Salmonella Bacteriophage-Resistance Genomic Analysis, Computational Approaches of Environmental Data, and Chicken Embryo Lethality Assay

by

Steven Ray Kitchens

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
May 4, 2024

Keywords: *Salmonella*, Bioinformatics, Environmental, Veterinary, Bacteriophage, Chicken Embryo Assay

Approved by

Stuart B. Price, Co-Chair, Associate Professor of Pathobiology
Chengming Wang, Co-Chair, Professor of Pathobiology
Nancy D. Merner, Associate Professor of Pathobiology
Elizabeth Hiltbold Schwartz, Associate Professor of Biological Sciences

Abstract

This dissertation is a coalescence of three individual projects with *Salmonella* being the only area that the projects have in common. Bacteriophage (phage) treatment for the reduction of multiple drug-resistant *Salmonella* Newport in dairy calves has been examined in our lab from a clinical disease and food safety perspective. Previously, our lab has examined the emergence of phage-resistant *Salmonella* as a potential consequence of phage treatment and the affect phage-resistance has on virulence in *Salmonella*. We generated a spontaneous mutant resistant to 4 of 5 lytic phages used in our treatment regimen. This study examined the mutation that conferred the mutation of phage-resistance in our *Salmonella* Newport. We also examined a chicken embryo lethality assay as a model for phage therapy against *Salmonella* and to investigate the virulence of *Salmonella* strains. In addition, we examined the application of novel statistical methods and whole-genome sequencing when investigating the prevalence of *Salmonella* in a multi-species animal facility. Regarding the phage-resistant mutation, we found that short-read sequencing alone was not a valid option to locate single-nucleotide polymorphisms (SNPs) that could be attributed to the phage-resistant phenotype. By using short-read and long-read sequencing with a hybrid assembly, we found a SNP in the *rfbM* gene that could explain a phage-resistant phenotype. Regarding the chicken embryo lethality assay, differences in the survival of embryos were found between different phage isolates. However, we found that this assay should be used cautiously with the understanding that *Salmonella*'s virulence and effect on survival can be very dramatic. The third project utilized previous studies on environmental surveillance of Salmonella, with an additional year of surveillance to investigate the prevalence of *Salmonella* with novel statistical methods and whole-genome sequencing. Comparing supervised machine learning algorithms (logistic regression, random forest analysis, and Markov Chain Monte Carlo (MCMC)), we found that these models may be beneficial to epidemiologists investigating widespread environmental *Salmonella* contamination. All three models found that bovine, summer, and the dairy barns and pastures were variables of importance for our study of environmental *Salmonella* prevalence. We found three strains of *Salmonella* Muenster out of ten isolates sequenced, but all isolates appear to be genetically linked and were derived from a common ancestor. Isolates within our strain cluster have less than eight SNPs, with some clusters spanning timelines up to 1493 days. These studies contained methods that have been used before but have not been applied in the areas of research

of our interest. These studies are processes of the application of methods of interest to our questions pertaining to *Salmonella*.

Acknowledgments

Firstly, I would like to express my sincere gratitude to my major professor, Dr. Stuart Price, for the support of my dissertation study and related research and for his understanding, guidance, motivation, and immense knowledge. His guidance helped me in all aspects of the research and preparation of this dissertation. I am grateful to have such a great advisor and mentor for my dissertation study. I would like to thank the rest of my dissertation committee: Dr. Wang, Dr. Merner, and Dr. Schwartz for their insightful comments, encouragement, and guidance in my research. I would like to thank Dr. Diana Bourassa for being the University Reader. I want to give a special thanks to Zheran (Rachel) Wang for helping me understand the principles of machine learning algorithms. I want to thank Dr. Xu Wang for helping me troubleshoot complications with whole-genome sequencing. I want to thank all my supportive co-workers in the Auburn University College of Veterinary Medicine Department of Pathobiology. I want to thank Dr. Jim Wright, Dr. Sue Duran, Dr. Paul Walz, Dr. Stephanie Ostrowski, Cassandra Kitchens, Andrea Pietruska, Ashley Williams, Shelby Osburn, Chidozie Ugochuckwu, Stephen Tansie, Lindsey Morris, Regan Morrow, MacKenzie Micheals, Ramone Zegpi, and Raimundo Espejo for their support and contributions to this project.

I would like to give thanks to Auburn University and the Employee Education Benefit for providing a tuition waiver benefit to allow me to enroll in graduate coursework. I would also like to thank my mother, Lorie, and my siblings, David, Michael, and Velora, for providing me with love, support, and continuous encouragement. I must express gratitude to my children, Abel, Hannah, and Collin, and their many nights of missing me. Finally, I am most grateful for the greatest tag-team partner and loving wife, Cassandra. This accomplishment would not have been possible without her sacrifice, support, patience, and encouragement. Thank you, Beautiful!

Table of Contents

Abstract ... ii
Acknowledgments ... iv
List of Tables ... viii
List of Figures ... ix
List of Abbreviations ... xi
Chapter 1 ... 1
1.1 *Salmonella* Introduction ... 1
1.2 Salmonellosis and Host-Specificity ... 3
1.3 *Salmonella* Virulence Factors Associated with Gastroenteritis ... 5
1.4.1 *Salmonella* Pathogenicity Islands ... 5
1.4.2 Surface Structure – LPS and the "O" Antigen ... 6
1.4.3 Surface Structure – Flagella the "H" Antigen ... 7
1.4.4 Surface Structure – The Capsular or "Vi" Antigen ... 7
***1.4.5* *Salmonella* and the Microbiota** ... 7
1.4 Bacteriophage ... 9
2.3.2 Introduction and Phage Therapy ... 9
2.3.3 Bacteriophage-Resistance ... 10
1.5 *Salmonella* Epidemiology ... 12
1.5.1 *Salmonella* in People ... 12
1.5.2 Environmental *Salmonella* in Animal Facilities ... 13
1.5.3 *Salmonella* in Animals ... 14
1.5.4 *Salmonella* Outbreaks in Veterinary Hospitals ... 15
1.6 Computational Analysis in Epidemiology ... 16
1.6.1 Machine-Learning Algorithms ... 16
1.6.2 Whole-Genome Sequencing in Bacterial Epidemiology ... 18
1.7 A Study of a Bacteriophage-Resistant *Salmonella* Mutant and Factors Influencing Prevalence of *Salmonella* in a Multi-Species Animal Facility (Kitchens, 2016) ... 20
1.7.1 Factors Influencing Prevalence of *Salmonella* in a Multi-Species Animal Facility ... 20
1.7.2 A Study of a Bacteriophage-Resistant *Salmonella* Mutant and Infection in Calves ... 21
1.8 References ... 24
Chapter 2 ... 41
2.1. Abstract ... 41
2.2. Introduction ... 41
2.3. Materials and Methods ... 43
2.3.1 *Salmonella* Strains ... 43
2.3.2 Variant Detection GATK Best Practices Workflow ... 43
2.3.3 Variant Detection with Snippy ... 44
2.3.4 Comparison of Whole Genome Alignments between Short-Read Assemblies and Hybrid (short-read and long-read sequencing) Assemblies for Variant Detection ... 47

2.3.5 Characterizing Bacteriophage on *Sal*. Newport Parent and *Sal*. Newport Mut$^{\Phi}$ with Adsorption Kinetics, Antisera Agglutination, and Motility. 47
2.4. Results 48
2.4.1 Variant Detection GATK Best Practices Workflow 48
2.4.2 Variant Detection Snippy Workflow 53
2.4.3 Comparison of Whole Genome Alignments between Short-Read Assemblies and Hybrid (short-read and long-read sequencing) Assemblies for Variant Detection 59
2.4.4 Characterizing Bacteriophage on *Sal*. Newport Parent and *Sal*. Newport Mut$^{\Phi}$ with Adsorption Kinetics, Antisera Agglutination, and Motility. 62
2.5. Discussion 63
2.6. References 66
Chapter 3 72
3.1. Abstract 72
3.2. Introduction 72
3.3. Materials and Methods 73
3.3.1. *Salmonella* Strains 73
3.3.2. Bacteriophage Amplification 74
3.3.3. Chicken Egg Embryo Lethality Assay 74
3.4. Results 75
3.4.1. Chicken Embryo Lethality Assay: *Sal*. Newport Control Experiment 75
3.4.2. Chicken Embryo Lethality Assay: *Sal*. Newport treated with MOI≈10 or MOI≈100 of bacteriophage S50 75
3.4.3. Chicken Embryo Lethality Assay: *Sal*. Newport treated with MOI≈10^5 or MOI≈10^6 of bacteriophage S50 76
3.4.4. Chicken Embryo Lethality Assay: *Sal*. Newport treated with MOI≈10^7 77
3.4.5. Chicken Embryo Lethality Assay: *Sal*. Newport treated with MOI≈10^2 or MOI≈10^3 of a cocktail of phage (S11, S40, S41, S44, S50). 78
3.4.6. Chicken Embryo Lethality Assay: *Sal*. Newport 3596 and *Sal.* Newport 3596 Mut$^{\Phi}$. 79
3.4.7. Chicken Embryo Lethality Assay: *Sal*. Enteritidis treated with S7 at MOI≈10^4 or S10 at MOI≈10^4 82
3.4.8. Chicken Embryo Lethality Assay: *Sal*. Enteritidis treated with S7 at MOI≈10^3, S7 at MOI≈10^4, or S10 at MOI≈10^4 83
3.4.9. Chicken Embryo Lethality Assay: Comparisons of a rough isolate of *Salmonella* (O-:r:1,5) with *Sal*. Typhimurium, *Sal*. Typhimurium monophasic variant 1,4,[5],12:i-, and a *Sal*. Infantis 85
3.5. Discussion 87
3.6. References 91
Chapter 4 95
4.1. Abstract 95
4.2. Introduction 95
4.3. Materials and Methods 98
4.3.1. Sampling from Facilities 98
4.3.2. Sampling from Pasture 98
4.3.3. Water Sampling 98

4.3.4. Water Sample Culturing ... 98
4.3.5. *Salmonella* Culture and Detection ... 99
4.3.6. Data Collection ... 99
4.3.7. Analysis of Variables ... 100
4.3.8. Whole-Genome Sequencing ... 101
4.3.9. WGS Data Analysis ... 102
4.4. Results ... 105
4.4.1. Analysis of Variables ... 105
4.4.2. WGS of *Salmonella* Muenster ... 112
4.5. Discussion ... 121
4.6. References ... 128
Chapter 5 ... 135
5.1 *Salmonella* Introduction ... 135
5.2 Genomic Analysis of a Bacteriophage-Resistant Mutant of *Salmonella* ... 135
5.3 Chicken Embryo Lethality Assay with *Salmonella* ... 139
5.4 Computational Approaches in *Salmonella* Outbreak Investigation ... 141
5.5 Conclusions ... 147
5.6 References ... 148
Chapter 6 ... 153
Appendice. Supplemental Data ... 181

List of Tables

Table 2.1 49
Table 2.2 53
Table 2.3 62
Table 2.4 63
Table 4.1 101
Table 4.2 102
Table 4.3 103-104
Table 4.4 109
Table 4.5 110
Table 4.6 110
Table 4.7 110
Table 4.8 112

List of Figures

Figure 1.1 24
Figure 2.1 45
Figure 2.2 46
Figure 2.3 49
Figure 2.4 50
Figure 2.5 51
Figure 2.6 51
Figure 2.7 52
Figure 2.8 52
Figure 2.9 53
Figure 2.10 54
Figure 2.11 54
Figure 2.12 55
Figure 2.13 55
Figure 2.14 56
Figure 2.15 57
Figure 2.16 57
Figure 2.17 58
Figure 2.18 58
Figure 2.19 59
Figure 2.20 60
Figure 2.21 60
Figure 2.22 61
Figure 2.23 61
Figure 3.1 75
Figure 3.2 76
Figure 3.3 77
Figure 3.4 78
Figure 3.5 79

Figure 3.6 80
Figure 3.7 81
Figure 3.8 82
Figure 3.9 83
Figure 3.10 84
Figure 3.11 85
Figure 3.12 86
Figure 3.13 87
Figure 4.1 100
Figure 4.2 101
Figure 4.3 106
Figure 4.4 106
Figure 4.5 107
Figure 4.6 107
Figure 4.7 111
Figure 4.8 113
Figure 4.9 114
Figure 4.10 114
Figure 4.11 115
Figure 4.12 116
Figure 4.13 116
Figure 4.14 117
Figure 4.15 118
Figure 4.16 119
Figure 4.17 120
Figure 4.17 125
Figure 5.1 137
Figure 5.2 139
Supplemental Figure 1 181
Supplemental Figure 2 182

List of Abbreviations

Salmonella	*Salmonella enterica spp. enterica*
Salmonella	*Salmonella* species
Sal.	*Salmonella*
Phage	Bacteriophage
Sal. Newport Mut$^{\Phi}$	Phage-Resistant Mutant of *Salmonella* Newport
WKLM	White-Kauffman-Le Minor Scheme
USA	United States of America
CDC	Centers for Disease Control and Prevention
USDA	United States Department of Agriculture
FDA	Food and Drug Administration
NVSL	National Veterinary Service Laboratory
AUCVM	Auburn University College of Veterinary Medicine
GRAS	Generally Recognized as Safe
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
SNP	Single Nucleotide Polymorphism
PFGE	Pulsed-Field Gel Electrophoresis
MLST	MultiLocus Sequence Typing
cgMLST	Core Gene MultiLocus Sequence Typing
wgMLST	Whole-Genome MultiLocus Sequence Typing
MLEE	MultiLocus Enzyme Electrophoresis
WGS	Whole-Genome Sequencing
NGS	Next-Generation Sequencing
Pacbio	Pacific Biosciences
ONT	Oxford Nanopore Technology
ML	Machine Learning
MLE	Maximum-Likelihood Estimation
MCMC	Markov Chain Monte Carlo
SPI	*Salmonella* Pathogenicity Islands

T3SS	Type III secretion system
TLR4	Toll-like receptor 4
Sie	Superinfection Exclusion
CFU	Colony Forming Unit
PFU	Plaque Forming Unit
µm	Micrometre
µL	Microliter
mL	Milliliter
MOI	Multiplicity of infection
SM	Salt Magnesium Buffer
TTh	Tetrathionate Enrichment Broth
RV	Rappaport Vassiliadis
XLT4	Xylose Lysine Agar Supplemented with Tergitol 4
TSI	Triple Sugar Iron Agar
LIA	Lysine Iron Agar
LBM	Luria-Bertani broth containing 1mM magnesium
LB agar$^{0.35\%}$	Luria-Bertani broth, Miller with 0.35% agar
SPF	Specific-Pathogen Free
MDR	Multidrug Resistant
LPS	Lipopolysaccharide
IM	Inner Membrane
OM	Outer Membrane
ROS	Reactive Oxygen Species
HAI	Hospital Acquired Infection
WKLM	White-Kauffmann-Le Minor Scheme

Chapter 1

Literature Review

1.1 *Salmonella* Introduction and Taxonomy

The genus *Salmonella* is a Gram-negative facultatively anaerobic and peritrichously flagellated bacilli. *Salmonella* can be distinguished from members of other genera of the *Enterobacteriaceae* by a combination of biochemical reactions such as the production of hydrogen sulfide, citrate metabolism, lysine as a nitrogen source, and tetrathionate as a terminal electron acceptor (Sterzenbach et al., 2013). It was named after the American veterinarian Daniel E. Salmon, who first isolated Bacillus cholera-suis from a pig suffering from hog cholera (Salmon and Smith, 1885; Smith, 1894). B. cholera-suis has since been renamed *Salmonella enterica* subspecies *enterica* serovar Choleraesuis, abbreviated to *Sal*. *enterica* serovar Choleraesuis or *Sal*. Choleraesuis.

The history of the taxonomy of *Salmonella* is complicated and controversial with phenotypic, serologic, and genotypic methods used to determine the phylogeny of *Salmonella* (Grimont and Weill, 2007; Liu et al., 2014). Originally, each serovar of *Salmonella* was classified as a separate species. *Salmonella* is now comprised of two species, *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* contains six subspecies (*enterica, indica, salamae, houtenae, diarizonae,* and *arizonae*). *Salmonella enterica* subspecies *enterica* comprises over 2,600 serovars (Brown et al., 2021; Cobo-Simón et al., 2023). As an example of the nomenclature for *Salmonella* (*Sal*.) *enterica* subspecies *enterica* serovars, the *Salmonella enterica* subspecies *enterica* serovar Typhimurium can be simplified with the synonyms *Sal*. *enterica* sub. *enterica* Typhimurium or simply *Sal*. Typhimurium. These serovars are designated by the antigenic formula, which incorporates the antigenic properties of their lipopolysaccharide (LPS) sugar repeat units (O-antigens) and their flagellar structural protein subunits (H-antigens). In a few serotypes, *Sal*. Typhi, *Sal*. Dublin, and *Sal*. Paratyphi C, a capsular polysaccharide antigen (Vi-antigen) can be found. The method of deriving antigenic formulas for serovars is called the White-Kauffmann-Le Minor (WKLM) scheme (Grimont and Weill, 2007). As an example, the antigenic formula for *Salmonella* Newport is 6,8,20 :e,h :1,2 :[z67],[z78] and the antigenic formula for *Salmonella* Typhimurium is 1,4,[5],12 :i :1,2. These antigenic formulas can be broken down with the example of *Salmonella* Newport where the 6, 8 , and 20 are the O-antigens, the e,h represents the phase 1

H-antigens, the 1,2 represents the phase 2 H-antigens, and [z67],[z78] are considered special antigens. *Salmonella* Typhimurium antigenic formula is broken down to where the 1, 4, [5], and 12 are the O-antigens, the i represents the phase 1 H-antigen, and the 1,2 represents the phase 2 H-antigens.

