2019)" )
```

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

## Warning: Use of ‘cdc$No..Reported.Pertussis.Cases‘ is discouraged. Use
## ‘No..Reported.Pertussis.Cases‘ instead.

## Warning: Use of ‘cdc$Year‘ is discouraged. Use ‘Year‘ instead.

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



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?

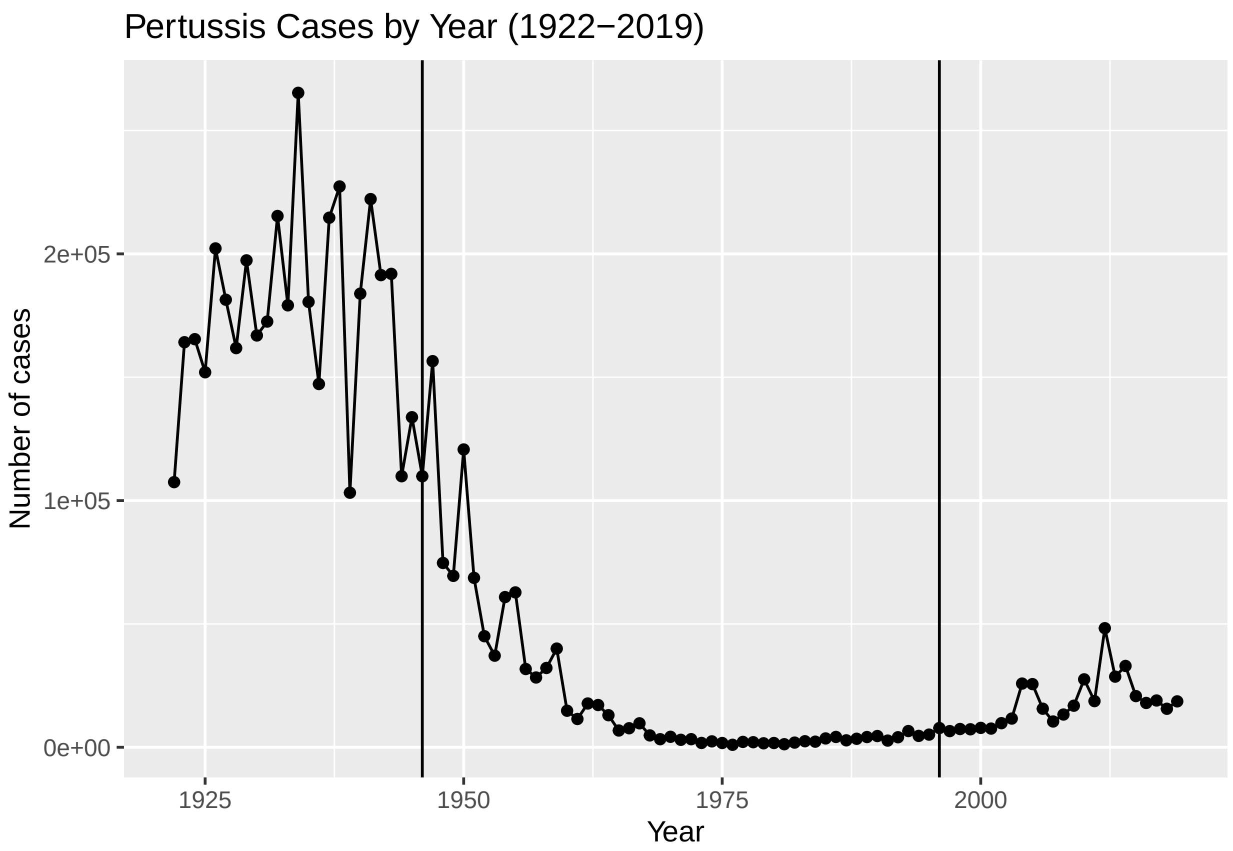
```
library(ggplot2)
ggplot(cdc) +
  aes(x = cdc$Year, y= cdc$No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  labs(x = "Year", y= "Number of cases", title= "Pertussis Cases by Year (1922-2019)" ) +
  geom_vline(xintercept= 1946) +
  geom_vline(xintercept= 1996)
```

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

## Warning: Use of ‘cdc$No..Reported.Pertussis.Cases‘ is discouraged. Use
## ‘No..Reported.Pertussis.Cases‘ instead.

## Warning: Use of ‘cdc$Year‘ is discouraged. Use ‘Year‘ instead.

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



> 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, significant increase in number of cases appeared.

3. Exploring CMI-PB data

