Project_ospC

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Introduction

A majority of infectious diseases are zoonotic, they often originate from human-animal interactions both with domestic animals and wildlife (Marie, Gordon, 2023). As humans increasingly encroach on previously untouched habitats through urbanization, changes of land use, etc., their risk of zoonoses increases significantly. Lyme disease is the most common vector-borne disease in North America, with approximately 476,000 people being diagnosed with the disease each year in the United States (Center for Disease Control [CDC], 2023). Since the Lyme disease pathogen Borrelia burgdorferi (Bb) is spread by a vector, Ixodid spp. ticks, the spirochete maintains great genotypic diversity in order to maximize its number of competent hosts. Genetic studies of Bb find that the pathogen's genetic diversity in its outer surface protein C (ospC) is not only required for host infection but that the pathogen has unique ospC genotypes that are adapted to specific host species for easier infection (Combs, et al., 2023). There are currently 20 known genotypes for ospC, but only four are known to be human-invasive (Earnhart, et al., 2005). As different regions in North America have different host and vector species, climates, and habitats, there are also different strains and genotypes of Bb in each region.

There is currently no information on whether different genotypes of Bb are more infectious than others, specifically if human-invasive genotypes yield higher loads of bacteria in infected hosts. Using data collected from oak woodland forests in the California Bay Area from 2018 to 2022, this study utilized digital droplet PCR to quantify absolute counts of Bb from nymphal Ixodes pacificus ticks. Taking into account various factors, such as site and year, we sought to identify whether there was a change in the bacterial burdens of the nymphal ticks depending on the genotype of Bb they were infected with and whether or not that genotype was human invasive.

Aims

Aim 1: Determine the accuracy of the protocol's ability to assess bacterial load using digital droplet PCR.

There is currently no known protocol for detecting and quantifying Bb in a DNA sample using ddPCR, but the Swei lab at San

```r
#Aim 1
library(ggplot2)
```

```
## Warning: package 'ggplot2' was built under R version 4.3.3
```

```r
library(dplyr)
```

```
## Warning: package 'dplyr' was built under R version 4.3.2
```

```
##
## 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
```

```r
library(tidyverse)
```

```
## Warning: package 'tidyverse' was built under R version 4.3.2
```

```
## Warning: package 'tidyr' was built under R version 4.3.2
```

```
## Warning: package 'readr' was built under R version 4.3.2
```

```
## Warning: package 'purrr' was built under R version 4.3.2
```

```
## Warning: package 'stringr' was built under R version 4.3.2
```

```
## Warning: package 'forcats' was built under R version 4.3.1
```

```
## Warning: package 'lubridate' was built under R version 4.3.2
```

```
## -- Attaching core tidyverse packages ------------------------ tidyverse 2.0.0 --
## 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     1.3.1
## v readr     2.1.5
```

```
## -- 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 become errors
```

```r
setwd("C:/Users/14085/Desktop/working_directory")
OSPC<-read.csv("ospC_bacterial_load.csv")
restructured_ospC<-read.csv("Restructured_ospc_2.csv")

#View(OSPC)
#View(restructured_ospC)

#Creating table that will calculate the % difference each replicate is from each other
OSPC %>% group_by(TICK_ID) %>% dplyr::summarise(AVERAGE,REP1=abs(DDPCR1-AVERAGE),REP2=abs(DDPCR2-AVERAGE

mean(stats_summary$PRECISION)
```