The standard serotyping method involves using rabbit antisera with antibodies specific to the individual antigens that comprise the WKLM scheme. The WKLM scheme has not been updated since 2007, and there is no consensus on how to replace it (Chattaway et al., 2021). Serologic *Salmonella* serotyping reagents are expensive, laboratories are highly specialized, the method is laborious and time-consuming, it requires well-trained technicians, and results can be open to interpretation error (Abatcha et al., 2014; Chattaway et al., 2019). Researchers at the United States of America (USA) Centers for Disease Control and Prevention (CDC) started sequencing the alleles on the genes that encode the flagellin proteins *fliB*, *fliC*, and *flpA* (McQuiston et al., 2004). From this work, polymerase chain reaction (PCR) primers to specific genes encoding the flagellar antigens (*fliB* and *fliC*). These probes can detect the 36 flagellar antigen genes found in *Salmonella* (Mcquiston et al., 2011). Fitzgerald *et al.* (2007) developed a related strategy for serogroup identification based on the O-antigen *rfb* genes, from which signature probes were derived and integrated into a suspension bead (Luminex Technology) fluorescence assay (Fitzgerald et al., 2007).

Salmonella serotyping is in transition. Genomic typing tools have become increasingly popular with the rise of next-generation sequencing (NGS) techniques (Banerji *et al.*, 2020; Chattaway *et al.*, 2021). In 1988, MultiLocus Enzyme Electrophoresis (MLEE) was used to identify natural groupings of *Salmonella*. These groupings could correspond to serovars, and a sequence-based alternative, MultiLocus Sequence Typing (MLST), was developed. MLST was like MLEE but was based on sequences of multiple housekeeping gene fragments as opposed to electrophoretic migration of proteins (Achtman et al., 2012). Zhang *et al.* (2015), developed a sequence-based method of serotyping ("SeqSero") that incorporated a curated database that included *rfb* gene clusters responsible for somatic O-antigen synthesis; *wzx* O-antigen flippase gene; *wzy* O-antigen polymerase gene; additional genes from *rfb* cluster that is useful for O-group determination; sequence-specific genetic markers for additional O-antigen groups; and the *fliC* and *fliB* genes that encode *Salmonella* flagellar antigens. Raw sequence reads are mapped against the curated database, or with genome assemblies, and genes of interest are extracted and mapped

against the curated database (Zhang et al., 2015). Yoshida *et al.* (2016) developed a core gene MLST (cgMLST) method called *Salmonella In Silico* Typing Resource (SISTER). Yoshida *et al.* characterized the method as a genoserotyping approach that would incorporate queried genome assemblies into cgMLST-based phylogenetic clusters (Yoshida et al., 2016). Zhang *et al.* (2019) developed an updated tool ("SeqSero2") with an expanded database to serotype raw sequence reads or assemblies. SeqSero2 would generate k-mers, a contiguous subsequence of length *k* within a deoxyribonucleic acid (DNA) sequence, from assemblies or Oxford Nanopore Technology (ONT) reads. The query genome's O or H antigen genes were matched to a database that yielded the highest similarity score. With raw sequence reads, micro-assemblies were generated and mapped to the curated database (Zhang et al., 2019). SISTR and SeqSero2 are well-recognized bioinformatic tools and the original SeqSero is widely used as it is accessible through the Center for Genomic Epidemiology (https://cge.food.dtu.dk/services/SeqSero/) and on the BioNumerics software platform (https://www.applied-maths.com/bionumerics) (Zhang et al., 2019; Wu et al., 2023).

1.2 Salmonellosis and Host-Specificity

The nomenclature of *Salmonella* is very complex, but the classification of *Salmonella* serovars that differ in the host range of clinical salmonellosis is also very complicated. *Salmonella* serovars are genetically closely related, but there are wide variations in host-specificity, virulence, and disease manifestations.

Salmonellosis can manifest as a range of symptoms from the asymptomatic carrier stage, enterocolitis/diarrhea, to the life-threatening bacteremia/septicemia and enteric/typhoid fever (Coburn et al., 2007; Tennant et al., 2016; Stevens and Kingsley, 2021; Fierer, 2022; Marchello et al., 2022). *Salmonella* can also be categorized as typhoidal and non-typhoidal *Salmonella*, which is whether the *Salmonella* serovar manifests a typhoid-like fever or a more common, self-limiting gastroenteritis that accounts for the foodborne illness typically seen in the USA (Schultz et al., 2021; Delahoy et al., 2023). Typhoidal serovars are serovars such as *Sal.* Typhi, *Sal*. Paratyphi A, *Sal*. Paratyphi B, *Sal*. Paratyphi C, and *Sal*. Sendai that only infect humans and higher primates (Feng et al., 2019; Gal-Mor, 2019). Several serovars cause typhoid-like bacteremia in specific animal hosts such as *Sal*. Cholerasuis in pigs, *Sal*. Dublin in cattle, *Sal*. Typhimurium in mice, *Sal*. Gallinarium in poultry, *Sal.* Pullorum in poultry, and *Sal.* Abortusovis in sheep. Some typhoid-like serovars are considered non-typhoidal serovars in different animal hosts, such as *Sal*.

Typhimurium, which causes a typhoid-like fever in mice but causes gastroenteritis in humans, cattle, and horses(Costa et al., 2012; Gal-Mor, 2019; Soza-Ossandón et al., 2020; Stevens and Kingsley, 2021).

Salmonella is also divided into groups based on their host range: "non-adapted" (broad), "host-adapted", and "host-restricted" (Uzzau et al., 2000; Evangelopoulou et al., 2013; Foster et al., 2021; Fierer, 2022). Host-restricted *Salmonella* has a very narrow host range (usually one specific host species) and causes typhoid or typhoid-like disease, with examples that include *Sal.* Typhi (humans and higher primates), *Sal*. Gallinarum (chickens), *Sal*. Abortusovis (sheep), *Sal*. Typhisuis (pigs), and *Sal*. Abortusequi (horses) (Uzzau et al., 2000; Agbaje et al., 2011; Evangelopoulou et al., 2013; Stevens and Kingsley, 2021). Host-adapted *Salmonella* has a narrow host range with the ability to disseminate beyond the gastrointestinal tract, colonize systemic sites, persist systemically for long periods, possibly persistent asymptomatic infections, and often are vertically transmitted in their preferred hosts(Monack, 2012; Foster et al., 2021; Stevens and Kingsley, 2021; Fierer, 2022). *Sal*. Dublin (cattle) and *Sal*. Cholerasuis (pigs) can cause systemic disease and bacteremia and be vertically transmitted in their preferred host but can accidentally infect other species, such as humans (Stevens and Kingsley, 2021; Fierer, 2022). The non-preferred host usually exhibits subclinical infections (Evangelopoulou et al., 2013). Some infections in non-preferred hosts might be localized to unusual locations, such as human cases involving a chest wall abscess (*Sal*. Cholerasuis) and a thyroid abscess (*Sal*. Dublin) (Alyousef et al., 2020; Mokraoui et al., 2023).

Non-adapted *Salmonella* are serovars that infect and cause disease in a wide range of host species and exhibit what is characterized as non-typhoidal disease. This type of disease is self-limiting, with acute gastroenteritis, and watery diarrhea. The host's inflammatory response is responsible for the symptoms of diarrhea, nausea, vomiting, intestinal cramping, and fever (Sterzenbach et al., 2013; Gal-Mor et al., 2014). Gastroenteritis found in humans can also be seen in infected animals. Subclinical infections in animals are common. Subclinical signs can be reduced milk or egg production, reduced weight gain, and persistent carrier states (Galán-Relaño et al., 2023). *Salmonella* in humans is primarily a food-borne pathogen associated with food-producing animal sources (Naushad et al., 2023). Animals can be infected by close contact with infected animals, contaminated water or direct contact with feces or feces contaminated equipment, contaminated feed or environment, or potential transmission by arthropods (Galán-

Relaño et al., 2023). Recirculation of *Salmonella* in the environment can lead to animals being reinfected and the appearance of a persistent carrier state in animals (Sterzenbach et al., 2013).

1.3 *Salmonella* Virulence Factors Associated with Salmonellosis

1.3.1 *Salmonella* Pathogenicity Islands

Salmonella Pathogenicity Islands (SPIs) are clusters of virulence genes found on the chromosome. These SPIs encode factors essential for adhesion, invasion, survival, and replication within a host (Han et al., 2024). There are twenty-four known SPIs with SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-6, SPI-9, and SPI-11 being conserved across all *Salmonella* (Li et al., 2021).

The SPI-1 encodes the type III secretion system (T3SS-1), regulators, effector proteins, and chaperone proteins (Que et al., 2013). Type III secretion systems (T3SS) are complex membrane molecular machines also called injectisomes. These inject bacterial effector proteins into a eukaryotic host cell (Wagner et al., 2018). SPI-1, T3SS-1, and T3SS-2 effector proteins are essential for host cytoskeleton rearrangement and invasion of epithelial cells (Han et al., 2024). The T3SS-1 effector proteins are encoded on SPI-1 (SipA, SipB, SipC, SipD, SptP) and SPI-5 (SopB) (Sterzenbach et al., 2013).

The SPI-2 and its encoded T3SS-2 are a crucial virulence factor required for survival in macrophages (Sterzenbach et al., 2013). *Salmonella* will be contained within the *Salmonella*-containing vacuole (SCV) in infected host cells such as epithelial cells and macrophages. The T3SS-2 is responsible for injecting effector proteins across the membrane of the SCV (Figueira and Holden, 2012). Twenty-eight known effector proteins can be translocated by the T3SS-2, with only a few encoded on the SPI-2 (Han et al., 2024). SPI-2 is essential for virulence in *Salmonella*. Grant *et al.* (2012) found that *Salmonella* with a mutation in the SPI-2 T3SS-2 could not replicate inside or escape from an infected cell (Grant et al., 2012).

The SPI-1 T3SS effectors trigger the production of proinflammatory cytokines and the less well-characterized SPI-2 T3SS-2 proinflammatory activity, which stimulates the rapid recruitment of neutrophils and induces acute intestinal inflammation and gastroenteritis (Andrews-Polymenis et al., 2010; Figueira and Holden, 2012). This response is exacerbated by SPI-1-dependent induction of macrophage cell death (Figueira and Holden, 2012). The host cell death is induced by SPI-1 effectors as well as SPI-2 effectors; this results in programmed cell death and further dissemination of Salmonellae (López et al., 2012). Some T3SS-secreted effectors have the potential to reduce inflammatory responses and halt over-activated innate immune responses,

which may help avoid detrimental endpoints for the host upon infection. The T3SSs of *Salmonella* are very complex in the ability to provoke strong inflammatory responses and suppress the inflammatory response to provide the best environment for *Salmonella* to replicate (Tang et al., 2024).

1.3.2 Surface Structure – LPS and the "O" Antigen

LPS is a molecule associated with Gram-negative bacteria. It is an outer membrane (OM) molecule comprised of three structural regions: the hydrophobic region called lipid A (or endotoxin), the nonrepeating core oligosaccharide, and the distal O side-chain polysaccharide (or the O-antigen). LPS is anchored to the OM by lipid A. The core oligosaccharide is highly conserved among *Enterobacteriaceae* and is an attachment site for the variable O-antigen. It is encoded on the *rfa* gene cluster (Raetz and Whitfield, 2002). *Salmonella* colonies with the full O side-chain have a smooth appearance and are referred to as smooth. While *Salmonella* mutant colonies that have lost their O side-chain have dull surfaces and are referred to as rough mutants.

LPS establishes a permeable barrier that protects the cell from toxic molecules such as antibiotics and bile salts. LPS is the primary bacterial component encountered by the host immune system. Toll-like Receptor 4 (TLR4) recognizes and binds to TLR4, which activates the expression of pro-inflammatory cytokine genes and apoptosis (Raetz and Whitfield, 2002). The core oligosaccharide is important for serum, antimicrobial peptide, and bile salts resistance (Kong et al., 2011).

The O-antigen is encoded on the *rfb* gene cluster. The O-antigen side chain is the outermost portion of the LPS. This portion of the LPS is the O-antigen used for serovar identification by the WKLM scheme. Many antigenic factors exist, but only 67 O-antigens are used for serological identification (Al-Hamadany, 2021). The O-antigen repeat unit contains three sugars and is present in variable numbers of repeat units ranging up to 40 units (Hong et al., 2023). The O-antigen is critical in resistance to complement-mediated lysis with true rough isolates with no O-antigen side chain being much more sensitive to serum killing (Bjanes and Nizet, 2021; Han et al., 2024). At the same time, complement can bind to the O-antigen, complement C3 is important for marking the cell for phagocytosis, and complement C5 is an important proinflammatory chemoattractant (Krzyżewska-Dudek et al., 2022). Modification of the LPS structures can impair complement recognition and is a common resistance mechanism of Gram-negative bacteria (Bjanes and Nizet, 2021). For instance, it has been shown that mutations that produce a truncated O-antigen might

have reduced resistance to complement. Murray *et al.* found that O-antigens greater than fifteen sugar repeat units were necessary for complement activation and at least four sugar repeat units were necessary for complement mediated lysis (Murray et al., 2006).

1.3.3 Surface Structure – Flagella the "H" Antigen

The flagella, also known as the "H" antigen, is the motility structure for *Salmonella*. *Salmonella* has two distinct H-antigens, phase 1 (H_1-antigen) and phase 2 (H_2-antigen). These are encoded on the *fliC* gene (H_1-antigen) and *fljB* gene (H_2-antigen) (McQuiston et al., 2004). *Salmonella* can alternately express between the two flagellar genes known as phase variation (Liang et al., 2016). This ability to change its flagellar proteins helps it avoid being cleared by the host's immune system (Al-Hamadany, 2021). Serovars that can express both H_1-antigen(s) and H_2-antigen(s) are called diphasic, while serovars that only express the H_1-antigen(s) are called monophasic (McQuiston et al., 2004; Liang et al., 2016).

1.3.4 Surface Structure – The Capsular or "Vi" Antigen

The virulence or "Vi" antigen is a polysaccharide capsule encoded on SPI-7 (Seth-smith, 2008; Seth-smith et al., 2012; Gunn et al., 2014). SPI-7 is one of *Salmonella*'s largest excisable pathogenicity islands but is only found in three serovars (*Sal*. Typhi, *Sal*. Paratyphi C, and *Sal*. Dublin) (Nieto et al., 2016). The main difference between the SPI-7 of *Sal*. Typhi versus *Sal*. Paratyphi C, and *Sal*. Dublin is that, *Sal*. Typhi SPI-7 encodes a T3SS-1 effector protein SopE (Han et al., 2024). The Vi-antigen capsule is thought to enhance systemic virulence by increasing bacterial resistance to complement, reducing phagocytic killing by protecting the bacterium from reactive oxygen species (ROS), and interfering with pathogen-associated molecular patterns (PAMPs) activation of the innate immune system (Gunn et al., 2014). Vi antigen expression represses expression of T3SS-1 and flagella proteins (Sande and Whitfield, 2021).

1.3.5 Salmonella and the Microbiota

The gut microbiota relies on fermentation to produce energy for growth. Epithelial cells detoxify microbiota-derived hydrogen sulfide (H_2S) by conversion into thiosulfate (Winter and Bäumler, 2011). Microbes depend on the nutrients present in the mucous layer for growth. To outcompete the microbiota, *Salmonella* must utilize nutrients generated because of the host inflammatory response (Santos et al., 2009). *Salmonella* uses T3SS-1 and T3SS-2 mediated intestinal inflammation to engineer a nutrient niche characterized by increased availability of monosaccharides, amino acids, and respiratory electron acceptors (Rogers et al., 2021).

During gastroenteritis, neutrophils transmigrate into the intestinal lumen in large numbers, giving rise to an abundance of fecal leukocytes, characteristic of inflammatory diarrhea. Neutrophils generate ROS that oxidize thiosulfate ($S_4O_3^{2-}$) into tetrathionate ($S_4O_6^{2-}$). The *ttrBCA ttrRS* gene cluster codes for tetrathionate reductases, enabling *Salmonella* to use tetrathionate as a terminal electron acceptor (Hensel et al., 1999). Through this mechanism, inflammation provides a respiratory electron acceptor that allows *Salmonella* to use anaerobic respiration instead of fermentation to produce energy for growth (Winter et al., 2010). *Salmonella* can use tetrathionate as an electron receptor in anaerobic respiration, which gives them an advantage in the intestinal environment. Tetrathionate respiration enables *Salmonella* to utilize fermentation end products that the fermenting microbiota cannot consume. Using tetrathionate respiration for energy production presents *S*. Typhimurium with a vital growth advantage over competing microbes that rely on fermentation. Inactivation of genes required for tetrathionate respiration removes the ability of *S*. Typhimurium to outgrow the microbiota during intestinal inflammation (Winter et al., 2010). This data indicates that tetrathionate respiration is one of the primary mechanisms enhancing the outgrowth of *Salmonella* in the inflamed gut.

