

class19 Pertussis and the CMI-PB project

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Pertussis is a a sever lung infection also known a whooping cough.

We will begin by investigating the number of Pertussis cases per year in the US.

This data is availabe on the CDC website [here](#)

```
#1 echo=FALSE

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)
```

```
)
```

Lets have a wee look at this table

```
head(cdc)
```

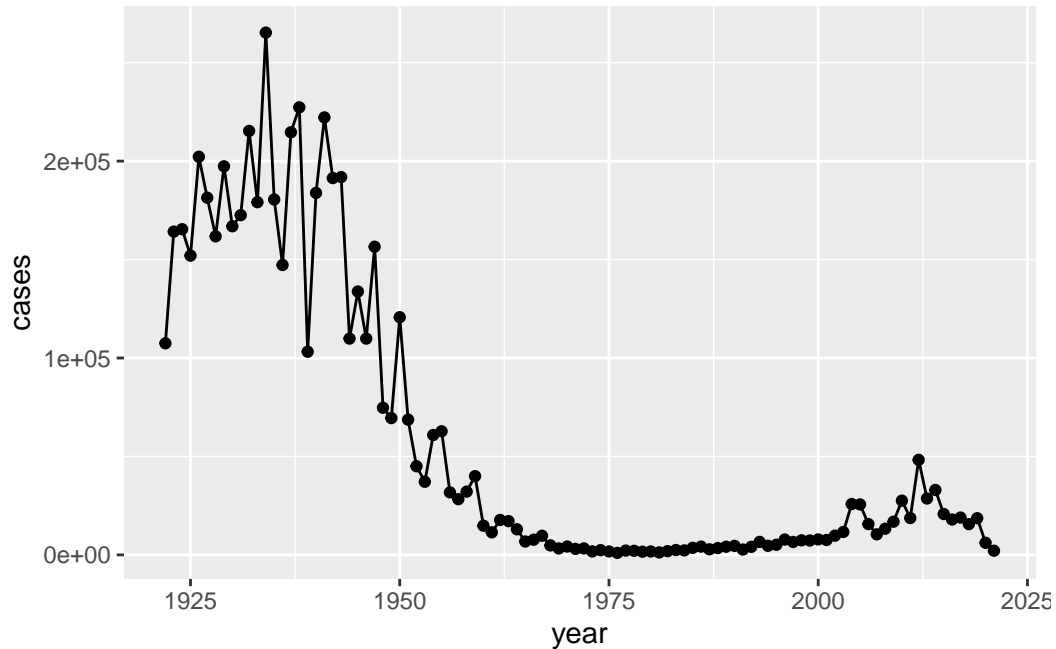
```
year  cases
1 1922 107473
2 1923 164191
3 1924 165418
4 1925 152003
5 1926 202210
6 1927 181411
```

I want a nice plot of the number of cases per year.

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 = year, y = cases) +
  geom_point() +
  geom_line()
```



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?

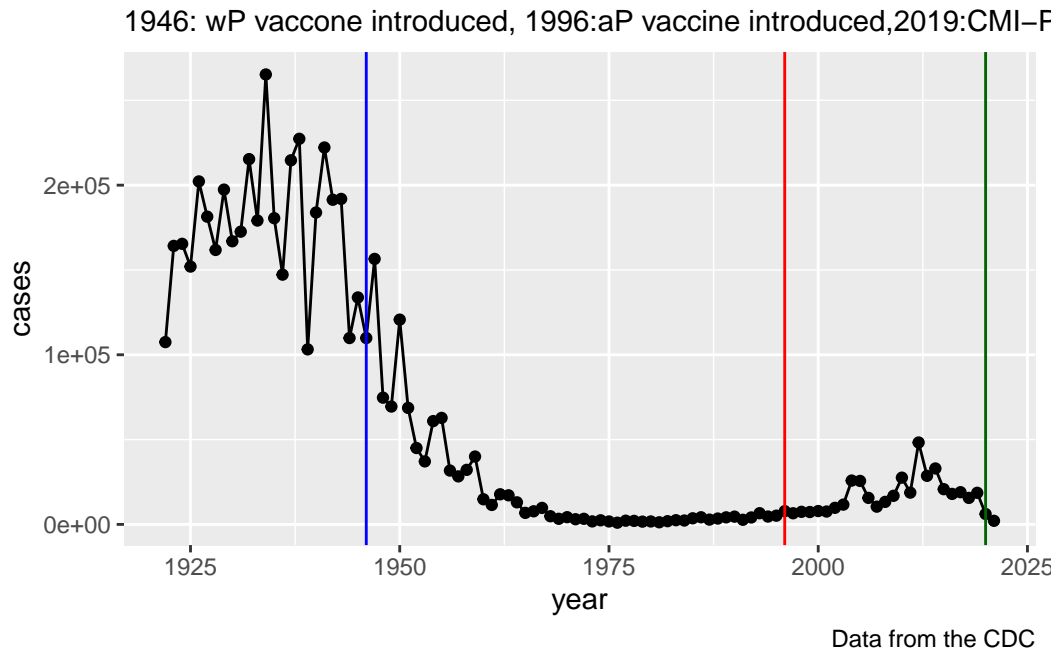
```
ggplot(cdc) +
  aes(x = year, y = cases) +
  geom_point() +
  geom_line() +
  geom_vline(xintercept = 1946, Linetype="dashed", col="blue") +
  geom_vline(xintercept = 1996, Linetype="dashed", col="red") +
  geom_vline(xintercept = 2020, Linetype="dashed", col="darkgreen") +
  labs(title="Pertussis cases per year in the US",
       subtitle="1946: wP vaccine introduced, 1996:aP vaccine introduced, 2019:CMI-PB project",
       caption="Data from the CDC")
```

Warning in `geom_vline(xintercept = 1946, Linetype = "dashed", col = "blue")`:
Ignoring unknown parameters: ``Linetype``

Warning in `geom_vline(xintercept = 1996, Linetype = "dashed", col = "red")`:
Ignoring unknown parameters: ``Linetype``

Warning in `geom_vline(xintercept = 2020, Linetype = "dashed", col =`

