# class19

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```
library(ggplot2)
library(tidyverse)
```

```
-- Attaching core tidyverse packages ------------------------ tidyverse 2.0.0 --
v dplyr     1.1.3     v readr     2.1.4
v forcats   1.0.0     v stringr   1.5.0
v lubridate 1.9.3     v tibble    3.2.1
v purrr     1.0.2     v tidyr     1.3.0
-- Conflicts ------------------------------------------ tidyverse_conflicts() --
x dplyr::filter() masks stats::filter()
x dplyr::lag()    masks stats::lag()
i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to becom
```

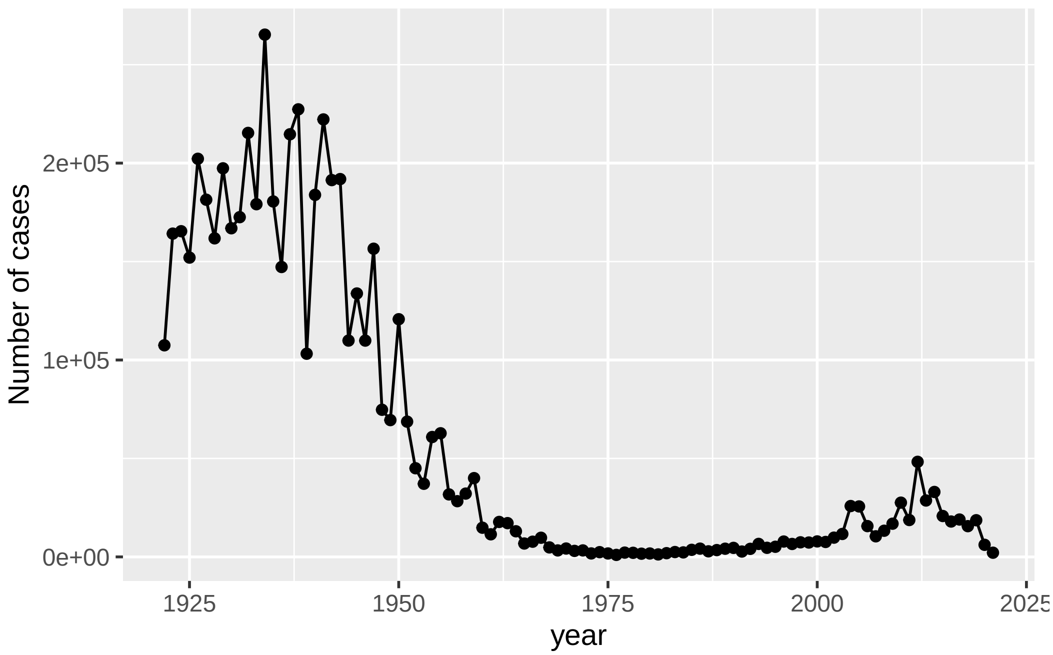
Perstussis is a bacterial infection that causes a severe cough. Often named “whooping cough”

Case numbers of Perstussis in the US.

```
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),
    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)
  )
```

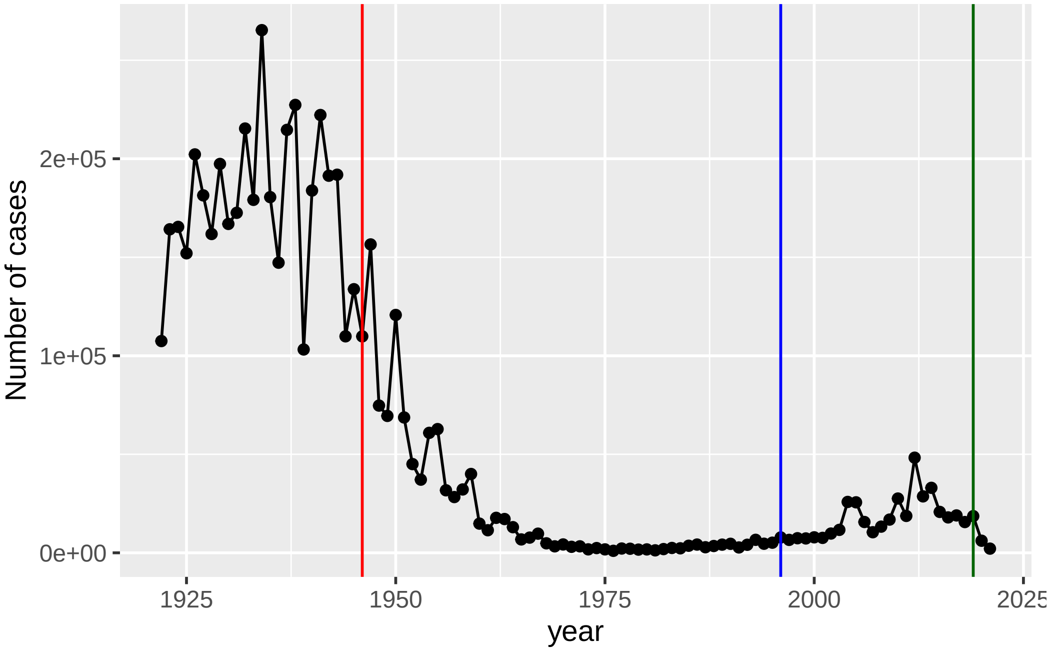
Q1. With the help of the R “addin” package datapasta assign the CDC pertussis case number data to a data frame called cdc and use ggplot to make a plot of cases numbers over time.