The pathogenic strategy of *Salmonella* associated with gastroenteritis is to use virulence factors (T3SS-1, T3SS-2, and others) to elicit acute intestinal inflammation. This host response provides a new respiratory electron acceptor in the gut, enabling the pathogen to outgrow the microbiota in the lumen, thereby enhancing its transmission to the next host by fecal shedding of the organism. *Salmonella* thus uses the host to provide them with a substance that allows them to outgrow their competition (Bäumler et al., 2011).

Salmonella has evolved ways to subvert, mimic, antagonize, and exploit the defense strategy of vertebrate hosts with their virulence factors creating a novel niche that favors the growth of *Salmonella* to outcompete the resident microbiota (Hallstrom and McCormick, 2011; Rivera-Chávez and Bäumler, 2015). *Salmonella* residing in the tissue face death by the host's innate immune system, but acute inflammation changes the environment of the gut lumen to favor *Salmonella* growth (Rivera-Chávez and Bäumler, 2015). Luminal outgrowth is required to increase their abundance in intestinal contents during gastroenteritis for successful transmission to the next naïve host by the fecal-oral route. Diarrheal disease (gastroenteritis) flushes the intestinal lumen, removing the intestinal contents and the Salmonellae (Sterzenbach et al., 2013; Rivera-Chávez and Bäumler, 2015).

1.4 Bacteriophage

1.4.1 Introduction and Phage Therapy

The French-Canadian microbiologist Félix D'Hérelle devised the term "bacteriophage" which means bacteria-eater (Salmond and Fineran, 2015). Phages are viruses; like all other viruses, they are obligate intracellular parasites of cellular organisms with their life cycle within a host cell. The basic life cycle involves using bacterial cellular metabolism to produce new phage particles, release them from their cellular confines, and infect new cells (Hyman and Abedon, 2010a).

This cycle of infection, replication, and release from bacterial cells gives phage the opportunity to be used as highly specific antimicrobial agents (Monk et al., 2010). This final step of the lytic life cycle, in which phages kill the bacterial cells, is the cornerstone of the idea of using phages as an antimicrobial agent (Skurnik and Strauch, 2006). This replication cycle is why the term "self-replicating pharmaceuticals" was coined for phage (Abedon and Thomas-Abedon, 2010).

Antibiotics have been the main treatment for bacterial diseases, but antibiotic resistance has been a growing concern. It is estimated that by 2050, ten million people a year will die from multidrug-resistant (MDR) bacterial pathogens. This has renewed interest in phage and phage therapy (Penziner et al., 2021). There are several case reports of emergency use of phage therapy, but studies have been limited on the safety and toxicity of phage therapy (Liu et al., 2021). Phage is widely regarded as safe, with the Federal Bureau of Drugs and Administration (FDA) approving phage preparations as "Generally Recognized as Safe" (GRAS) in food preparations (Sarhan and Azzazy, 2015; Podlacha et al., 2021; Ge et al., 2022).

Phage can be very effective in disseminating throughout the body and can even cross the blood-brain barrier (Sulakvelidze and Kutter, 2005; Podlacha et al., 2021). When treating with phage, where the bacterium is found, so can the phage be found. The proposed mechanism is called the "trojan horse" mechanism, by which the phage-infected bacterium moves through the body and thus carries the phage with them (Podlacha et al., 2021). Phage have a narrow specificity to their host bacteria and will not disrupt the host microbiota; they increase in number after administration due to replicating in host cells, they can have lytic activity against MDR bacterial pathogens, and can penetrate biofilms (Liu et al., 2021; Penziner et al., 2021). Phage has also been proposed as a treatment in conjunction with antibiotics. Phage has been shown to re-sensitize MDR

bacterial pathogens to antibiotics. This phage-antibiotic synergy could combat many antibiotic-resistant pathogens (Yosef et al., 2015; Segall et al., 2019).

No harmful effects have been observed with phage treatment, but the purity of phages has been a problem. Safety issues involve crude phage preparations containing LPS, peptidoglycan, and additional inflammatory components from lysed bacteria. This can be alleviated with phage purification by density gradient purification or column chromatography (Sulakvelidze and Kutter, 2005; Drulis-Kawa et al., 2015; Ranveer et al., 2024). Potential downsides of phage therapy could be that phage could undergo an alternative lifecycle called the lysogenic cycle, in which the phage DNA integrates into the host bacterium's DNA (Penziner et al., 2021). This lysogenic conversion could transfer undesirable genetic material, such as virulence factors or antibiotic resistance (Penziner et al., 2021; Podlacha et al., 2021; Ge et al., 2022). Another major concern with the use of phage is the emergence of phage-resistant variants. To combat the problem of phage resistance, it has been proposed to use multiple non-identical phages or a "phage cocktail". The phage cocktail could prevent the emergence of phage-resistance (Abedon, 2010). A study by Dalmasso *et al.* (2016) found that a cocktail of three phages inhibited *Escherichia coli*'s growth while preventing phage-resistant mutants' emergence (Dalmasso et al., 2016). Phage resistance should not be underestimated, and understanding phage resistance costs or benefits to a host bacterium is critical in the progression of phage therapy (Oechslin, 2018).

1.4.2 Bacteriophage-Resistance

Bacteria and phage are in a permanent arms race with co-evolution driving mechanisms to evade and the latter adapting and avoiding evolved defense systems (Bleriot et al., 2024). There are several steps of phage infection in a host bacterium. The phage attaches to the surface of the cell, injects the phage genome into the bacterial cell, assembly phage proteins, and releases progeny phages. At any of these stages, phage infection can be inhibited or aborted (Azam and Tanji, 2019). The most common mechanism of phage-resistance is to prevent phage adsorption.

Phage-resistance by preventing phage adsorption occurs by the modification of surface phage receptors, hindering access to phage receptors, or by producing competitive inhibitors (Oechslin, 2018). Phage can use many different surface molecules as phage receptors; this includes outer membrane proteins, flagella, pili, capsule, and teichoic acids in Gram-positive bacteria and LPS in Gram-negative bacteria (León and Bastías, 2015; Egido et al., 2022). The O-antigen region of LPS is also a receptor for numerous phage (León and Bastías, 2015). Modifications to these

structures can lead to phage-resistance if any of these are the phage's receptor (Labrie et al., 2010). Outer membrane vehicles can also be a mechanism by being a non-replicating nanostructure composed of a membrane and membrane structures produced during bacterial growth. These molecules can act as decoys for phage to bind, thus reducing phage titers (phage numbers) (Azam and Tanji, 2019). Bacteria can produce extracellular matrixes that are a physical barrier to phage, or masking proteins that block phage adhesion, or even flagellar phase variation as seen in *Salmonella* can be mechanisms to prevent phage attachment and adsorption (Labrie et al., 2010; Azam and Tanji, 2019; Egido et al., 2022; Bleriot et al., 2024).

The mechanism by which bacteria can block phage DNA injection is superinfection exclusion (Sies) systems. These systems are proteins that block the entry of phage DNA into the host bacterium. Many of these systems are encoded by prophage or lysogenic phage genes, and they protect phage by preventing other phages from infecting the same cell (Labrie et al., 2010; Azam and Tanji, 2019).

If phage DNA is injected, an innate defense system can cleave injected DNA. These systems include the restriction-modification system, the defense island system associated with restriction-modification, and prokaryote argonaute proteins. Adaptive immune systems include the CRISPR-Cas system, where foreign DNA is inserted into the CRISPR loci, and when CRISPR ribonucleic acids (RNAs) bind to a complementary nucleic acid that has entered the cell, the DNA is degraded by nucleases (Labrie et al., 2010; Oechslin, 2018; Azam and Tanji, 2019; Zhang and Cheng, 2022).

Phage-inducible chromosomal islands (PICI) can detect infection by phage. The PICI will be excised, circularized, replicated, and packaged. These PICI are packaged into the capsid of the phage particle instead of the phage genome. The cell will lyse, but the particles released will carry the PICIs instead of the phage genome. These will infect other cells, and the PICI will incorporate itself into the new cell (Carpena et al., 2016; Azam and Tanji, 2019).

The final mechanism of phage-resistance is abortive infection (Abi) systems. These systems are not fully understood, but it is known that these systems cause bacterial cells to die (Labrie et al., 2010; Drulis-Kawa et al., 2015; Azam and Tanji, 2019; Egido et al., 2022). These systems can be triggered at many different stages of the infection cycle (Azam and Tanji, 2019). These altruistic bacterial systems trigger the cell to commit suicide, in which the surrounding

bacterial population is protected by the phage being contained within the dead cell (Fineran et al., 2009; Blower et al., 2011; Shabbir et al., 2016).

Many of these mechanisms can affect the virulence of the bacterium. These modifications can lower the fitness compared to non-resistant strains (León and Bastías, 2015). There have been many different studies that have concluded that phage-resistance lead to decreased virulence in *Listeria monocytogenes*, *Bacillus thuringiensis*, *Vibrio cholerae*, *Dickeya solani*, *Flavobacterium columnare*, *Staphylococcus aureus, Serratia marcescens, Shigella flexneri*, *Klebsiella pneumoniae*, and *Salmonella* serovars (Flyg et al., 1980; Heierson et al., 1986; Shamim Hasan Zahid et al., 2008; Capparelli et al., 2010b; Laanto et al., 2012; Kitchens, 2016; Sumrall et al., 2019; Wang et al., 2019; Bartnik et al., 2022; Kortright et al., 2022; Tang et al., 2023). Capparelli *et al.* (2010) found that *Salmonella* resistant to phage was due to the lack of the O-antigen on the LPS, which conferred attenuation in mice (Capparelli et al., 2010a). It appears that the phage-resistance can help the bacterium survive viral infection, but the trade-off is a fitness cost that typically affects the virulence of the bacterium in a host organism.

1.5 *Salmonella* Epidemiology

1.5.1 *Salmonella* in People

Salmonella infections affect roughly a million people in the USA each year. The CDC estimates that *Salmonella* infections can range from 645,000 to 1.7 million cases yearly, but only approximately 42,000 cases are laboratory-confirmed and reported to the surveillance system (Scallan et al., 2011). CDC Foodnet Annual Report for 2021 has *Salmonella* ranked second among laboratory-confirmed bacterial foodborne pathogens but the highest in hospitalizations, deaths, and outbreak-associated infections (Delahoy et al., 2023). The United States Department of Agriculture (USDA) Economic Research Service reports that in 2013, *Salmonella* was responsible for approximately 11% of all foodborne illnesses, second only to Norovirus infections. Non-typhoidal *Salmonella* is estimated as the leading cause of hospitalizations (35% or 19,000 cases) and deaths (28% or 378 deaths) caused by foodborne illnesses linked to a specific pathogen. *Salmonella* is ranked first among 15 pathogens in terms of economic burden, estimated at $3.7 billion in a typical year. Ninety percent of the burden is due to deaths ($3.3 billion), 8 percent due to hospitalization ($294 million), and the remaining 2 percent to non-hospitalized cases. The economic burden can range from $193 million to $9.5 billion annually (Hoffmann *et al.*, 2015).

The most recent Laboratory-based Enteric Disease Surveillance System's *Salmonella* Annual Report reported there were 46,623 laboratory-confirmed human *Salmonella* infections. Approximately 22% of these laboratory-confirmed *Salmonella* infections were in children four years of age or younger. The most frequently reported serovars were *Sal.* Enteritidis (16.8%), *Sal.* Newport (10.1%), *Sal.* Typhimurium (9.8%), *S.* Javiana (5.8%), *S. enterica* serovar I 4,[5],12:i:- (4.7%), *S.* Infantis (2.7%), *S.* Muenchen (2.6%), *S.* Montevideo (2.2%), *S.* Braenderup (2.1%), and *Sal.* Thompson (1.7%) (CDC, 2018). The US Department of Health and Human Services' "Healthy People 2030" made it a national health objective to reduce *Salmonella* infections by 25% by 2030. The 2030 objective target is an incidence rate of 11.5 per 10^6 people; in 2021, the incidence rate was reported as 13.3 per 10^6 people, which is a decrease from the 2016-2018 report (US HHS, 2023).

1.5.2 Environmental *Salmonella* in Animal Facilities

Salmonella has been extensively studied in animals and environmental sites. There is a wide array of environmental niches in which *Salmonella* can survive. *Salmonella* may be present in various water sources such as effluent discharges, agricultural runoff, excretions by wild animals, and freshwater. Sediments may protect enteric organisms like *Salmonella* from stresses in aquatic environments and provide some nutrients. Water contaminated with animal waste has the potential to proliferate and disseminate *Salmonella* by wild animals (Murray, 2000).

Farm environments can easily be affected if there are outbreaks of *Salmonella* among animals or some of the animals on the farm are asymptomatic carriers. Other than animal-to-animal transmission, additional factors for the on-farm environmental spread of *Salmonella* include recycling of (manure) lagoon wastewater for flushing, contaminated feeds, inadequately controlled rodent and wild bird populations, contaminated rendering trucks being driven into animal areas and use of the same loader for transporting dead animals and moving feeds without appropriate cleaning and decontamination (Murray, 2000). Movement of animals can also lead to the spread of *Salmonella* by introducing an infected or carrier animal into a population of non-infected animals (Wray et al., 1990). Due to the stress of being moved or transported between premises, animals are at risk of being more susceptible to infection (De Lucia et al., 2018). Many production animals have subclinical infections that lead to widespread environmental contamination. This makes internal and external biosecurity measures critical to restrict *Salmonella* movement within a farm (Smith et al., 2018). Some of the best practices for the reduction of environmental

Salmonella are worker education in biosafety and cleaning practices (Smith, 2004; Pandya et al., 2009; Burgess and Morley, 2014; Cummings et al., 2014; Morley and Weese, 2015; Hwang et al., 2020). Sources for contamination of environmental sites are so diverse that absolute elimination of *Salmonella* in the outdoor farm environment is impossible. However, addressing efforts to prevent introduction, minimize pathogen load, and prevent unintended distribution spread may assist in reductions into the food-chain (Murray, 2000).

1.5.3 *Salmonella* in Animals

The host-host transmission of *Salmonella* is primarily by the fecal-oral route. After a host becomes infected, most of the time, the host will resolve the salmonellosis, and shedding will stop. Still, a few infected individuals will become carriers and will intermittently shed *Salmonella* in their feces for long periods. These animals can act as reservoirs for the pathogen. Food animals can be sources of food contamination for humans by fecal contamination of vegetables, fruit, and nuts or from fecal contamination of carcasses upon slaughter (Gopinath *et al.*, 2012). It is also possible that wildlife can serve as reservoir hosts by being asymptomatic carriers, causing sporadic cases of salmonellosis by contamination of feeding places (Oludairo et al., 2023). It has been observed that supershedders can lead to persistent shedding of *Salmonella*. However, in low-shedding animals, constant reinfection and host-to-host transmission led to persistent *Salmonella* shedding, and persistent shedding can be interrupted by breaking the cycle of reinfection (Menanteau et al., 2018).

Salmonella can be found in many different domestic and wild animals. Poultry, swine, cattle, horses, wild birds, rodents, pets, and exotic animals can all be reservoirs for *Salmonella*. Animals such as companion animals (dogs and cats) and exotic animals (reptiles, birds, and amphibians) can pose a risk of infecting humans or other animals in the environment (Galán-Relaño et al., 2023). A study looking at bacterial species associated with hospital-acquired infections (HAIs) (both human and veterinary HAIs) found *Salmonella* as the fourth highest (15% of reported HAIs) bacterial pathogen reported (Sebola et al., 2023). *Salmonella* is insidious, with animals being asymptomatic shedders where they can shed the bacterium in high numbers. This poses a risk for nosocomial infections and zoonotic infections to the personnel working close to these animals (Sebola et al., 2023).

Salmonella can generate different symptoms in animals. Common symptoms for cattle are diarrhea, fever, and dehydration. Cattle can suffer from abortions and subclinical symptoms like

reduced milk production (Ragione et al., 2013). Clinical salmonellosis in pigs is usually enterocolitis or septicemia, as with *Sal*. Cholerasuis infections (Stevens and Gray, 2013). Horses usually exhibit colic from salmonellosis (Timoney, 2013). Chickens typically are asymptomatic with most *Salmonella* serovars except for *Sal*. Gallinarium and *Sal*. Pullorum, both of which cause high levels of mortality—chickens infected with *Sal*. Gallinarium usually appear normal 24-36 hours prior to death. With all other serovars, infections are asymptomatic. Chickens pose the highest risk to public health because domestic fowl constitute one of the largest reservoirs of *Salmonella* (Shivaprasad et al., 2013). An additional concern with chickens is that *Salmonella* serovars such as *Sal*. Enteritidis can be vertically transmitted from hen to eggs by transovarian infection in the laying hen (Howard et al., 2012).

