# Class 15: Investigating Pertussis Resurgence

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## Investigate Pertussis case 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

```
library(datapasta)
library(ggplot2)
```

```
## Warning in register(): Can’t find generic ‘scale_type‘ in package ggplot2 to
## register S3 method.
```

```
library(dplyr)
```

```
##
## Attaching package: ’dplyr’
```

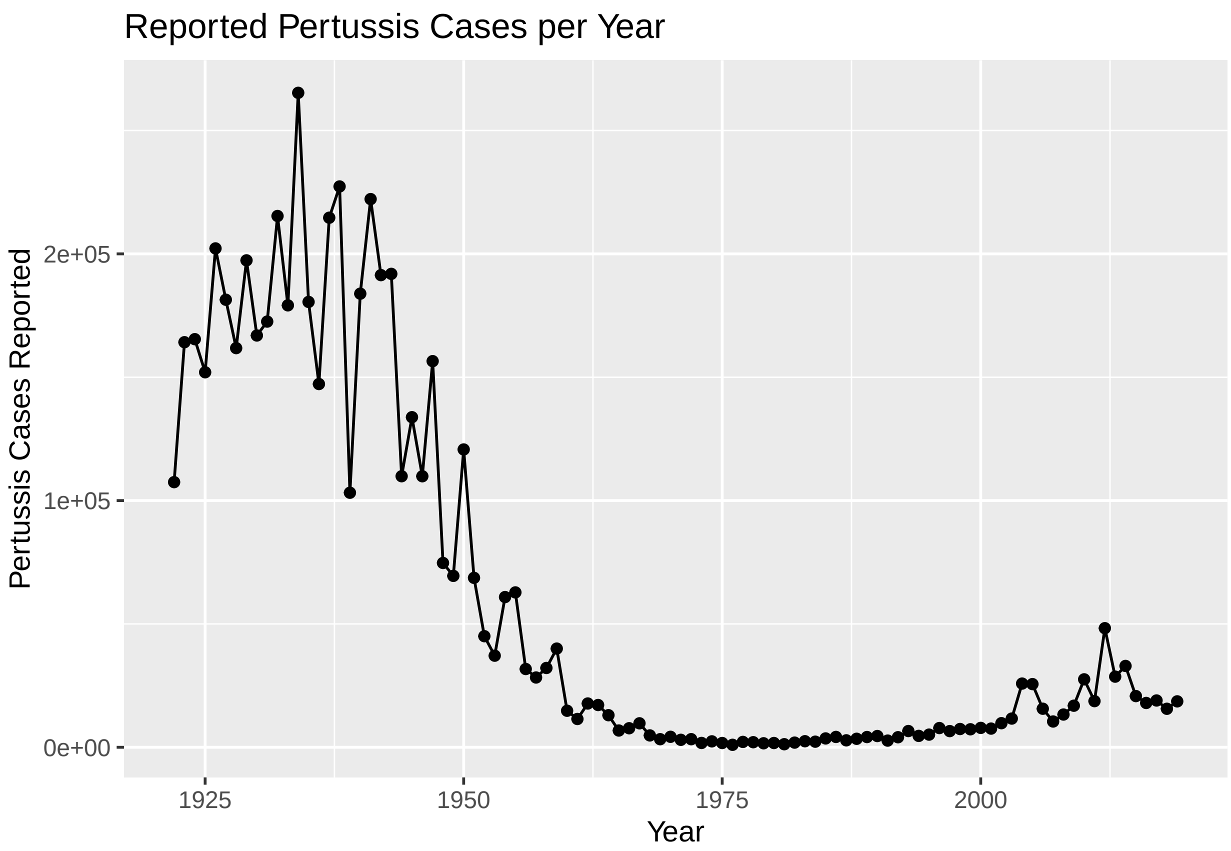
```
## The following objects are masked from ’package:stats’:
##
##     filter, lag
```

```
## The following objects are masked from ’package:base’:
##
##     intersect, setdiff, setequal, union
```

```
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)
)
```

