# Investigating Pertussis Resurgence Mini-Project

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## Investigate Pertussis cas numbers over time in the US

The CDC has tracked case numbers since the early 1920s. https://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html

> 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.

```
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)
       )
```

```
library(tidyverse)
```

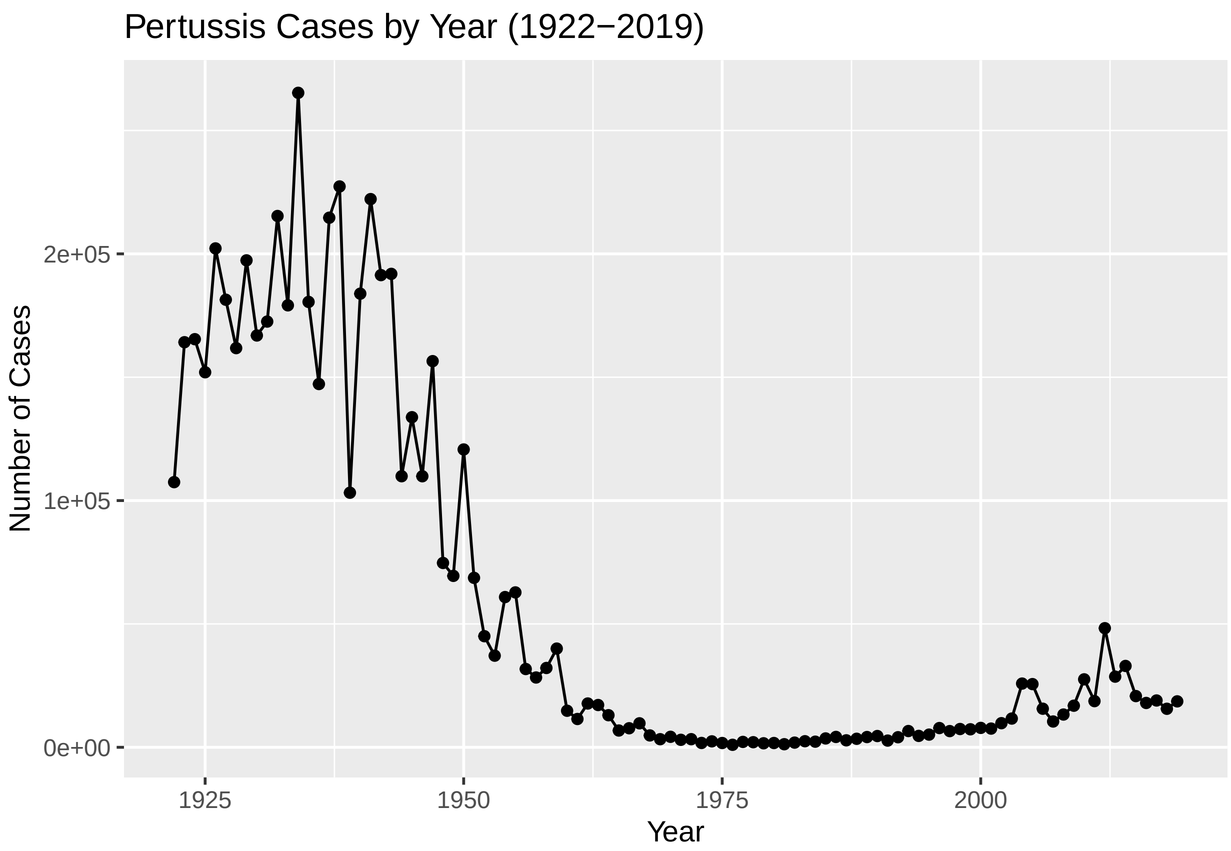
```
## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --
```

```
## v ggplot2 3.3.5     v purrr   0.3.4
## v tibble  3.1.6     v dplyr   1.0.8
## v tidyr   1.2.0     v stringr 1.4.0
## v readr   2.1.2     v forcats 0.5.1
```

```
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

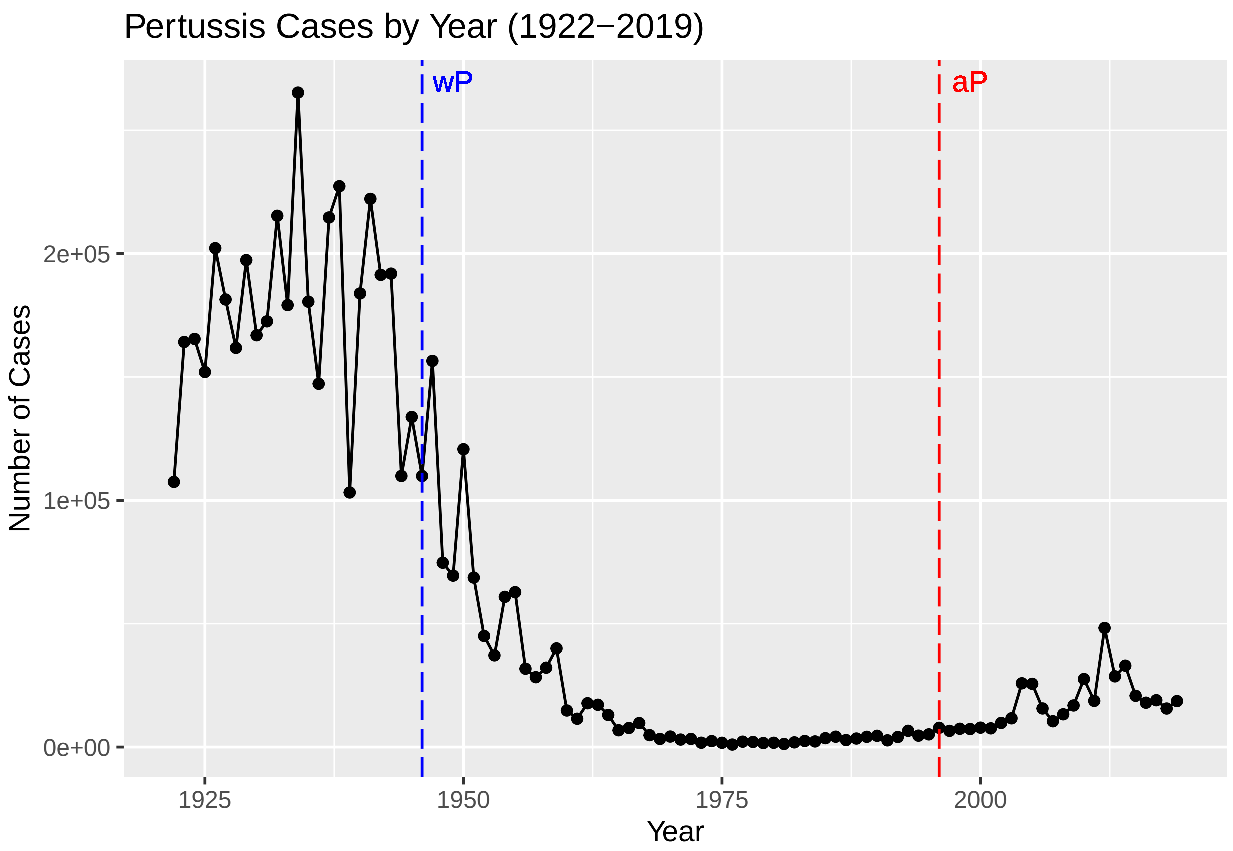
```
#ggplot()
```

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



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, No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  geom_vline(xintercept = 1946, col="blue", linetype="longdash") +
  geom_vline(xintercept = 1996, col="red", linetype="longdash") +
  geom_text(label = "wP", x =1949, y=270000, col="blue") +
  geom_text(label = "aP", x =1999, y=270000, col="red") +
  labs(x="Year", y="Number of Cases", title="Pertussis Cases by Year (1922-2019)")
```