```
library(jsonlite)
```

```
## Warning: package 'jsonlite' was built under R version 4.1.3
```

```
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   study_name
## 1    1986-01-01    2016-09-12 2020_dataset
## 2    1968-01-01    2019-01-28 2020_dataset
## 3    1983-01-01    2016-10-10 2020_dataset
```

Q4. How may aP and wP infancy vaccinated subjects are in the dataset?

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

```
##
## aP wP
## 47 49
```

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

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

```
##
## Female   Male
##     66     30
```

Q6. What is the breakdown of race and biological sex (e.g. number of Asian females, White males etc…)?

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

```
##
##                                              Female Male
##   American Indian/Alaska Native                   0    1
##   Asian                                          18    9
##   Black or African American                       2    0
##   More Than One Race                              8    2
##   Native Hawaiian or Other Pacific Islander       1    1
##   Unknown or Not Reported                        10    4
##   White                                          27   13
```

```
library(lubridate)
```

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

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

```
today()
```

```
## [1] "2022-03-18"
```

```
#HOw many days have passed since new year 2000
today() - ymd("2000-01-01")
```

```
## Time difference of 8112 days
```

```
#What is this in years?
time_length( today() - ymd("2000-01-01"), "years")
```

```
## [1] 22.20945
```

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      24      25      25      25      26
```

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

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##      27      31      34      35      39      54
```

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)
```

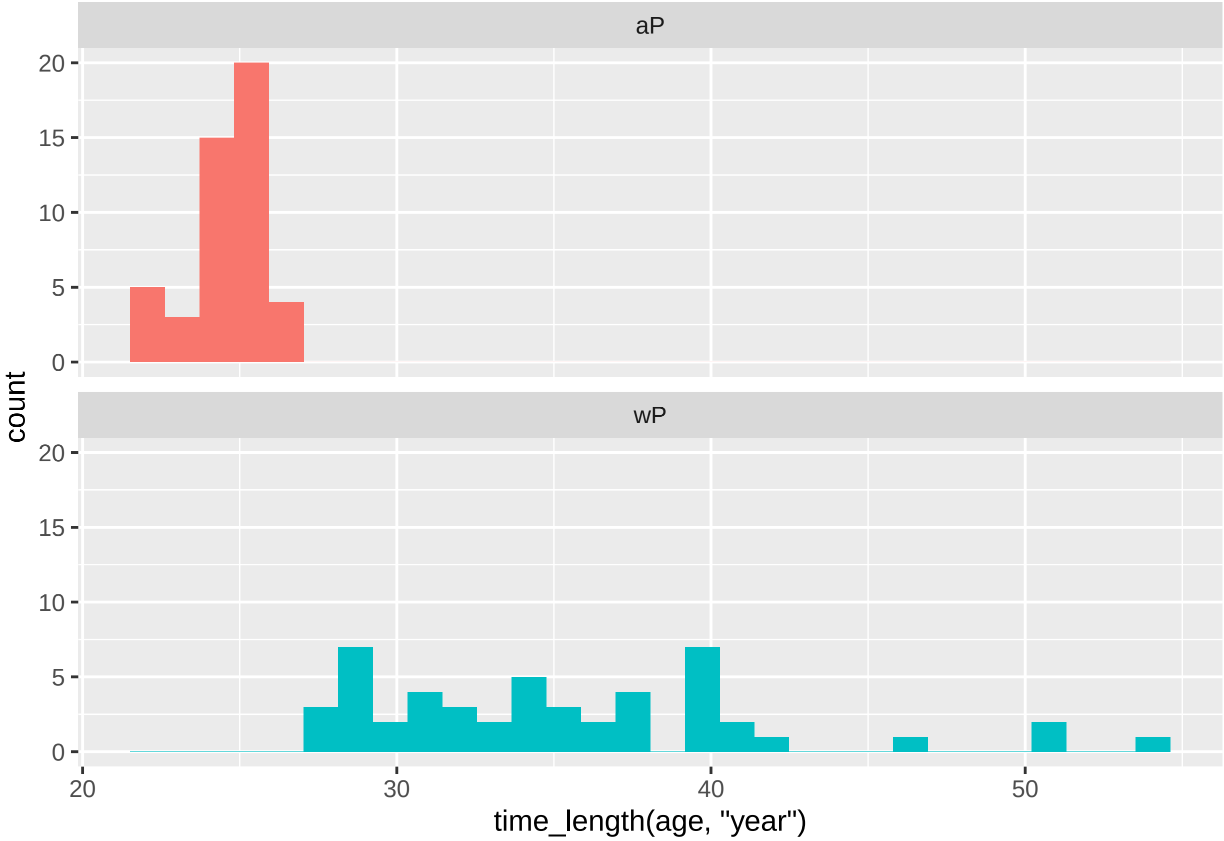
```
## [1] 30.69678 51.07461 33.77413 28.65982 25.65914 28.77481
```

