THE AGE EFFECT ON SALMONELLA ENTERICA INDUCE MORTALITY IN CAENORHABDITIS ELEGANS

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ABSTRACT

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Salmonella has been known to cause illnesses across the United States. The severity of the infection is based on age and overall health of the innate immune system. *Caenorhabditis elegans* has been shown to be infected with *Salmonella enterica* and can age quickly, which makes it a good model organism for aging studies. This study tests 3 different ages (day 1, day 3 and day 5) of *C. elegans* with 7 different *S. enterica* serovars. Each age group was infected with either *Escherichia coli* OP50 or *S. enterica* serovar. The plates were checked twice a day until there were no more surviving *C. elegans*. The time of death in hours was determined for each age and bacteria. The median survival +/- standard error post infection of each age and bacteria was calculated. The main effect of age, bacteria and age with bacteria was calculated. Each serovar tested revealed decrease in the life span of the *C. elegans*, however age did not show a significant effect on the life span of *C. elegans*. To gain more knowledge if aging effects the life span of *C. elegans*, older ages need to be tested. Also, other bacteria can be tested to determine if aging effects induced mortality.

KEY WORDS: Aging, *Salmonella enterica* infection, *Caenorhabditis elegans*.

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CHAPTER I

Introduction

Aging is a process of changes that occur over time (McDonald, 2014). It has been shown that age has an influence on how the immune system responds to infection (Dorshkind & Linton, 2004). The process of aging causes dysregulation of the host response (Hajishengallis, 2014). This can lead to a gain or loss in the immune response. Some cells activity decline, some can be maintained and some can increase (Gomez, Boehmer, & Kovacs, 2005). The increase in activity can cause inflammation.

Salmonella enterica is a gram-negative, rod-shaped, bacilli facultative anaerobe ("CDC - Salmonella," 2014). *Salmonella* species are known to infect about one million individuals and cause about 400 deaths per year in the United States ("CDC - General Information on Salmonella," 2014). Infection is often caused by the consumption of raw or undercooked, contaminated meat products, produce, nuts and other food products (Marrero-Ortiz et al., 2012). Infection can also be transmitted by human-to-human or animal-to-human contact (Darwin & Miller, 1999). *Salmonella* is classified by two species, *Salmonella enterica* and *Salmonella bongori*, with about 2,500 subspecies, also known as serovars (Agbaje, Begum, Oyekunle, Ojo, & Adenubi, 2011).

Caenorhabditis elegans is a soil-dwelling nematode that feds on bacteria that has been used as an experimental model (Leung et al., 2008). *C. elegans* is used as a model organism for infection because it is relatively cheap, it can self-fertilize, it has a short life cycle, is relatively small in size, and has an innate immune system similar to a mouse (Kurz & Ewbank, 2000). *C. elegans* has an average life span of 18-20 days in a laboratory setting, making it an ideal model to study the process of aging within

(Volovik, Marques, & Cohen, 2014). Many pathogenies, such as *S. enterica* has been known to infect and kill *C. elegans* (A. Aballay, Yorgey, & Ausubel, 2000). The goal of this study is to determine if aging affects *S. enterica* induced mortality of *C. elegans*.

Innate immune system and *Caenorhabditis elegans*

The innate immune system is the first line of immune response in humans. There are physical barriers such as skin and mucus layer (Alberts et al., 2002). There is also cellular components to this response. This response differs from adaptive immune response and does not have cellular memory. *C. elegans* only has an innate immune response. *C. elegans* have physical barriers like other animals such as cuticle, grinder in the pharynx and defecation (Sifri, Begun, & Ausubel, 2005). The grinder can destroy bacteria by grinding up the cells, this way no living cells make it to the intestines. *C. elegans* have also shown to have antibacterial peptides that act against pathogen (Millet & Ewbank, 2004). Studies on *Caenorhabditis elegans* have shown that as host life span increases, the rate of death from infection also rapidly increase (Laws, Harding, Smith, Atkins, & Titball, 2004). As *C. elegans* age, many body functions decline. The body slows down in movement and the pharyngeal pumping slows down (Antebi, 2007). Defecation also declines which keeps the ingested bacteria in the intestine, which leads to death (Collins, Huang, Hughes, & Kornfeld, 2005). Antibacterial properties that are present within the model organism are also decreased as a result of aging process (Laws et al., 2004).

***Salmonella enterica* as a pathogen**

Salmonella enterica is a gram-negative, rod-shaped, bacilli facultative anaerobe ("CDC - Salmonella," 2014). *Salmonella* species are known to infect about one million

individuals and cause about 400 deaths per year in the United States ("CDC - General Information on Salmonella," 2014). Infection is often caused by the consumption of raw or undercooked, contaminated meat products, produce, nuts and other food products (Marrero-Ortiz et al., 2012). Infection can also be transmitted by human-to-human or animal-to-human contact (Darwin & Miller, 1999). This pathogen can infect both humans and animals (Agbaje et al., 2011). The most common symptoms of *Salmonella* infection are diarrhea, fever and abdominal cramps. These symptoms are usually self-limiting and last about 4 to 7 days but can become severe. Because of the self-limiting nature of the infection, many people do not get treated or consult a physician when they are infected (Darwin & Miller, 1999). The severity of the infection is often based on the individual's age and immune system ("Signs & Symptoms Salmonella," 2014).