Several animal models have been used to study *Salmonella*. Mice, rabbits, zebrafish, rats, cattle, chickens, and rhesus macaque have all been used to study the salmonellosis (Tsolis et al., 1999; Santos et al., 2001; van der Sar et al., 2003; Van Immerseel et al., 2004; Simon et al., 2011; Panda et al., 2014; Higginson et al., 2016). Mice are used for studies of *Salmonella*, but disease involving serovar Typhimurium tends to model typhoid fever in humans. This is because *Sal*. Typhimurium causes a systemic, sometimes fatal disease in mice very similar to *Sal*. Typhi disease in humans (Santos et al., 2001). The calf animal model tends to be a very good model for *Salmonella*-induced enteritis except for *Sal*. Dublin, which causes a systemic disease with high mortality in calves and abortion in cows. (Tsolis et al., 1999; Santos et al., 2001). *Salmonella* and phage interventions in animal models have included mice, chickens, and calves (Hyland et al., n.d.; Fiorentin et al., 2005; Toro et al., 2005; Capparelli et al., 2010a; Bardina et al., 2012; Wottlin et al., 2022).

1.5.4 *Salmonella* Outbreaks in Veterinary Hospitals

Salmonella outbreaks have repeatedly been shown to be a constant risk to all types of veterinary hospitals. The resulting infections have been costly, both financially and in significant morbidity and mortality among patients and zoonotic disease among hospital personnel (Tillotson et al., 1997; Schott et al., 2001; Wright et al., 2005; Schaer et al., 2010; Steneroden et al., 2010; Cummings et al., 2014). Most of these nosocomial outbreaks involved equine patients, which is not surprising because horses exhibit severe symptoms such as colic, while other animals, like cattle, can be asymptomatic (Ragione et al., 2013; Timoney, 2013). The most recent nosocomial

outbreak was reported in 2014 and was discovered by retrospective analysis that concluded the outbreak lasted from January 1, 2006, to June 1, 2011 (Cummings et al., 2014).

Sal. Infantis, *Sal*. Newport (two outbreaks), *Sal*. Oranienberg, *Sal*. Typhimurium (six outbreaks) were the serovars responsible for the more recently reported *Salmonella* nosocomial infections involving veterinary hospitals (Tillotson et al., 1997; Schott et al., 2001; Ward et al., 2005; Wright et al., 2005; Schaer et al., 2010; Steneroden et al., 2010; Cummings et al., 2014). Veterinary hospital *Salmonella* outbreaks all have the universal feature of widespread environmental *Salmonella* contamination (Burgess and Morley, 2018, 2019; Burgess, 2023). Animals are likely the initial source of environmental contamination, which leads to continued infection in new patients (Wright et al., 2005; Burgess and Morley, 2019). Biosecurity is critical to managing the transmission of *Salmonella* in veterinary settings (Sebola et al., 2023). For this reason, people play a crucial role in the transmission of HAIs. Education programs for personnel in hygiene, proper use of personal protection equipment, movement control, cleanliness of equipment, managing high-risk groups, and the benefits of continued compliance are the best practices to reduce transmission to a baseline or acceptable endemic level (Wright et al., 2005; Anderson, 2015; Burgess and Morley, 2018; Burgess and Weese, 2022; Burgess, 2023; Sebola et al., 2023).

1.6 Computational Analysis in Epidemiology

1.6.1 Machine-Learning Algorithms

To quote Leonard M Schuman, “Any science is as objective as its capability of measuring the events which it purports to be observing and relating. Epidemiology has not been exempt from the usual evolutionary development of this necessary aspect of its methodology.” (Lilienfeld, 1980). Abraham Lilienfeld (1980) did a review on “Advances in Quantitative Methods in Epidemiology,” in which he discussed that in the 1950s, the new statistical tool was the 2x2 contingency table and estimates of relative risk and odds ratios. He discussed the development of a “logistic regression” method that uses many factors that might influence the occurrence of a disease to calculate estimates of relative risk and tests of significance. Lilienfeld (1980) stated that the “entry of the computer” into data analysis makes a “logistic regression” possible, and the “current” problem is epidemiologists’ unfamiliarity with using computers and the need for training on computer usage (Lilienfeld, 1980). A publication in 1997 discussed the usefulness of a

computer program to calculate 2x2 contingency table data as the program is an easy and quick "epidemiological calculator" (Štefek et al., 1997). Zocchetti *et al.* (1997) discussed using "some algebra" for 2x2 contingency table calculations for prevalence risk ratios versus the not-so-easy prevalence risk ratios calculations with advanced statistical tools like a logistic regression (Zocchetti et al., 1997). Even an article published in 2017 discusses the usefulness of manually calculating odds ratios and relative risk with 2x2 contingency tables (Albert, 2017). Lilienfeld hoped that the introduction of computers would bring the transition from more classical epidemiological methods to more advanced methods, but epidemiologists can be stubborn with the adherence to simple analytical methods with the concern that readers could not understand more complex methods (Porta and Bolúmar, 2016; Olsen et al., 2017).

Novel computational modeling strategies are being utilized in scientific literature. One area of interest is in "machine learning" algorithms, and these algorithms could be beneficial to epidemiologists (Wiemken and Kelley, 2019). These models "learn" from the data to improve its performance, and the analysis allows for the identification of "important" variables (Dharma et al., 2023). Machine learning shines within descriptive epidemiology, which is the field to describe associations between multiple variables and identify patterns within the data (Vilne et al., 2019; Dharma et al., 2023). Machine learning algorithms have three methods of learning: supervised, unsupervised, and semisupervised. Supervised learning is when the outcome is known for each observation. Unsupervised learning attempts to identify relationships and groups within the data, but the outcomes are not known. Semisupervised learning is a mixture of supervised and unsupervised where some outcomes are known and others might have missing data (Bi et al., 2019; Hamilton et al., 2021). Data is usually split into two groups: training data and test data. Training data is a randomly selected subset of data that is used to train the machine learning algorithm. The test data set is used to evaluate the performance of the model to predict the outcome (Vilne et al., 2019; Wiemken and Kelley, 2019; Hamilton et al., 2021; Serghiou and Rough, 2023)

Markov Chain Monte Carlo (MCMC) analysis is a method used to simulate parameter distributions of interest, such as generalized linear model parameters. It is particularly useful for handling difficult types of analyses and is commonly used for Bayesian analysis. MCMC methods involve repeatedly querying datasets to determine the probability distribution function of quantities of interest. The resulting sequence of values forms a Markov chain that can be analyzed to find best-fit values and confidence intervals. MCMC approaches can provide advantages over methods

based on standard maximum-likelihood estimation (MLE) and allow for the simultaneous estimation of parameters for complex models (Serghiou and Rough, 2023). MCMC in a Bayesian framework allows the posterior probability distribution to be approximated computationally, which revolutionizes infectious disease modeling (Lope and Demirhan, 2024).

Another type of machine learning algorithm is a decision tree model. A decision tree is a model that separates data into smaller and smaller partitions until each observation is classified according to the outcome of interest(Hamilton et al., 2021). Random Forest analysis is a machine-learning technique that combines multiple decision trees to make predictions for the outcome (Hamilton et al., 2021; Aawar and Srivastava, 2022). It is a useful tool for epidemiologists as it allows for the interpretation of complex association patterns in epidemiological data. Using random forests, researchers can identify relevant features and understand their relationships with the outcome of interest. Combining random forests with Bayesian network surrogate models further enhances interpretability by providing a deeper understanding of the association patterns (Aawar and Srivastava, 2022). The random forest model provides predictive accuracy, but they are considered black-box models. This is because it is difficult to retrace how the model came to a specific prediction (Becker et al., 2022).

Epidemiologists should transition to machine learning because it offers new tools to tackle problems for which classical methods are not well-suited (Barapatre et al., 2023). Machine learning can be used for descriptive epidemiology to identify important associations and predictors of outcomes (Dharma et al., 2023). Integrating machine learning algorithms with existing methods can improve the understanding of health and disease (Wiemken and Kelley, 2019). However, language and technical barriers between the fields of epidemiology and machine learning need to be addressed (Serghiou and Rough, 2023). Epidemiologists can benefit from learning the concepts and terminology used in machine learning literature (Serghiou and Rough, 2023). By incorporating machine learning into epidemiologic research, there are opportunities to enhance the field and improve the safety and efficacy of applications (Russo and Bonassi, 2015).

1.6.2 Whole-Genome Sequencing in Bacterial Epidemiology

During the 2000s, next-generation sequencing greatly increased sequencing capabilities, allowing the ability to sequence the whole genome of microorganisms (Timsit et al., 2023). Whole-genome sequencing (WGS) technology has revolutionized the field of bacterial epidemiology by providing valuable insights into the epidemiology and pathogenesis of infectious diseases. WGS

has become more affordable and accessible for microbiological laboratories, allowing for an improved understanding of disease ecology and control strategies (Garaizar and Laorden, 2023). WGS has significantly increased the amount of information available for studying infectious diseases and has improved the precision of epidemiological inferences. The use of WGS in the surveillance of bacterial pathogens has proven effective in outbreak investigations, source tracking, and variant analysis (Schürch et al., 2018; Thomas et al., 2023). Whole genome sequencing (WGS) technology is increasingly used for the epidemiology of *Salmonella*. WGS allows for the analysis of *Salmonella* isolates obtained from various sources, such as food products, animals, and humans, to better understand their genetic characteristics and relationships (Garaizar and Laorden, 2023; Thomas et al., 2023).

Short-read sequencing was the next advancement in sequencing technology after first-generation traditional Sanger sequencing. Short-read technology is the sequencing of short (250-800 basepair (bp)), clonally amplified DNA molecules sequenced in parallel (Hu et al., 2021). Illumina sequencing platform (36 – 300 bp) is based on the "sequencing by synthesis" (SBS) approach that involves DNA-polymerase-dependent nucleotide incorporation on the extended DNA chain (Hu et al., 2021; Satam et al., 2023). Illumina's technology is based on the SBS with a fluorescent-labeled reversible terminator technology. This, along with paired-end sequencing, makes it the most accurate base-by-base sequencing technology, with an error rate of 0.1% (Hu et al., 2021).

Long-read sequencing technology (third-generation sequencing technology) can generate sequences greater than 10,000 bp directly from native DNA. Inaccuracies plagued early iterations, but recent modifications have improved the accuracy. The two primary long-read technologies are Pacific Biosciences (PacBio) and Oxford Nanopore Technology (ONT) (Hu et al., 2021). The two technologies operate on different principles. PacBio can be used for whole genome *de novo* assembly due to the read length being up to 300 kb, but it has the disadvantage of the high cost associated with sequencing. ONT reads average from 10 to 30 kb but has a very high error rate (Hu et al., 2021; Satam et al., 2023). ONT long-read technology is based on a nanopore technology where single-stranded DNA is passed through a biological pore, and the electrical current is measured as each base is passed through (Hu et al., 2021; Satam et al., 2023). Deep learning algorithms are used to translate the electrical signals into a DNA sequence (Lin et al., 2021).

Short-read sequencing technology results in incomplete genome assemblies only considered as draft genomes. Long-read sequencing technology is highly error-prone. The new frontier in genome assembly is to make a hybrid assembly based on short-read and long-read sequencing (Giani et al., 2020). With hybrid assembly, the genome is assembled first with the long reads, and then the short reads are used to polish the long-read assembly (Wick et al., 2023). This provides a more polished complete reference genome because the short reads are higher quality than the long reads, while the long reads provide a backbone for the short reads and fill in gaps (Eagle et al., 2023).

Short-read sequencing technology's incomplete draft genomes have been commonly used to identify and characterize *Salmonella* bacteria (Chen and Meng, 2022; Zhao et al., 2023). Serotyping, detecting antimicrobial resistance and virulence genes, plasmid detection, and phylogenetics can be done with short reads. Phylogeny approaches are core-genome MLST (cgMLST) (aligns core genes), whole-genome MLST (wgMLST) (aligns core and accessory genes), single nucleotide polymorphism (SNP) (core SNP alignment and SNP matrix), and K-mer based methods (pair-wise comparison of nucleotide blocks) (Uelze et al., 2020). SNP phylogeny or SNP detection first requires a closely related genome reference, which the choice of reference is critical. Draft genomes contain too many contigs and are poor at calling accurate SNP positions. A reference that is too distant will provide fewer reference positions, and fewer SNPs will be discovered (Uelze et al., 2020). Reads are aligned to a reference, and variants are detected (Hu et al., 2021). Variants are SNPs, insertions, deletions, and structural variants such as duplications, inversions, and translocations (Danecek et al., 2011; Giani et al., 2020). Tools available for SNP calling are SAMtools, GATK, and Freebayes. Popular specialized pipelines for SNP calling from bacterial genomes are Snippy, CFSAN SNP Pipeline, NASP, and BactSNP (Schürch et al., 2018; Uelze et al., 2020; Rossi et al., 2023; Zhao et al., 2023).

1.7 A Study of a Bacteriophage-Resistant *Salmonella* Mutant and Factors Influencing Prevalence of *Salmonella* in a Multi-Species Animal Facility

(Kitchens, 2016)

1.7.1 Factors Influencing Prevalence of *Salmonella* in a Multi-Species Animal Facility

Over a two-year period, 631 samples were collected from various large animal facilities and pastures at the Auburn University College of Veterinary Medicine John Thomas Vaughan

Large Animal Teaching Hospital. The study aimed to identify environmental *Salmonella* contamination risk factors in the teaching hospital to understand environmental *Salmonella* in a multi-species animal facility. The overall prevalence of *Salmonella* at the J.T. Vaughn Teaching Hospital (excluding samples from Animal Health and Research Pastures and the off-site beef herd) was 50.33% (n=449) of all environmental samples collected. This prevalence level suggests widespread *Salmonella* contamination at the J.T. Vaughn Teaching Hospital at Auburn University. The sections with the highest prevalence in the study were the dairy barns and pastures, as well as the food animal barns, which had 69% and 60% positive *Salmonella* prevalence respectively. Summer season, water samples, drains swabs, buildings, the dairy barns and pastures, and the food animal barns were associated with the isolation of *Salmonella spp.* by risk ratio. The two major serovars isolated from environmental samples over the two-year span were *Salmonella* Muenster and *Salmonella* Cerro.

The initial aim of the study was to determine if proximal movement of *Salmonella* occurs within a multi-species animal facility. The goal was to understand if *Salmonella* could move from something such as a beef cattle herd to nearby chicken flocks or vice versa, to develop interventions to reduce *Salmonella* burden among food-producing animals. Overall, this project found that certain factors do increase the chances of recovering *Salmonella* from the environment in a multi-species animal facility, including season, resident animal species, sample source, and the facility. The study was not able to determine proximal movement due to a lack of evidence, such as pulse-field gel electrophoresis, wgMLST, or WGS, that could identify strains between proximally located facilities.

1.7.2 A Study of a Bacteriophage-Resistant *Salmonella* Mutant and Infection in Calves

Hyland (N.D.) studied phage treatment for the reduction of multiple drug-resistant *Salmonella* Newport in dairy calves from a clinical disease and food safety perspective (Hyland et al., n.d.). An unintended consequence of phage treatment could be the emergence of fully virulent but phage-resistant *Salmonella*. Using one phage for selective pressure to select for a spontaneous mutation that confers bacteriophage resistance in *Sal.* Newport also conferred resistance to four out of five cocktail phage (by spot lysis). The bacteriophage-resistant mutant of *Sal*. Newport selected using bacteriophage S50 was resistant to phages S11, S40, S41, and S50.

The bacteriophage-resistant mutant was administered orally at a dose of 10^9 colony-forming units (CFUs) to two calves to determine if the mutant alone could colonize. In this pair of

calves given the bacteriophage-resistant mutant *S.* Newport alone, fecal shedding of the mutant by one calf ceased by day three post-inoculation and by the other calf by post-inoculation day four. Neither calf inoculated with the bacteriophage-resistant mutant *Sal*. Newport strain showed disease signs. The second pair received 10^{10} CFUs of phage-resistant mutants. The fecal shedding of the mutant by one calf ceased by day nine post-inoculation and by the other calf by post-inoculation day ten. Both calves inoculated with the higher dose of the bacteriophage-resistant mutant *S*. Newport strain showed abbreviated signs of diarrhea and neither showed any signs of fever. A difference in disease was observed with the dose of the bacteriophage-resistant mutant of *S.* Newport with the higher inoculum. The phage-resistant mutant persisted longer in feces, and diarrhea was observed during the experiment. The fecal shedding and diarrhea observed were not what is typically seen in a case of salmonellosis, which would be a milder form of salmonellosis.