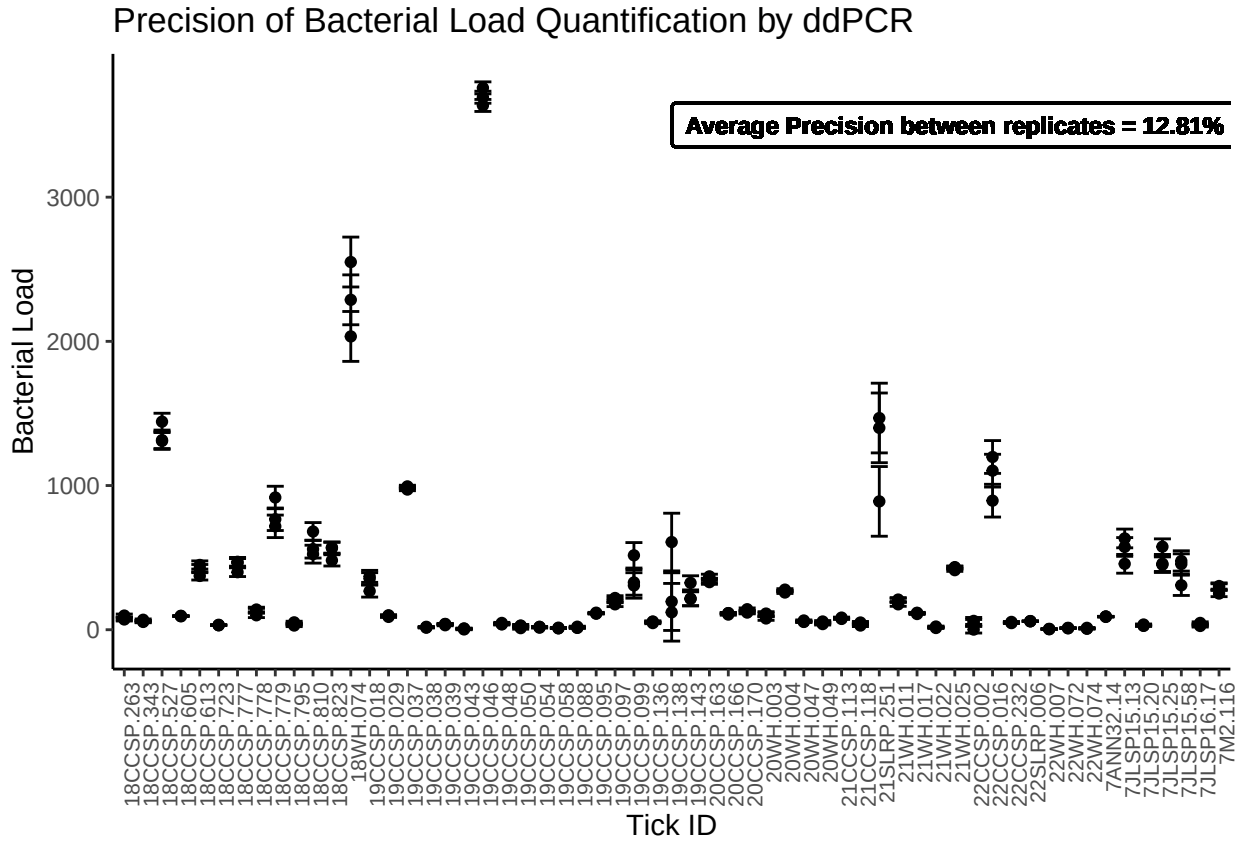
```
## [1] 0.1281421
```

```r
#0.1281421
#On average, each replicate is within 12.81% of each other

merged_tables<- merge(restructured_ospC, stats_summary, by = "TICK_ID")
#View(merged_tables)

precise_plot<- ggplot(data=merged_tables,aes(x=TICK_ID,y=BB_LOAD,fill(REPLICATE)))+geom_point()+geom_er


precise_plot
```



Aim 2: Assess whether there is a difference in bacterial load in nymphal Ixodes pacificus ticks due to differences in ospC genotypes, human-invasive status, and other factors.

The Swei lab currently has DNA samples from Ixodes pacificus ticks collected from six different field sites around the California Bay Area that have already been tested for Bb prevalence, using nested PCR, and ospC genotype, using a 16S PCR and genomic sequencing. These samples were run in triplicates using the aforementioned ddPCR protocol. Rstudio was then used to determine through one-way and two-way ANOVA, whether ospC genotype or human invasive status had any affect on the bacterial burden of the samples. This study also took into account other factors, such as site, year, and county, when determining the factors that may play a role in bacterial burden found in the nymphal ticks.

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