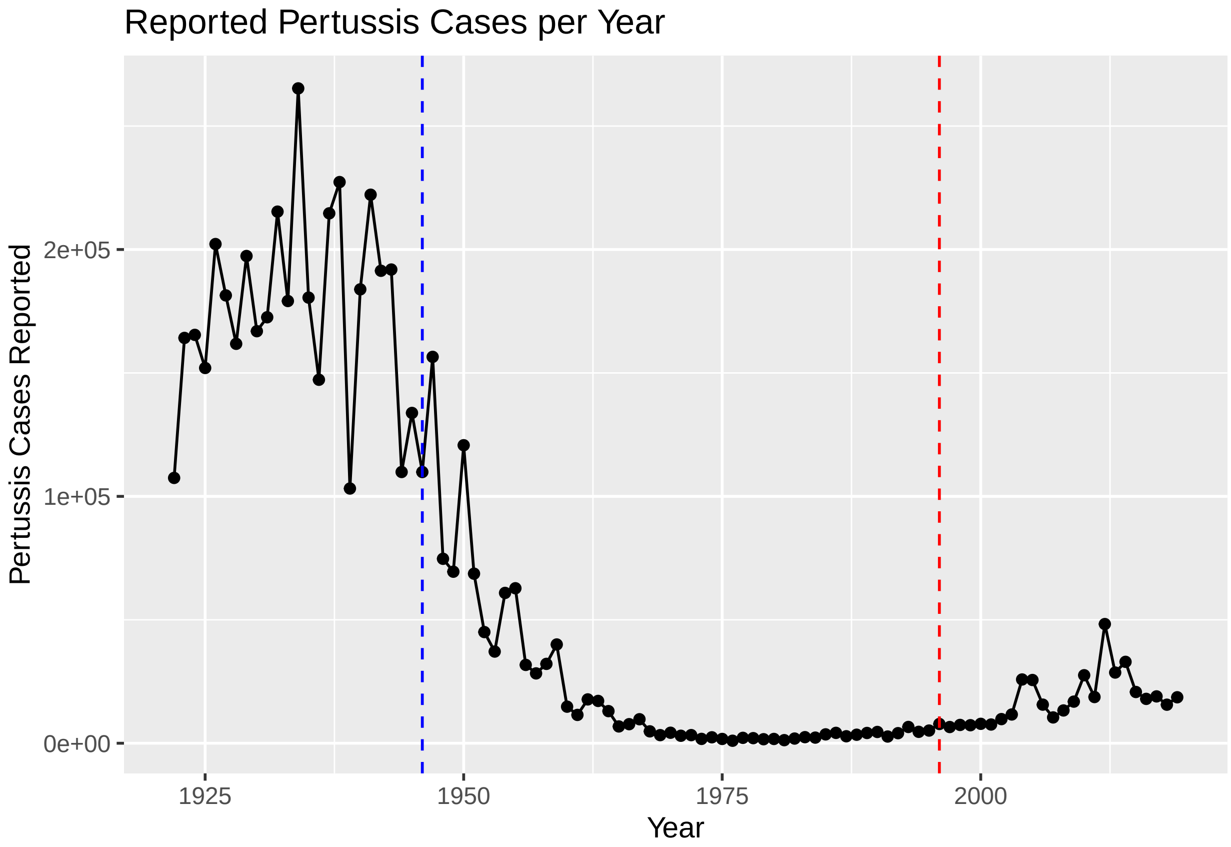
Q. Make a figure of cases (y) per year (x) using ggplot

```
ggplot(cdc) +
  aes(Year, No..Reported.Pertussis.Cases) +
  geom_point() +
  geom_line() +
  labs(title= "Reported Pertussis Cases per Year",
       x = "Year",
       y = "Pertussis Cases Reported")
```