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

Key-point: Despite high levels of acellular pertussis (aP) vaccination, the United States and other countries are now experiencing a significant resurgence in pertussis cases with large outbreaks now once again a major public health concern.

## 3. Exploring CMI-PB data

We will use the **jsonlite** package to read from the CMI database API.

```
library(jsonlite)
```

```
##
## Attaching package: 'jsonlite'

## The following object is masked from 'package:purrr':
##
##     flatten
```

```
url <- "https://www.cmi-pb.org/api/subject"

subject <- read_json(url, 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
```

How many total?

```
nrow(subject)
```

```
## [1] 96
```

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] "2022-03-08"
```

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

```
## Time difference of 8102 days
```

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

```
## [1] 22.18207
```

> Q7 and Q8 optional.

# Join datasets

Read the specimen and ab_titer tables into R and store the data as specimen and titer named data frames.

```
# 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)
```

Have a quick look

```
head(specimen, 3)
```

```
##   specimen_id subject_id actual_day_relative_to_boost
## 1           1          1                           -3
## 2           2          1                          736
## 3           3          1                            1
##   planned_day_relative_to_boost specimen_type visit
## 1                             0         Blood     1
## 2                           736         Blood    10
## 3                             1         Blood     2
```

```
library(dplyr)
```

> 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:

I need to use inner_join() here

```
dim(subject)
```

```
## [1] 96  8
```

```
dim(specimen)
```

```
## [1] 729   6
```

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

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

```
dim(meta)
```

```
## [1] 729  13
```

```
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
```

> 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    19
```

```
head(abdata, 3)
```

```
##   specimen_id isotype is_antigen_specific antigen   ab_titer  unit
## 1           1     IgE               FALSE   Total 1110.21154 UG/ML
## 2           1     IgE               FALSE   Total 2708.91616 IU/ML
## 3           1     IgG                TRUE      PT   68.56614 IU/ML
##   lower_limit_of_detection subject_id actual_day_relative_to_boost
## 1                      NaN          1                           -3
## 2                    29.17          1                           -3
## 3                     0.53          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
##                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
```

> 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? It's a lot lower than the other visits.