Salmonella is classified by two species, *Salmonella enterica* and *Salmonella bongori* (Agbaje et al., 2011). Within *S. enterica* there are about 2,500 subspecies, also known as serovars. These serovars are classified by the O (somatic) and H (flagellar) antigens (Brenner, Villar, Angulo, Tauxe, & Swaminathan, 2000). Some serovars can be broad-range and infect multiple hosts. Some are can be narrow-range and infect specific hosts (Gerlach & Hensel, 2007). Studying different types of *S. enterica* serovars in a host can increase the overall understanding of how the infection can affect the host ("CDC - General Information on Salmonella," 2014). The virulence of a serovar is based on the pathogenicity islands (PAIs). PAIs are cluster of genes that are 10-100 kb in size that encode for the different virulence factors (Nieto et al., 2016). Non-pathogen bacteria do not have these PAIs (Gal-Mor & Finlay, 2006). Testing different *S. enterica* serovars in hosts of different ages is a new concept for this research.

In this study, the *S. enterica* serovars that were used included, *S. enterica* serovar Typhimurium, *S. enertica* serovar Paratyphi A, *S. enterica* serovar Heidelberg, *S. enterica* serovar Enteritidis, *S. enterica* serovar Saintpaul and *S. enterica* serovar Muenchen. Many of these serovars have been the most common *S. enterica* infection in the United States, with some of being responsible for recent outbreaks ("FoodNet 2014 Annual Foodborne Illness Surveillance Report FoodNet CDC," 2016, "Outbreak-associated Salmonella enterica Serotypes," 2013; Switt & Ho, 2016). In 2014, out of the 7,439 reported cases, 6,805 serovars were identified out of the reported cases of infection ("FoodNet 2014 Annual Foodborne Illness Surveillance Report FoodNet CDC," 2016). The serovars are seen in Table 1. Since Enteritidis, Typhimurium and Heidelberg were within the top six serovars infectious serovars identified, they were chosen for this study. In 2016, Muenchen was reported to be one of the top ten serovars isolated from humans in the United States (Switt & Ho, 2016) and thus was also chosen as an isolate to study within this experiment. Saintpaul was also chosen because it was one of the top ten serovars identified within the time period of 1998-2008 ("Outbreak-associated Salmonella enterica Serotypes," 2013). Since many of these serovars have been seen to infect humans and have been in the top ten isolates over the years, they were chosen to be tested.

Table 1

2014 Reported Serotype Information

Salmonella enterica Serovars	Reported Cases (6,805)
Enteritidis	19%
Typhimurium	11%
Newport	10%
Javiana	9%
l 4,[5],12,i-	5%
Heidelberg	3%
All other	34%

Note. FoodNet 2014:reported serotypes of known cases of S. enterica("FoodNet 2014 Annual Foodborne Illness Surveillance Report FoodNet CDC," 2016)

Previous studies

Studies have shown different *S. enterica* serovars can infect and kill *C. elegans.* In 2002, it was shown that *S. enterica* serovar Typhimurium is intact in the intestine and pharynx of *C. elegans* after a 5 days of contact (Labrousse, Chauvet, Couillault, Léopold Kurz, & Ewbank, 2000). Other serovars have been shown to decrease the life span of C. elegans. These serovars include: Choleraesius, Agona, Newport, Typhi, Heidelberg, Enteritidis, Paratyphi A, Dublin, Schwarzengrund and Gallinarum (Alejandro Aballay, Yorgey, & Ausubel, 2000; Powell, 2013). These studies did look at *S. enterica* serovar infections in *C. elegans* but did not look at different ages of *C. elegans*.

In 2000, it was suggested that 1 day old worms compared to L4 worms died faster within the first couple of days of *S. enterica* serovar Typhimurium exposure (Alejandro Aballay et al., 2000). Both 1 day old adult and L4 worms died faster than those exposed to *E. coli* OP50. This study only looked at 1 day old adults and Typhimurium infection.

One study has looked at aging and bacterial infection in *C. elegans* (Laws et al., 2004). This study looked at *Pseudomonas aeruginosa* PA14 and *Yersinia pseudotuberculosis* YP3 infection in L4, 1, 2, and 4 day old adults. The older aged worms did die more rapidly than the younger aged worms. This study did not go past 4 day old worms. It also did not look at *S. enterica* infection with these age groups.

Study goals

In this research study, the influence of host age to six different types of *Salmonella enterica* serovars in *C. elegans* was examined. The *C. elegans* were exposed chronically to six different types of *S. enterica* serovars. The specific questions were addressed by this study included: (1) Does host age influence the infection severity of different *S. enterica* serovars in *C. elegans*? (2) Do different *S. enterica* serovars influence the infection severity in *C. elegans*?

CHAPTER II

Methods

Salmonella enterica serovar maintenance

S. enterica serovars were obtained from Michael McClelland at the San Diego Institute of Biological Research. These serovars are standard laboratory samples. The *S. enterica* serovars that were used are *S. enterica* serovar Typhimurium, *S. enterica* serovar Paratyphi A, *S. enterica* serovar Heidelberg, *S. enterica* serovar Enteritidis, *S. enterica* serovar Saintpaul and *S. enterica* serovar Muenchen. The *S. enterica* samples were streaked onto Xylose lysine deoxycholate (XLD) agar and maintained at 37°C.