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, color = "blue", linetype = "dashed") +
  geom_vline(xintercept= 1996, color = "red", linetype = "dashed") +
  labs(title= "Reported Pertussis Cases per Year",
       x = "Year",
       y = "Pertussis Cases Reported")
```



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

After 1946 vaccination, the reported cases significantly decreased. That said, after 1996, there is a slight increase in cases. We could attribute this trend to the increase in population. With greater population, the bacteria could mutate and become resistant to the vaccine. As such, younger generations would not be immune to the newly evolved bacteria. There is also a growing portion of vaccine hesitancy. Overtime, vaccines also wane in efficacy over time, and the aP vaccine could have waned faster than the wP vaccine.

## Explore CMI-PB data

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

```
# Allows us to read, write and process JSON data
library(jsonlite)
```

```
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
```

> 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
```

## Working with dates

```
library(lubridate)
```

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

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

> We skipped Q7 and Q8.

# Join datasets.

```
# Complete the API URLs...
specimen <- read_json("http://cmi-pb.org/api/specimen",
                      simplifyVector = TRUE)

titer <- read_json("http://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
```

```
head(titer, 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
## 1                      NaN
## 2                    29.17
## 3                     0.53
```

I need to join

> 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.

```
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?

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

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

There are noticeably less visit 8 specimens compared to the other visits. We're still collecting data for visit 8, which is more than a month after.

## Examine IgG1 Ab titer levels

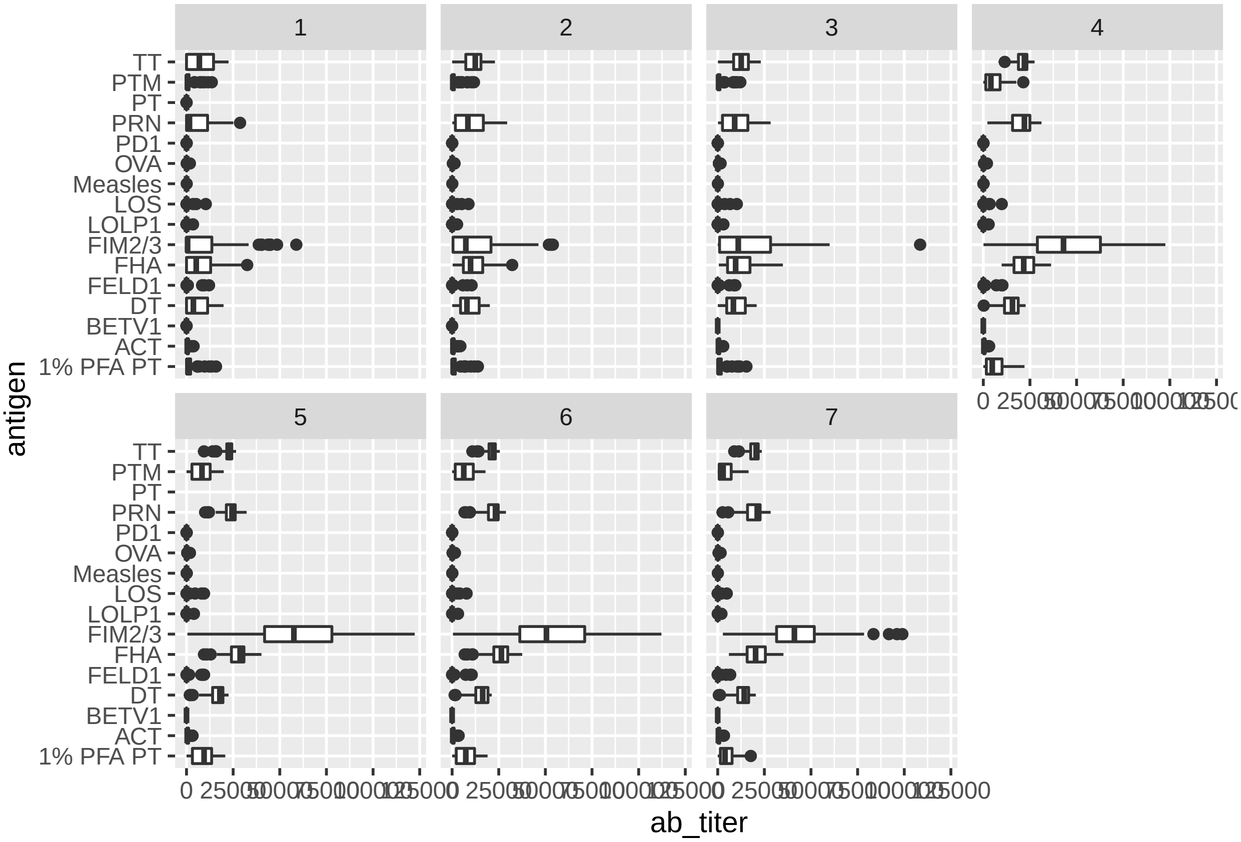
```
ig1 <- abdata %>% filter(isotype == "IgG1", visit!=8)
head(ig1)
```