```
"darkgreen"): Ignoring unknown parameters: `Linetype`
```



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

There is a lag and then cases rise with a ~ 3 year cycle perhaps similar to that observed prior to the first wP vaccine introduction.

3.Exploring CMI-PB data

Why is this vaccine-preventable disease on the upswing? To answer this question we need to investigate the mechanisms underlying waning protection against pertussis. This requires evaluation of pertussis-specific immune responses over time in wP and aP vaccinated individuals.

This is the goals of the CMI-PB project: <https://www.cmi-pb.org/>

The CMI-PB project makes its data available via “API-endpoint” that return JSON format.

We will use the ‘jsonlite package to access this data. The main function in this package is called ‘read_json()’

```
library(jsonlite)
```

```
# Subject table
```

```
subject <- read_json("https://www.cmi-pb.org/api/subject", simplifyVector = TRUE)
specimen <- read_json("http://cmi-pb.org/api/specimen", simplifyVector = TRUE)
titer <- read_json("http://cmi-pb.org/api/v4/plasma_ab_titer", simplifyVector = TRUE )
```

Have a wee peak at these new object

```
head(subject)
```

	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
4	4	wP	Male Not Hispanic or Latino	Asian	
5	5	wP	Male Not Hispanic or Latino	Asian	
6	6	wP	Female Not Hispanic or Latino	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
4	1988-01-01	2016-08-29	2020_dataset
5	1991-01-01	2016-08-29	2020_dataset
6	1988-01-01	2016-10-10	2020_dataset

```
head(titer)
```

	specimen_id	isotype	is_antigen_specific	antigen	MFI	MFI_normalised
1	1	IgE	FALSE	Total	1110.21154	2.493425
2	1	IgE	FALSE	Total	2708.91616	2.493425
3	1	IgG	TRUE	PT	68.56614	3.736992
4	1	IgG	TRUE	PRN	332.12718	2.602350
5	1	IgG	TRUE	FHA	1887.12263	34.050956
6	1	IgE	TRUE	ACT	0.10000	1.000000

	unit	lower_limit_of_detection
1	UG/ML	2.096133
2	IU/ML	29.170000
3	IU/ML	0.530000
4	IU/ML	6.205949

```
5 IU/ML          4.679535
6 IU/ML          2.816431
```

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

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

```
aP wP
60 58
```

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

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

```
Female  Male
    79    39
```

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

```
#1 echo=FALSE, message=FALSE,warning=FALSE
library(tidyverse)
```

```
-- Attaching core tidyverse packages ----- tidyverse 2.0.0 --
v dplyr      1.1.4      v readr      2.1.4
v forcats    1.0.0      v stringr    1.5.1
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 purrr::flatten() masks jsonlite::flatten()
x dplyr::lag()     masks stats::lag()
i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become
```

working with dates

dates can really suck to work with and do math. The lubridate package makes this much easier. It is part of the **tidyverse** that includes dplyr, ggplots, etc

```
today()
```

```
[1] "2023-12-05"
```

```
today() - mdy("11-23-2001")
```

Time difference of 8047 days

```
time_length( today() - ymd("2001-11-23"), "years")
```

```
[1] 22.03149
```

Now add the age of each subject to the subject table.