Q9. Q9. With the help of a faceted boxplot (see below), do you think these two groups are significantly different?

```
ggplot(subject) +
  aes(time_length(age, "year"),
      fill=as.factor(subject$infancy_vac)) +
  geom_histogram(show.legend=FALSE) +
  facet_wrap(vars(subject$infancy_vac), nrow=2)
```

```
## Warning: Use of ‘subject$infancy_vac‘ is discouraged. Use ‘infancy_vac‘ instead.

## ‘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] 1.316045e-16
```

There is significant difference.

#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/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 <- full_join(specimen, subject)
```

```
## Joining, by = "subject_id"
```

```
dim(meta)
```

```
## [1] 729  14
```

```
head(meta)
```

```
##   specimen_id subject_id actual_day_relative_to_boost
## 1           1          1                           -3
## 2           2          1                          736
## 3           3          1                            1
## 4           4          1                            3
## 5           5          1                            7
## 6           6          1                           11
##   planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
## 1                             0         Blood     1          wP         Female
## 2                           736         Blood    10          wP         Female
## 3                             1         Blood     2          wP         Female
## 4                             3         Blood     3          wP         Female
## 5                             7         Blood     4          wP         Female
## 6                            14         Blood     5          wP         Female
##                ethnicity  race year_of_birth date_of_boost   study_name
## 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 13225 days
## 2 13225 days
## 3 13225 days
## 4 13225 days
## 5 13225 days
## 6 13225 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, by = "specimen_id"
```

```
dim(abdata)
```

```
## [1] 32675    20
```

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

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

```
## 
##  IgE  IgG IgG1 IgG2 IgG3 IgG4 
## 6698 1413 6141 6141 6141 6141
```

Q12. What do you notice about the number of visit 8 specimens compared to other visits?