Caenorhabditis elegans strain maintenance

Strains of *C. elegans* were purchased from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis, MN). The standard wild-type N2 stain was used in this study. The worms were grown and maintained at 25 °C on Nematode Growth Media (NGM) (Cold Spring Harbor Laboratory, 2008). Thirty-five mm x ten mm NGM agar plates were seeded with *Escherichia coli* OP50. *E. coli* OP50 was maintain on Eosin methylene blue agar (EMB) which is selective and differential for *E. coli*. The plates with *E. coli* and worms were sealed with Parfilm and placed in a Ziploc© bag to prevent contamination.

Caenorhabditis elegans Age-synchronization

Four NGM plate were chunked with a mixed-age population of N2 worms, then incubated at 25° C for 2 days to accumulate eggs. Chunking refers to cutting a small piece of agar out of the plates and transferring it to another agar plate. The worms were then washed off with 5 mL of M9 buffer into a 15 mL tube. The tube was then

centrifuged at 1500 rpm for 1 minute. The supernatant was removed and 5 mL of lysis solution (20% Clorox ® bleach and 25% 1 M NaOH) was added to the tube (Hope, 1999). The tube was vigorously shaken for 3 minutes, and 10 mL of M9 buffer was added (MIT, 2015). The tube was centrifuged at 1500 rpm for 1 minute. The lysis solution/M9 buffer supernatant was removed and 10 mL of fresh M9 buffer was added to the tube. The tube was centrifuged at 1500 rpm for 1 minute, this was done one more time to wash the eggs. The eggs were then resuspended in 5 mL of M9 buffer and incubated at room temperature overnight to allow the eggs to hatch. After incubation, the tube containing the eggs was centrifuged at 3000xg for 1 minute and 4 mL of the supernatant was removed. The pellet was vortexed to mix with the remaining buffer and the first larval stage (L1) was plated on an OP50 seeded plate following the methodology of (Hope, 1999).

Salmonella enterica **Serovar Infection Assays**

The L1 worms were left on an OP50 plate for two days to reach the adult stage. Then the plates had an additional 5, 3 or 1 day of incubation to reach 5, 3 and 1 day old adults. *C. elegans* goes through 4 larva stages, L1, L2, L3 and L4 before going into the adult stage. On either the 5^{th}, 3^{rd} or 1^{st} day of adulthood, 10 adult aged worms were picked and placed onto each *S. enterica* serovar plate or the OP50 control plate. Each plate was checked twice a day to look for dead of worms. After the first two days, the adults were picked and placed onto a new *S. enterica* serovar plate or OP50 control plate. The first couple of days the worms reproduce rapidly, this can crowd the plate. Moving the adults allows for only the aged worms to be counted and not the progeny. After the first two days, the reproduction of eggs slows down, so moving the aged worms can be

done every three days to allow there to be fresh bacteria to feed on. During the three days, if any progeny is seen, they are killed by a heated pick to ensure that only the aged worms are counted. This is done until there is no surviving L4 worms. This is repeated 10 times. The infection assay can be seen in Figure 1.