```
subject$age <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)

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

head(subject)
```

	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
4	4	wP	Male	Not Hispanic or Latino	Asian
5	5	wP	Male	Not Hispanic or Latino	Asian
6	6	wP	Female	Not Hispanic or Latino	White

	year_of_birth	date_of_boost	dataset	age	age_years
1	1986-01-01	2016-09-12	2020_dataset	11212 days	30.69678
2	1968-01-01	2019-01-28	2020_dataset	18655 days	51.07461
3	1983-01-01	2016-10-10	2020_dataset	12336 days	33.77413
4	1988-01-01	2016-08-29	2020_dataset	10468 days	28.65982
5	1991-01-01	2016-08-29	2020_dataset	9372 days	25.65914
6	1988-01-01	2016-10-10	2020_dataset	10510 days	28.77481

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?

The mean age for the aP group was 21, while for the wP group, it was 31, indicating a difference of 10 years. Both the median and mean for the wP group exceeded those for the aP group.

```
library(dplyr)

ap <- subject %>% filter(infancy_vac == "aP")

round( summary( time_length( ap$age, "years" ) ) )
```

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
19	20	20	21	21	28

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

wp_age_summary <- round(summary(time_length(wp$age, "years")))
print(wp_age_summary)
```

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
23	26	29	31	34	51

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


Welch Two Sample t-test

```
data: ap$age and wp$age
t = -12.088 days, df = 69.647, p-value < 2.2e-16
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -4186.413 days -3000.486 days
sample estimates:
Time differences in days
mean of x mean of y
 7608.533 11201.983
```

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

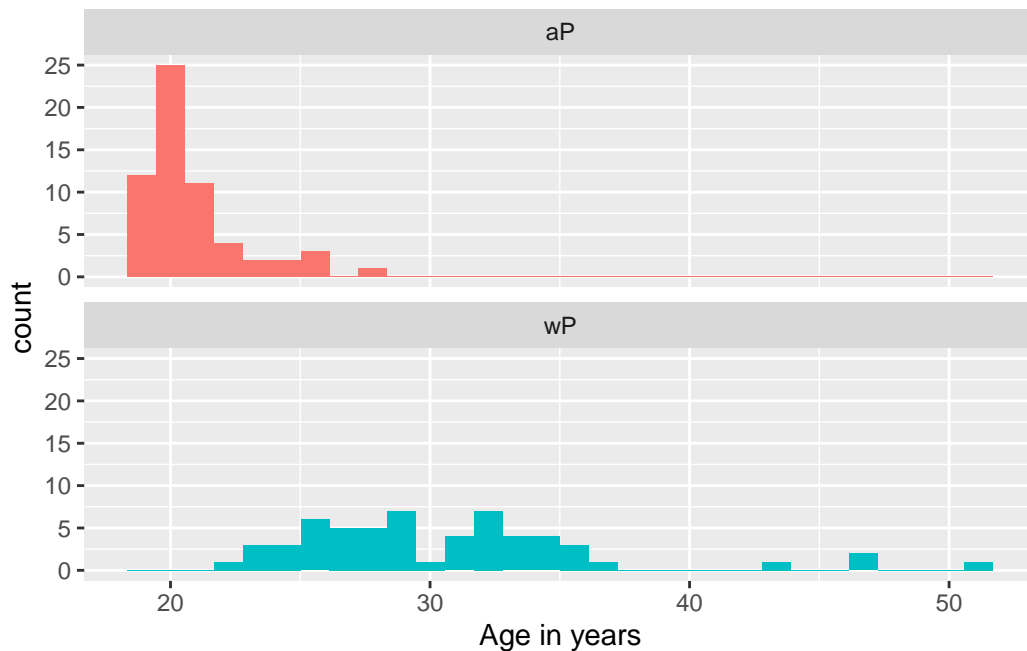
```
int <- ymd(subject$date_of_boost) - ymd(subject$year_of_birth)
age_at_boost <- time_length(int, "year")
head(age_at_boost)
```

```
[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`.



```
# Or use wilcox.test()
x <- t.test(time_length( wp$age, "years" ),
            time_length( ap$age, "years" ))

x$p.value
```

```
[1] 9.121472e-19
```

Merge or join tables

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 <- inner_join(specimen,subject)
```

Joining with `by = join_by(subject_id)`

```
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	11212 days	30.69678
2	11212 days	30.69678
3	11212 days	30.69678
4	11212 days	30.69678
5	11212 days	30.69678
6	11212 days	30.69678

Antiboday measurments in the blood

Q10. Now using the same procedure join meta with titer data so we can further analyze this data in terms of time of

```
abdata <- inner_join(titer, meta)
```

Joining with `by = join_by(specimen_id)`

```
dim(abdata)
```

```
[1] 41810    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 3240 7968 7968 7968 7968

```

Q12. What are the different \$dataset values in abdata and what do you notice about the number of rows for the most “recent” dataset?