```
table(abdata$visit)
```

```
## 
##    1    2    3    4    5    6    7    8 
## 5795 4640 4640 4640 4640 4320 3920   80
```

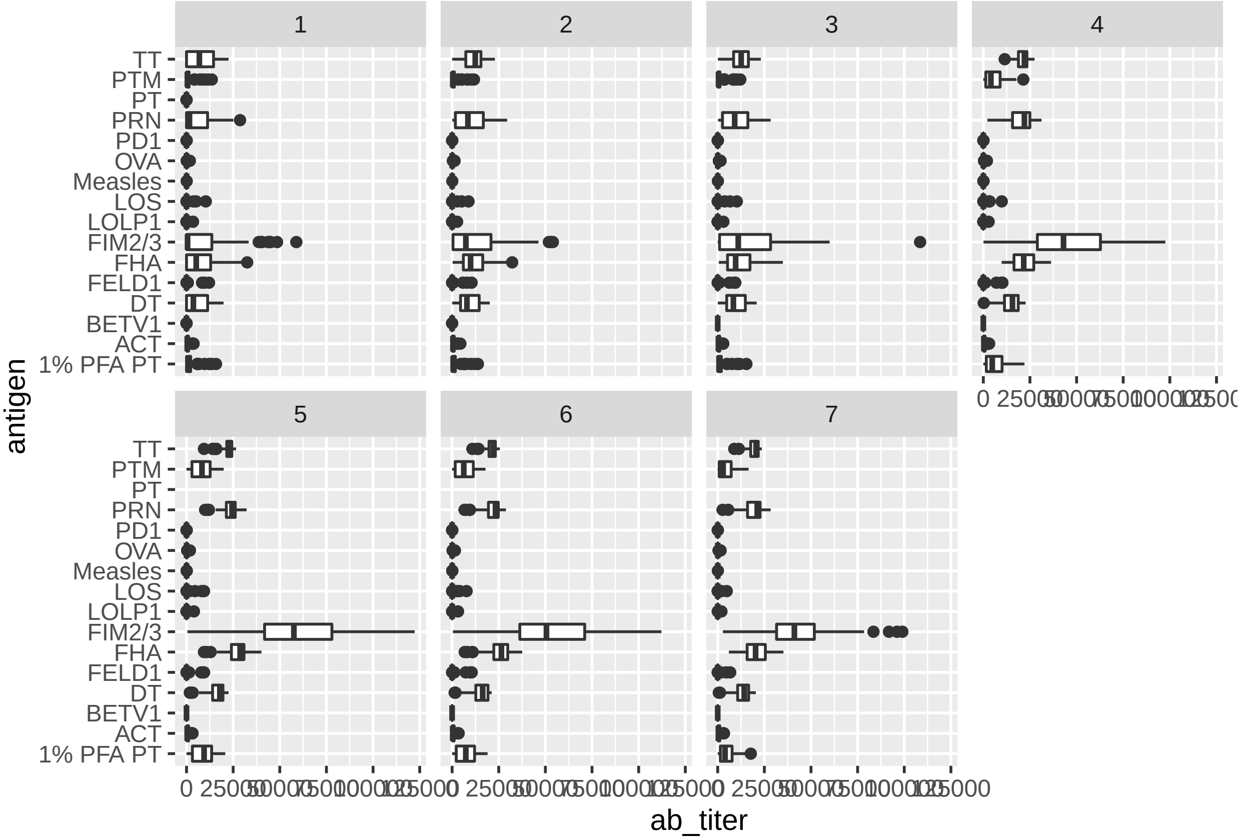
4. Examine IgG1 Ab titer levels

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

```
##   specimen_id isotype is_antigen_specific antigen   ab_titer  unit
## 1           1    IgG1                TRUE     ACT 274.355068 IU/ML
## 2           1    IgG1                TRUE     LOS  10.974026 IU/ML
## 3           1    IgG1                TRUE   FELD1   1.448796 IU/ML
## 4           1    IgG1                TRUE   BETV1   0.100000 IU/ML
## 5           1    IgG1                TRUE   LOLP1   0.100000 IU/ML
## 6           1    IgG1                TRUE Measles  36.277417 IU/ML
##   lower_limit_of_detection subject_id actual_day_relative_to_boost
## 1                 3.848750          1                           -3
## 2                 4.357917          1                           -3
## 3                 2.699944          1                           -3
## 4                 1.734784          1                           -3
## 5                 2.550606          1                           -3
## 6                 4.438966          1                           -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   study_name
## 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 13225 days
## 2 13225 days
## 3 13225 days
## 4 13225 days
## 5 13225 days
## 6 13225 days
```

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

