Prelim-Data Analysis Semester Project

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Data Description

The dataset used for this analysis is from the second study of my MSc. thesis titled "Evaluation of the Efficacy of Bovine Adenovirus-Vectored Avian Influenza Vaccine in Poultry,". This study investigates how different mucosal vaccine routes influence protection against avian influenza. For this preliminary analysis, I used qPCR viral-load data **(Ct values and log10 genomic equivalents)** from tracheal swabs collected at three time points—2, 4, and 6 days post-challenge (DPC2, DPC4, DPC6)—in chickens that received a single dose of the BAdV-H5HA+H7NP vaccine ($1x10^8$pfu) in a prime-booster dose vaccine administration via two routes —**intraocular (IO)** and **intramuscular (IM)**—and challenged with two avian influenza virus strains (**H5N1** and **H7N2**).

Each Excel sheet corresponds to one **Virus × Timepoint** combination (e.g., DPC2-H5N1).
Key variables:

- **Vaccine_Group**: Mock, Mock Challenge, Empty-Vector, BAds-AIV
- **Route**: IM (intramuscular) or IO (intraocular)
- **Bird_ID**: unique sample identifier
- **Ct**: qPCR cycle threshold (continuous)
- **log10GE**: $\log_{10}$ genome equivalents/mL (continuous)

Since "Mock" birds were not challenged, they are excluded.
Data are nested: **Bird_IDs** are nested within **Vaccine_Group × Route** combinations, and each sheet (timepoint) is nested within each **Virus**.

```
#LOADING LIBRARIES
library(tidyverse)
```

```
── Attaching core tidyverse packages ──────────────────────── tidyverse 2.0.0 ──
```

```
✔ dplyr     1.1.4     ✔ readr     2.1.5
✔ forcats   1.0.0     ✔ stringr   1.5.1
✔ ggplot2   4.0.0     ✔ tibble    3.3.0
✔ lubridate 1.9.4     ✔ tidyr     1.3.1
✔ purrr     1.1.0
── Conflicts ─────────────────────────────────────────
tidyverse_conflicts() ──
✖ dplyr::filter() masks stats::filter()
✖ dplyr::lag()    masks stats::lag()
ℹ Use the conflicted package (<http://conflicted.r-lib.org/>)
to force all conflicts to become errors
```

```
library(stringr)
library(readxl)
library(dplyr)
library(tidyr)
library(ggplot2)
library(readr)   # for parse_number()
library(purrr)
library(broom)
library(multcomp) # for Tukey
```

```
Loading required package: mvtnorm
Loading required package: survival
Loading required package: TH.data
Loading required package: MASS

Attaching package: 'MASS'

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

    select


Attaching package: 'TH.data'

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

    geyser
```

```
library(car)
```

```
Loading required package: carData
```

```
Attaching package: 'car'

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

    recode

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

    some
```

```
library(emmeans)
```

```
Welcome to emmeans.
Caution: You lose important information if you filter this
package's results.
See '? untidy'
```

```
library(multcompView)
library(ggpubr)
library(glm2)
```

```
Attaching package: 'glm2'

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

    crabs

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

    heart
```

```
library(glmertree)
```

```
Loading required package: lme4
Loading required package: Matrix

Attaching package: 'Matrix'

The following objects are masked from 'package:tidyr':

    expand, pack, unpack
```

```
Loading required package: partykit
Loading required package: grid
Loading required package: libcoin
```

```
library(glmmTMB)
```

```
Warning in check_dep_version(dep_pkg = "TMB"): package version
mismatch:
glmmTMB was built with TMB package version 1.9.17
Current TMB package version is 1.9.18
Please re-install glmmTMB from source or restore original
'TMB' package (see '?reinstalling' for more information)
```

```
library(lme4)
library(pscl)
```

```
Classes and Methods for R originally developed in the
Political Science Computational Laboratory
Department of Political Science
Stanford University (2002-2015),
by and under the direction of Simon Jackman.
hurdle and zeroinfl functions by Achim Zeileis.
```

```
library(ZIM)
library(TMB)
library(bbmle)
```

```
Loading required package: stats4

Attaching package: 'bbmle'

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

    slice
```

```
library(DHARMa)
```

```
This is DHARMa 0.4.7. For overview type '?DHARMa'. For recent
changes, type news(package = 'DHARMa')
```

```
library(patchwork)
```

```
Attaching package: 'patchwork'

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

    area
```

```
# Loading dataset
# Importing my qPCR excel data file
excel_path <- path.expand("~/Desktop/Entomology tech-Fall 2025/
excel_path <- "ENT_Project_Tracheal_qPCR_Clean.xlsx .xlsx"

ENT_Project_Tracheal_qPCR_Clean_xlsx_ <- read_excel("ENT_Projec

#qPCR data wrangling process
excel_path
```

```
[1] "ENT_Project_Tracheal_qPCR_Clean.xlsx .xlsx"
```

```
#GETTING SHEET NAMES FROM THE CHOSEN FILE
sheets <- readxl::excel_sheets(excel_path)
#READING ALL SHEETS AND BINDING INTO ONE DATA FRAME
qpcr <- purrr::map_dfr(sheets, ~ readxl::read_excel(excel_path,
dplyr::glimpse(qpcr)        #quick peek into the selected data
```

```
Rows: 450
Columns: 8
$ Timepoint     <chr> "DPC2", "DPC2", "DPC2", "DPC2", "DPC2",
"DPC2", "DPC2", …
$ Virus         <chr> "H5N1", "H5N1", "H5N1", "H5N1", "H5N1",
"H5N1", "H5N1", …
$ Vaccine_Group <chr> "Mock", "Mock", "Mock", "Mock", "Mock",
"Mock", "Mock", …
$ Route         <chr> "NA", "NA", "NA", "NA", "NA", "NA",
"NA", "NA", "NA", "N…
$ Challenge     <chr> "No", "No", "No", "No", "No", "No",
"No", "No", "No", "N…
$ Ct            <dbl> 38.867, 0.000, 0.000, 0.000, 0.000,
0.000, 0.000, 0.000,…
$ log10GE       <dbl> 3.807920, 3.250908, 3.250908, 3.250908,
3.250908, 3.2509…
$ Bird_ID       <dbl> 712, 713, 714, 715, 716, 717, 718, 719,
```

```
720, 721, 722, 7…
```

```
# Checking counts per original sheet
qpcr %>% count(Timepoint, Virus)
```

```
# A tibble: 6 × 3
  Timepoint Virus     n
  <chr>     <chr> <int>
1 DPC2      H5N1     77
2 DPC2      H7N2     73
3 DPC4      H5N1     77
4 DPC4      H7N2     73
5 DPC6      H5N1     77
6 DPC6      H7N2     73
```

```
qpcr <- lapply(sheets, function(s) {
  df <- read_excel(excel_path, sheet = s)
  df$Sheet <- s
  return(df)
}) |> bind_rows()
```

```
# Clean and set factors
qpcr <- qpcr %>%
  filter(Vaccine_Group != "Mock") %>%
  mutate(
    Timepoint = factor(Timepoint, levels = c("DPC2","DPC4","DPC
    Virus = factor(Virus, levels = c("H5N1","H7N2")),
    Vaccine_Group = factor(Vaccine_Group, levels = c("Mock Chal
    Route = factor(Route, levels = c("IM","IO")),
    GroupRoute = interaction(Vaccine_Group, Route, sep = ":")
  )

glimpse(qpcr)
```