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

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

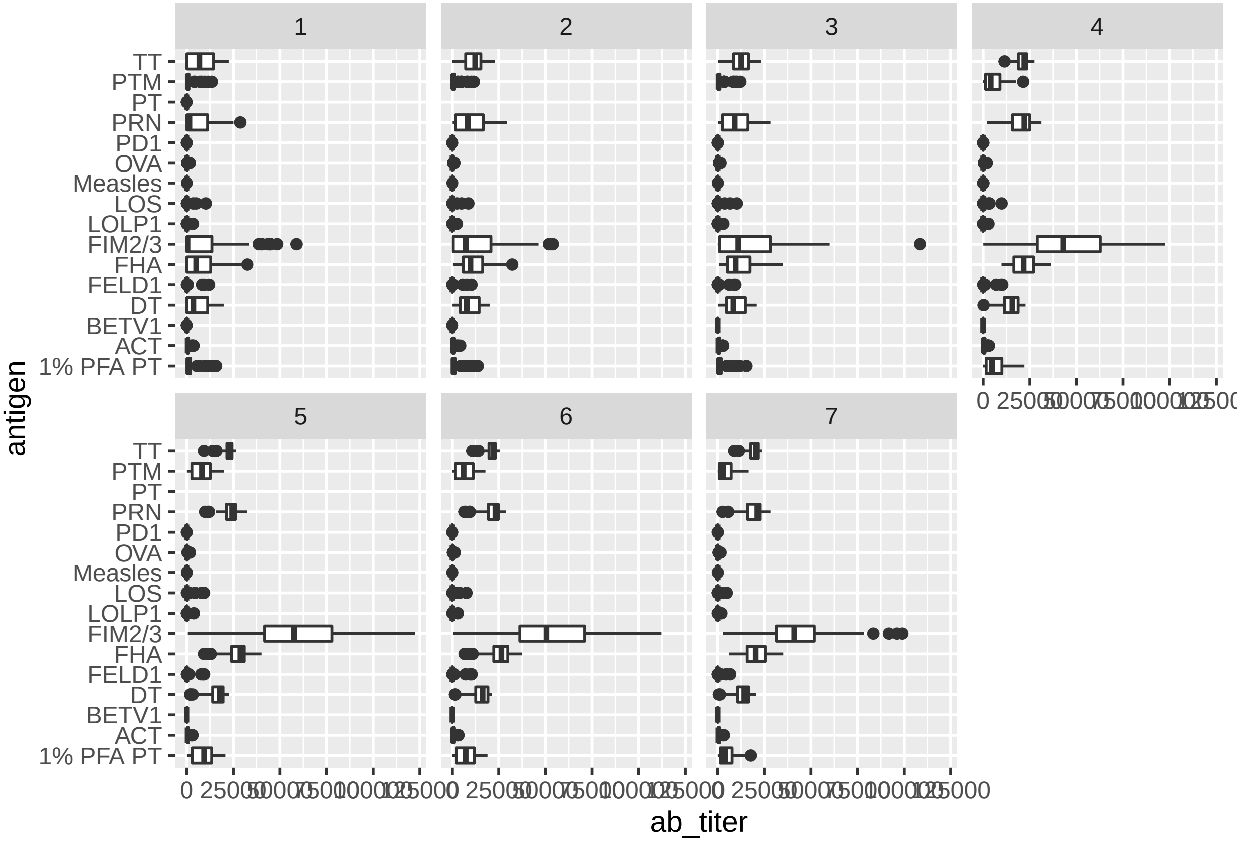
# Examine IgG1 Ab titer levels

```
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
```

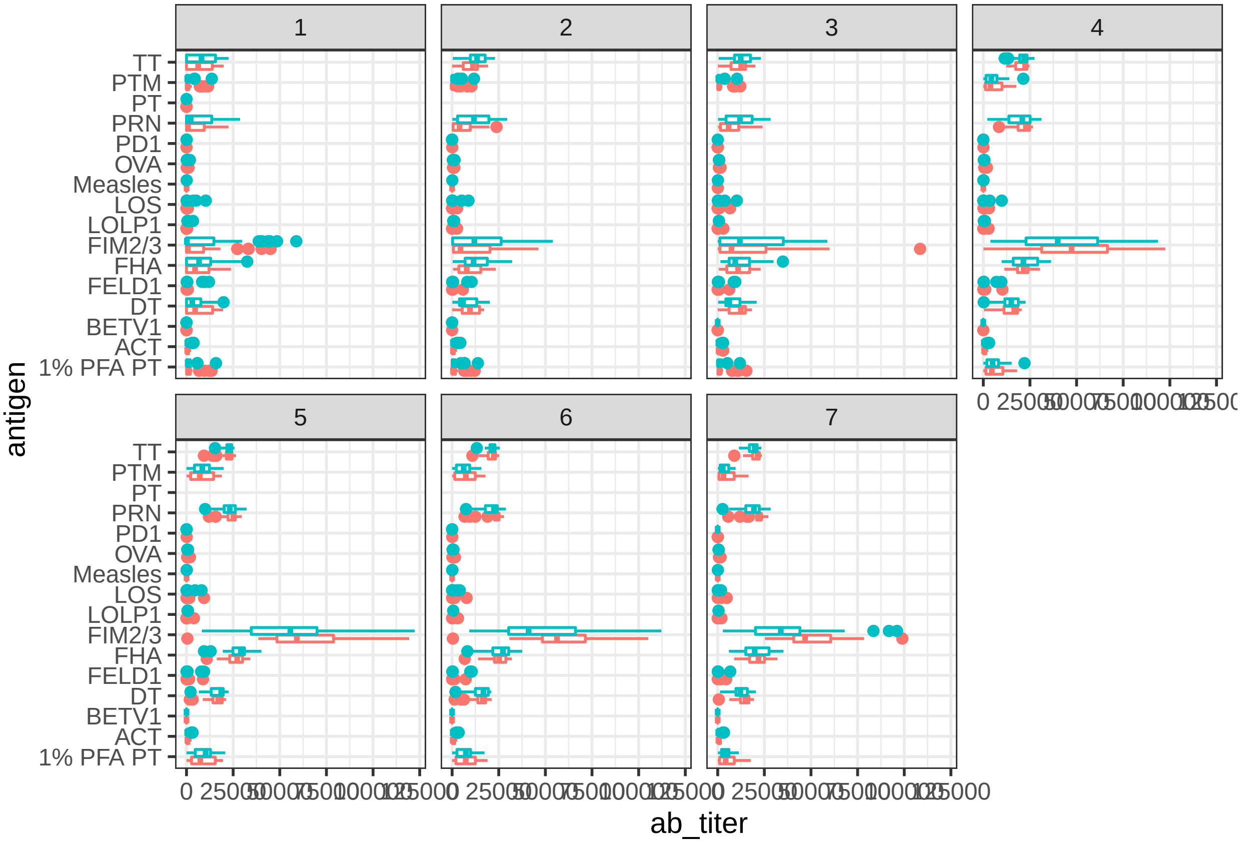
Q13. Complete the following code to make a summary boxplot of Ab titer levels for all antigens:

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

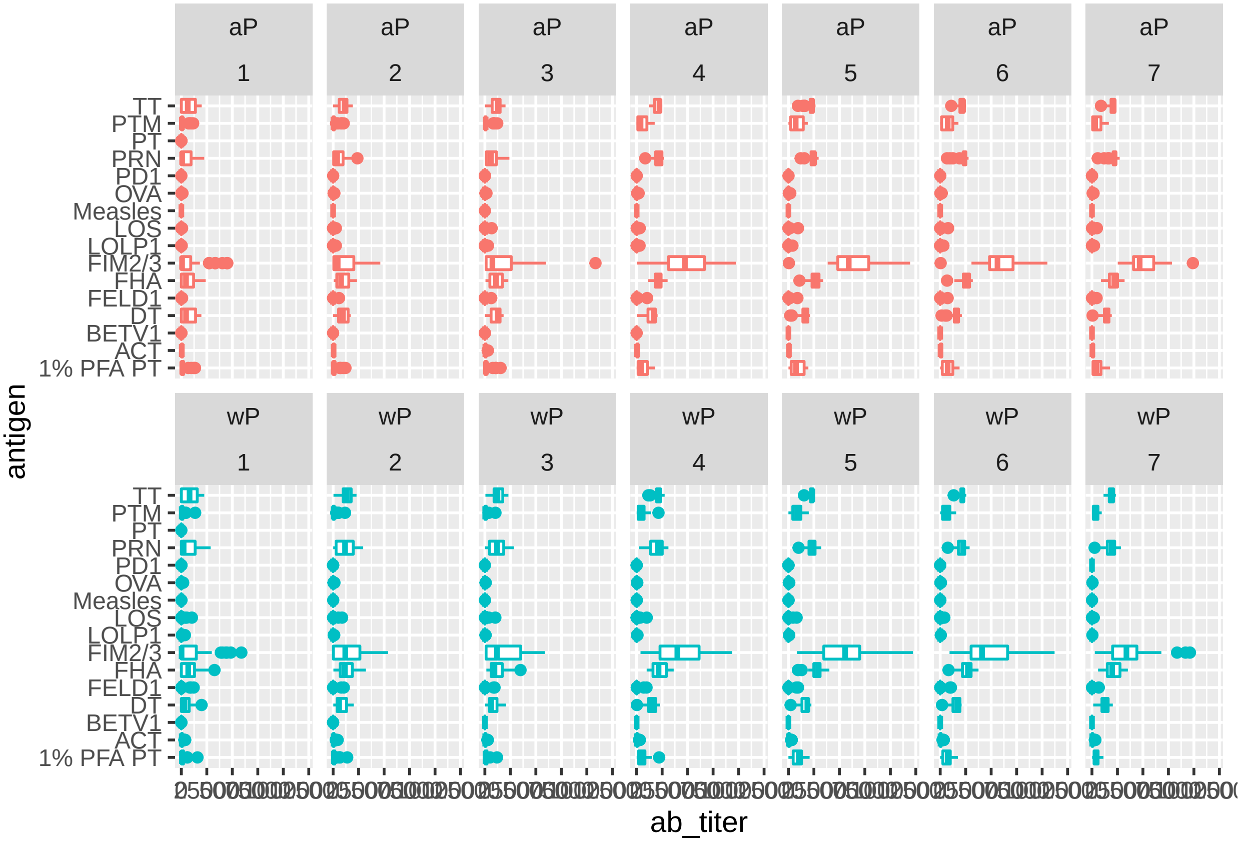


Q14. What antigens show differences in the level of IgG1 antibody titers recognizing them over time? Why these and not others? FIM2/3, FHA, DT, and TT change over time. Antibodies are specifically recognizing them.

```
ggplot(ig1) +
  aes(ab_titer, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  theme_bw()
```