```
ggplot(cdc) +
  aes(x=year, y=cases) +
  geom_point() +
  geom_line() +
  labs(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() +
  geom_line() +
  labs(x= "year", y="Number of cases") +
  geom_vline(xintercept = 1946, color= "red") +
  geom_vline(xintercept = 1996, color= "blue") +
  geom_vline(xintercept = 2019, color= "darkgreen")
```



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

After the aP vaccine, there is an increase in cases a couple years later, and shows that you need a booster (every ~10 yrs) after initially getting the first vaccine.

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

```

Attaching package: 'jsonlite'


The following object is masked from 'package:purrr':

    flatten
```

```
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 
```

aP= 60 wP= 58

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

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

```

Female   Male 
    79     39 
```

Male = 39 Female = 79

> 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                                         21   11
  Black or African American                      2    0
  More Than One Race                             9    2
  Native Hawaiian or Other Pacific Islander      1    1
  Unknown or Not Reported                       11    4
  White                                         35   20
```

### Working with dates

```
library(lubridate)
today()
```

```
[1] "2023-12-08"
```

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

```
Time difference of 8742 days
```

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

```
[1] 23.93429
```

> 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)
```

```
library(dplyr)

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 
```

The average age for aP is 26, the average age for wP is 36, and there's a 10 year difference.

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

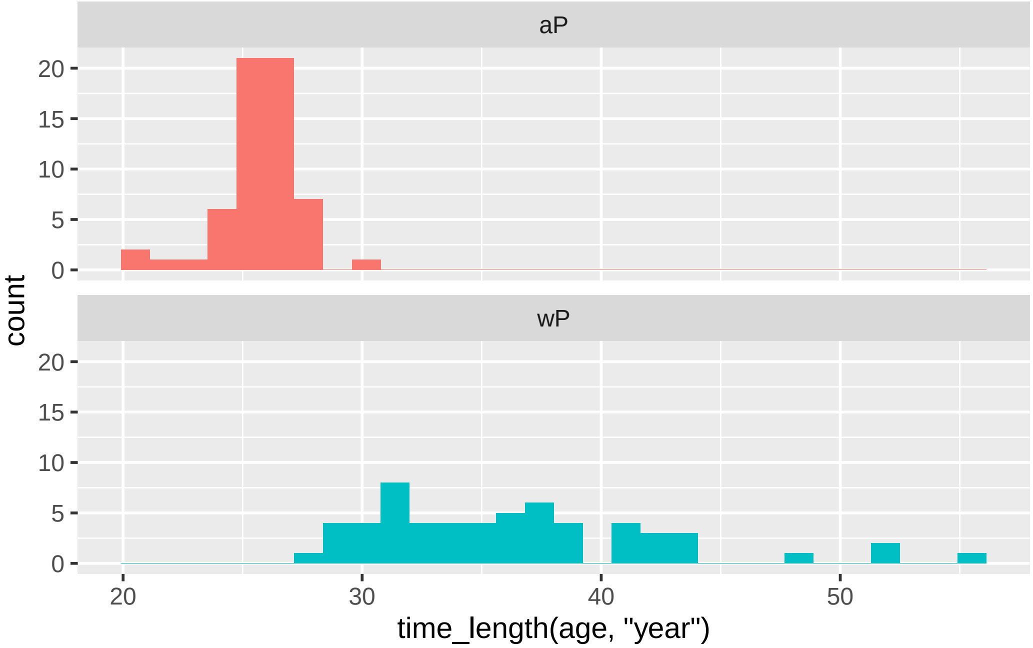
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
[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?

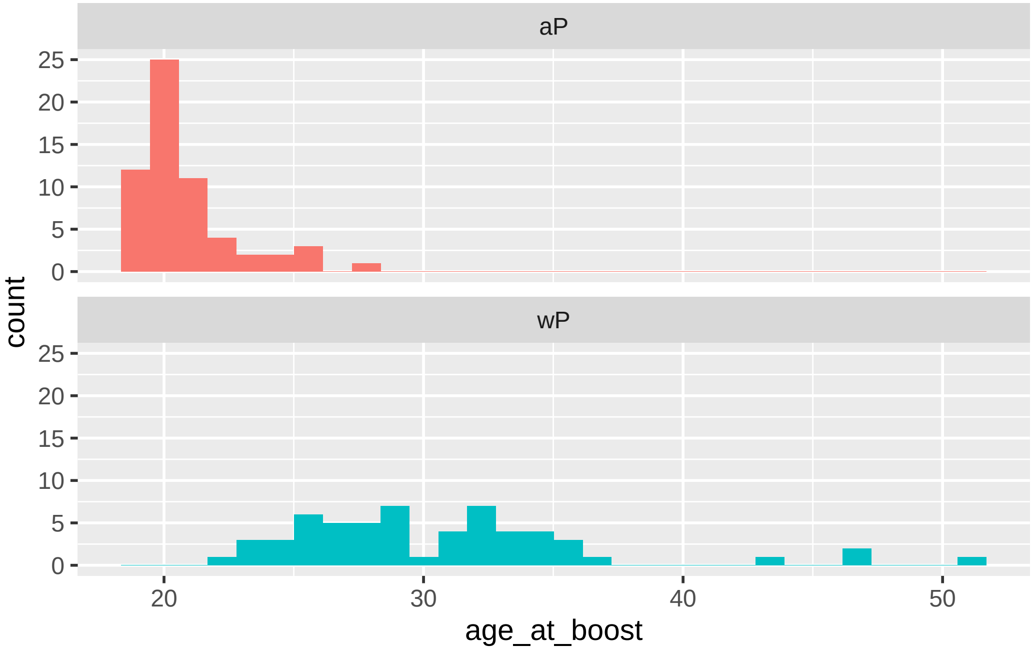
```
ggplot(subject) +
  aes(time_length(age, "year"),
      fill=as.factor(infancy_vac)) +
  geom_histogram(show.legend=FALSE) +
  facet_wrap(vars(infancy_vac), nrow=2)
```

```
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



```
ggplot(subject) +
  aes(age_at_boost,
      fill=as.factor(infancy_vac)) +
  geom_histogram(show.legend=FALSE) +
  facet_wrap(vars(infancy_vac), nrow=2)
```

```
`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] 6.813505e-19
```

This is 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-(2nd). 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 13855 days
2 13855 days
3 13855 days
4 13855 days
5 13855 days
6 13855 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 with `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?

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

```

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

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

```
table(abdata$dataset)
```

```

2020_dataset 2021_dataset 2022_dataset 
       31520         8085         2205 
```

The number of visits gradually decreased.

## Examine IgG1 Ab titer levels

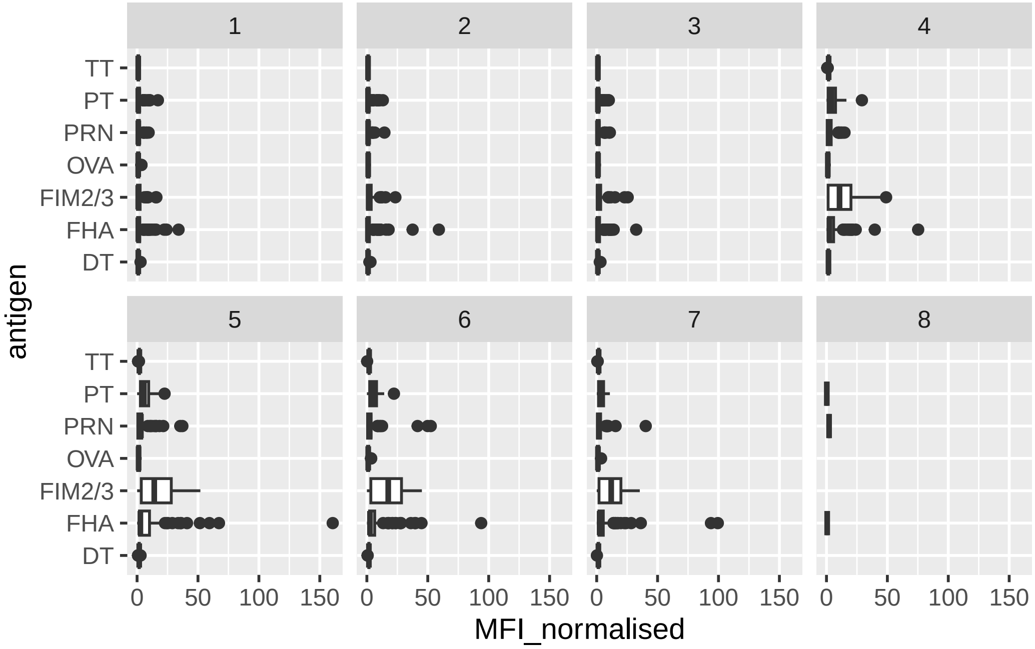
```
igg <- abdata %>% filter(isotype == "IgG")
head(igg)
```