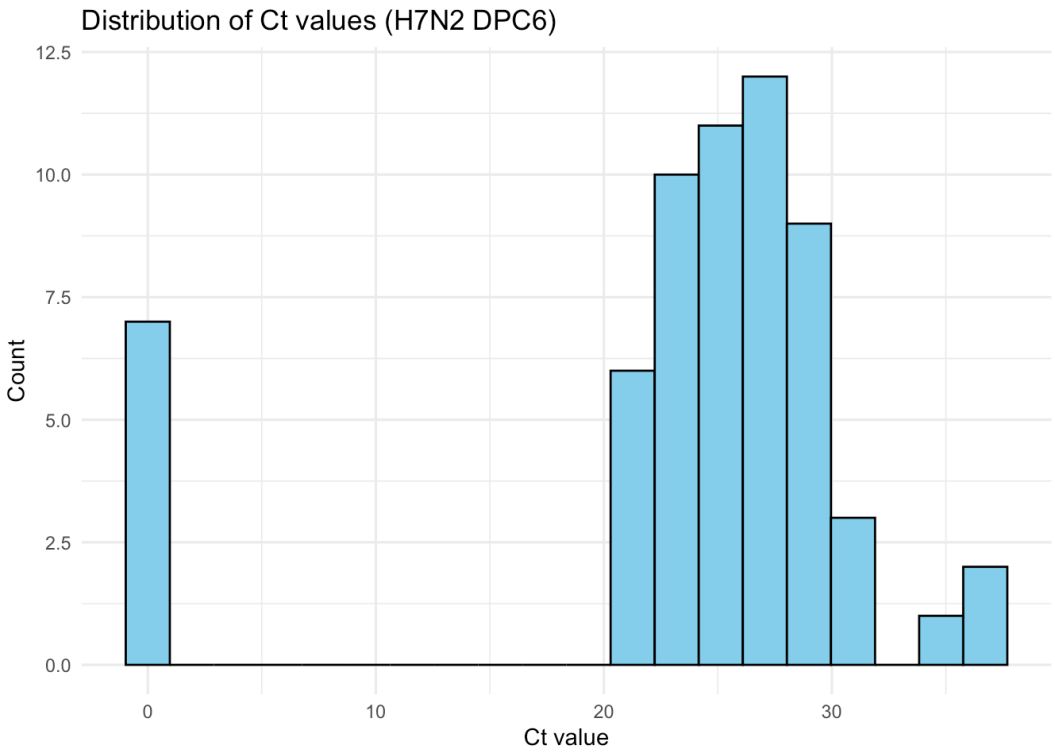
```
Rows: 378
Columns: 10
$ Timepoint     <fct> DPC2, DPC2, DPC2, DPC2, DPC2, DPC2,
DPC2, DPC2, DPC2, DP…
$ Virus         <fct> H5N1, H5N1, H5N1, H5N1, H5N1, H5N1,
H5N1, H5N1, H5N1, H5…
$ Vaccine_Group <fct> Mock Challenge, Mock Challenge, Mock
Challenge, Mock Cha…
```

```
$ Route         <fct> NA, NA, NA, NA, NA, NA, NA, NA, NA, NA,
NA, NA, NA, NA, …
$ Challenge     <chr> "Yes", "Yes", "Yes", "Yes", "Yes",
"Yes", "Yes", "Yes", …
$ Ct            <dbl> 28.294, 29.838, 30.844, 29.953, 29.932,
31.468, 28.799, …
$ log10GE       <dbl> 6.923950, 6.468909, 6.172425, 6.435017,
6.441206, 5.9885…
$ Bird_ID       <dbl> 775, 776, 777, 778, 779, 780, 781, 782,
783, 784, 785, 7…
$ Sheet         <chr> "DPC2-H5N1", "DPC2-H5N1", "DPC2-H5N1",
"DPC2-H5N1", "DPC…
$ GroupRoute    <fct> NA, NA, NA, NA, NA, NA, NA, NA, NA, NA,
NA, NA, NA, NA, …
```