```
##   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
```

We excluded visit 8 because the data is incomplete

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

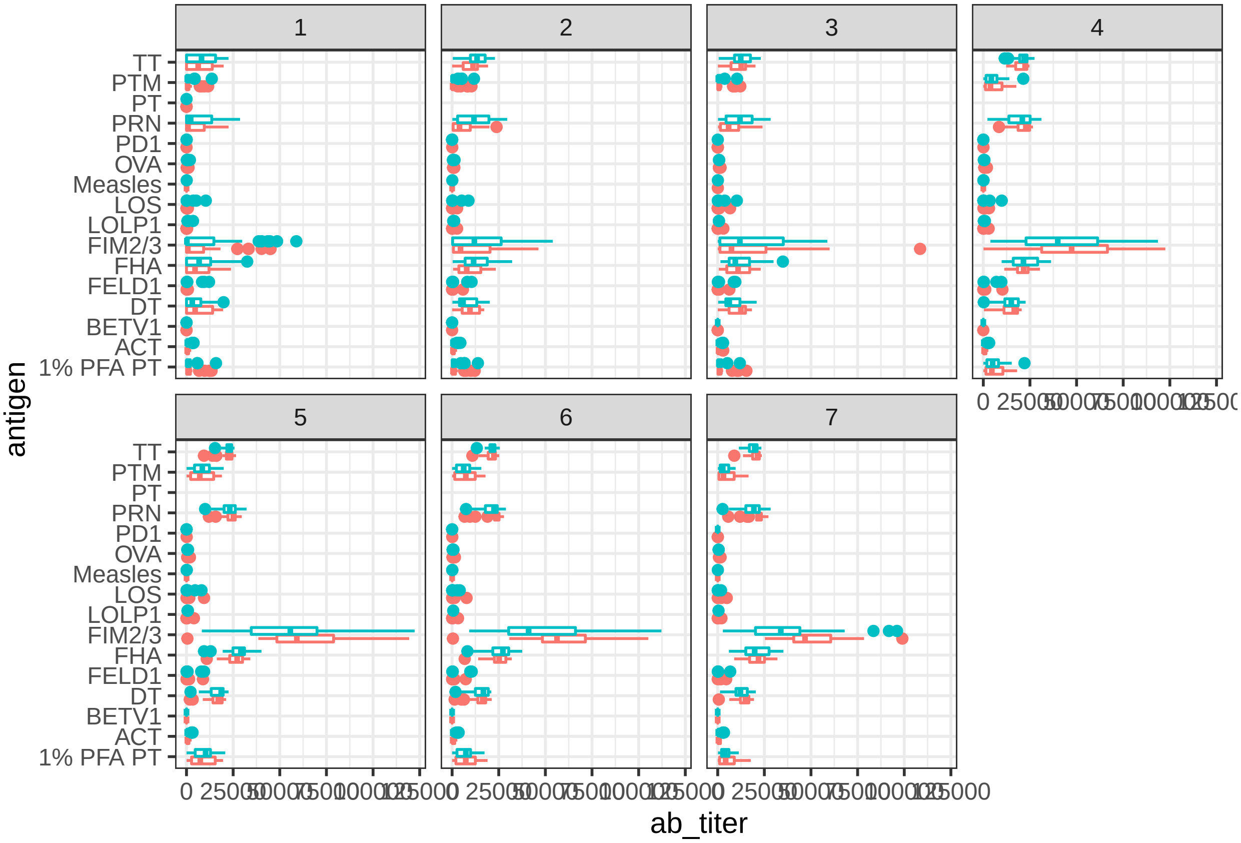
```
ggplot(ig1) +
  aes(ab_titer, antigen) +
  geom_boxplot() +
  facet_wrap(vars(visit), nrow=2)
```