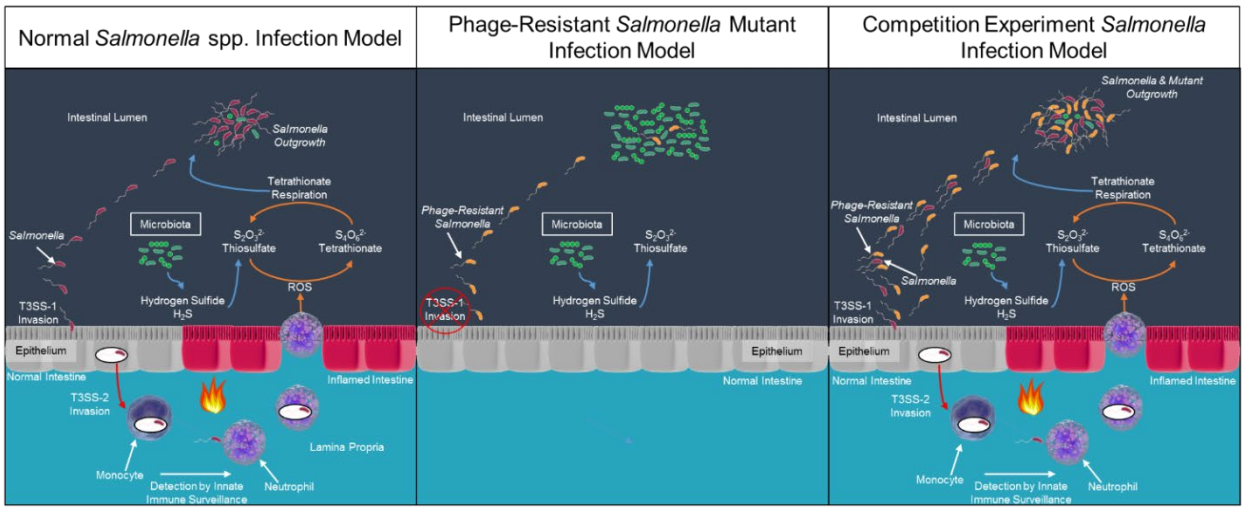
A third pair of calves was challenged with a total dose of 1.96×10^{10} CFUs composed of a 1:1.3 ratio of parent:mutant in a competition experiment designed to determine how well the mutant competed against the parent strain *in vivo*. This experiment had a very unusual shedding pattern. Typical calves infected with *Sal*. Newport has shed *Sal*. Newport at countable numbers out until day 12 when administered a dose of around 5×10^{9} CFUs. The calves in the competition experiment fecally shed high numbers of bacteriophage-resistant mutant and parent *Sal*. Newport, with clinical symptoms from day 3 to day 6 for one calf and from day 2 to day 4 for the second calf. The length of fecal shedding was also extended out to day 24 for both calves. The experimental infection during the competition experiment was unusual due to the initial disease that subsided and the prolonged *Salmonella* fecal shedding of both parent and mutant with mild to no clinical disease later on.

There appeared to be conflicting calf fecal shedding results between the bacteriophage-resistant mutant of *Sal.* Newport experiments. In the first two calves' experiment, the bacteriophage-resistant mutant of *Sal.* Newport was not able to survive and be shed in the experimental calf infections, which would suggest an attenuation. The competition experiment between the parent and bacteriophage-resistant mutant of *Sal.* Newport showed prolonged shedding of the bacteriophage-resistant mutant of *Sal.* Newport. It appeared that the parent strain was able to help the bacteriophage-resistant mutant of *Sal.* Newport survives and is excreted fecally.

A hypothetical model was developed to explain the findings. Normally *Salmonella* can induce intestinal inflammation by virulence factors (encoded on *Salmonella* Pathogenicity Islands 1 and 2, SPI-1 and SPI-2, respectively) that results in the production of large amounts of nitric oxide radicals and reactive oxygen species in the lumen of the gut. Thiosulfate ($S_2O_3^{2-}$) can be oxidized to tetrathionate ($S_4O_6^{2-}$), which *Salmonella* species, unlike other coliforms, can utilize for aerobic respiration in the anaerobic environment of the intestinal lumen because of the tetrathionate reductase found on SPI-2. Inflammation is required to generate intestine tetrathionate. This provides *Salmonella* species with a growth advantage in an inflamed gut. The hypothetical model explains why the competition experiment had a prolonged fecal shedding of *Salmonella*. The bacteriophage-resistant mutant of *Sal*. Newport may not be able to invade but can utilize tetrathionate as a terminal electron acceptor to outcompete the microbiota if the tetrathionate reductase is not compromised. The parent strain invades and induces inflammation, which helps the bacteriophage-resistant mutant of *Sal*. Newport to outcompete the microbiota in the intestinal lumen.

The hypothetical model predicts that the mutation is responsible for the bacteriophage-resistance in *Sal.* Newport and might interfere with one of the type three secretion systems (T3SSs) that is important for the pathogenesis of *Salmonella*. A model for what is hypothesized to have occurred in the lumen of the calves can be seen in **Figure 1.1**. The type three secretion system one (T3SS-1) located on SPI-1 is important for the invasion of epithelial cells, and the type three secretion system two (T3SS-2) located on SPI-2 is important for survival in macrophages. SPI-1-mediated colonization of intestinal tissues appears essential for bovine enteritis, but SPI-2 mutants are only mildly attenuated in calves. Due to the knowledge that mutations in SPI-2 cause only mild attenuation in calves, it is suggested that the mutation in the phage-resistance in the *Sal.* Newport must interfere with the SPI-1 encoded type three secretion system one injectisome.

Figure 1.1. Hypothetical model of dynamics in calf experimental infections.



1.8 References

Aawar, M. Al, Srivastava, A., 2022. Random Forest of Epidemiological Models for Influenza Forecasting, arXiv preprint. Association for Computing Machinery. doi:https://doi.org/10.48550/arXiv.2206.08967

Abatcha, M.G., Zakaria, Z., Kaur, D.G., Thong, K.L., 2014. Typing of *Salmonella* Species: A Mini-Review. Journal of Natural Sciences Research 4, 13–17.

Abedon, S., Thomas-Abedon, C., 2010. Phage Therapy Pharmacology. Curr Pharm Biotechnol 11, 28–47. doi:10.2174/138920110790725410

Abedon, S.T., 2010. The 'Nuts and Bolts' of Phage Therapy. Curr Pharm Biotechnol 11, 1.

Achtman, M., Wain, J., Weill, F.X., Nair, S., Zhou, Z., Sangal, V., Krauland, M.G., Hale, J.L., Harbottle, H., Uesbeck, A., Dougan, G., Harrison, L.H., Brisse, S., 2012. Multilocus Sequence Typing as a Replacement for Serotyping in *Salmonella enterica*. PLoS Pathog 8. doi:10.1371/journal.ppat.1002776

Agbaje, M., Begum, R.H., Oyekunle, M.A., Ojo, O.E., Adenubi, O.T., 2011. Evolution of *Salmonella* nomenclature : a critical note 497–503. doi:10.1007/s12223-011-0075-4

Albert, A., 2017. Biostatistics: Facing the Interpretation of 2 × 2 Tables. J Belg Soc Radiol 101, 1–5. doi:10.5334/jbr-btr.1399

Al-Hamadany, W.S., 2021. *Salmonella* and the Immune System, in: Lamas, A., Regal, P., Franco, C. (Eds.), *Salmonella Spp.* - A Global Challenge. IntechOpen, p. 13. doi:DOI: 10.5772/intechopen.95673

Alyousef, M.K., Al-Sayed, A.A., Al Afif, A., Alamoudi, U., Leblanc, J.M., Leblanc, R., 2020. A Pain in the Neck: *Salmonella spp.* as an Unusual Cause of a Thyroid Abscess. A Case Report and Review of the Literature. BMC Infect Dis 20, 1–8. doi:10.1186/s12879-020-05161-w

Anderson, M.E.C., 2015. Contact Precautions and Hand Hygiene in Veterinary Clinics. Veterinary Clinics of NA: Small Animal Practice 45, 343–360. doi:10.1016/j.cvsm.2014.11.003

Andrews-Polymenis, H.L., Bäumler, A.J., Beth, A., Fang, F.C., 2010. Taming the Elephant: *Salmonella* Biology, Pathogenesis, and Prevention. Infect Immun 78, 2356–2369. doi:10.1128/IAI.00096-10

Azam, A.H., Tanji, Y., 2019. Bacteriophage-Host Arm Race: An Update on the Mechanism of Phage Resistance in Bacteria and Revenge of the Phage with the Perspective for Phage Therapy. Appl Microbiol Biotechnol 103, 2121–2131. doi:10.1007/s00253-019-09629-x

Barapatre, H., Jangir, J., Bajpai, S., Chawla, B., Keswani, G., 2023. Analytics of Epidemiological Data Using Machine Learning Models. International Journal of Next-Generation Computing 14, 255–262.

Bardina, C., Spricigo, D.A., Cortés, P., Llagostera, M., 2012. Significance of the Bacteriophage Treatment Schedule in Reducing *Salmonella* Colonization of Poultry. Appl Environ Microbiol 78, 6600–6607. doi:10.1128/aem.01257-12

Bartnik, P., Lewtak, K., Fiołka, M., Czaplewska, P., Narajczyk, M., Czajkowski, R., 2022. Resistance of *Dickeya solani* Strain IPO 2222 to Lytic Bacteriophage ΦD5 Results in Fitness Tradeoffs for the Bacterium During Infection. Sci Rep 12, 1–23. doi:10.1038/s41598-022-14956-7

Bäumler, A.J., Winter, S.E., Thiennimitr, P., Casadesús, J., 2011. Intestinal and Chronic Infections: *Salmonella* Lifestyles. Environ Microbiol 3, 508–517. doi:10.1111/j.1758-2229.2011.00242.x

Becker, A.K., Ittermann, T., Dörr, M., Felix, S.B., Nauck, M., Teumer, A., Völker, U., Völzke, H., Kaderali, L., Nath, N., 2022. Analysis of Epidemiological Association Patterns of Serum Thyrotropin by Combining Random Forests and Bayesian Networks. PLoS One 17, 1–17. doi:10.1371/journal.pone.0271610

Bi, Q., Goodman, K.E., Kaminsky, J., Lessler, J., 2019. What is Machine Learning? A Primer for the Epidemiologist. Am J Epidemiol 188, 2222–2239. doi:10.1093/aje/kwz189

Bjanes, E., Nizet, V., 2021. More than a Pore: Nonlytic Antimicrobial Functions of Complement and Bacterial Strategies for Evasion. Microbiology and Molecular Biology Reviews 85. doi:10.1128/mmbr.00177-20

Bleriot, I., Pacios, O., Blasco, L., Fernández-García, L., López, M., Ortiz-Cartagena, C., Barrio-Pujante, A., García-Contreras, R., Pirnay, J.P., Wood, T.K., Tomás, M., 2024. Improving

Phage Therapy by Evasion of Phage Resistance Mechanisms. JAC Antimicrob Resist 6, 1–16. doi:10.1093/jacamr/dlae017

Blower, T.R., Pei, X.Y., Short, F.L., Fineran, P.C., Humphreys, D.P., Luisi, B.F., Salmond, G.P.C., 2011. A Processed Noncoding RNA Regulates an Altruistic Bacterial Antiviral System. Nat Struct Mol Biol 18, 185–191. doi:10.1038/nsmb.1981

Brown, E.W., Bell, R., Zhang, G., Timme, R., Zheng, J., Hammack, T.S., Allard, M.W., 2021. *Salmonella* Genomics in Public Health and Food Safety. EcoSal Plus 9. doi:10.1128/ecosalplus.esp-0008-2020

Burgess, B.A., 2023. *Salmonella* in Horses. Veterinary Clinics of North America - Equine Practice 39, 25–35. doi:10.1016/j.cveq.2022.11.005

Burgess, B.A., Morley, P.S., 2019. Risk Factors for Shedding of *Salmonella enterica* Among Hospitalized Large Animals Over a 10-year Period in a Veterinary Teaching Hospital. J Vet Intern Med 33, 2239–2248. doi:10.1111/jvim.15579

Burgess, B.A., Morley, P.S., 2018. Risk Factors for Veterinary Hospital Environmental Contamination with *Salmonella enterica*. Epidemiol Infect 146, 1282–1292. doi:10.1017/S0950268818001164

Burgess, B.A., Morley, P.S., 2014. Managing *Salmonella* in Equine Populations. Veterinary Clinics of NA: Equine Practice 30, 623–640. doi:10.1016/j.cveq.2014.08.005

Burgess, B.A., Weese, J.S., 2022. Prevention of Infectious Diseases in Hospital Environments, Fifth Edit. ed, Greene's Infectious Diseases of the Dog and Cat, Fifth Edition. Elsevier Inc., St. Louis, Missouri. doi:10.1016/B978-0-323-50934-3.00015-X

Capparelli, R., Nocerino, N., Lannaccone, M., Ercolini, D., Parlato, M., Chiara, M., Lannelli, D., 2010a. Bacteriophage Therapy of *Salmonella enterica*: A Fresh Appraisal of Bacteriophage Therapy. J Infect Dis 201, 52–61. doi:10.1086/648478

Capparelli, R., Nocerino, N., Lanzetta, R., Silipo, A., Amoresano, A., Giangrande, C., Becker, K., Blaiotta, G., Evidente, A., Cimmino, A., Iannaccone, M., Parlato, M., Medaglia, C., Roperto, S., Roperto, F., Ramunno, L., Iannelli, D., 2010b. Bacteriophage-Resistant *Staphylococcus aureus* Mutant Confers Broad Immunity Against Staphylococcal Infection in Mice. PLoS One 5, 1–13. doi:10.1371/journal.pone.0011720

Carpena, N., Manning, K.A., Dokland, T., Marina, A., Penadés, J.R., 2016. Convergent Evolution of Pathogenicity Islands in Helper *cos* Phage Interference. Philosophical Transactions of the Royal Society B: Biological Sciences 371. doi:10.1098/rstb.2015.0505

CDC, C. for D.C. and P., 2018. National Enteric Disease Surveillance: *Salmonella* Annual Report, 2016. US Department of Health and Human Services, Centers for Disease Control and Prevention: Atlanta, GA, USA http://www.cdc.gov/ncezid/dfwed/pdfs/*Salmonella*-an.

Chattaway, M.A., Dallman, T.J., Larkin, L., Nair, S., McCormick, J., Mikhail, A., Hartman, H., Godbole, G., Powell, D., Day, M., Smith, R., Grant, K., 2019. The Transformation of Reference Microbiology Methods and Surveillance for *Salmonella* with the Use of Whole Genome Sequencing in England and Wales. Front Public Health 7, 1–12. doi:10.3389/fpubh.2019.00317

Chattaway, M.A., Langridge, G.C., Wain, J., 2021. *Salmonella* Nomenclature in the Genomic Era: a Time for Change. Sci Rep 11, 1–8. doi:10.1038/s41598-021-86243-w

Chen, Z., Meng, J., 2022. Critical Assessment of Short-Read Assemblers for the Metagenomic Identification of Foodborne and Waterborne Pathogens Using Simulated Bacterial Communities. Microorganisms 10. doi:10.3390/microorganisms10122416

Cobo-Simón, M., Hart, R., Ochman, H., 2023. Gene Flow and Species Boundaries of the Genus *Salmonella*. mSystems 8, 1–12. doi:10.1128/msystems.00292-23

Coburn, B., Grassl, G.A., Finlay, B.B., 2007. *Salmonella*, the Host and Disease: a Brief Review. Immunol Cell Biol 85, 112–118. doi:10.1038/sj.icb.7100007

Costa, L.F., Paixão, T.A., Tsolis, R.M., Bäumler, A.J., Santos, R.L., 2012. Salmonellosis in Cattle: Advantages of Being an Experimental Model. Res Vet Sci 93, 1–6. doi:10.1016/j.rvsc.2012.03.002

Cummings, K.J., Rodriguez-Rivera, L.D., Mitchell, K.J., Hoelzer, K., Wiedmann, M., Mcdonough, P.L., Altier, C., Warnick, L.D., Perkins, G.A., 2014. *Salmonella enterica* Serovar Oranienburg Outbreak in a Veterinary Medical Teaching Hospital with Evidence of Nosocomial and On-Farm Transmission. Vector-Borne and Zoonotic Diseases 14, 496–502. doi:10.1089/vbz.2013.1467

Dalmasso, M., Strain, R., Neve, H., Franz, C.M.A.P.A.P., Cousin, F.J., Ross, R.P., Hill, C., Cousin, J., Ross, R.P., Hill, C., 2016. Three New *Escherichia coli* Phages from the Human Gut Show Promising Potential for Phage Therapy. PLoS One 11, 1–16. doi:10.1371/journal.pone.0156773

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 2011. The Variant Call Format and VCFtools. Bioinformatics 27, 2156–2158. doi:10.1093/bioinformatics/btr330

De Lucia, A., Rabie, A., Smith, R.P., Davies, R., Ostanello, F., Ajayi, D., Petrovska, L., Martelli, F., 2018. Role of Wild Birds and Environmental Contamination in the Epidemiology of *Salmonella* Infection in an Outdoor Pig Farm. Vet Microbiol 227, 148–154. doi:10.1016/j.vetmic.2018.11.003

Delahoy, M.J., Shah, H.J., Weller, D.L., Ray, L.C., Smith, K., McGuire, S., Trevejo, R.T., Scallan Walter, E., Wymore, K., Rissman, T., McMillian, M., Lathrop, S., LaClair, B., Boyle, M.M., Harris, S., Zablotsky-Kufel, J., Houck, K., Devine, C.J., Lau, C.E., Tauxe, R. V., Bruce, B.B., Griffin, P.M., Payne, D.C., 2023. Preliminary Incidence and Trends of Infections Caused by Pathogens Transmitted Commonly Through Food — Foodborne

Diseases Active Surveillance Network, 10 U.S. Sites, 2022. MMWR Morb Mortal Wkly Rep 72, 701–706. doi:10.15585/mmwr.mm7226a1

Dharma, C., Fu, R., Chaiton, M., 2023. Table 2 Fallacy in Descriptive Epidemiology: Bringing Machine Learning to the Table. Int J Environ Res Public Health 20. doi:10.3390/ijerph20136194

Drulis-Kawa, Z., Majkowska-Skrobek, G., Maciejewska, B., 2015. Bacteriophages and Phage-Derived Proteins – Application Approaches. Curr Med Chem 22, 1757–1773. doi:10.2174/0929867322666150209152851

Eagle, S.H.C., Robertson, J., Bastedo, D.P., Liu, K., Nash, J.H.E., 2023. Evaluation of Five Commercial DNA Extraction Kits Using *Salmonella* as a Model for Implementation of Rapid Nanopore Sequencing in Routine Diagnostic Laboratories. Access Microbiol 5, 1–20. doi:10.1099/acmi.0.000468.v3

Egido, J.E., Costa, A.R., Aparicio-Maldonado, C., Haas, P.J., Brouns, S.J.J., 2022. Mechanisms and Clinical Importance of Bacteriophage Resistance. FEMS Microbiol Rev 46, 1–16. doi:10.1093/femsre/fuab048

Evangelopoulou, G., Kritas, S., Govaris, A., Burriel, A.R., 2013. Animal Salmonelloses: a Brief Review of “Host Adaptation and Host Specificity" of *Salmonella spp.* Vet World 6, 703–708. doi:10.14202/vetworld.2013.703-708

Feng, Y., Lin, E., Zou, S., Chen, C.L., Chiu, C.H., 2019. Complete Genome Sequence of *Salmonella enterica* Serovar Sendai Shows H Antigen Convergence with S. Miami and Recent Divergence From *S*. Paratyphi A. BMC Genomics 20, 1–10. doi:10.1186/s12864-019-5798-7

Fierer, J., 2022. Invasive Non-typhoidal *Salmonella* (iNTS) Infections. Clinical Infectious Diseases 75, 732–738.