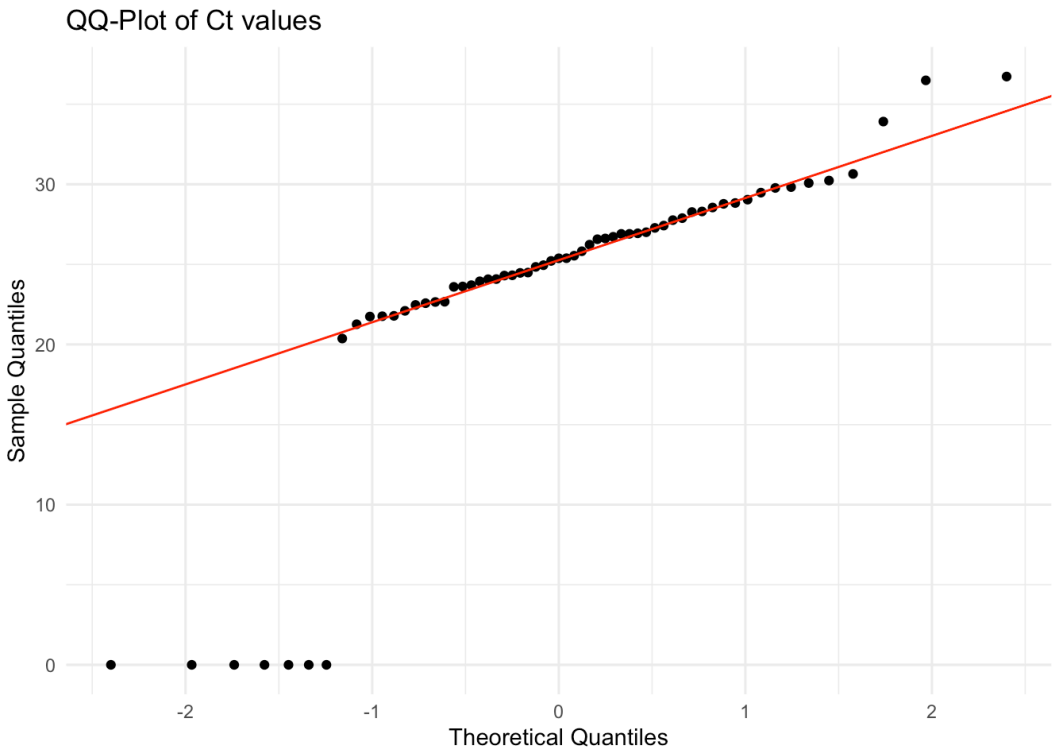
Checking Normality (Distribution)