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

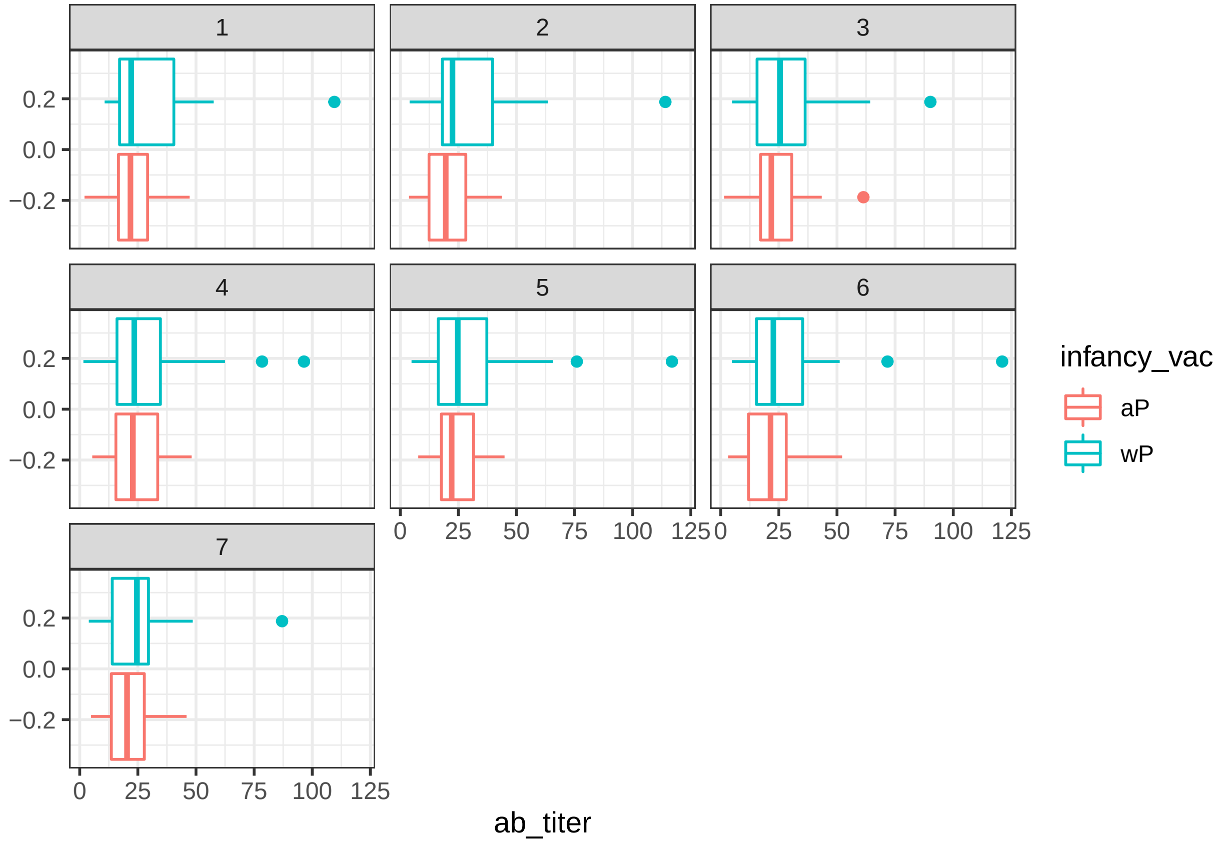
FIM2/3 shows difference in the level of IgG1 antibody titers recognizing them over time, where it actually gets larger over time. This could be because their antibodies specifically recognize them

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