```

dataset_counts <- table(abdata$dataset)

print(dataset_counts)

```

```

2020_dataset 2021_dataset 2022_dataset
      31520         8085         2205

```

```
abdata <- inner_join(titer, meta)
```

Joining with `by = join_by(specimen_id)`

```
head(abdata)
```

	specimen_id	isotype	is_antigen_specific	antigen	MFI	MFI_normalised
1	1	IgE	FALSE	Total	1110.21154	2.493425
2	1	IgE	FALSE	Total	2708.91616	2.493425
3	1	IgG	TRUE	PT	68.56614	3.736992
4	1	IgG	TRUE	PRN	332.12718	2.602350
5	1	IgG	TRUE	FHA	1887.12263	34.050956
6	1	IgE	TRUE	ACT	0.10000	1.000000

	unit	lower_limit_of_detection	subject_id	actual_day_relative_to_boost
1	UG/ML	2.096133	1	-3
2	IU/ML	29.170000	1	-3
3	IU/ML	0.530000	1	-3
4	IU/ML	6.205949	1	-3
5	IU/ML	4.679535	1	-3

```

6 IU/ML                2.816431                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      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 11212 days  30.69678
2 11212 days  30.69678
3 11212 days  30.69678
4 11212 days  30.69678
5 11212 days  30.69678
6 11212 days  30.69678

```

Q.How many isotypes are we measuring for all these individuals?

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

```

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

```

Lets focus on one of these IgG

```

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

```

```

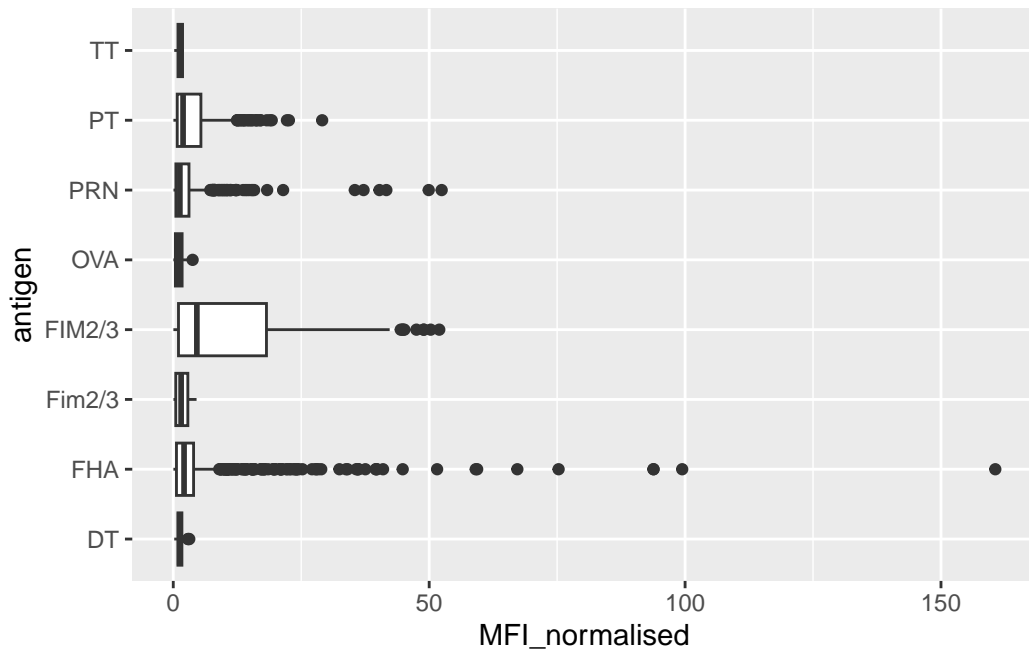
specimen_id isotype is_antigen_specific antigen      MFI MFI_normalised
1          1      IgG                TRUE      PT   68.56614        3.736992
2          1      IgG                TRUE     PRN  332.12718        2.602350
3          1      IgG                TRUE     FHA 1887.12263       34.050956
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	11212 days	30.69678				
2	11212 days	30.69678				
3	11212 days	30.69678				
4	12336 days	33.77413				
5	12336 days	33.77413				
6	12336 days	33.77413				