```
ggplot(ig1) +
  aes(ab_titer, antigen) +
  geom_boxplot() +
  facet_wrap(vars(visit), nrow=2)
```

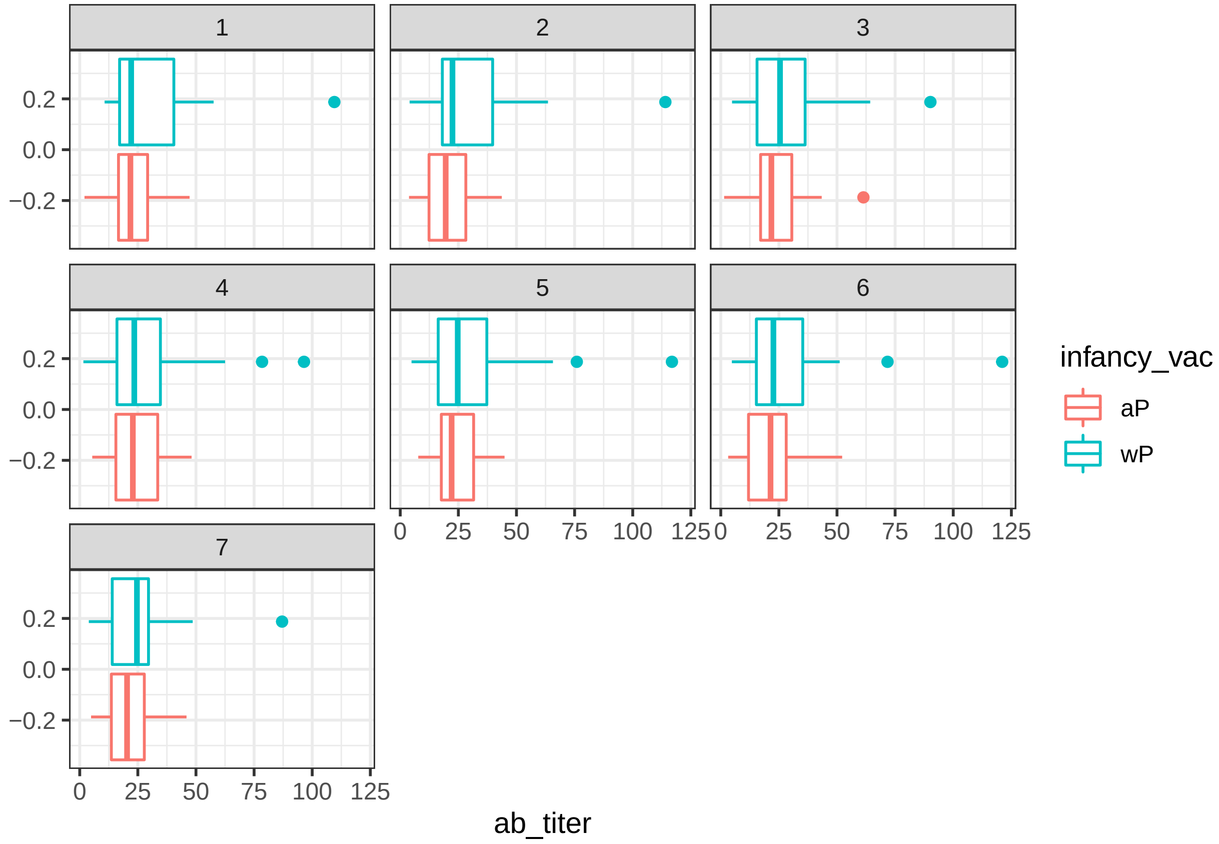


Q14. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others?

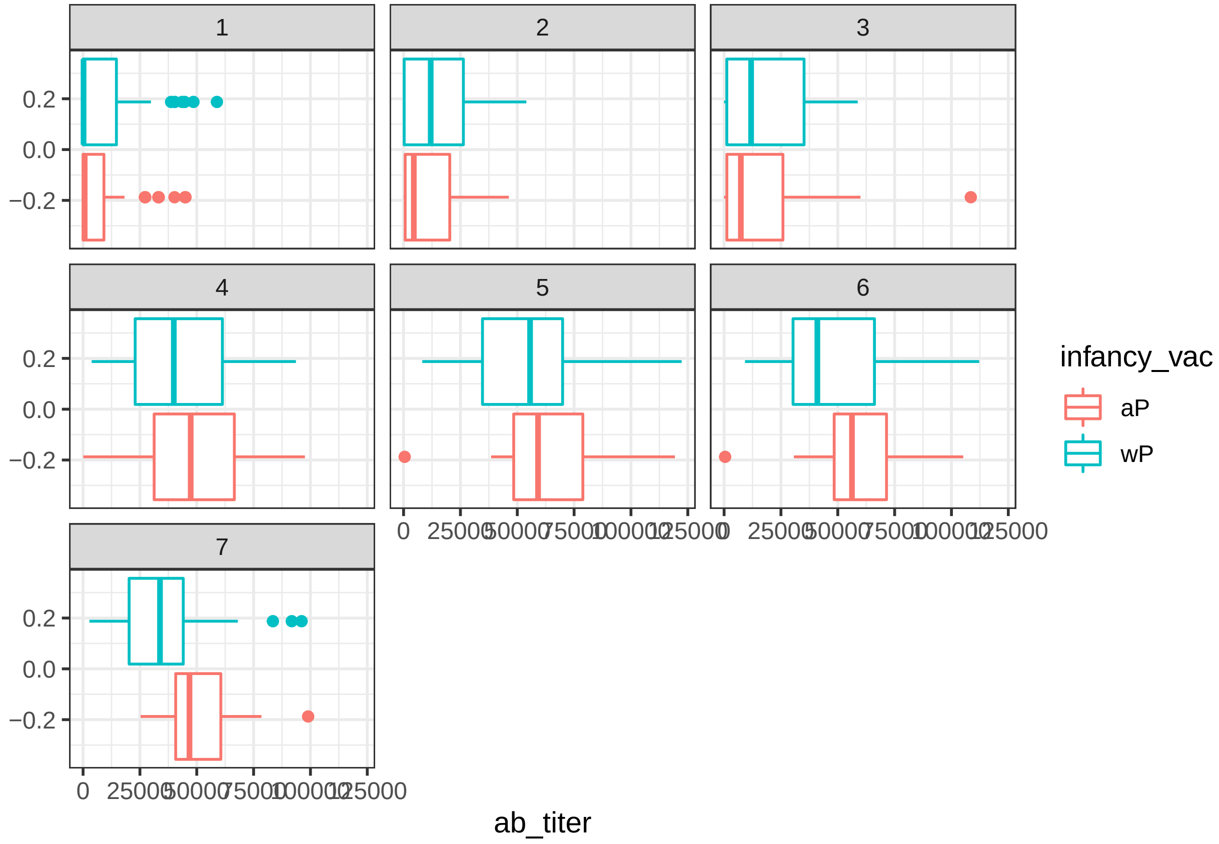
FIM2/3

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