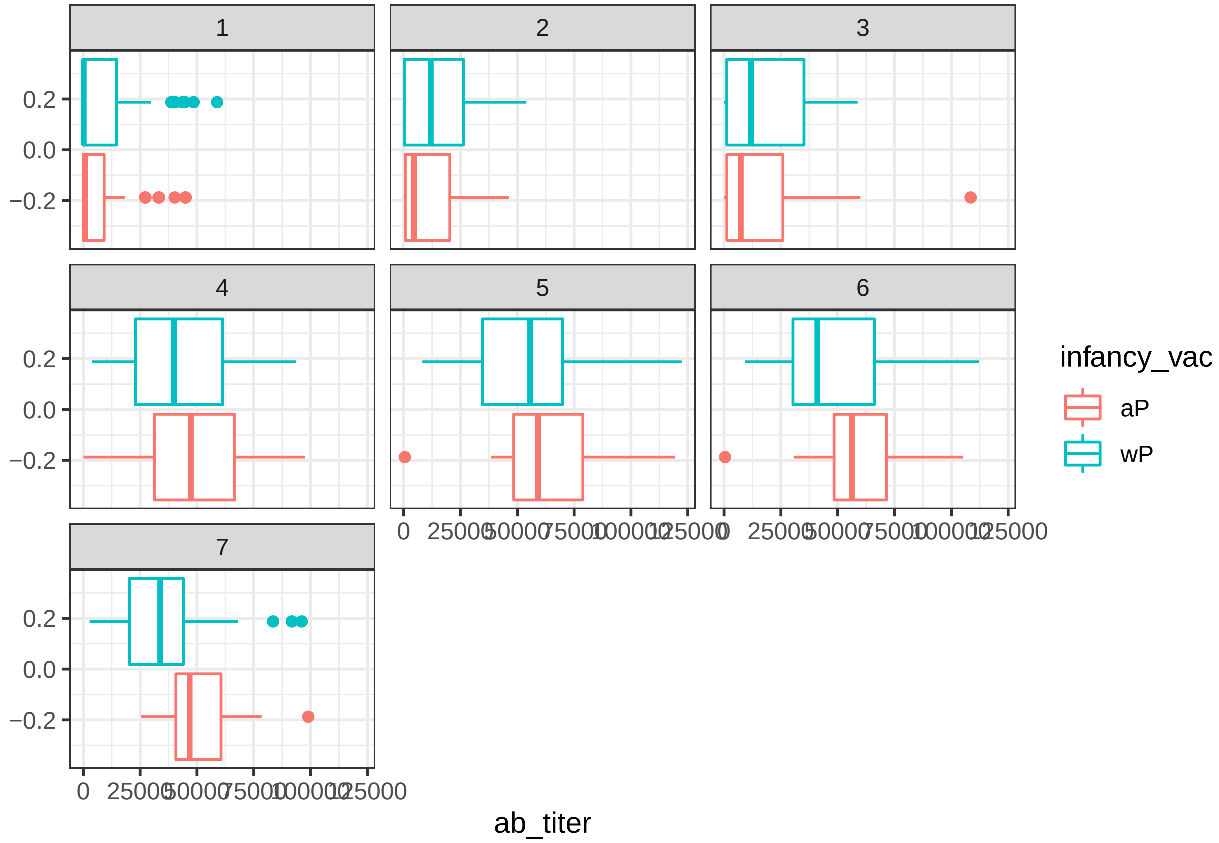


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("Measles", that is not in our vaccines) and a clear antigen of interest ("FIM2/3", extra-cellular fimbriae proteins from B. pertussis that participate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