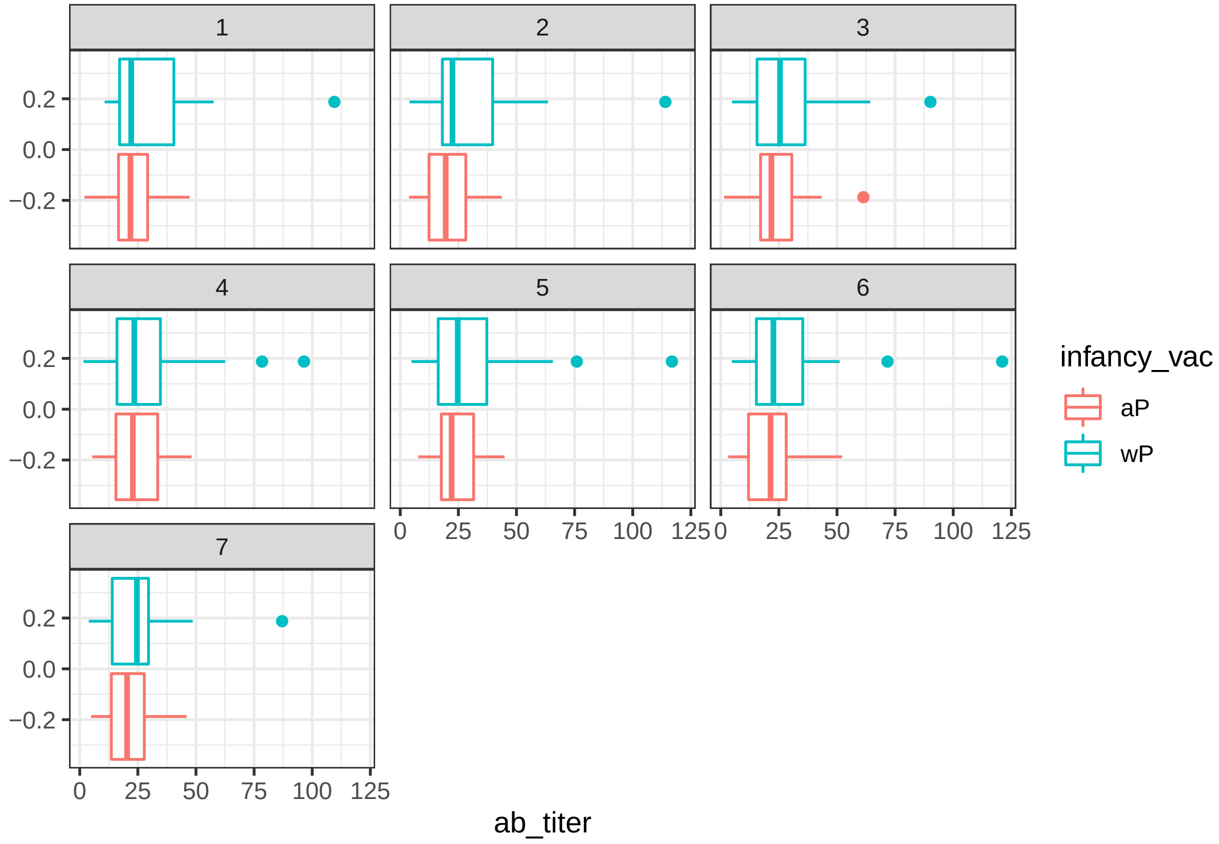


```
ggplot(ig1) +
  aes(ab_titer, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

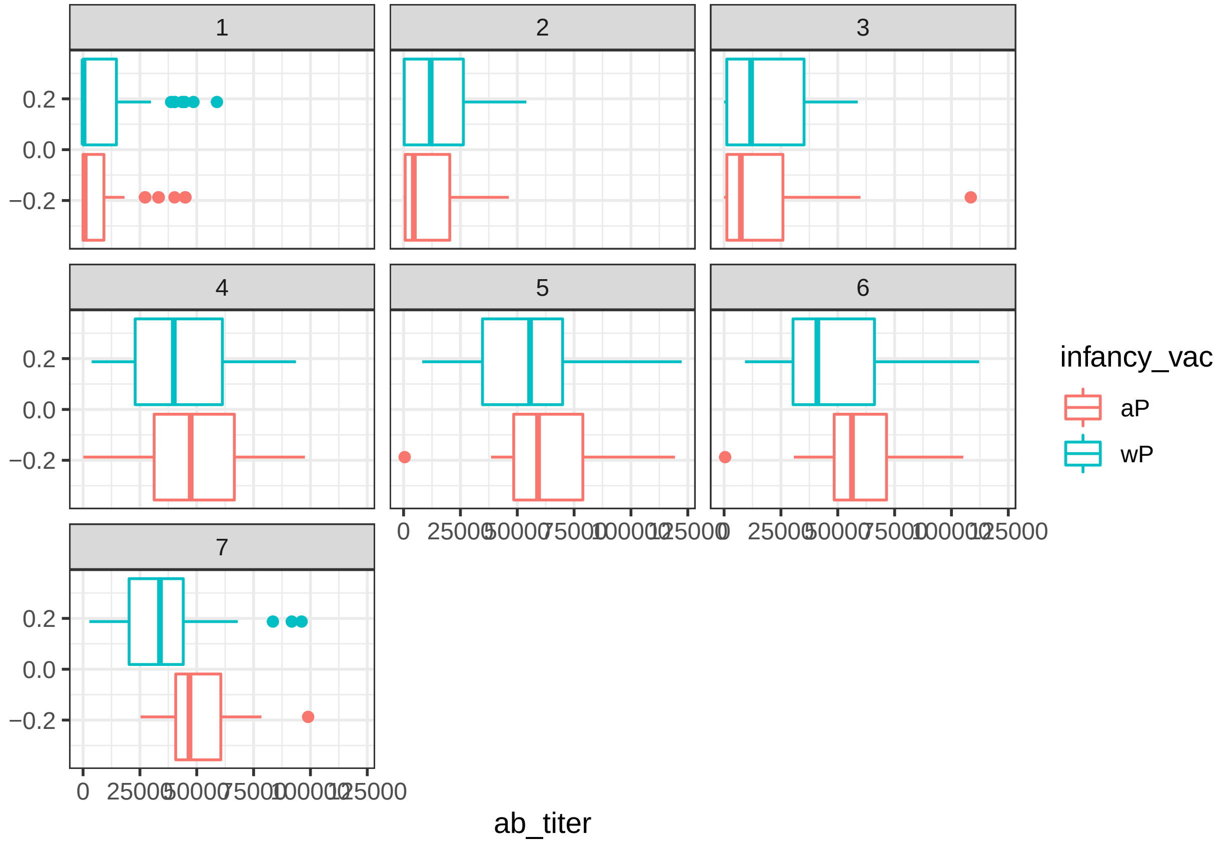


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("Measles", that is not in our vaccines) and a clear antigen of interest ("FIM2/3", extra-cellular fimbriae proteins from B. pertussis that participate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

