Atitkar_BIOF339 Final Project

Experiment/Data Description

I work on Shiga toxin-producing *Escherichia coli* (STEC). Strain 4944 is highly virulent in our mouse model, killing all of the mice by day 5: this virulence is likely mediated by the production of Shiga toxin.

Sub-lethal levels of the antibiotic ciprofloxacin causes induction of toxin in STEC. To better understand the ability of strain 4944 to induce toxin, we tested varying sub-lethal concentrations of ciprofloxacin for its ability to increase toxin production. Toxin production is measured using a cell-based assay and calculating the CD50: a higher CD50 correlates to increased toxin production.

Install Packages

ggplot2: to visualize data

ggpubr: for global statistical analysis/visualization

ggsignif: manually plot significance comparisons

```
install.packages("ggplot2",repos="http://cran.us.r-project.org")
```

```
##
## The downloaded binary packages are in
##  /var/folders/53/gryn6qk92319jmd_3wbyxc9r0000gn/T//Rtmpyfeki7/downloaded_packages
```

```
install.packages("ggpubr",repos="http://cran.us.r-project.org")
```

```
##
## The downloaded binary packages are in
##  /var/folders/53/gryn6qk92319jmd_3wbyxc9r0000gn/T//Rtmpyfeki7/downloaded_packages
```

```
install.packages("ggsignif", repos="http://cran.us.r-project.org")
```

```
##
## The downloaded binary packages are in
##  /var/folders/53/gryn6qk92319jmd_3wbyxc9r0000gn/T//Rtmpyfeki7/downloaded_packages
```

Load Data

From local CSV file

```
my_data <- read.csv("/Users/ratitkar/Documents/BIOF339/Atitkar_BIOF339 Data.csv", header = TRUE)
```