Before model fitting, I checked whether **Ct** and **log10GE** are approximately normally distributed.

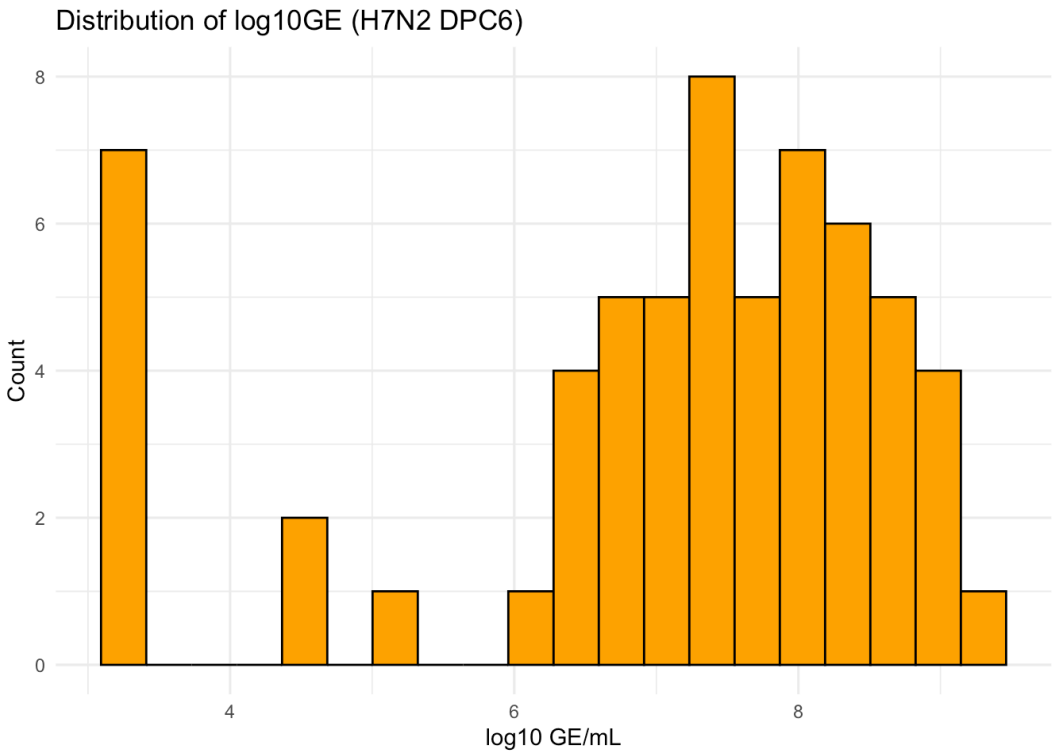
```
# Example: visualize H7N2 DPC6 data only

panel <- qpcr %>%
  filter(Virus == "H7N2", Timepoint == "DPC6")

# Histogram and QQ plot for Ct

ggplot(panel, aes(x = Ct)) +
  geom_histogram(bins = 20, fill = "skyblue", color = "black")
  labs(title = "Distribution of Ct values (H7N2 DPC6)", x = "Ct
  theme_minimal()
```