```r
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? See a big rise, hits peak around day 5 and goes back.

Q17. Do you see any clear difference in aP vs. wP responses? Not really, trend is pretty similar.

# Obtaining CMI-PB RNASeq data

## Pull RNA-Seq data from the CMI-PB

We can use CMI-PB API to obtain time-course RNA-Seq results for wP and aP subjects (i.e. patients).

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

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

```
## [1] 360   4
```

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

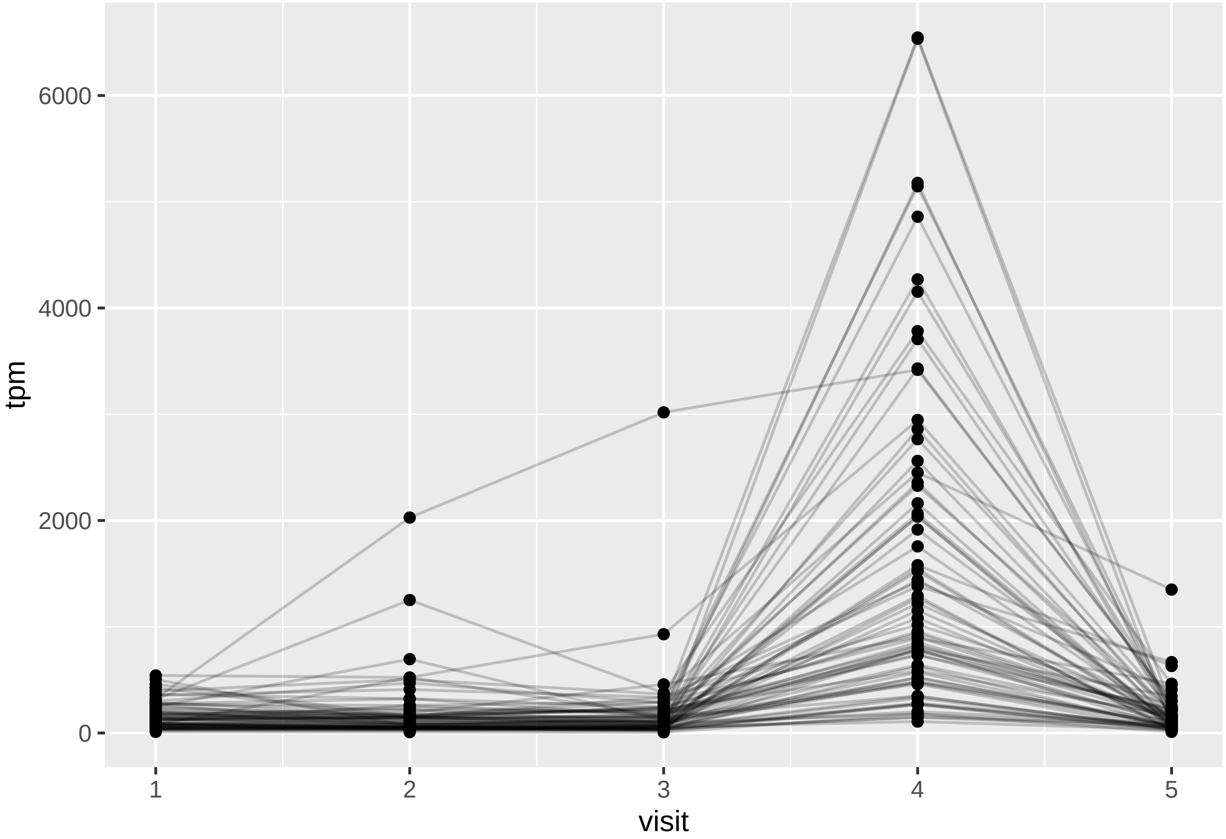
```
## Joining, by = "specimen_id"
```

```
dim(ssrna)
```

```
## [1] 360  16
```

Q18. Make a plot of the time course of gene expression for IGHG1 gene (i.e. a plot of visit vs. tpm).