Figueira, R., Holden, D.W., 2012. Functions of the *Salmonella* Pathogenicity Island 2 (SPI-2) Type III Secretion System Effectors. Microbiology (N Y) 158, 1147–1161. doi:10.1099/mic.0.058115-0

Fineran, P.C., Salmond, G.P.C., Blower, T.R., Lilley, K.S., Humphreys, D.P., Foulds, I.J., 2009. The Phage Abortive Infection System, ToxIN, Functions as a Protein–RNA Toxin–Antitoxin Pair. Proceedings of the National Academy of Sciences 106, 894–899. doi:10.1073/pnas.0808832106

Fiorentin, L., Vieira, N.D., Barioni Jr, W., 2005. Oral Treatment with Bacteriophages Reduces the Concentration of *Salmonella* Enteritidis PT4 in Caecal Contents of Broilers. Avian Dis 34, 258–263. doi:10.1080/01445340500112157

Fitzgerald, C., Collins, M., Duyne, S. Van, Mikoleit, M., Brown, T., Fields, P., 2007. Multiplex , Bead-Based Suspension Array for Molecular Determination of Common *Salmonella* Serogroups. J Clin Microbiol 45, 3323–3334. doi:10.1128/JCM.00025-07

Flyg, C., Kenne, K., Boman, H.G., 1980. Insect Pathogenic Properties of *Serratia marcescens*: Phage-Resistant Mutants with a Decreased Resistance to *Cecropia* Immunity and a Decreased Virulence to *Drosophila*. J Gen Microbiol 120, 173–181. doi:10.1099/00221287-120-1-173

Foster, N., Tang, Y., Berchieri, A., Geng, S., Jiao, X., Barrow, P., 2021. Revisiting Persistent *Salmonella* Infection and the Carrier State: What Do We Know? Pathogens 10, 1–21. doi:10.3390/pathogens10101299

Galán-Relaño, Á., Valero Díaz, A., Huerta Lorenzo, B., Gómez-Gascón, L., Mena Rodríguez, M.a. Á., Carrasco Jiménez, E., Pérez Rodríguez, F., Astorga Márquez, R.J., 2023. *Salmonella* and Salmonellosis: An Update on Public Health Implications and Control Strategies. Animals 13, 3–22. doi:10.3390/ani13233666

Gal-mor, O., 2019. Persistent Infection and Long-Term Carriage of Typhoidal and Nontyphoidal Salmonellae. Clin Microbiol Rev 32, 1–31.

Gal-mor, O., Boyle, E.C., Grassl, G.A., 2014. Same Species, Different Diseases: How and Why Typhoidal and Non-Typhoidal *Salmonella enterica* Serovars Differ. Front Microbiol 5, 1–10. doi:10.3389/fmicb.2014.00391

Garaizar, J., Laorden, L., 2023. Bacterial Genomics and Epidemiology. Microorganisms 11, 10–11. doi:10.3390/microorganisms11061428

Ge, H., Fu, S., Guo, H., Hu, M., Xu, Z., Zhou, X., Chen, X., Jiao, X., 2022. Application and Challenge of Bacteriophage in the Food Protection. Int J Food Microbiol 380, 109872. doi:10.1016/j.ijfoodmicro.2022.109872

Giani, A.M., Gallo, G.R., Gianfranceschi, L., Formenti, G., 2020. Long Walk to Genomics: History and Current Approaches to Genome Sequencing and Assembly. Comput Struct Biotechnol J 18, 9–19. doi:10.1016/j.csbj.2019.11.002

Grant, A.J., Morgan, F.J.E., McKinley, T.J., Foster, G.L., Maskell, D.J., Mastroeni, P., 2012. Attenuated *Salmonella* Typhimurium Lacking the Pathogenicity Island-2 Type 3 Secretion System Grow to High Bacterial Numbers inside Phagocytes in Mice. PLoS Pathog 8. doi:10.1371/journal.ppat.1003070

Grimont, P.A., Weill, F.-X., 2007. Antigenic Formulae of the *Salmonella* Serovars, 9th ed, WHO Collaborating Centre for Reference and Research on *Salmonella*.

Gunn, J.S., Marshall, J.M., Baker, S., Dongol, S., Charles, R.C., Ryan, E.T., 2014. *Salmonella* Chronic Carriage: Epidemiology, Diagnosis, and Gallbladder Persistence. Trends Microbiol 22, 648–655. doi:10.1016/j.tim.2014.06.007

Hallstrom, K., McCormick, B.A., 2011. *Salmonella* Interaction With and Passage Through the Intestinal Mucosa: Through the Lens of the Organism. Front Microbiol 2, 1–10. doi:10.3389/fmicb.2011.00088

Hamilton, A.J., Strauss, A.T., Martinez, D.A., Hinson, J.S., Levin, S., Lin, G., Klein, E.Y., 2021. Machine Learning and Artificial Intelligence: Applications in Healthcare Epidemiology. Antimicrobial Stewardship and Healthcare Epidemiology 1, 1–6. doi:10.1017/ash.2021.192

Han, J., Aljahdali, N., Zhao, S., Tang, H., Harbottle, H., Hoffmann, M., Frye, J.G., Foley, S.L., 2024. Infection Biology of *Salmonella enterica*. EcoSal Plus 0, 1–54. doi:10.1128/ecosalplus.esp-0001-2023

Heierson, A., Siden, I., Kivaisi, A., Boman, H.G., 1986. Bacteriophage-Resistant Mutants of *Bacillus thuringiensis* with Decreased Virulence in Pupae of *Hyalophora cecropia*. J Bacteriol 167, 18–24. doi:10.1128/jb.167.1.18-24.1986

Hensel, M., Hinsley, A.P., Sawers, G., Berks, B.C., 1999. The Genetic Basis of Tetrathionate Respiration in *Salmonella* Typhimurium. Mol Microbiol 32, 275–287.

Higginson, E.E., Simon, R., Tennant, S.M., 2016. Animal Models for Salmonellosis: Applications in Vaccine Research. Clinical and Vaccine Immunology 23, 746–756. doi:10.1128/CVI.00258-16.Editor

Hong, Y., Hu, D., Verderosa, A.D., Qin, J., Totsika, M., Reeves, P.R., 2023. Repeat-Unit Elongations To Produce Bacterial Complex Long Polysaccharide Chains, an O-Antigen Perspective. EcoSal Plus 11. doi:10.1128/ecosalplus.esp-0020-2022

Howard, Z.R., Bryan, C.A.O., Crandall, P.G., Ricke, S.C., 2012. *Salmonella* Enteritidis in Shell Eggs: Current Issues and Prospects for Control. Food Research International 45, 755–764. doi:10.1016/j.foodres.2011.04.030

Hu, T., Chitnis, N., Monos, D., Dinh, A., 2021. Next-Generation Sequencing Technologies: An Overview. Hum Immunol 82, 801–811. doi:10.1016/j.humimm.2021.02.012

Hwang, D., Rothrock, M.J., Pang, H., Dev Kumar, G., Mishra, A., 2020. Farm Management Practices that Affect the Prevalence of *Salmonella* in Pastured Poultry Farms. LWT- Food Science and Technology 127, 109423. doi:10.1016/j.lwt.2020.109423

Hyland, J., Kitchens, S., Wright, J., Walz, P., Price, S., n.d. Oral Bacteriophage Treatment to Reduce Fecal Shedding of *Salmonella enterica* Serotype Newport from Calves. Manuscript in Preparation.

Hyman, P., Abedon, S.T., 2010. Bacteriophage Host Range and Bacterial Resistance., in: Advances in Applied Microbiology. Elsevier Inc., San Diego, CA, pp. 217–248. doi:10.1016/S0065-2164(10)70007-1

Kitchens, S.R., 2016. A Study of a Bacteriophage-Resistant *Salmonella* Mutant and Factors Influencing Prevalence of *Salmonella* in a Multi-Species Animal Facility (Master's Thesis). Auburn University.

Kong, Q., Yang, J., Liu, Q., Alamuri, P., Roland, K.L., Curtiss, R., 2011. Effect of Deletion of Genes Involved in Lipopolysaccharide Core and O-Antigen Synthesis on Virulence and Immunogenicity of *Salmonella enterica* Serovar Typhimurium. Infect Immun 79, 4227–4239. doi:10.1128/IAI.05398-11

Kortright, K.E., Done, R.E., Chan, B.K., Souza, V., Turner, P.E., 2022. Selection for Phage Resistance Reduces Virulence of *Shigella flexneri*. Appl Environ Microbiol 88, 1–15. doi:10.1128/AEM.01514-21

Krzyżewska-Dudek, E., Kotimaa, J., Kapczyńska, K., Rybka, J., Meri, S., 2022. Lipopolysaccharides and Outer Membrane Proteins as Main Structures Involved in Complement Evasion Strategies of Non-Typhoidal *Salmonella* Strains. Mol Immunol 150, 67–77. doi:10.1016/j.molimm.2022.08.009

Laanto, E., Bamford, J.K.H., Laakso, J., Sundberg, L.R., 2012. Phage-Driven Loss of Virulence in a Fish Pathogenic Bacterium. PLoS One 7. doi:10.1371/journal.pone.0053157

Labrie, S.J., Samson, J.E., Moineau, S., 2010. Bacteriophage Resistance Mechanisms. Nat Rev Microbiol 8, 317–327. doi:10.1038/nrmicro2315

León, M., Bastías, R., 2015. Virulence Reduction in Bacteriophage Resistant Bacteria. Front Microbiol 6, 1–7. doi:10.3389/fmicb.2015.00343

Li, C., Tyson, G.H., Hsu, C.H., Harrison, L., Strain, E., Tran, T.T., Tillman, G.E., Dessai, U., McDermott, P.F., Zhao, S., 2021. Long-Read Sequencing Reveals Evolution and Acquisition of Antimicrobial Resistance and Virulence Genes in *Salmonella enterica*. Front Microbiol 12, 1–14. doi:10.3389/fmicb.2021.777817

Liang, D.W., Lu, J.H., Wu, Q., Ke, B.X., Jiang, C.H., Long, J., Fang, Y.P., Lin, L.J., Zeng, N.Y., 2016. Comparing the Ability of Luminex xMAP *Salmonella* Serotyping Assay and Traditional Serotyping Method for Serotyping *Salmonella* Isolated from Southern Chinese Population. J Appl Microbiol 120, 1668–1676. doi:10.1111/jam.13106

Lilienfeld, A.M., 1980. Advances in Quantitative Methods in Epidemiology. Public Health Reports 95, 462–469.

Lin, B., Hui, J., Mao, H., 2021. Nanopore Technology and Its Applications in Gene Sequencing. Biosensors (Basel) 11. doi:10.3390/bios11070214

Liu, B., Knirel, Y.A., Feng, L., Perepelov, A. V, Senchenkova, S.N., Reeves, P.R., Wang, L., 2014. Structural Diversity in *Salmonella* O Antigens and Its Genetic Basis. FEMS Microbiol Rev 38, 56–89. doi:10.1111/1574-6976.12034

Liu, D., Van Belleghem, J.D., de Vries, C.R., Burgener, E., Chen, Q., Manasherob, R., Aronson, J.R., Amanatullah, D.F., Tamma, P.D., Suh, G.A., 2021. The Safety and Toxicity of Phage Therapy: A Review of Animal and Clinical Studies. Viruses 13, 1–20. doi:10.3390/v13071268

Lope, D.J., Demirhan, H., 2024. Spatial and Spatio-Temporal Epidemiology JAGS Model Specification for Spatiotemporal Epidemiological Modelling. Spat Spatiotemporal Epidemiol 49, 100645. doi:10.1016/j.sste.2024.100645

López, F.E., De, M., Pescaretti, M., Morero, R., Delgado, M.A., 2012. *Salmonella* Typhimurium General Virulence Factors: A Battle of David Against Goliath? Food Research International 45, 842–851. doi:10.1016/j.foodres.2011.08.009

Marchello, C.S., Birkhold, M., Crump, J.A., Martin, L.B., Ansah, M.O., Breghi, G., Canals, R., Fiorino, F., Gordon, M.A., Kim, J.H., Hamaluba, M., Hanumunthadu, B., Jacobs, J., Kariuki, S., Malvolti, S., Mantel, C., Marks, F., Medaglini, D., Mogasale, V., Msefula, C.L., Muthumbi, E., Niyrenda, T.S., Onsare, R., Owusu-Dabo, E., Pettini, E., Ramasamy, M.N., Soura, B.A., Spadafina, T., Tack, B., 2022. Complications and Mortality of Non-Typhoidal *Salmonella* Invasive Disease: A Global Systematic Review and Meta-Analysis. Lancet Infect Dis 22, 692–705. doi:10.1016/S1473-3099(21)00615-0

McQuiston, J.R., Parrenas, R., Gheesling, L., Brenner, F., Fields, P.I., 2004. Sequencing and Comparative Analysis of Flagellin Genes fliC, fljB, and flpA from *Salmonella*. J Clin Microbiol 42, 1923–1932. doi:10.1128/JCM.42.5.1923

Mcquiston, J.R., Waters, R.J., Dinsmore, B.A., Mikoleit, M.L., Fields, P.I., 2011. Molecular Determination of H Antigens of *Salmonella* by Use of a Microsphere-Based Liquid Array. J Clin Microbiol 49, 565–573. doi:10.1128/JCM.01323-10

Menanteau, P., Kempf, F., Trotereau, J., Virlogeux-Payant, I., Gitton, E., Dalifard, J., Gabriel, I., Rychlik, I., Velge, P., 2018. Role of Systemic Infection, Cross Contaminations and Super-Shedders in *Salmonella* Carrier State in Chicken. Environ Microbiol 20, 3246–3260. doi:10.1111/1462-2920.14294

Mokraoui, N.R., Ganim, R., Paez, A., 2023. Chest Wall Abscess and Costochondritis Due to *Salmonella enterica* Serotype Choleraesuis: a Case Report. J Med Case Rep 17, 1–7. doi:10.1186/s13256-023-04229-w

Monack, D.M., 2012. *Salmonella* Persistence and Transmission Strategies. Curr Opin Microbiol 15, 100–107. doi:10.1016/j.mib.2011.10.013

Monk, A.B., Rees, C.D., Barrow, P., Hagens, S., Harper, D.R., 2010. Bacteriophage applications: where are we now? Lett Appl Microbiol 51, 363–369. doi:10.1111/j.1472-765X.2010.02916.x

Morley, P.S., Weese, J.S., 2015. Biosecurity and Infection Control for Large Animal Practices, in: Smith, B.P. (Ed.), Large Animal Internal Medicine. Elsevier, New York, NY, pp. 1407–1431.

Murray, C.J., 2000. Environmental Aspects of *Salmonella*, in: *Salmonella* in Domestic Animals. pp. 265–283.