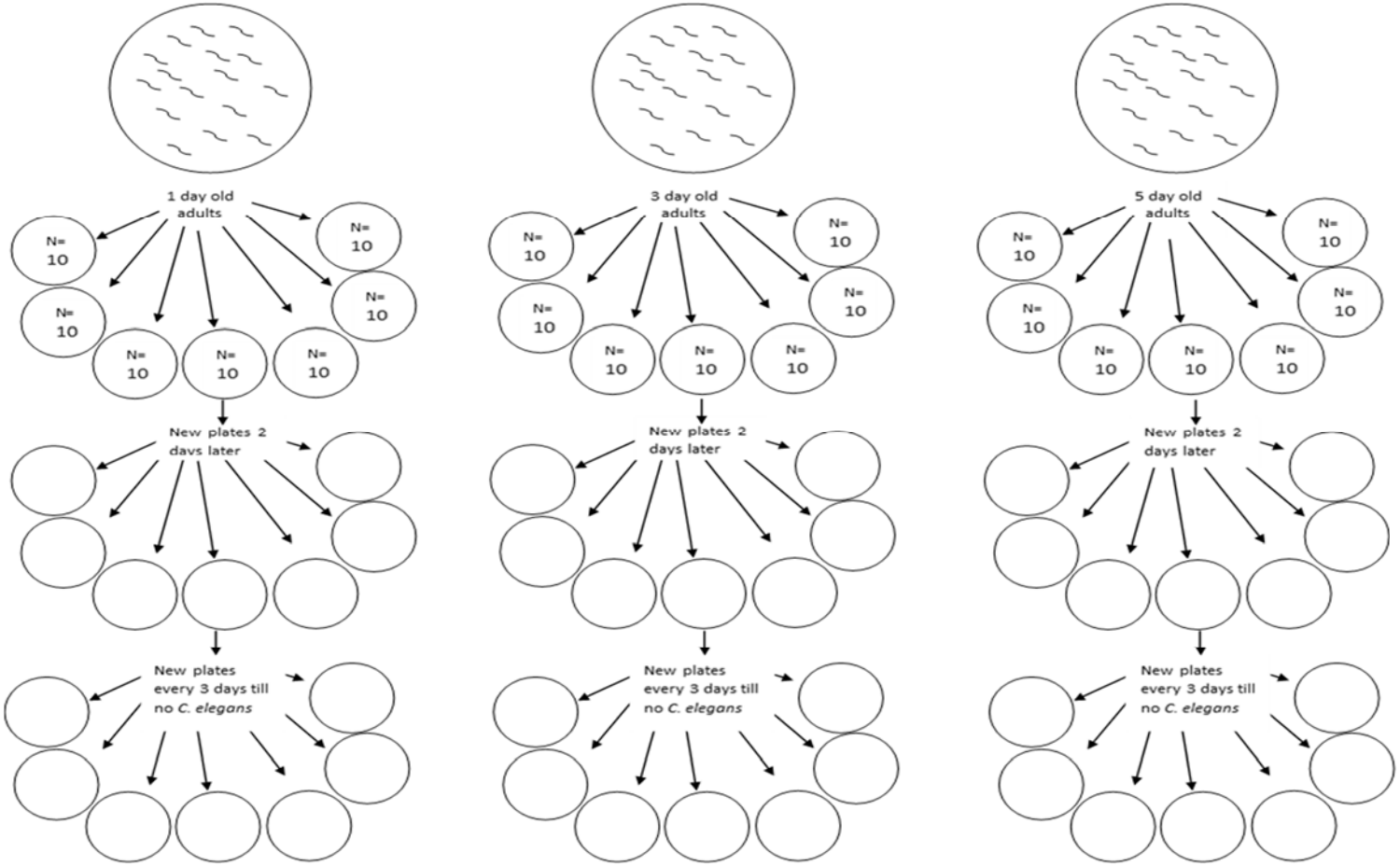

Figure 1. Depiction of infection assay experiment.

Statistics

Each death was scored, 0 or 1. The worms labeled 1 were the worms that were killed by picking. The data was censored to account of the picking death instead of taking those worms out of the data.

Due to screening methods, such as Cox-Regression, it was determined that the median longevity of each bacteria, and age group would be used. The mean of the median longevity was calculated using Kaplan-Meier. These mean longevities were used to create a two-way ANOVA with age and serovar. The significance of the main effect was then calculated using Tukey-Kramer Multiple-Comparison Test. All stats were run on NCSS 11.

CHAPTER III

Results

Figure 2 compares each serovar to the reference point, *E. coli* for this model to show that each bacterium varies in risk ratios over the replications. *E. coli* was used at the reference because it was the control for this study. The risk ratios were calculated by Cox-Regression. Since there is drastic variation within the serovars over the replicates, the data could not be pooled.

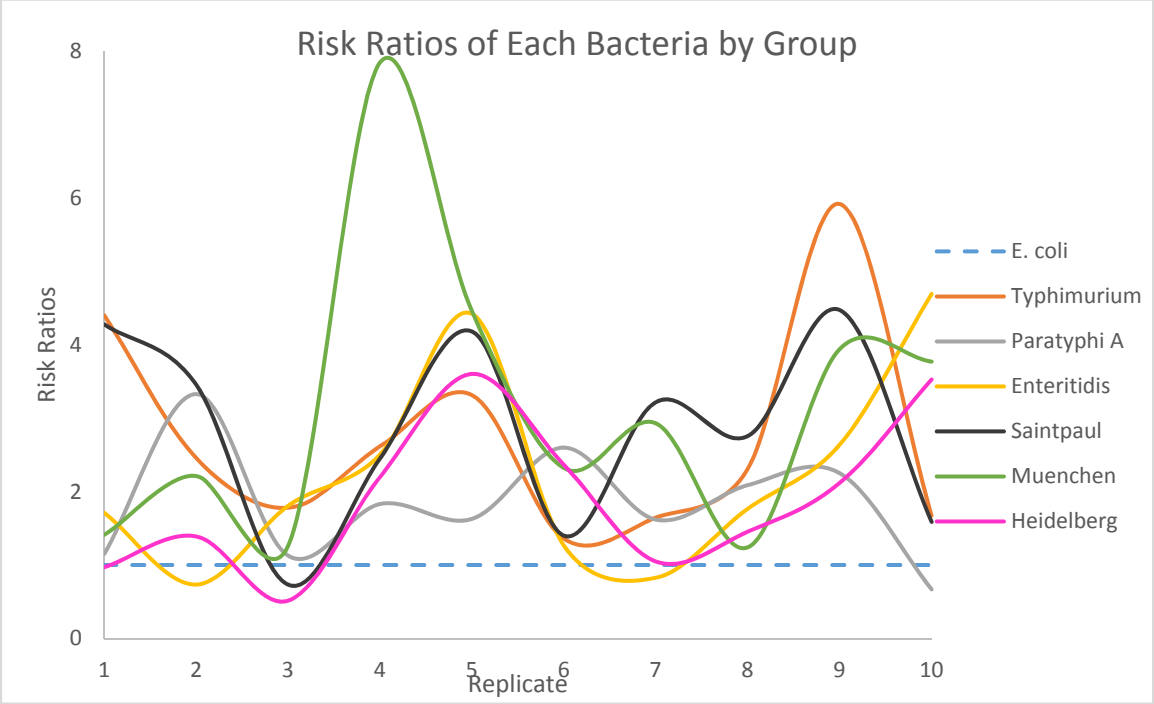

Figure 2. Risk ratios of each bacterium by replicate.