```r
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?

FIM2/3 demonstrates a greater rise over time in comparison to measles. Measles stays fairly consistent. FIM2/3 increases, but specifically peaks at visit 5.

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

There are more differences in aP vs wP responses for FIM2/3, with aP having slightly greater. That said, they aren't that significant since they are overlapping in range.

### Obtaining CMI-PB RNASeq data

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

## For example use the following URL

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

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

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

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

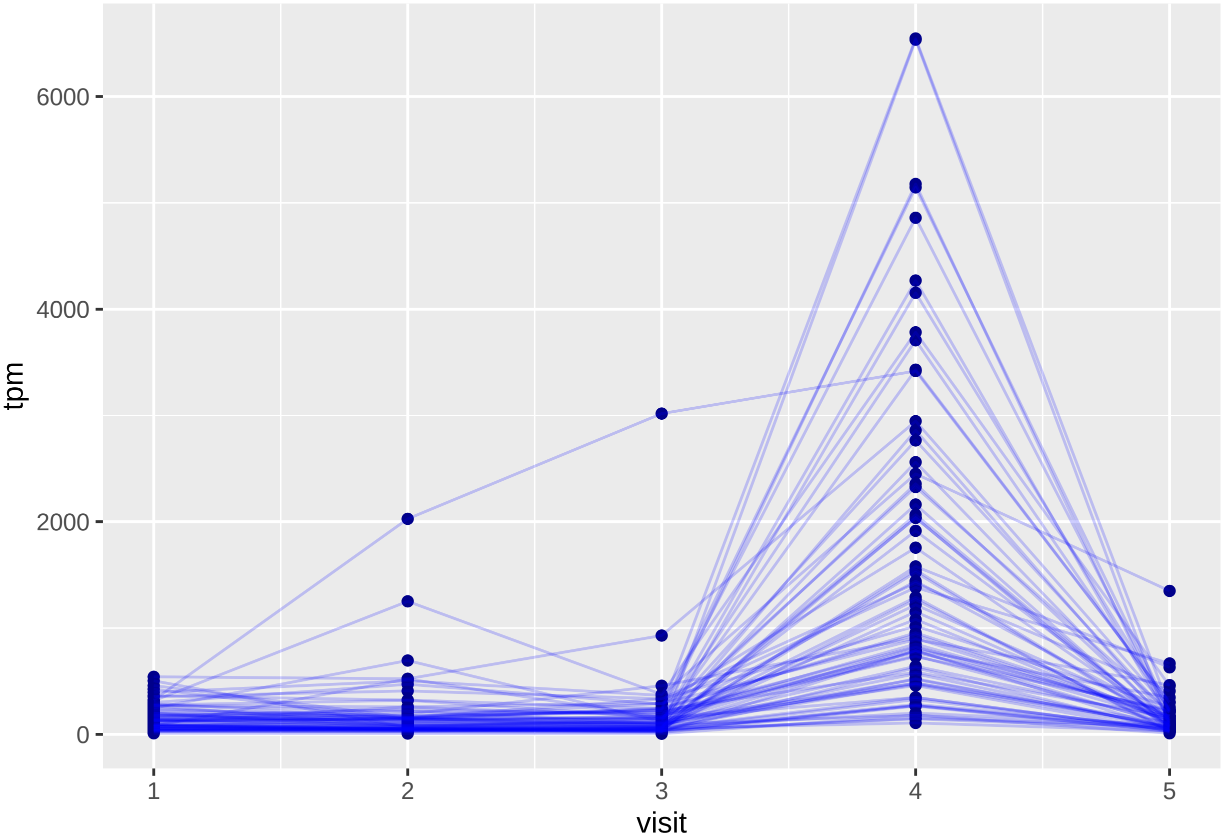
```
## Joining, by = "subject_id"
```

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

```
## 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(color = "blue4") +
  geom_line(alpha=0.2, color = "blue")
```



We see a rise at visit 4.

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

At its maximum level around vist 4, it appear to be over 6000 tpm.

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

Yes, the antobody titer data does appear to match.