```
filter(ig1, antigen=="Measles") %>%
  ggplot() +
  aes(ab_titer, col=infancy_vac) +
  geom_boxplot(show.legend = TRUE) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



```
filter(ig1, antigen=="FIM2/3") %>%
  ggplot() +
  aes(ab_titer, col=infancy_vac) +
  geom_boxplot(show.legend = TRUE) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



Q16. What do you notice about these two antigens time course and the FIM2/3 data in particular?

The FIM2/3 levels rise over time trend is very similary for wP and aP subjects

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

No there is no clear difference, some parts, wP has higher values, some aP has higher values.

5. Obtaining CMI-PB RNA Seq data

```
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)
```

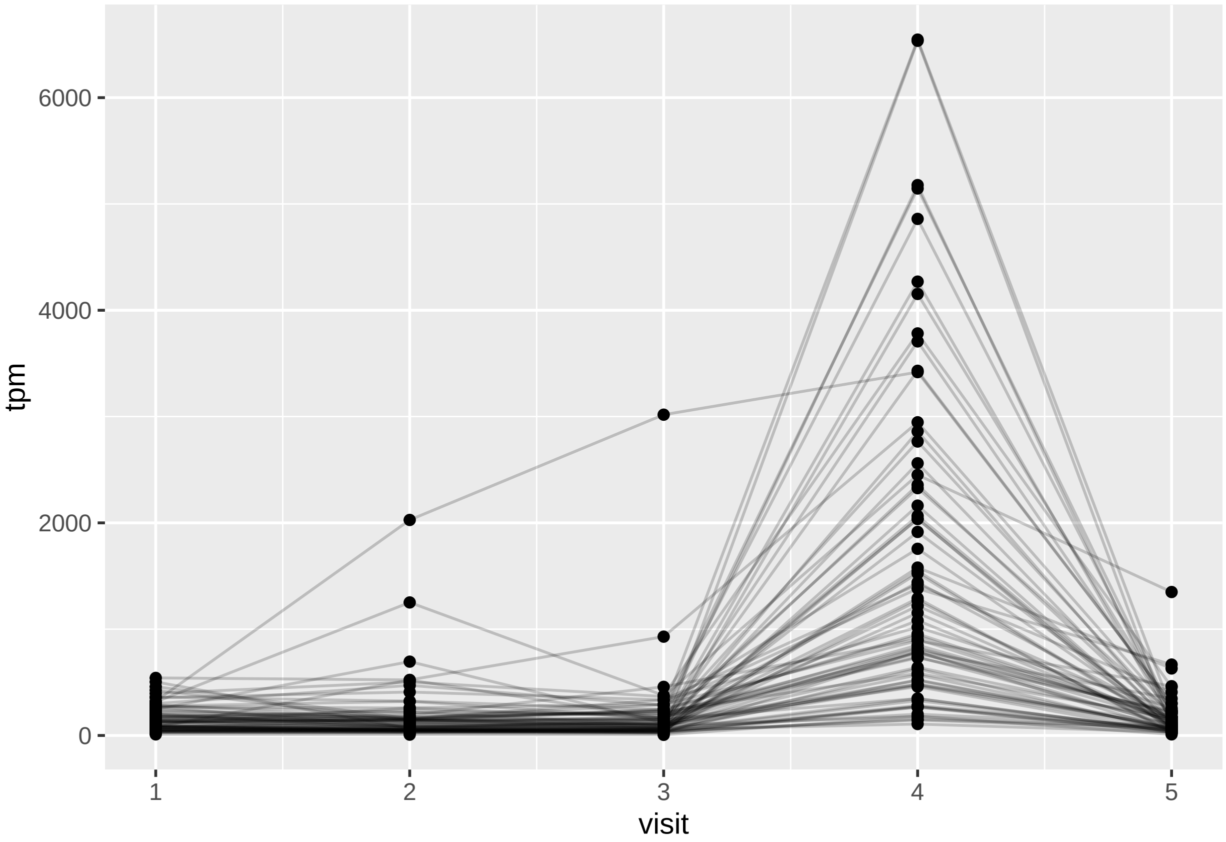
```
## Joining, by = "subject_id"
```

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