```
  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 13855 days
2 13855 days
3 13855 days
4 14951 days
5 14951 days
6 14951 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() +
  facet_wrap(vars(visit), nrow=2)
```

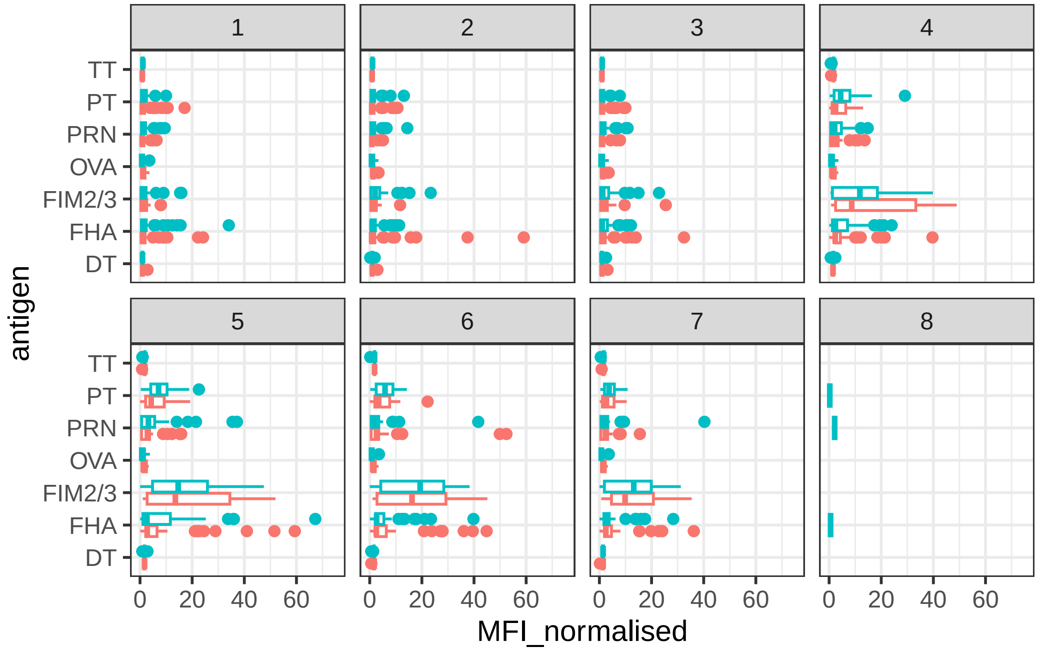


Q14. What antigens show differences in the level of IgG antibody titers recognizing them over time?

FIM2/3 show differences.

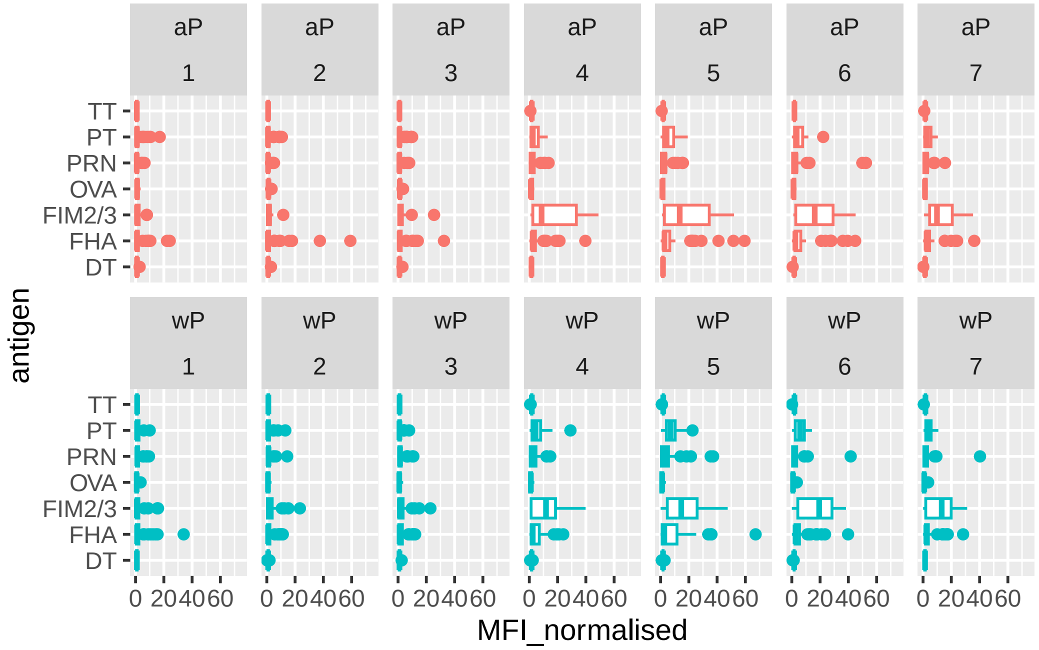
```
ggplot(igg) +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  xlim(0,75) +
  theme_bw()
```