Box plot of MFI_normalised vs antigen

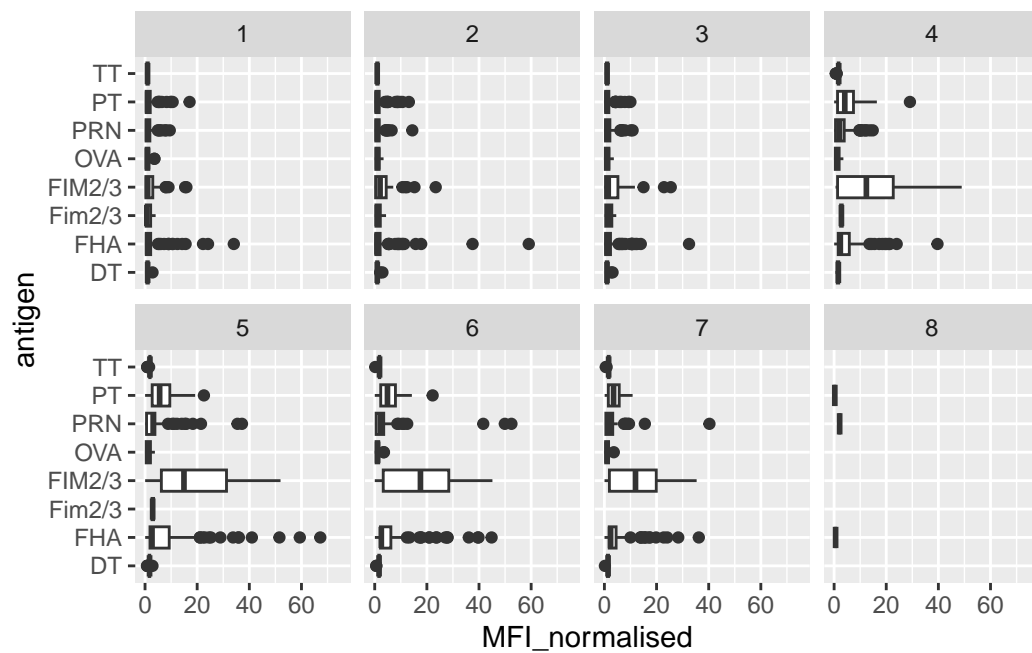
```
ggplot(igg)+
  aes(MFI_normalised ,
      antigen) +
  geom_boxplot()
```



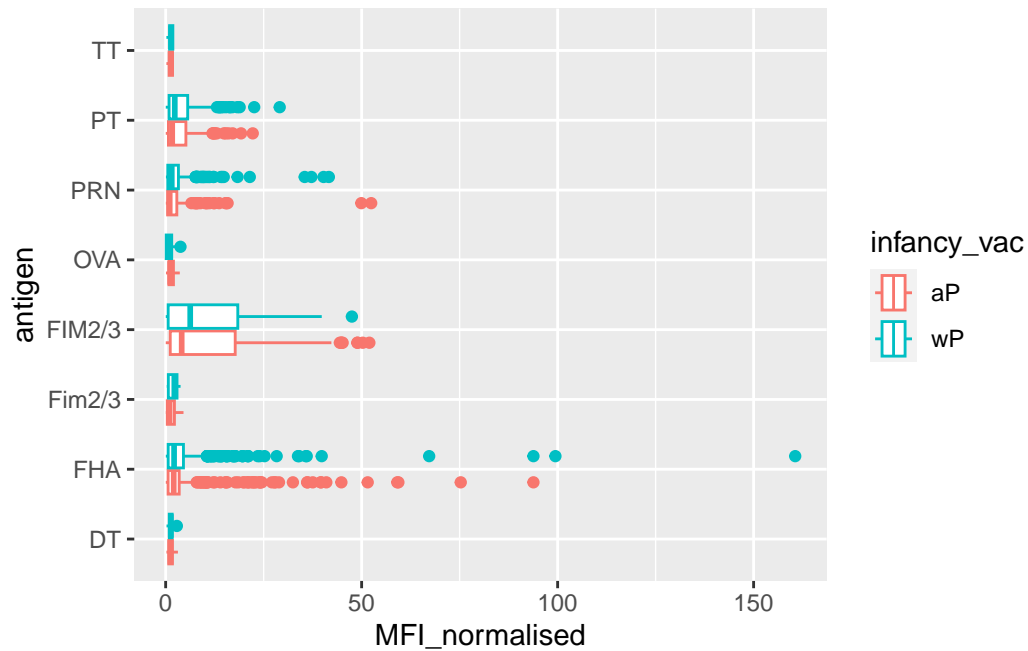
Q13. Complete the following code to make a summary boxplot of Ab titer levels (MFI) for all antigens:

```
ggplot(igg)+
  aes(MFI_normalised ,
      antigen) +
  geom_boxplot() +
  xlim(0,75) +
  facet_wrap(vars(visit),nrow = 2)
```