Figure 3 depicts the mean +/- the standard error of survival post infection. This is only the survival post infection based on the age of the worm, not looking at each serovar. Using a two-way ANOVA, the main effect that aging had on the survival longevity of *C. elegans* was calculated and showed that age was not significant ($p > .07$). When each age was compared to one and another using Tukey-Kramer Multiple-Comparison Test, it was shown that each age group did not differ from one another. This means that the longevity of each age group was relativity the same no matter the age, determining that age did not affect *S. enterica* induced mortality in *C. elegans*.

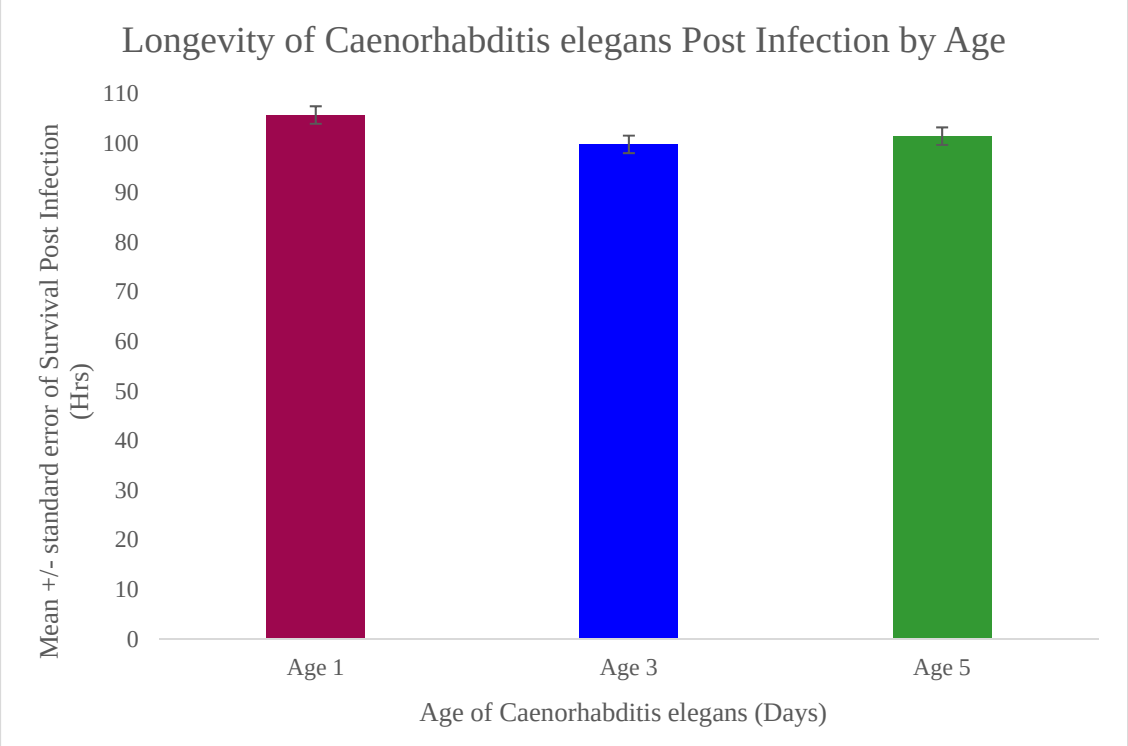

*Figure 3.*The survival longevity +/- standard error of *C. elegans* based only on age.

Consistent with Powell's results, Figure 4 shows that the bacterium did affect the survival longevity of *C. elegans* (Powell, 2013). Figure 4 depicts the mean +/- the standard error of survival longevity post infection by bacteria. This is only looking at the bacteria not age of each group. Using a two-way ANOVA, the main effect of the bacteria had on the survival longevity had on *C. elegans* was calculated and showed it was significant ($p<.01$). When each age was compared to one another using Tukey-Kramer, it was shown that *E. coli* differed from all of the *S. enterica* serovars tested. This indicates that the worms grown on *E. coli* live longer than worms grown on *S. enterica* serovars. Typhimurium, Enteritidis and Heidelberg only differed from *E. coli*. Paratyphi A differs from E. coli and Saintpaul and Muenchen. It is suggested that the worms grown on Heidelberg and Paratyphi A outlive those worms that grow on other serovars.