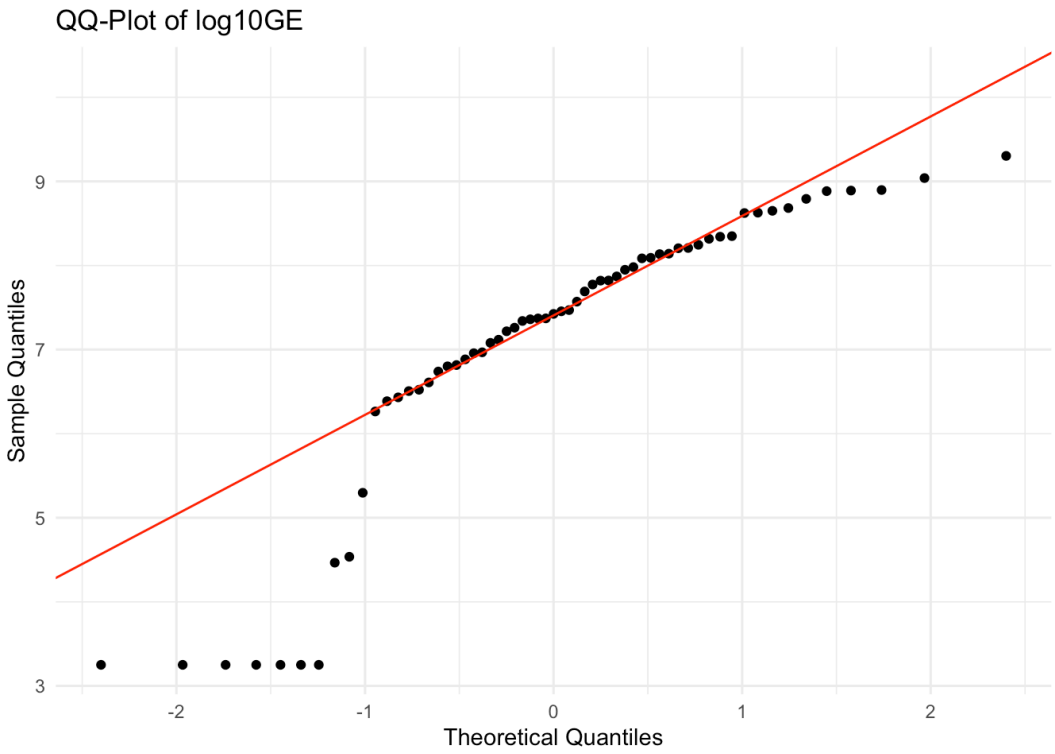

```
ggplot(panel, aes(sample = Ct)) +
  stat_qq() + stat_qq_line(color = "red") +
  labs(title = "QQ-Plot of Ct values", x = "Theoretical Quantil
  theme_minimal()
```




```
# Histogram and QQ plot for log10GE

ggplot(panel, aes(x = log10GE)) +
  geom_histogram(bins = 20, fill = "orange", color = "black") +
  labs(title = "Distribution of log10GE (H7N2 DPC6)", x = "log1
  theme_minimal()
```




```
ggplot(panel, aes(sample = log10GE)) +
  stat_qq() + stat_qq_line(color = "red") +
  labs(title = "QQ-Plot of log10GE", x = "Theoretical Quantiles
  theme_minimal()
```



Interpretation:

The histograms show approximately continuous, right-skewed distributions—typical of qPCR data. log10GE values are closer to normal than raw Ct values but still slightly skewed.

Model Structure and Selection Rationale

Data Nesting: Each measurement is **nested** as follows:

Nesting structure: Bird_ID ⊂ (Vaccine_Group × Route) ⊂ Timepoint ⊂ Virus (i.e., multiple birds belong to each treatment group (combination of vaccine and route), within each virus and timepoint.

Example model formula (for next stage)

Continuous response (log10GE) ~ fixed effects (Vaccine_Group, Route) + random effects (Bird_ID nested within Timepoint). Not yet running this model; just specifying for rationale

model_example <- “lmer(log10GE ~ Vaccine_Group * Route + (1 | Timepoint/Bird_ID), data = qpcr)” model_example

Chosen Model

Since my data contain repeated measurements of viral load (Ct and log10GE) across multiple timepoints, birds, and routes, a **nested or mixed-effects model** is appropriate.

The hierarchical structure (*Bird_ID nested within Timepoint within Virus*) requires random effects to account for correlation. Residuals for log10GE appear approximately normal, so a **Linear Mixed Model (LMM)** with Gaussian error is suitable. So, If future analysis shows strong skewness or heteroscedasticity, a **Tweedie GLMM** will be considered to model zero-inflated or right-skewed data. Thus, I will proceed using a **Gaussian LMM** framework with fixed effects for *Vaccine_Group*, *Route*, and *Virus*, and random effects for *Timepoint* and *Bird_ID*.