```
## Joining, by = "specimen_id"
```

Q18. 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)
```



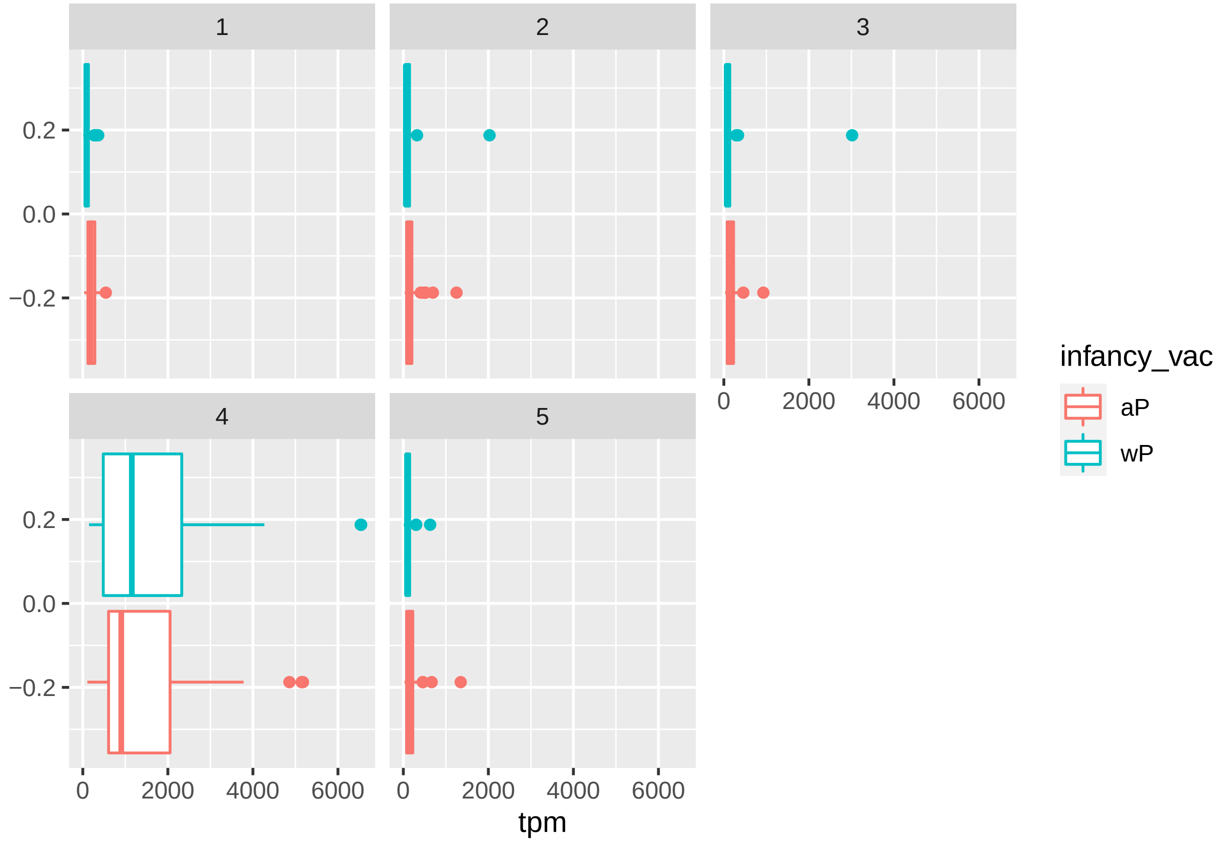
Q19.: What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