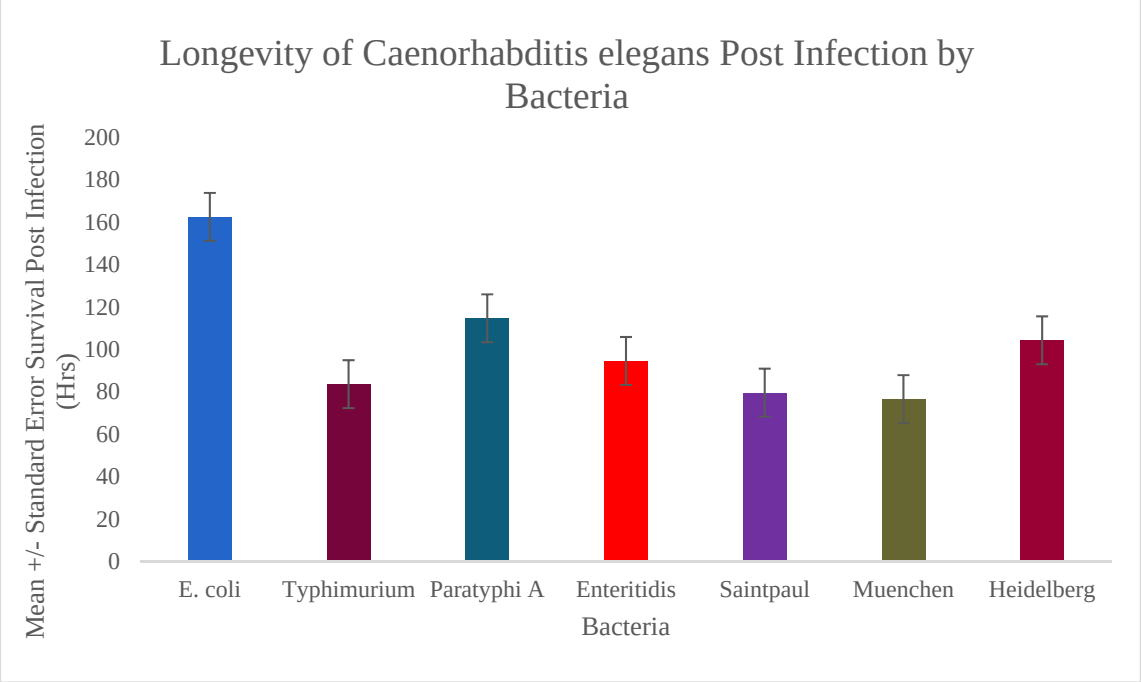

Figure 4. The survival longevity +/- standard error of *C. elegans* based only bacteria.

Figure 5 depicts the mean survival post infection based on age of the worm and bacteria the worms were grown on. It was shown that no matter the age, *E. coli* live longer than the other bacterium. Each age/bacteria group are clustered together showing there is not difference in survival based on age. Using a two-way ANOVA the interactions between age and bacteria was shown to have no significant interactions ($p>.08$). The longevity of each serovar and all three age group seems to be cluster together.

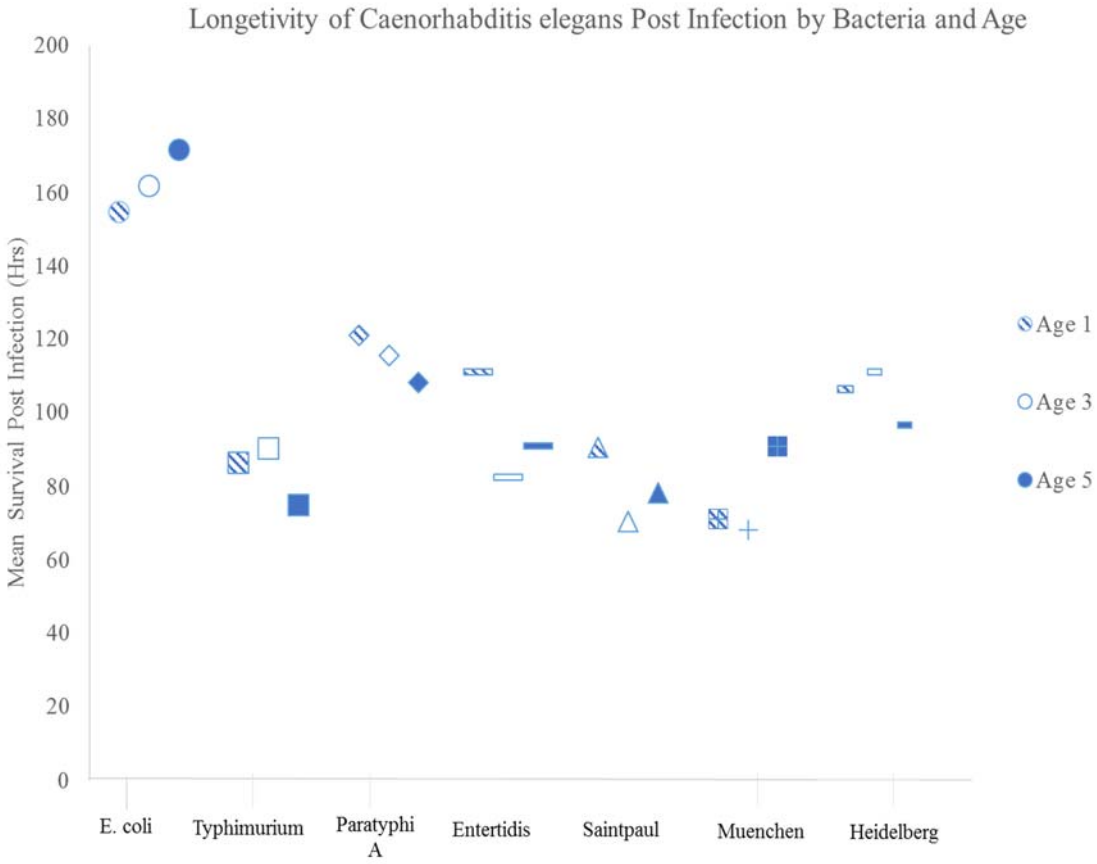

Figure 5. Survival longevity of *C. elegans* based on age and bacteria.