Data Manipulation

1. Convert "group" variable from numeric to factor

2. Log10 transformation of the "CD50" variable

```
"Original Factors"
```

```
## [1] "Original Factors"
levels(my_data$group)
```

```
## NULL
my_data$group <- factor(my_data$group)
"New Factors"
```

```
## [1] "New Factors"
levels(my_data$group)
```

```
## [1] "0"  "3"  "7"  "10"
"Original CD50 Values"
```

```
## [1] "Original CD50 Values"
head(my_data)
```

```
##        CD50 group
## 1  80000.0     0
## 2  69800.0     0
## 3  57000.0     0
## 4  93800.0     0
## 5 223103.3     0
## 6 116227.3     0
my_data$CD50 <- log10(my_data$CD50)
"Log Transformation of CD50"
```

```
## [1] "Log Transformation of CD50"
head(my_data)
```
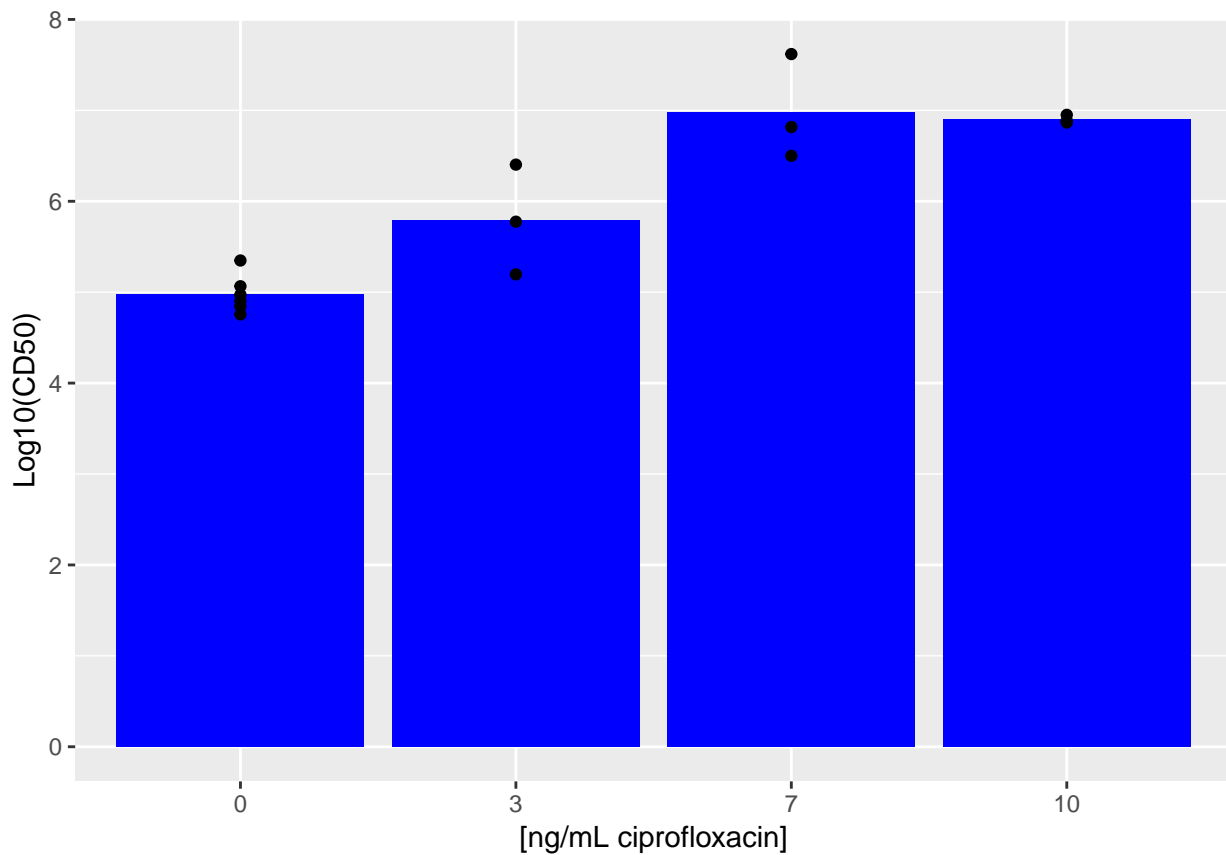
```
##       CD50 group
## 1 4.903090     0
## 2 4.843855     0
## 3 4.755875     0
## 4 4.972203     0
## 5 5.348506     0
## 6 5.065308     0
```

Data Visualization

Scatter plot with bar graph of means using ggplot

```
library(ggplot2)
ggplot(my_data, aes(x=group, y=CD50)) + geom_bar(fill = "blue",stat = "summary", fun.y = "mean") + geom_
```

Statistical Analysis

One-Way Anova: Compare means between independent variable groups

```
anova <- aov(CD50 ~ group, data = my_data)
summary(anova)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## group        3 11.585   3.862   26.27 2.58e-05 ***
## Residuals   11  1.617   0.147
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Result: Means of the ciprofloxacin-treated groups are statistically different (p<0.05)

Tukey's Test:adjusted pairwise comparison between factors to determine which comparisons are statistically significant

```
TukeyHSD(anova)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
```

```
Fit: aov(formula = CD50 ~ group, data = my_data)
##
$group
diff   lwr   upr   p adj
3-0   0.80994927 -0.005891154 1.625790 0.0518386
7-0   1.99686770 1.181027273 2.812708 0.0000715
10-0   1.92018335 1.104342927 2.736024 0.0001023
7-3   1.18691843 0.244867050 2.128970 0.0135334
10-3   1.11023408 0.168182705 2.052285 0.0203638
10-7 -0.07668435 -1.018735722 0.865367 0.9945265
```

**Visualize ANOVA/Tukey's analysis**