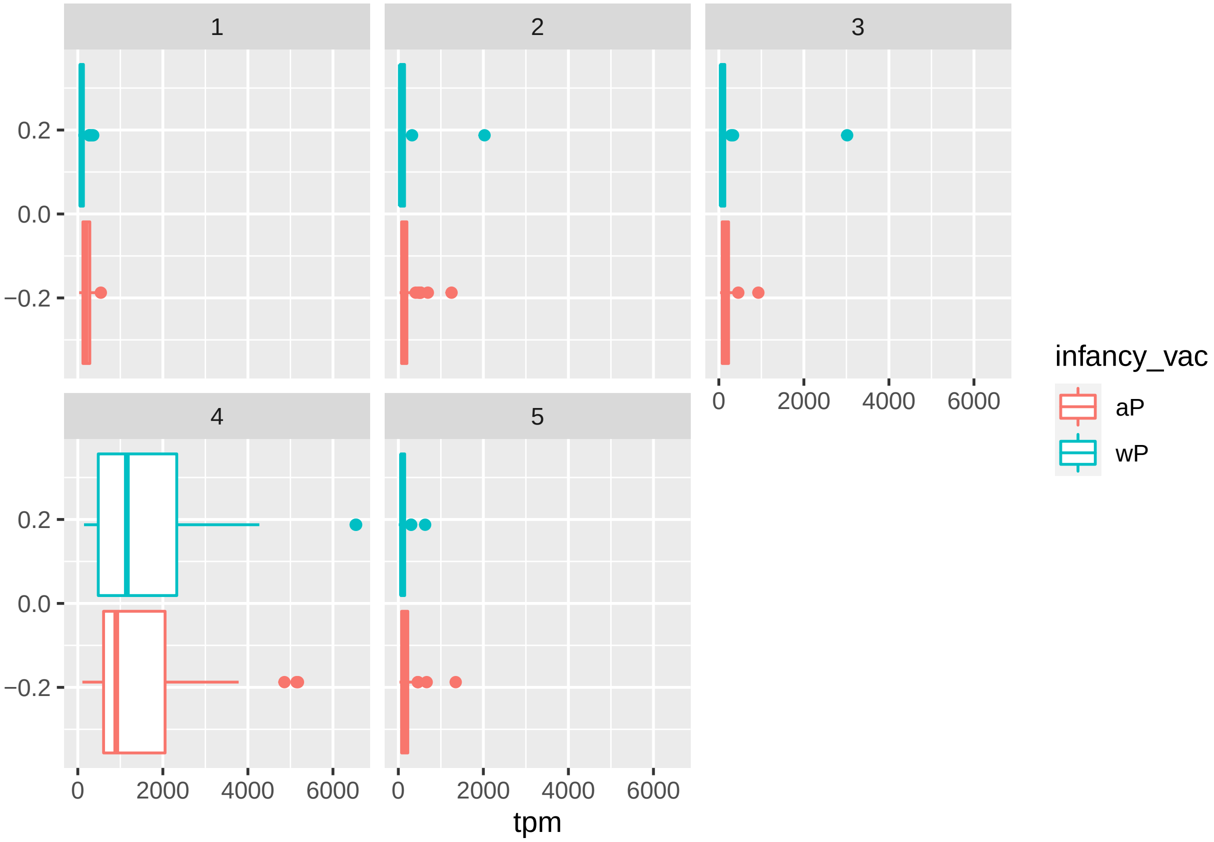
```
ggplot(ssrna) +
  aes(visit, tpm, group=subject_id) +
  geom_point() +
  geom_line(alpha=0.2)
```