CHAPTER IV

Discussion

The results of the experiment showed that the age of the worm at the time of infection had not effect. This study only looked at 1, 3 and 5 day old worms, *C. elegans* has a life span about 18 to 20 days in the laboratory with 5 day being about 25% of the worms life span (Volovik et al., 2014). Aging studies that have used mice, have seen the immune system decline in the 16-18 months age group and a dramatic decline in 22-24 month age group (Miller, 1992). The decrease in immune function is seen about 50-60% and the dramatic decline about 73-80% of the mice life. The decrease in immune response might occur later in the worms' life. Five day old worms could be too young to see if age does play a role in declining immune system. A decrease in immune system in *C. elegans* could be later in the life span than tested. Testing older aged *C. elegans* with the same *S. enterica* serovars could see if age does affect the longevity of *C. elegans*.

Other studies have shown that many human pathogens can infect and kill *C. elegans* such as *Pseudomonas* and *Staphylococci* (Alegado, Campbell, Chen, Slutz, & Tan, 2003). *Pseudomonas aeruginosa* is a gram-negative bacterium and can infect humans and animals like *S. enterica*. It has been shown that over serval days, this bacterium can cause infection in the worms, very similar to *S. enterica* (Marsh & May, 2012). *Staphylococci aureus* differs from *S. enterica* by being gram-positive bacteria and causing decline in movement in the worms as little as 24 hours (Sifri, Begun, Ausubel, & Calderwood, 2003). To increase the knowledge of aging and infection, other pathogens might need to be tested to see if age does affect the survival longevity. In 2013, Powell showed that different *S. enterica* serovars affected the longevity and each serovar

affected *C. elegans* about the same (Powell, 2013). This was consistent with the data collected in this study. Some serovars such as Heidelberg and Paratyphi A suggested to have killed the *C. elegans* slower than the other serovars. Mostly the serovars seem to have followed the same trend in infecting and killing the *C. elegans*. While this study focused solely on *S. enterica* serovars, other bacteria might affect the longevity the model differently.

S. enterica serovar Muenchen and Saintpaul have not been used in *C. elegans* studies before. This study does demonstrate that these serovars can infect *C. elegans* which can increase the list of known human pathogens that infect and kill *C. elegans*. As previously stated, these serovars were selected due to being in the top ten serovars that have infected humans over the years.

Each replicate showed varying results, the data could not be pooled. Figure 2 compares each serovar to the reference point, which is *E. coli* OP50. Each serovar showed variations from replicate to replicate, determining the data from each replicate had too much variation and could not be pooled. Each age with each bacteria only had a n=10. A bigger sample size could give a better picture if the age effect the survival longevity. The severity of the infection can also help determine if there is a difference in each age group. This is done by collecting the bacteria in the gut. This will give a better idea of the differences of each age group and determine if *C. elegans* is dying faster due to *S. enterica* infection or old age. It has been shown that the virulence of the bacteria can vary depend on the media it has been grown on (Alegado et al., 2003). The bacteria may not be at it most pathogenic state on the NGM agar. Different bacteria might need

different media to become pathogenic or toxic to *C. elegans*. Changing the media might show different results for each of the serovars tested

In conclusion, it was found that age did not affect the longevity of *C. elegans*. It was also found that the serovars did affect the longevity of *C. elegans*. Muenchen and Saintpaul are now known to infect and kill *C. elegans*.

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VITA

Education

M.S. Biology, Sam Houston State University, Huntsville, Texas.
Advisor: Dr. Aaron M. Lynne
Projected Graduation Date: December 2016.

B.S. Biology and Chemistry, Sam Houston State University, Huntsville, Texas.
Advisor: Dr. Rick E. Norman
Graduation Date: August 2014.

Research Experience

Sam Houston State University, Huntsville, TX
Undergraduate Research Assistant, Department of Chemistry
Studying the use of synthetic and garlic sulfur donors to treat cyanide intoxication. (Summer 2013- Summer 2014)

Sam Houston State University, Huntsville, TX
Undergraduate Research Assistant, Department of Biological Sciences
Studying the phenotypic and genotypic profiles of *Salmonella enterica* serovar Typhimurium and serovar Heidelberg human isolates. (Spring 2013-Fall 2014, Fall 2015)

Sam Houston State University, Huntsville, TX
Undergraduate/Graduate Research Assistant, Department of Biological Sciences Microbiome of Decomposing bodies in East Texas/ Bone scavenging. (Spring 2014, Fall 2015, Spring 2016)

Sam Houston State University, Huntsville, TX
Graduate Research Assistant, Department of Biological Sciences. The age effect on *Salmonella enterica* induced mortality in *Caenorhabditis elegans* (Spring 2015-Current)

Professional Experience

Teaching Assistant, **General Microbiology (Bio 3470)**, Sam Houston State University, Department of Biological Sciences, Huntsville, Texas (Spring 2014-current)

Helped write the **General Microbiology (3470) Lab Manual** (Fall 2015)

Teaching Assistant, **Molecular Biology (Bio 4440)**, Sam Houston State University, Department of Biological Sciences, Huntsville, Texas (Fall 2014, Fall 2015)