```
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)
```

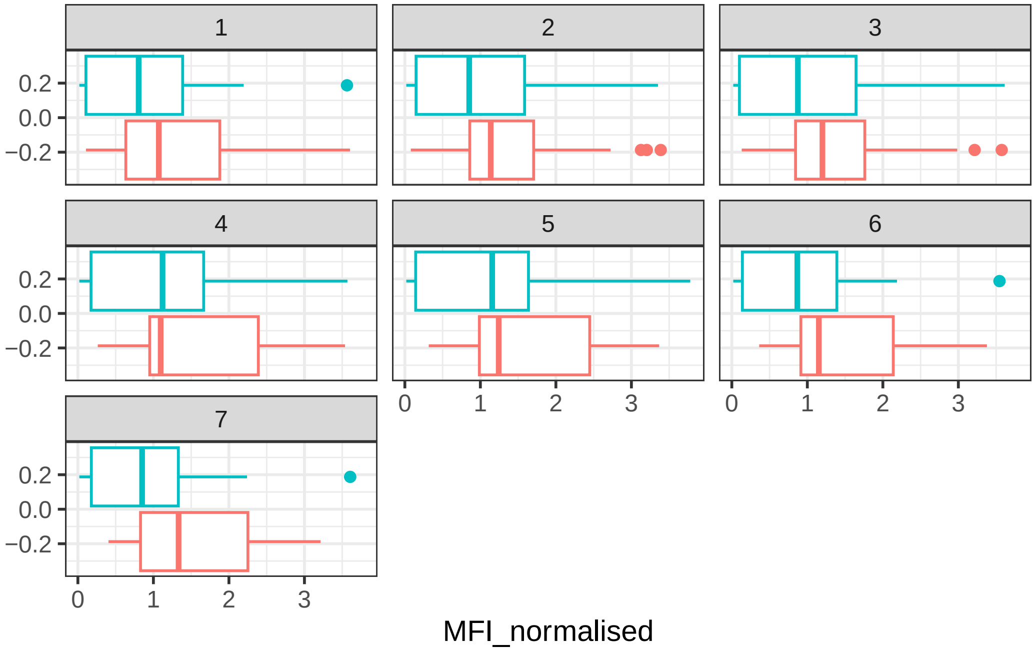
```
Warning: Removed 5 rows containing non-finite values (`stat_boxplot()`).
```



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).

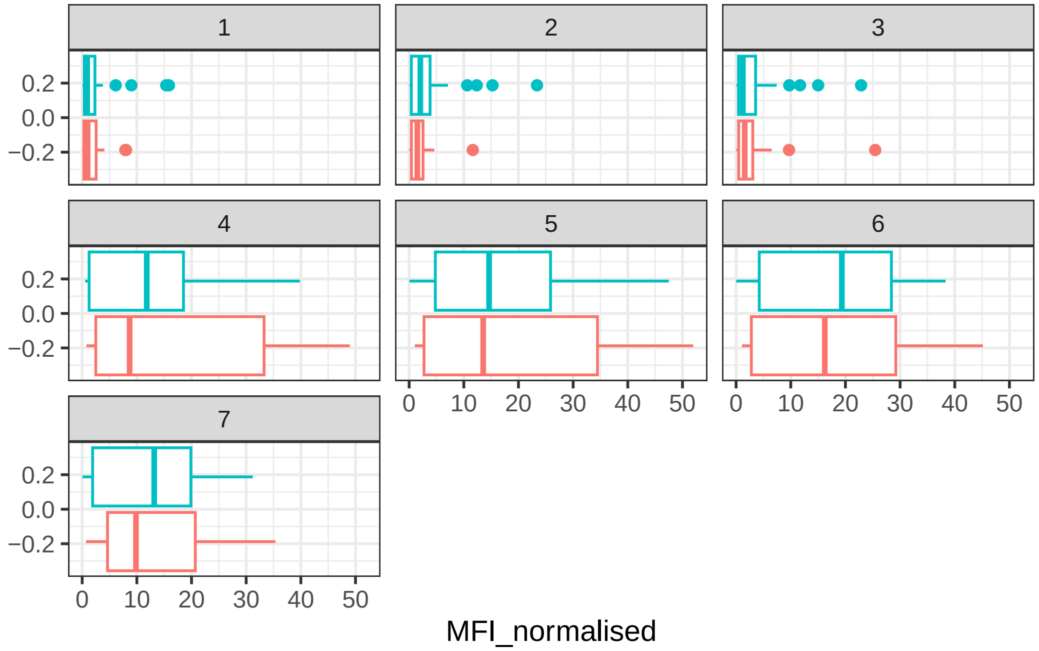
```
filter(igg, antigen=="OVA") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = "antigen") +
  facet_wrap(vars(visit)) +
  theme_bw()
```