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

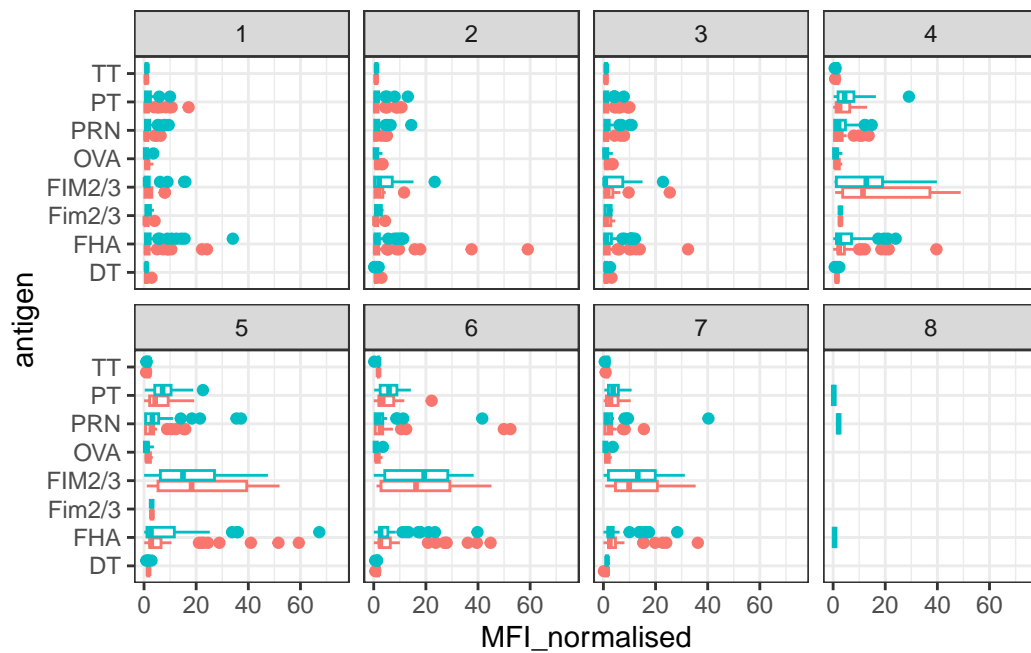


```
ggplot(igg)+
  aes(MFI_normalised ,
      antigen,
      col=infancy_vac) +
  geom_boxplot()
```

```
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()`).

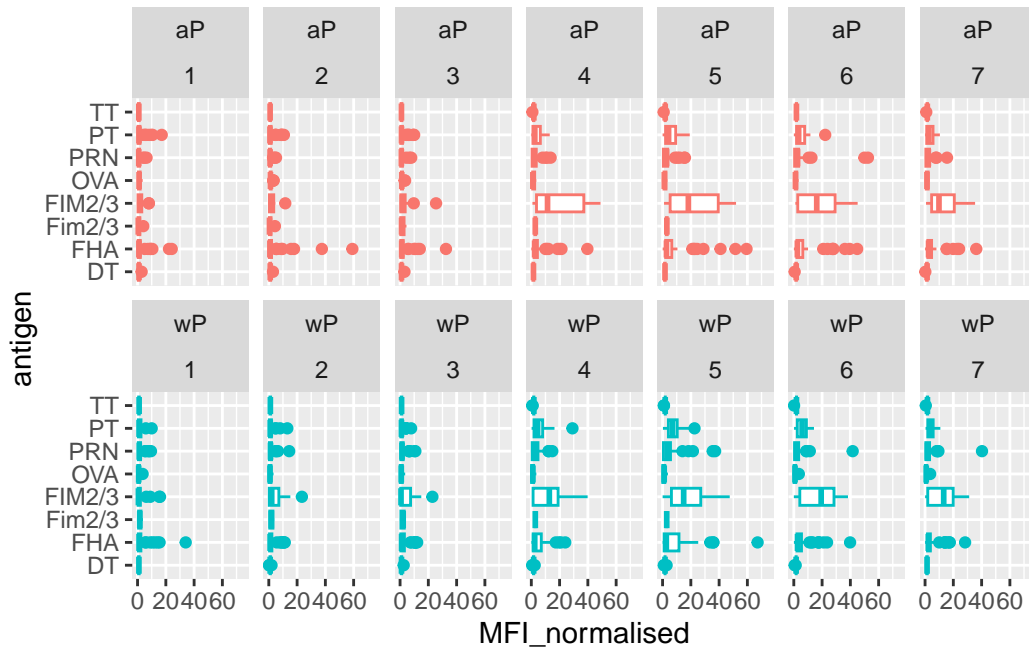