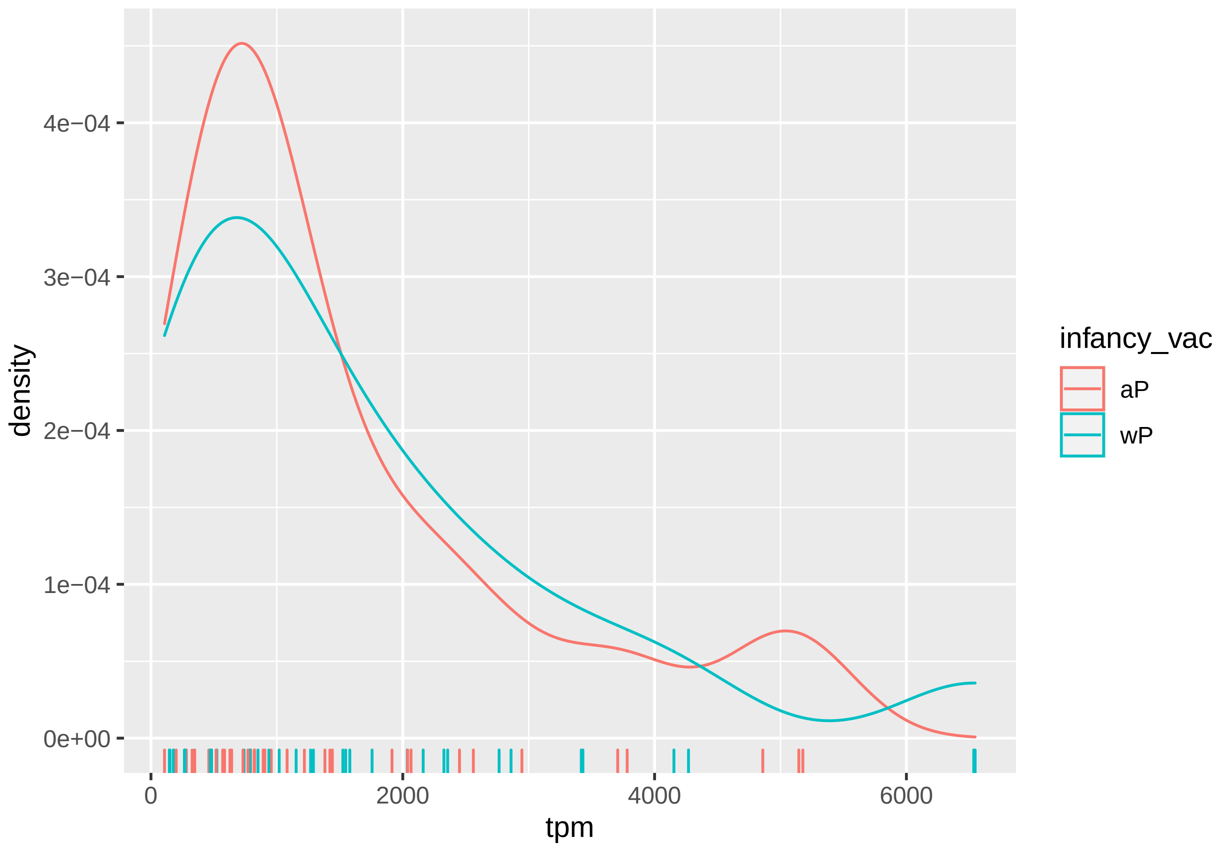
Q19. What do you notice about the expression of this gene (i.e. when is it at it's maximum level)? At visit 4.

Q20. Does this pattern in time match the trend of antibody titer data? If not, why not? Overall yes, as over time it increases, peaks, then drops.

```
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()
```



## Working with larger datasets [OPTIONAL]

```
rnaseq <- read.csv("~/Desktop/BIMM143/week10/2020LD_rnaseq.csv")
head(rnaseq,3)
```

```
##   versioned_ensembl_gene_id specimen_id raw_count tpm
## 1         ENSG00000229704.1         209         0   0
## 2         ENSG00000229707.1         209         0   0
## 3         ENSG00000229708.1         209         0   0
```

```
dim(rnaseq)
```

```
## [1] 1048575       4
```

```
n_genes <- table(rnaseq$specimen_id)
head( n_genes , 10)
```

```
## 
##    74    81    82   102   103   104   105   106   114   115 
## 20509 22200 34298 49183 58347 54747 58347 37615 58347 54790
```

```
length(n_genes)
```

```
## [1] 24
```

```
sessionInfo()
```

```
## R version 4.1.2 (2021-11-01)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
## 
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib
## 
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
## 
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base     
## 
## other attached packages:
##  [1] lubridate_1.8.0 jsonlite_1.8.0  forcats_0.5.1   stringr_1.4.0  
##  [5] dplyr_1.0.8     purrr_0.3.4     readr_2.1.2     tidyr_1.2.0    
##  [9] tibble_3.1.6    ggplot2_3.3.5   tidyverse_1.3.1
## 
## loaded via a namespace (and not attached):
##  [1] tidyselect_1.1.1 xfun_0.29        haven_2.4.3      colorspace_2.0-2
##  [5] vctrs_0.3.8      generics_0.1.2   htmltools_0.5.2  yaml_2.2.2      
##  [9] utf8_1.2.2       rlang_1.0.0      pillar_1.7.0     withr_2.4.3     
## [13] glue_1.6.1       DBI_1.1.2        dbplyr_2.1.1     modelr_0.1.8    
## [17] readxl_1.3.1     lifecycle_1.0.1  munsell_0.5.0    gtable_0.3.0    
## [21] cellranger_1.1.0 rvest_1.0.2      evaluate_0.14    labeling_0.4.2  
## [25] knitr_1.37       tzdb_0.2.0       fastmap_1.1.0    fansi_1.0.2     
## [29] highr_0.9        broom_0.7.12     Rcpp_1.0.8       backports_1.4.1 
## [33] scales_1.1.1     farver_2.1.0     fs_1.5.2         hms_1.1.1       
## [37] digest_0.6.29    stringi_1.7.6    grid_4.1.2       cli_3.1.1       
## [41] tools_4.1.2      magrittr_2.0.2   crayon_1.4.2     pkgconfig_2.0.3 
## [45] ellipsis_0.3.2   xml2_1.3.3       reprex_2.0.1     assertthat_0.2.1
## [49] rmarkdown_2.11   httr_1.4.2       rstudioapi_0.13  R6_2.5.1        
## [53] compiler_4.1.2
```