```
Warning: `show.legend` must be a logical vector.
```



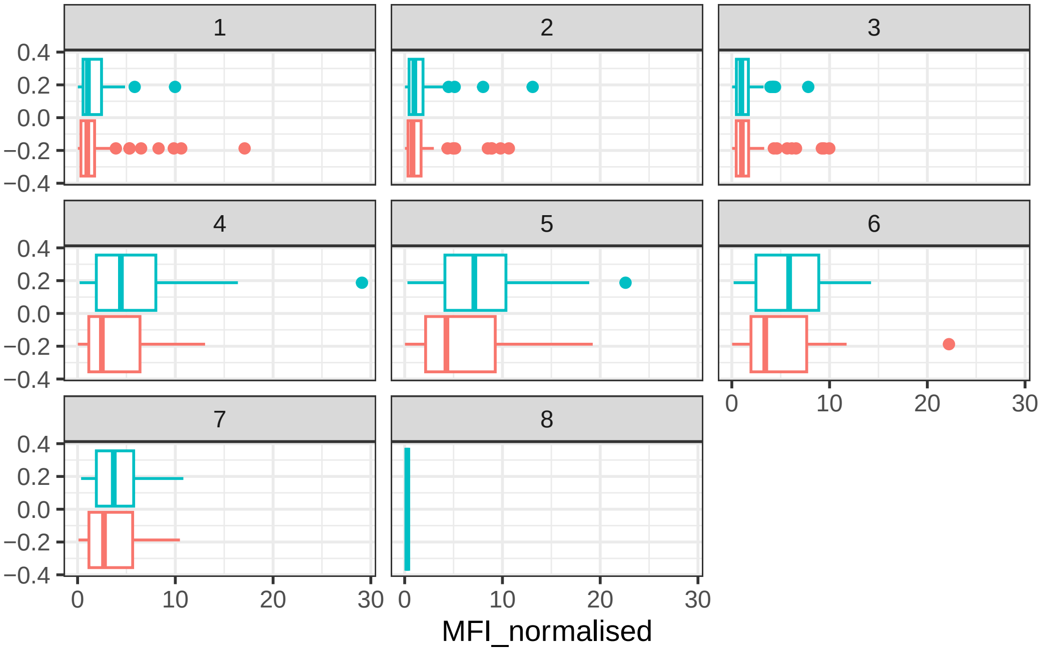
```
filter(igg, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = "antigen") +
  facet_wrap(vars(visit)) +
  theme_bw()
```