Murray, G.L., Attridge, S.R., Morona, R., 2006. Altering the Length of the Lipopolysaccharide O antigen has an Impact on the Interaction of *Salmonella enterica* Serovar typhimurium with Macrophages and Complement. J Bacteriol 188, 2735–2739. doi:10.1128/JB.188.7.2735-2739.2006

Naushad, S., Ogunremi, D., Huang, H., 2023. *Salmonella*: A Brief Review, in: Huang, H., Naushad, S. (Eds.), *Salmonella* - Perspectives for Low-Cost Prevention, Control and Treatment. IntechOpen, pp. 1–22. doi:DOI: 10.5772/intechopen.112948

Nieto, P.A., Pardo-roa, C., Salazar-echegarai, F.J., Tobar, H.E., Coronado-arr, I., Riedel, C.A., Kalergis, A.M., Bueno, S.M., 2016. New Insights About Excisable Pathogenicity Islands in *Salmonella* and Their Contribution to Virulence. Microbes Infect 18, 302–309. doi:10.1016/j.micinf.2016.02.001

Oechslin, F., 2018. Resistance Development to Bacteriophages Occurring During Bacteriophage Therapy. Viruses 10, 1–23. doi:10.3390/v10070351

Olsen, J., Sudan, M., Arah, O.A., Kheifets, L., 2017. The Methodological 'Revolution': Caution Accepted. Eur J Epidemiol 32, 165–166. doi:10.1007/s10654-016-0194-y

Oludairo, O.O., Kwaga, J.K.P., Kabir, J., Abdu, P.A., Gitanjali, A., Perrets, A., Cibin, V., Lettini, A.A., Aiyedun, J.O., 2023. Ecology and Epidemiology of *Salmonella spp.* Isolated From the Environment and the Roles Played by Wild Animals in Their Maintenance. Int J One Health 9, 1–9. doi:10.14202/IJOH.2023.1-9

Panda, A., Tatarov, I., Masek, B.J., Hardick, J., Crusan, A., Wakefield, T., Carroll, K., Yang, S., Hsieh, Y.H., Lipsky, M.M., McLeod, C.G., Levine, M.M., Rothman, R.E., Gaydos, C.A., DeTolla, L.J., 2014. A Rabbit Model of Non-Typhoidal *Salmonella* Bacteremia. Comp Immunol Microbiol Infect Dis 37, 211–220. doi:10.1016/j.cimid.2014.05.004

Pandya, M., Wittum, T., Tadesse, D.A., Gebreyes, W., Hoet, A., 2009. Environmental *Salmonella* Surveillance in The Ohio State University Veterinary Teaching Hospital. Vector-Borne and Zoonotic Diseases 9, 649–654. doi:10.1089/vbz.2008.0120

Penziner, S., Schooley, R.T., Pride, D.T., 2021. Animal Models of Phage Therapy. Front Microbiol 12, 1–13. doi:10.3389/fmicb.2021.631794

Podlacha, M., Grabowski, Ł., Kosznik-Kawśnicka, K., Zdrojewska, K., Stasiłojć, M., Węgrzyn, G., Węgrzyn, A., 2021. Interactions of Bacteriophages with Animal and Human Organisms—Safety Issues in the Light of Phage Therapy. Int J Mol Sci 22, 1–40. doi:10.3390/ijms22168937

Porta, M., Bolúmar, F., 2016. Caution: Work in Progress: While the Methodological "Revolution" Deserves In-Depth Study, Clinical Researchers and Senior Epidemiologists Should Not Be Disenfranchised. Eur J Epidemiol 31, 535–539. doi:10.1007/s10654-016-0181-3

Que, F., Wu, S., Huang, R., 2013. *Salmonella* Pathogenicity Island 1 (SPI-1) at Work. Curr Opin Microbiol 66, 582–587. doi:10.1007/s00284-013-0307-8

Raetz, C.R.H., Whitfield, C., 2002. Lipopolysaccharide Endotoxins. Annu Rev Biochem 71, 635–700. doi:10.1146/annurev.biochem.71.110601.135414

Ragione, R.M. La, Metcalfe, H.J., Villarreal-Ramos, B., Werling, D., 2013. *Salmonella* Infections in Cattle, in: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, Boston, MA, pp. 233–262.

Ranveer, S.A., Dasriya, V., Ahmad, M.F., Dhillon, H.S., Samtiya, M., Shama, E., Anand, T., Dhewa, T., Chaudhary, V., Chaudhary, P., Behare, P., Ram, C., Puniya, D.V., Khedkar, G.D., Raposo, A., Han, H., Puniya, A.K., 2024. Positive and Negative Aspects of Bacteriophages and Their Immense Role in the Food Chain. NPJ Sci Food 8. doi:10.1038/s41538-023-00245-8

Rivera-Chávez, F., Bäumler, A.J., 2015. The Pyromaniac Inside You: *Salmonella* Metabolism in the Host Gut. Annu Rev Microbiol 69, 31–48. doi:10.1146/annurev-micro-091014-104108

Rogers, A.W.L., Tsolis, R.M., Bäumler, A.J., 2021. *Salmonella* versus the Microbiome . Microbiology and Molecular Biology Reviews 85. doi:10.1128/mmbr.00027-19

Rossi, G., Shih, B.B.J., Egbe, N.F., Motta, P., Duchatel, F., Kelly, R.F., Ndip, L., Sander, M., Tanya, V.N., Lycett, S.J., Bronsvoort, B.M., Muwonge, A., 2023. Unraveling the Epidemiology of *Mycobacterium bovis* Using Whole-Genome Sequencing Combined with Environmental and Demographic Data. Front Vet Sci 10. doi:10.3389/fvets.2023.1086001

Russo, S., Bonassi, S., 2015. Prospects and Pitfalls of Machine Learning in Nutritional Epidemiology. Encyclopedia of Food and Health 14, 1–17. doi:10.1016/B978-0-12-384947-2.00494-3

Salmon, D.E., Smith, T., 1885. Report on Swine Plague, in: Second Annual Report of the Bureau of Animal Industry for the Year. House document (United States. Congress. House), Washington, D.C., pp. 184–246.

Salmond, G.P.C., Fineran, P.C., 2015. A century of the phage: past, present and future. Nat Rev Microbiol 13, 777–786. doi:10.1038/nrmicro3564

Sande, C., Whitfield, C., 2021. Capsules and Extracellular Polysaccharides in *Escherichia coli* and *Salmonella*. EcoSal Plus 9. doi:10.1128/ecosalplus.esp-0033-2020

Santos, R.L., Raffatellu, M., Bevins, C.L., Adams, L.G., Tükel, Ç., Tsolis, M., Bäumler, A.J., 2009. Life in the Inflamed Intestine, *Salmonella* Style. Trends Microbiol 17, 498–506. doi:10.1016/j.tim.2009.08.008

Santos, R.L., Zhang, S., Tsolis, R.M., Kingsley, R.A., 2001. Animal Models of *Salmonella* Infections: Enteritis Versus Typhoid Fever. Microbes Infect 3, 1335–1344.

Sarhan, W.A., Azzazy, H.M.E., 2015. Phage Approved in Food, Why Not as a Therapeutic? Expert Rev Anti Infect Ther 13, 91–101. doi:10.1586/14787210.2015.990383

Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., Thakare, R.P., Banday, S., Mishra, A.K., Das, G., Malonia, S.K., 2023. Next-Generation Sequencing Technology: Current Trends and Advancements. Biology (Basel) 12, 1–25. doi:10.3390/biology12070997

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R. V, Widdowson, M., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne Illness Acquired in the United States — Major Pathogens 1–21. doi:10.3201/eid1701.P11101

Schaer, B.L.D., Aceto, H., Rankin, S.C., 2010. Outbreak of Salmonellosis Caused by *Salmonella enterica* Serovar Newport MDR-AmpC in a Large Animal Veterinary Teaching Hospital. J Vet Intern Med 24, 1138–1146.

Schott, H.C., Ewart, S.L., Walker, R.D., Dwyer, R.M., Dietrich, S., Eberhart, S.W., Kusey, J., Stick, J. a, Derksen, F.J., 2001. An Outbreak of Salmonellosis Among Horses at a Veterinary Teaching Hospital. J Am Vet Med Assoc 218, 1152–1159. doi:10.2460/javma.2001.218.1152

Schultz, B.M., Melo-Gonzalez, F., Salazar, G.A., Porto, B.N., Riedel, C.A., Kalergis, A.M., Bueno, S.M., 2021. New Insights on the Early Interaction Between Typhoid and Non-typhoid *Salmonella* Serovars and the Host Cells. Front Microbiol 12, 1–20. doi:10.3389/fmicb.2021.647044

Schürch, A.C., Arredondo-Alonso, S., Willems, R.J.L., Goering, R. V., 2018. Whole Genome Sequencing Options for Bacterial Strain Typing and Epidemiologic Analysis Based on Single Nucleotide Polymorphism Versus Gene-by-Gene–Based Approaches. Clinical Microbiology and Infection 24, 350–354. doi:10.1016/j.cmi.2017.12.016

Sebola, D.C., Oguttu, J.W., Kock, M.M., Qekwana, D.N., 2023. Hospital-Acquired and Zoonotic Bacteria from a Veterinary Hospital and Their Associated Antimicrobial-Susceptibility Profiles: A Systematic Review. Front Vet Sci 9. doi:10.3389/fvets.2022.1087052

Segall, A.M., Roach, D.R., Strathdee, S.A., 2019. Stronger Together? Perspectives on Phage-Antibiotic Synergy in Clinical Applications of Phage Therapy. Curr Opin Microbiol 51, 46–50. doi:10.1016/j.mib.2019.03.005

Serghiou, S., Rough, K., 2023. Deep Learning for Epidemiologists: An Introduction to Neural Networks. Am J Epidemiol 192, 1904–1916. doi:10.1093/aje/kwad107

Seth-smith, H.M.B., 2008. SPI-7: *Salmonella*'s Vi-Encoding Pathogenicity Island. J Infect Dev Ctries 2, 267–271.

Seth-smith, H.M.B., Fookes, M.C., Okoro, C.K., Baker, S., Harris, S.R., Scott, P., Pickard, D., Quail, M.A., Churcher, C., Sanders, M., Harmse, J., Dougan, G., Parkhill, J., Thomson, N.R., 2012. Structure, Diversity, and Mobility of the *Salmonella* Pathogenicity Island 7 Family of Integrative and Conjugative Elements within *Enterobacteriaceae*. J Bacteriol 194, 1494–1504. doi:10.1128/JB.06403-11

Shabbir, M.A.B., Hao, H., Shabbir, M.Z., Wu, Q., Sattar, A., Yuan, Z., 2016. Bacteria vs. Bacteriophages: Parallel Evolution of Immune Arsenals. Front Microbiol 7, 1–8. doi:10.3389/fmicb.2016.01292

Shamim Hasan Zahid, M., Nashir Udden, S.M., Faruque, A.S.G., Calderwood, S.B., Mekalanos, J.J., Faruque, S.M., 2008. Effect of Phage on the Infectivity of *Vibrio cholerae* and Emergence of Genetic Variants. Infect Immun 76, 5266–5273. doi:10.1128/IAI.00578-08

Shivaprasad, H.L., Methner, U., Barrow, P.A., 2013. *Salmonella* Infections in the Domestic Fowl, in: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, Boston, MA, p. 192.

Simon, R., Tennant, S.M., Galen, J.E., Levine, M.M., 2011. Mouse Models to Assess the Efficacy of Non-Typhoidal *Salmonella* Vaccines: Revisiting the Role of Host Innate Susceptibility and Routes of Challenge. Vaccine 29, 5094–5106. doi:10.1016/j.vaccine.2011.05.022

Skurnik, M., Strauch, E., 2006. Phage therapy: Facts and fiction. International Journal of Medical Microbiology 296, 5–14. doi:10.1016/j.ijmm.2005.09.002

Smith, B.P., 2004. Evolution of Equine Infection Control Programs. Veterinary Clinics Equine Practice 20, 521–530. doi:10.1016/j.cveq.2004.07.002

Smith, R.P., Andres, V., Cheney, T.E., Martelli, F., Gosling, R., Marier, E., Rabie, A., Gilson, D., Davies, R.H., 2018. How Do Pig Farms Maintain Low *Salmonella* Prevalence: A Case-Control Study. Epidemiol Infect 146, 1909–1915. doi:10.1017/S0950268818002248

Smith, T., 1894. The Hog-Cholera Group of Bacteria. U.S. Department of Agriculture. Bureau of Animal Industry Bulletin 6, 6–40.

Soza-Ossandón, P., Rivera, D., Tardone, R., Riquelme-Neira, R., García, P., Hamilton-West, C., Adell, A.D., González-Rocha, G., Moreno-Switt, A.I., 2020. Widespread Environmental Presence of Multidrug-Resistant *Salmonella* in an Equine Veterinary Hospital That Received Local and International Horses. Front Vet Sci 7, 1–9. doi:10.3389/fvets.2020.00346

Štefek, M., Švejda, D., Zvárová, J., Tomečková, M., 1997. Epidemiology Tools Program and Selected Medical Applications. Int J Med Inform 45, 35–41.

Steneroden, K.K., Metre, D.C. Van, Jackson, C., Morley, P.S., Van Metre, D.C., Jackson, C., Morley, P.S., 2010. Detection and Control of a Nosocomial Outbreak Caused by *Salmonella* Newport at a Large Animal Hospital. J Vet Intern Med 24, 606–616. doi:https://doi.org/10.1111/j.1939-1676.2010.0484.x

Sterzenbach, T., Crawford, R.W., Winter, S.E., Bäumler, A.J., 2013. *Salmonella* Virulence Mechanisms and their Genetic Basis, in: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, Boston, MA, pp. 80–103.

Stevens, M.P., Gray, J.T., 2013. *Salmonella* Infections in Pigs, in: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, Boston, MA, pp. 263–294.

Stevens, M.P., Kingsley, R.A., 2021. *Salmonella* Pathogenesis and Host-Adaptation in Farmed Animals. Curr Opin Microbiol 63, 52–58. doi:10.1016/j.mib.2021.05.013

Sulakvelidze, A., Kutter, E., 2005. Bacteriophage Therapy in Humans, in: Kutter, E., Sulakvelidze, A. (Eds.), Bacteriophages: Biology and Applications. CRC Press, Boca Raton, Florida, pp. 381–436.

Sumrall, E.T., Shen, Y., Keller, A.P., Rismondo, J., Pavlou, M., Eugster, M.R., Boulos, S., Disson, O., Thouvenot, P., Kilcher, S., Wollscheid, B., Cabanes, D., Lecuit, M., Gründling, A., Loessner, M.J., 2019. Phage Resistance at the Cost of Virulence: *Listeria monocytogenes* Serovar 4b Requires Galactosylated Teichoic Acids for InlB-Mediated Invasion. PLoS Pathog 15, 1–29. doi:10.1371/journal.ppat.1008032

Tang, J., Gu, Y., Wang, X., Luo, Y., Zhang, F., Zheng, J., Wang, Y., Shen, X., Xu, L., 2024. *Salmonella* T3SS-Elicited Inflammatory Innate Immune Response Inhibits Type I IFN Response in Macrophages. Vet Microbiol 289, 109970. doi:10.1016/j.vetmic.2023.109970

Tang, M., Huang, Z., Zhang, X., Kong, J., Zhou, B., Han, Y., Zhang, Y., Chen, L., Zhou, T., 2023. Phage Resistance Formation and Fitness Costs of Hypervirulent *Klebsiella pneumoniae* Mediated by K2 Capsule-Specific Phage and the Corresponding Mechanisms. Front Microbiol 14, 1–18. doi:10.3389/fmicb.2023.1156292

Tennant, S.M., Maclennan, C.A., Simon, R., Martin, L.B., Khan, M.I., 2016. Nontyphoidal *Salmonella* Disease: Current Status of Vaccine Research and Development. Vaccine 34, 2907–2910. doi:10.1016/j.vaccine.2016.03.072

Thomas, C., Methner, U., Marz, M., Linde, J., 2023. Oxford Nanopore Technologies—A Valuable Tool to Generate Whole-Genome Sequencing Data for in silico Serotyping and the Detection of Genetic Markers in *Salmonella*. Front Vet Sci 10, 1–11. doi:10.3389/fvets.2023.1178922

Tillotson, K., Savage, C.J., Salman, M.D., Gentry-Weeks, C., Rice, D., Fedorka-Cray, P.J., Hendricksen, D.A., Jones, R.L., Nelson, A.W., Traub-Dargatz, J.L., 1997. Outbreak of *Salmonella* Infantis Infection in a Large Animal Veterinary Teachin Hospital. Journal of American Veterinary Medical Association 211, 1554–1557.

Timoney, J., 2013. *Salmonella* Infections in Horses, in: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, Boston, MA, pp. 305–317.

Timsit, S., Armand-Lefèvre, L., Le Goff, J., Salmona, M., 2023. The Clinical and Epidemiological Impacts of Whole Genomic Sequencing on Bacterial and Virological Agents. Infect Dis Now 54, 104844. doi:10.1016/j.idnow.2023.104844

Toro, H., Price, S.B., McKee, S., Hoerr, F.J., Krehling, J., Perdue, M., Bauermeister, L., 2005. Use of Bacteriophages in Combination with Competitive Exclusion to Reduce *Salmonella* from Infected Chickens. Avian Dis 49, 118–124. doi:10.1637/7286-100404R

Tsolis, E.M., Adams, L.G., Ficht, T.A., Ba, A.J., 1999. Contribution of *Salmonella* Typhimurium Virulence Factors to Diarrheal Disease in Calves. Infect Immun 67, 4879–4885.

Uelze, L., Grützke, J., Borowiak, M., Hammerl, J.A., Juraschek, K., Deneke, C., Tausch, S.H., Malorny, B., 2020. Typing Methods Based on Whole Genome Sequencing Data. One Health Outlook 2, 1–19. doi:10.1186/s42522-020-0010-1

US HHS, U.D. of H. and H.S., 2023. Healthy People 2030: Foodborne Illness [WWW Document]. US Department of Health and Human Services, Centers for Disease Control and Prevention: Atlanta, GA, USA. URL https://health.gov/healthypeople/objectives-and-data/browse-objectives/foodborne-illness/reduce-infections-caused-*Salmonella*-fs-04 (accessed 3.3.24).