```
library(ggsignif)
library(ggpubr)
```

```
## Loading required package: magrittr
```

```
ggplot(my_data, aes(x=group, y=CD50)) + geom_bar(fill = "blue", stat = "summary", fun.y = "mean") + ylim
  stat_compare_means(method = "anova", label.y = 10) +
  geom_signif(comparisons = list(c("0","3")), annotations="NS",y_position=6.2, tip_length = 0) +
  geom_signif(comparisons=list(c("0","7")), annotations = "***", y_position = 7.5, tip_length = 0) +
  geom_signif(comparisons = list(c("0","10")),annotations = "***", y_position = 9, tip_length = 0)
```
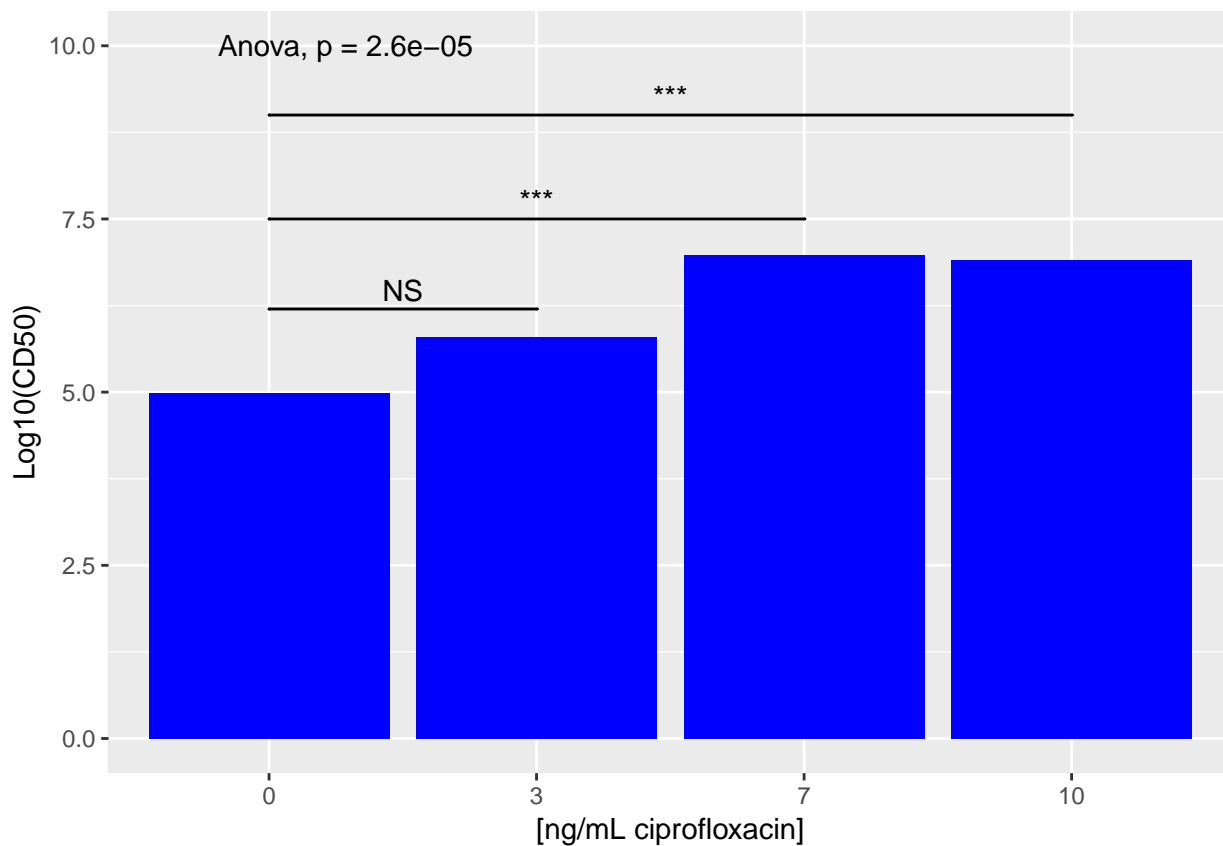
Results/Conclusions

Result: The mean of the 7 and 10 ng/mL-treated groups are significantly different than the mean of the 0 ng/mL-treated group.

Conclusion: This suggests that at least 7 ng/mL of ciprofloxacin is needed to get a significant increase in toxin production from baseline levels in strain 4944.