```
Warning: `show.legend` must be a logical vector.
```



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

```
Warning: `show.legend` must be a logical vector.
```

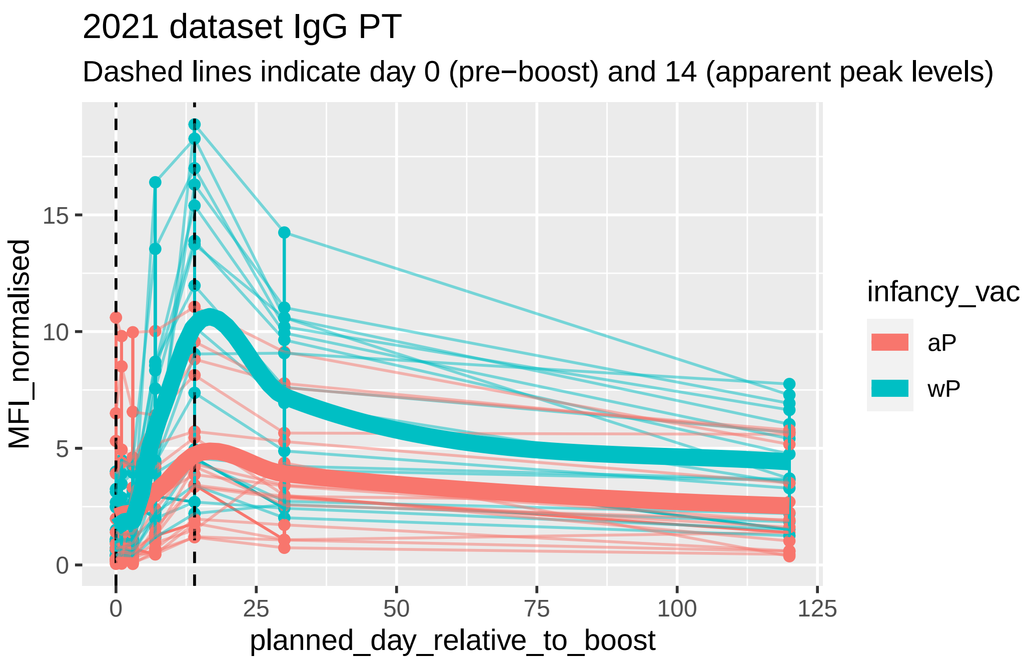


Select (or filter) for the 2021 dataset and isotype IgG. I want a time course (‘planned_day_relative_to_boost’) of IgG levels (‘MFI_normalised’) for “PT” antigen.

```
#abdata$planned_day_relative_to_boost
```

```
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) +
    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)")
  geom_line(aes(group=subject_id), linewidth= 0.5, alpha= 0.5) +
  geom_smooth(se=F, span = 0.4, linewidth=3)
```

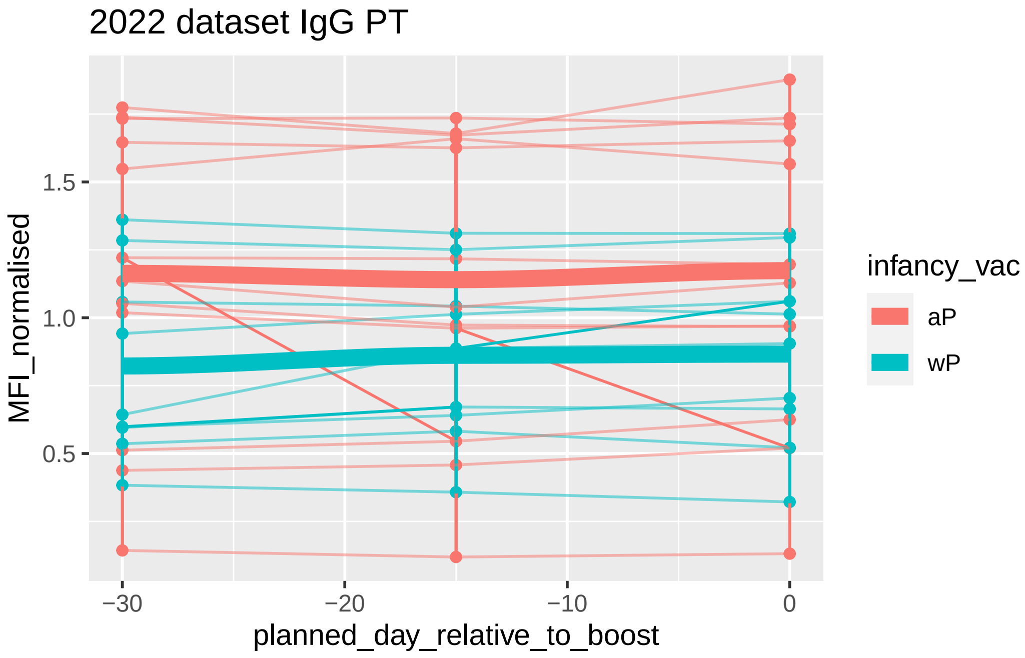
```
`geom_smooth()` using method = 'loess' and formula = 'y ~ x'