When it is at maximum level, the visit number is 4.

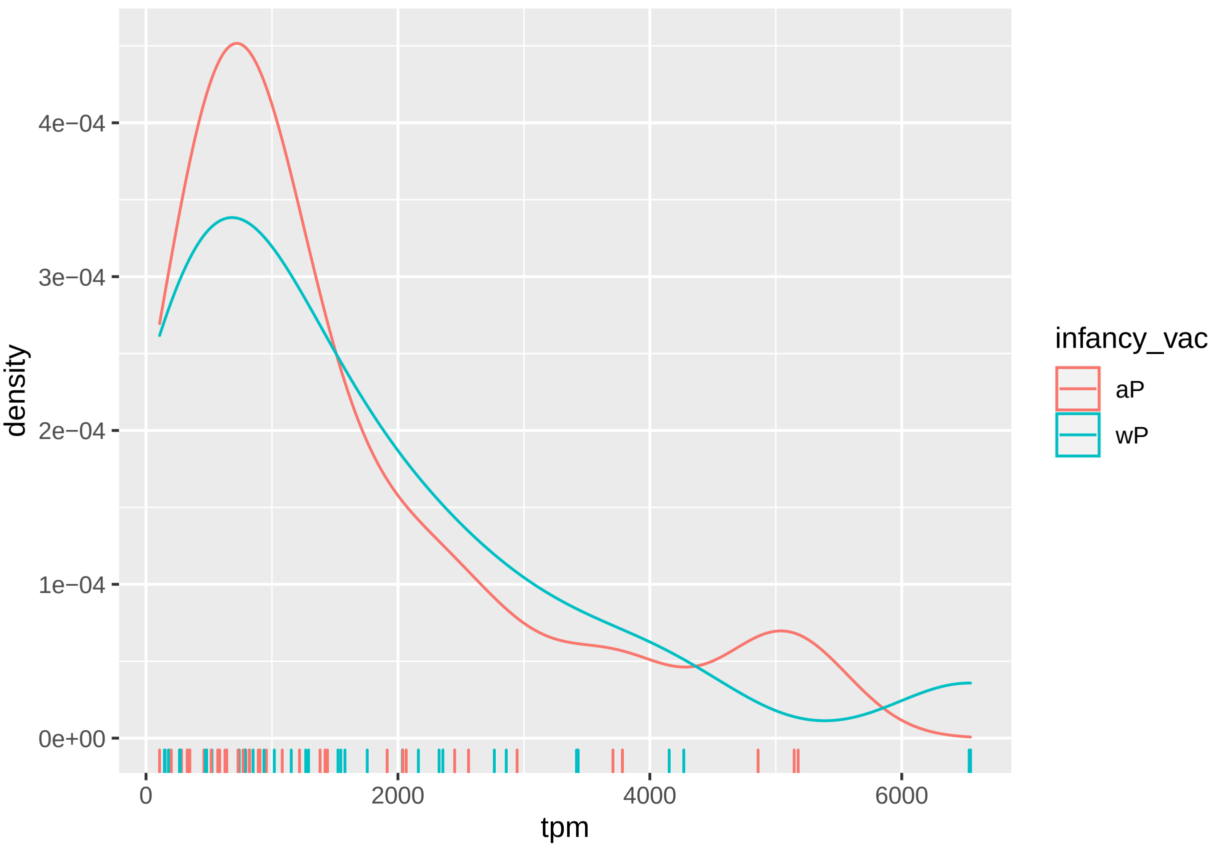
Q20. Does this pattern in time match the trend of antibody titer data? If not, why not?

Yes, it matches in a way where it takes time for it to reach peak.

```
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()
```


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