Undergraduate Research Assistant, **Use of synthetic and garlic sulfur donors to treat cyanide intoxication**, Sam Houston State University, Department of Chemistry, Huntsville, Texas. (Fall 2013-Summer 2014)

Teaching Assistant, **Inorganic Chemistry for non-majors**, Sam Houston State University, Department of Chemistry, Huntsville Texas. (Fall 2012)

Stock Room/Prep Teaching Assistant, Sam Houston State University, Department of Chemistry, Huntsville Texas. (Spring 2011-Summer 2014)

Graduate Student Moderator, Undergraduate Research Symposium, Huntsville, Texas. (Spring 2015, Spring 2016)

Skill Sets

- Molecular, Microbiology and Chemistry Stock and culture Lab preparation
- Conducting Antibiotic Resistance Assays
- Various Molecular Techniques Such as PCR, Gel/2D-electrophoresis, Western blot, ELISA
- Operating various instruments such as HPLC, GC-MS
- Running analyzes on QIIME
- Mice handling experience
- *C. elegans* handling experience

Certification

- IACCU-Animal Models (2013, 2016)
- Blood Borne Pathogen (2014, 2015)
- ACS (2014)

Society Membership

Member, Biological Graduate Student Organization, Sam Houston State University, (Fall 2014-Current)

Member, Project Sunshine, Sam Houston State University, (Fall 2012-Summer 2016)

Member, Chemistry Club, Sam Houston State University, (Fall 2013-Spring 2014)

Member, Lambda Theta Alpha Latin Sorority Inc., Sam Houston State University, (Spring 2011-Fall 2014)

Professional Society Memberships

American Society for Microbiology (Fall 2013-Current)

American Society for Microbiology Texas Branch (Fall 2014-Current)

Texas Academy of Science (Fall 2014)

Research Funding

Undergraduate Summer Research Grant for $500
This was used to fund the Characterizations of Antimicrobial Resistance Phenotypes and Genotypes in *Salmonella enterica* serovar Typhimurium Human Isolates (Summer 2014)

Mentorships

Peter Quach
Characterizations of Antimicrobial Resistance Phenotypes and Genotypes in *Salmonella enterica* serovar Heildberg Human Isolates (Summer 2016-Current)

Poster Presentations

Fisher, D.M., L.R. Smith, D. P. Haarmann, A.M. Lynne. Characterizations of Antimicrobial Resistance Phenotypes and Genotypes in *Salmonella enterica* serovar Typhimurium Human Isolates. National American Society for Microbiology. New Orleans, La. Summer 2015

Fisher, D.M., L.R. Smith, D. P. Haarmann, A.M. Lynne. Characterizations of Antimicrobial Resistance Phenotypes and Genotypes in *Salmonella enterica* serovar Typhimurium Human Isolates. American Society for Microbiology. New Braunfels, Texas. Spring 2015.

Fisher, D.M., A.M. Lynne, J. Harper**.** The Host-pathogen Interactions between *Salmonella enterica* serovars and Aging *Caenorhabditis elegans*. Professional Aspect Symposium. Huntsville, Texas. Fall 2014.

Fisher, D.M., L.R. Smith, D. P. Haarmann, A.M. Lynne. Characterizations of Antimicrobial Resistance Phenotypes and Genotypes in *Salmonella enterica* serovar Typhimurium Human Isolates. American Society for Microbiology. Houston, Texas. Fall 2014.

Fisher, D.M., L.R. Smith, D. P. Haarmann, A.M. Lynne. Characterizations of Antimicrobial Resistance Phenotypes and Genotypes in *Salmonella enterica* serovar Typhimurium Human Isolates. University of Texas San Antonio Science Research Conference. San Antonio, Texas. Fall 2014.

Fisher, D.M., L.R. Smith, D. P. Haarmann, A.M. Lynne. Characterizations of Antimicrobial Resistance Phenotypes in *Salmonella enterica* serovar

Typhimurium and serovar Heildberg Human Isolates. Undergraduate Research Symposium, Huntsville, Texas. Spring 2014

Smith, L. R., **D. M. Fisher**, D. P. Haarmann, A.M. Lynne. Characterizations of Antimicrobial Genes in *Salmonella enterica* serovar Typhimurium Human Isolates. Undergraduate Research Symposium, Huntsville, Texas. Spring 2014

Fisher, D.M., L.R. Smith, D. P. Haarmann, A.M. Lynne. Characterizations of Antimicrobial Resistance Phenotypes and Genotypes in *Salmonella enterica* serovar Typhimurium Human Isolates. American Society for Microbiology. New Braunfels, Texas. Spring 2014.

Fisher, D.M., L.R. Smith, D. P. Haarmann, A.M. Lynne. Characterizations of Antimicrobial Resistance Phenotypes in *Salmonella enterica* serovar Typhimurium and serovar Heildberg Human Isolates. American Society for Microbiology, New Orleans, La. Fall 2013.

Smith, L. R., **D. M. Fisher**, D. P. Haarmann, A.M. Lynne. Characterizations of Antimicrobial Genes in *Salmonella enterica* serovar Typhimurium Human Isolates. American Society for Microbiology, New Orleans, La. Fall 2013.