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: pseudoinverse used at -0.6

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: neighborhood radius 3.6

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: reciprocal condition number 1.7596e-16

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: There are other near singularities as well. 11364

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: pseudoinverse used at -0.6

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: neighborhood radius 3.6

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: reciprocal condition number 1.6196e-16

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: There are other near singularities as well. 11364
```



```
abdata.22 <- abdata %>% filter(dataset == "2022_dataset")

abdata.22 %>%
  filter(isotype == "IgG",  antigen == "PT") %>%
  ggplot() +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
        col=infancy_vac) +
    geom_point() +
    geom_line() +
  labs(title="2022 dataset IgG PT") +
  geom_line(aes(group=subject_id), linewidth= 0.5, alpha= 0.5) +
  geom_smooth(se=F, span = 0.4, linewidth=3)
```

```
`geom_smooth()` using method = 'loess' and formula = 'y ~ x'

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: pseudoinverse used at -30.15

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: neighborhood radius 15.15
```

```
Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: reciprocal condition number 0

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: There are other near singularities as well. 229.52

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: pseudoinverse used at -30.15

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: neighborhood radius 15.15

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: reciprocal condition number 0

Warning in simpleLoess(y, x, w, span, degree = degree, parametric = parametric,
: There are other near singularities as well. 229.52
```



### Obtaining CMI-PB RNAseq data

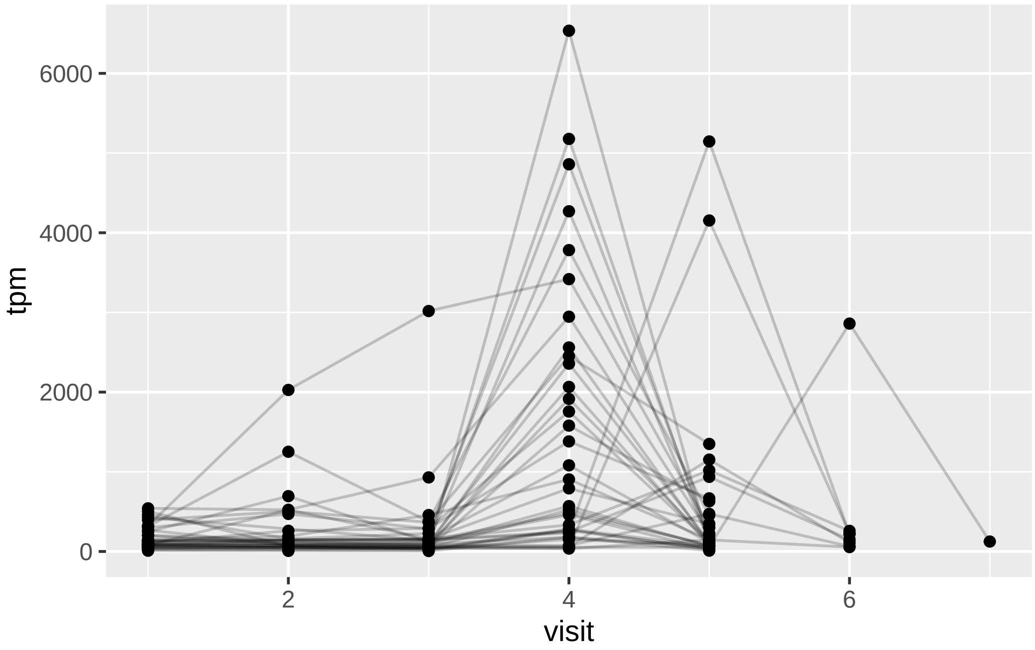
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.

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)
```


tpm
visit