```

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)

```

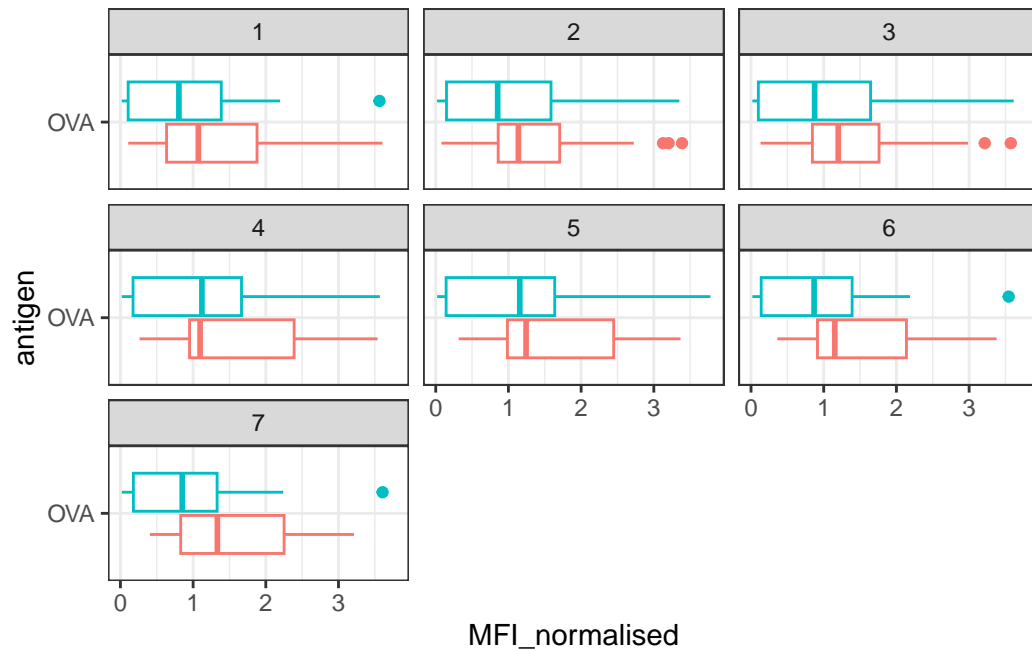
Warning: Removed 5 rows containing non-finite values (`stat_boxplot()`).



Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can choose 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*).

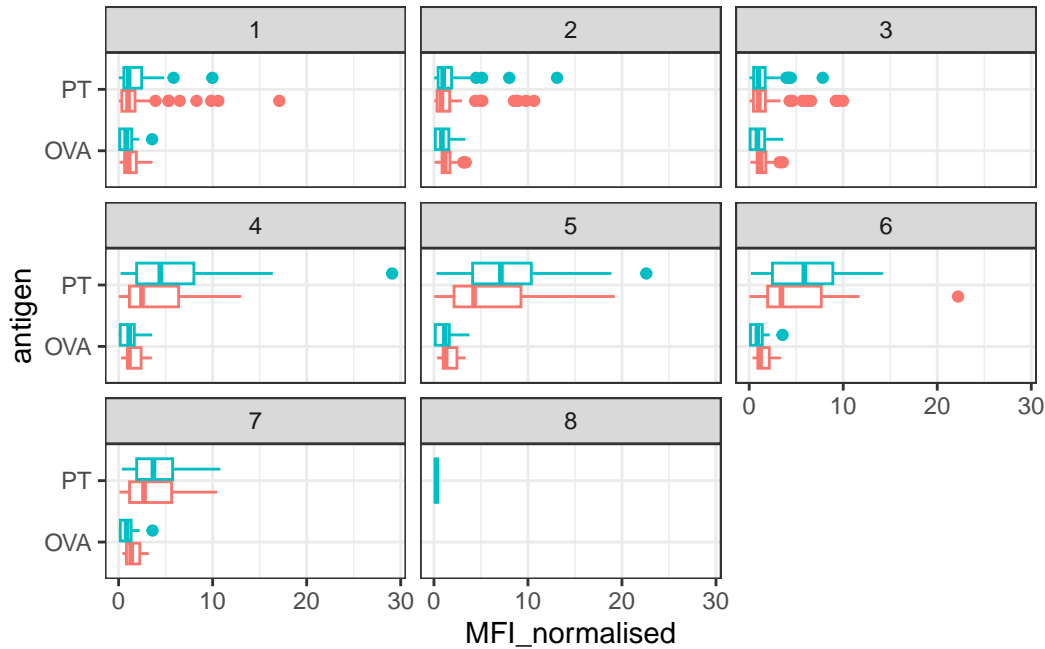
```
filter(igg, antigen=="OVA") %>%

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



```
filtered_igg <- filter(igg, antigen %in% c("OVA", "PT"))
```

```
ggplot(filtered_igg) +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw()
```



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

Over time, both aP and wP vaccines elicited immune responses to PT antigens, with aP vaccines (red) generally showing a higher median response than the wP vaccine (teal), potentially indicating a stronger immune reaction to the acellular pertussis vaccine. Additionally, the OVA antigen levels remained consistent over time for both vaccine types, confirming its suitability as a control.

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

The aP vaccine response (red) shows a generally higher median level of MFI compared to the wP vaccine (teal). The response to PT increased over time for both vaccines, peaking at around 5, but the peak for aP seems to be higher, perhaps the aP vaccine has a stronger response. aP and wP vaccines show very similar trends of increase and decrease. Also, both vaccine types showed outliers, indicating individual differences in immune response.

```
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	11212 days	30.69678
2	11212 days	30.69678
3	11212 days	30.69678
4	12336 days	33.77413
5	12336 days	33.77413
6	12336 days	33.77413

Focus in on IgG to the pertussis Toxin(PT) antigen in the 2021 dataset

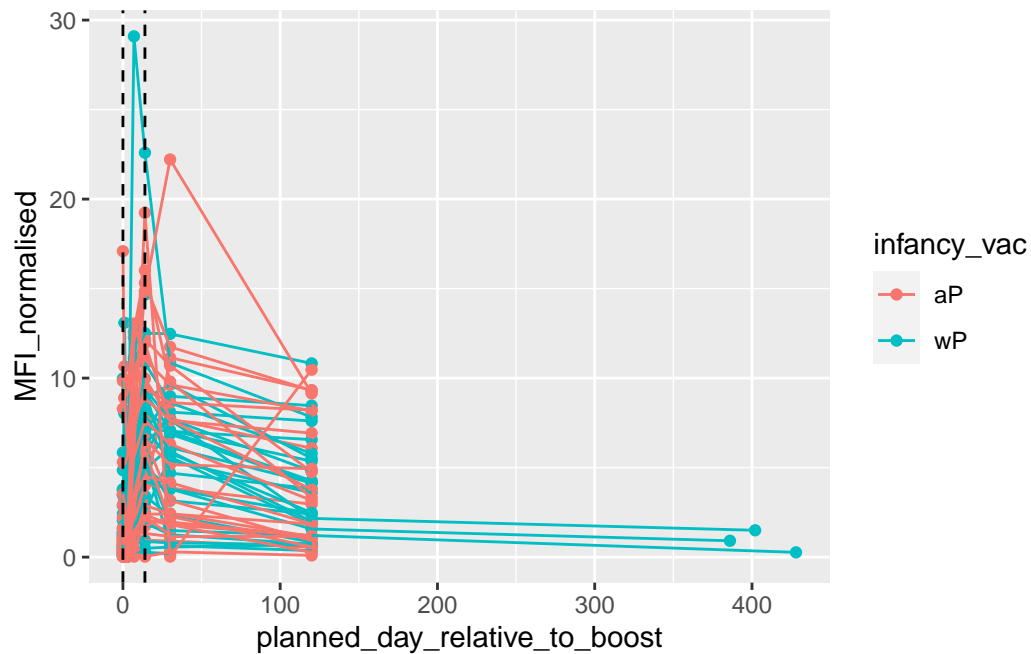
```

igg.pt <- igg %>% filter(antigen == "PT", dataset=="2020_dataset")

ggplot(igg.pt) +
  aes(planned_day_relative_to_boost,
      MFI_normalised,
      col=infancy_vac,
      group=subject_id) +

```

```
geom_point() +
geom_line() +
geom_vline(xintercept = 0, linetype = "dashed", col = "black") +
geom_vline(xintercept = 14, linetype = "dashed", col = "black")
```



```
labs(title="2020 dataset IgG PT",
      subtitle = "Dashed lines indicate day 0 (pre-boost) and 14 (apparent peak levels)")
```

```
$title
```

```
[1] "2020 dataset IgG PT"
```

```
$subtitle
```

```
[1] "Dashed lines indicate day 0 (pre-boost) and 14 (apparent peak levels)"
```

```
attr(,"class")
```

```
[1] "labels"
```

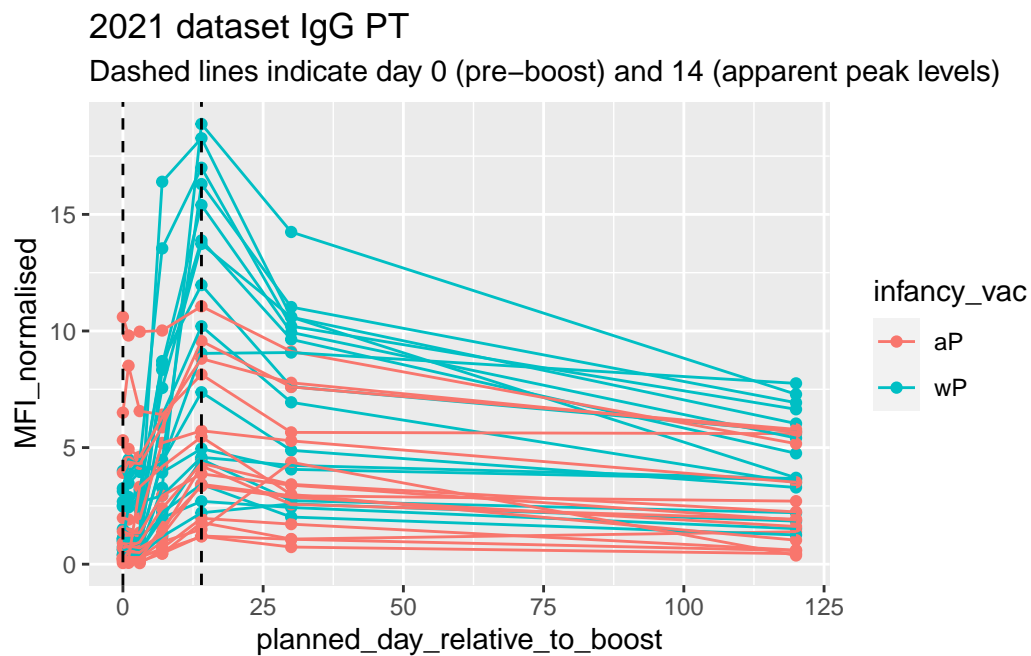
```
abdata.21 <- abdata %>% filter(dataset == "2021_dataset")
```

```
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?

No, they looks not similar.