Uzzau, S., Brown, D.J., Wallis, T., Rubino, S., Leori, G., Bernard, S., Casadesús, J., Platt, D.J., Olsen, J.E., 2000. Host Adapted Serotypes of *Salmonella enterica*. Epidemiol Infect 125, 229–255. doi:10.1017/S0950268899004379

van der Sar, A.M., Musters, R.J.P., van Eeden, F.J.M., Appelmelk, B.J., Vandenbroucke-Grauls, C.M.J.E., Bitter, W., 2003. Zebrafish Embryos as a Model Host for the Real Time Analysis of *Salmonella* Typhimurium Infections. Cell Microbiol 5, 601–611. doi:10.1046/j.1462-5822.2003.00303.x

Van Immerseel, F., De Buck, J., Pasmans, F., Bohez, L., Boyen, F., Haesebrouck, F., Ducatelle, R., Immerseel, F. Van, Buck, J. De, Pasmans, F., Bohez, L., Boyen, F., Haesebrouck, F., Ducatelle, R., 2004. Intermittent Long-Term Shedding and Induction of Carrier Birds after Infection of Chickens Early Posthatch with a Low or High Dose of *Salmonella* Enteritidis. Poult Sci 83, 1911–1916. doi:10.1093/ps/83.11.1911

Vilne, B., Meistere, I., Grantiņa-Ieviņa, L., Ķibilds, J., 2019. Machine Learning Approaches for Epidemiological Investigations of Food-Borne Disease Outbreaks. Front Microbiol 10. doi:10.3389/fmicb.2019.01722

Wagner, S., Grin, I., Malmsheimer, S., Singh, N., Torres-Vargas, C.E., Westerhausen, S., 2018. Bacterial type III Secretion Systems: A Complex Device for the Delivery of Bacterial Effector Proteins into Eukaryotic Host Cells. FEMS Microbiol Lett 365, 1–13. doi:10.1093/femsle/fny201

Wang, C., Nie, T., Lin, F., Connerton, I.F., Lu, Z., Zhou, S., Hang, H., 2019. Resistance Mechanisms Adopted by a *Salmonella* Typhimurium Mutant Against Bacteriophage. Virus Res 273, 197759. doi:10.1016/j.virusres.2019.197759

Ward, M.P., Brady, T.H., Coue, L.L., Liljebjelke, K., Maurer, J.J., Ching, C., 2005. Investigation and Control of an Outbreak of Salmonellosis Caused by Multidrug-Resistant *Salmonella*

Typhimurium in a Population of Hospitalized Horses. Vet Microbiol 107, 233–240. doi:10.1016/j.vetmic.2005.01.019

Wick, R.R., Judd, L.M., Holt, K.E., 2023. Assembling the Perfect Bacterial Genome using Oxford Nanopore and Illumina sequencing. PLoS Comput Biol 19, 1–12. doi:10.1371/journal.pcbi.1010905

Wiemken, T.L., Kelley, R.R., 2019. Machine Learning in Epidemiology and Health Outcomes Research. Annu Rev Public Health 41, 21–36. doi:10.1146/annurev-publhealth-040119-094437

Winter, S.E., Bäumler, A.J., 2011. A Breathtaking Feat: To Compete with the Gut Microbiota, *Salmonella* Drives its Host to Provide a Respiratory Electron Acceptor. Gut Microbes 2, 58–60. doi:10.4161/gmic.2.1.14911

Winter, S.E., Thiennimitr, P., Winter, M.G., Butler, B.P., Huseby, D.L., Crawford, R.W., Tsolis, M., Roth, J.R., Ba, A.J., Russell, J.M., Bevins, C.L., Adams, L.G., Tsolis, R.M., Roth, J.R., Bäumler, A.J., 2010. Gut Inflammation Provides a Respiratory Electron Acceptor for *Salmonella*. Nature 467, 426–429. doi:10.1038/nature09415

Wottlin, L.R., Edrington, T.S., Brown, T.R., Zook, C.A., Sulakvelidze, A., Droleskey, R., Genovese, K.J., Nisbet, D.J., 2022. Evaluation of a Preharvest Bacteriophage Therapy for Control of *Salmonella* within Bovine Peripheral Lymph Nodes. J Food Prot 85, 254–260. doi:10.4315/JFP-21-292

Wray, C., Todd, N., Mclaren, I., Beedell, Y., Rowe, B., 1990. The Epidemiology of *Salmonella* Infection of Calves: The Role of Dealers. Epidemiol Infect 105, 295–305.

Wright, J.G., Tengelsen, L.A., Smith, K.E., Bender, J.B., Frank, R.K., Grendon, J.H., Rice, D.H., Thiessen, A.M.B., Gilbertson, C.J., Sivapalasingam, S., Barrett, T.J., Besser, T.E., Hancock, D.D., Angulo, F.J., 2005. *Salmonella* Typhimurium in Four Animal Facilities. Emerg Infect Dis 11, 1235–1241.

Wu, X., Luo, H., Ge, C., Xu, F., Deng, X., Wiedmann, M., Baker, R.C., Stevenson, A.E., Zhang, G., Tang, S., 2023. Evaluation of Multiplex Nanopore Sequencing for *Salmonella* Serotype Prediction and Antimicrobial Resistance Gene and Virulence Gene Detection. Front Microbiol 13, 1–14. doi:10.3389/fmicb.2022.1073057

Yosef, I., Manor, M., Kiro, R., Qimron, U., 2015. Temperate and Lytic Bacteriophages Programmed to Sensitize and Kill Antibiotic-Resistant Bacteria. Proceedings of the National Academy of Sciences 112, 7267–7272. doi:10.1073/pnas.1500107112

Yoshida, C.E., Kruczkiewicz, P., Laing, C.R., Lingohr, E.J., Gannon, V.P.J., Nash, J.H.E., Taboada, E.N., 2016. The *Salmonella* In Silico Typing Resource (SISTR): An Open Web-Accessible Tool for Rapidly Typing and Subtyping Draft *Salmonella* Genome Assemblies. PLoS One 11, 1–17. doi:10.1371/journal.pone.0147101

Zhang, F., Cheng, W., 2022. The Mechanism of Bacterial Resistance and Potential Bacteriostatic Strategies. Antibiotics 11. doi:10.3390/antibiotics11091215

Zhang, S., den Bakker, H.C., Li, S., Chen, J., Dinsmore, B.A., Lane, C., Lauer, A.C., Fields, P.I., Deng, X., 2019. SeqSero2: Rapid and Improved *Salmonella* Serotype Determination Using Whole-Genome Sequencing Data. Appl Environ Microbiol 85. doi:10.1128/AEM.01746-19

Zhang, S., Yin, Y., Jones, M.B., Zhang, Z., Kaiser, B.L.D., Dinsmore, B.A., Fitzgerald, C., Fields, P.I., Deng, X., 2015. *Salmonella* Serotype Determination Utilizing High-Throughput Genome Sequencing Data. J Clin Microbiol 53, 1685–1692. doi:10.1128/JCM.00323-15

Zhao, W., Zeng, W., Pang, B., Luo, M., Peng, Y., Xu, J., Kan, B., Li, Z., Lu, X., 2023. Oxford Nanopore Long-Read Sequencing Enables the Generation of Complete Bacterial and Plasmid Genomes Without Short-Read Sequencing. Front Microbiol 14, 1–9. doi:10.3389/fmicb.2023.1179966

Zocchetti, C., Consonni, D., Bertazzi, P.A., 1997. Relationship Between Prevalence Rate Ratios and Odds Ratios in Cross-Sectional Studies. Int J Epidemiol 26, 220–223. doi:10.1093/ije/26.1.220

Chapter 2

Genomic Analysis of a Bacteriophage-Resistant Mutant of *Salmonella*

2.1. Abstract

Bacteriophages (phage), a virus of bacteria, have been proposed as a preharvest intervention to reduce or eliminate common foodborne pathogens such as *Salmonella* Newport. Concerns with phage-resistance emerging from the use of phage have led to further studies of phage-resistance in a calf model. A previous study of a phage-resistant mutant of *Salmonella* Newport exhibited attenuation. When co-inoculated with the phage-sensitive parent *Salmonella* Newport, both phage-sensitive and phage-resistant *Salmonella* Newport had a prolonged, high shed of *Salmonella*. The parent and phage-resistant *Salmonella* Newport were sequenced to identify the mutation responsible for the phage-resistance. After different approaches, a hybrid assembly combining short-read and long-read sequencing identified a mutation in the *rfbM* gene, which is important in LPS biosynthesis. This detected mutation is proposed to be responsible for the resistance to five out of five of the phage cocktail used in the *Salmonella* Newport calf model for preharvest interventions.

2.2. Introduction

Salmonella (*Sal*.) Newport is a bacterial pathogen that can cause disease in humans and animals. *Sal*. Newport is the third most common serotype responsible for *Salmonella*-associated foodborne disease in humans (Delahoy et al., 2023). *Sal*. Newport is a problem for humans through the food production chain and is commonly linked to cattle and beef (Marshall et al., 2018; Canning et al., 2023; Ford et al., 2023). *Sal*. Newport is also a problematic pathogen because it has acquired multiple drug resistances such as the *Sal*. Newport-MDRAmpC, which is resistant to ampicillin, amoxicillin/clavulanic acid, cephalothin, cefoxitin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline, and ceftiofur, and exhibits decreased susceptibility to ceftriaxone. This has necessitated the search for novel approaches to pathogen reduction (Gupta et al., 2003; Varma et al., 2006; Laufer et al., 2015).

The increase in antibiotic resistance and the U.S. Food and Drug Administration's Veterinary Feed Directive, which removed sub-therapeutic antibiotics from animal feed effective January 1st, 2017, requires the development of alternatives to conventional antibiotics (FDA, 2015). Bacteriophages (phages) are one such proposed pre-harvest intervention that could reduce

or eliminate susceptible foodborne bacterial pathogens prior to entering the food production chain (Wall et al., 2010; Endersen et al., 2014; Wottlin et al., 2022). Phages are viruses of bacteria and their natural process of infecting, replicating, and killing their bacterial host could make a lytic phage a very good alternative to current antimicrobials (Henry and Debarbieux, 2012). As the use of phages to combat these bacterial pathogens is being pursued, it is important to determine the efficacy of phage on bacterial pathogens and to investigate the impacts that phage have on the fitness of bacterial pathogens from all perspectives (Bicalho et al., 2012; Abedon, 2014). One challenge to preharvest interventions with phage is the rapid development of phage resistance (Capparelli et al., 2010a; Mahony et al., 2011; Sulakvelidze, 2013; León and Bastías, 2015). Phage-resistance in a bacterium will emerge over time when in the presence of phage (Faruque et al., 2005b, 2005a). Modifications to many different surface molecules can be responsible for preventing phage from initially binding to a bacterial host cell. Phage receptors could be any surface molecule such as lipopolysaccharide (LPS), teichoic acid, pili, outer membrane proteins, efflux pumps, flagella, and polysaccharide (Hyman and Abedon, 2010b; Luong et al., 2020). It is possible that a bacterial pathogen could evolve resistance to phage that could in turn increase the bacterium's virulence (Meaden and Koskella, 2013; Obeng et al., 2016).

To address the concern of phage-resistance, Kitchens (2016) isolated a phage-resistant mutant of *Sal*. Newport. The phage-resistant mutant, *Sal*. Newport Mut$^{\Phi}$ was resistant to four out of five cocktail phages by spot lysis after an overnight susceptible culture of *Sal*. Newport was exposed to a single phage. The phage-resistant *Sal*. Newport Mut$^{\Phi}$ appeared to be attenuated when inoculated in a calf model. However, when co-inoculated with the *Sal*. Newport parent, both *Sal*. Newport Mut$^{\Phi}$ and *Sal*. Newport parent had an extended *Salmonella* shed (Kitchens, 2016). It was hypothesized by Kitchens (2016) that the *Sal*. Newport Mut$^{\Phi}$ may not have been able to invade the intestinal epithelium and, therefore, could not stimulate the immune system to outcompete the normal gut microbiota (Kitchens, 2016). In immunocompetent individuals, *Salmonella* will outcompete the microbiota by invasion and surviving with its virulence factors until there is a host inflammatory response. This inflammation boosts the availability of resources and creates a new niche that *Salmonella* can use in the gut to outcompete the gut microbiota (Winter et al., 2010; Thiennimitr et al., 2012; Rivera-Chávez and Bäumler, 2015; Rogers et al., 2021). Kitchens (2016) hypothesized that the *Sal*. Newport parent was able to elicit the host inflammatory response and, therefore, both the *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ was able to proliferate (Kitchens,

2016). This study aims to identify the mutation that contributed to the phage-resistance seen in *Sal*. Newport Mut$^{\Phi}$. This study also explores different approaches with common sequencing technologies and various bioinformatics workflows to refine an approach when trying to identify mutations or genetic variations (variants) that might be responsible for phage-resistance.

2.3. Materials and Methods

2.3.1 *Salmonella* Strains

Salmonella Newport 3596 (originally isolated from a colistrum-deprived calf at the Auburn University College of Veterinary Medicine's Teaching Hospital) and a phage-resistant mutant of *Sal.* Newport 3596 S50.1.3.1 (*S.* Newport Mut$^{\Phi}$) short-read sequencing with Illumina Miseq by the lab of Dr. Anita Wright from the University of Florida Department of Food Science and Human Nutrition. The parent *Sal*. Newport reads were uploaded to NCBI under SRR2104591, Biosample SAMN03269318. It had an average read length of 222 bases and a coverage of 97x. The phage-resistant mutant *Sal*. Newport reads were uploaded to NCBI under SRR1752832, Biosample SAMN03269321. It had an average read length of 208 bases and a coverage of 96x. Reads were not trimmed based on the evaluation of quality by FastQC (v.0.10.1).

Salmonella Newport 3596 (parent) and a phage-resistant mutant of *Sal.* Newport 3596 S50.1.3.1 (*Sal.* Newport Mut$^{\Phi}$) was hybrid sequenced (short-read and long-read) on Illumina Miseq and Oxford Nanopore Technologies (ONT) by Plasmidsaurus. Hybrid sequence data was received as a circularized hybrid assembly (.fasta).

Reference *Salmonella* Newport strain BCW_3376 – GCF_002060435.1 was used as the reference sequence when necessary. All scripts used for analysis can be found at https://github.com/srk0002/Dissertation-Spring-2024.

2.3.2 Variant Detection GATK Best Practices Workflow

GATK Best Practices workflow was used with two different variations for variant detection (vcf file) between *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ (**Figure 2.1**). Variants will be defined as DNA polymorphism data such as single-nucleotide polymorphisms (SNPs), insertions, deletions, and structural variants (Danecek et al., 2011). Untrimmed raw reads from *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ was mapped to reference sequence (GCF_002060435.1) with BWA-MEM (v.0.7.12) and sorted with SAMtools (v.1.13) (Li and Durbin, 2009; Danecek et al., 2021). GATK (4.1.0.0) AddorReplaceReadGroups was used to add read group information to bam files. GATK HaplotypeCaller called variants from analysis-ready reads (.bam) to generate gvcf

files files. GATK SelectVariants selected single-nucleotide polymorphisms (SNPs) from gvcf files (McKenna et al., 2010; Depristo et al., 2011). Variants filtered and VCFtools (v.0.1.16) used to compare variants from *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ (Danecek et al., 2011). IGV Viewer (v.2.12.2) was used to inspect any called variants that were found only in either *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ vcf file (Robinson et al., 2011). Variants only found in one and not the other are considered variants of interest.

The second GATK Best Practices workflow approach utilizes a *Sal*. Newport parent assembly as the reference sequence (**Figure 2.2**). The *Sal*. Newport parent fastq files were assembled with SPAdes (v.3.13.0) (Prjibelski et al., 2020). This draft assembly (scaffolds.fasta) was used as the reference sequence and the *Sal*. Newport Mut$^{\Phi}$ was mapped to the *Sal*. Newport parent. The GATK Best Practices workflow was followed as previously stated, without VCFtools being necessary due to the *Sal*. Newport Mut$^{\Phi}$ variants detected against the *Sal*. Newport parent directly (McKenna et al., 2010; Danecek et al., 2011, 2021; Depristo et al., 2011; Li, 2013). IGV Viewer was used to inspect called variants.

2.3.3 Variant Detection with Snippy

This workflow uses the tool Snippy (v.4.6.0) for variant calling (**Figure 2.1**). Snippy detects variants between a reference genome (GCF_002060435.1) and raw fastq files (Seemann, 2020). This was done for both *Sal*. Newport Mut$^{\Phi}$ and *Sal*. Newport parent fastq reads. VCFtools was used to compare the output Snippy vcf file between *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ (Danecek et al., 2011). IGV Viewer was used to inspect any called variants that were found only in either *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ vcf file (Robinson et al., 2011). Variants only found in one and not the other were considered variants of interest.

The second approach with the tool Snippy for variant calling utilizes *Sal*. Newport parent assembly as the reference sequence (**Figure 2.2**) (Seemann, 2020). The *Sal*. Newport parent SPAdes assembly (scaffolds.fasta) was used as the input reference and the raw sequence read fastq files (Prjibelski et al., 2020). The output snippy vcf file was examined with IGV Viewer to inspect called variants (Robinson et al., 2011).

Figure 2.1. Workflows with Reference Genome (GCF_002060435.1). This flowchart displays the two approaches, GATK Best Practices workflow and Snippy tool, where the reference genome was GCF_002060435.1. Output vcf files for *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ was compared with VCFtools and unique variants were investigated with IGV viewer.

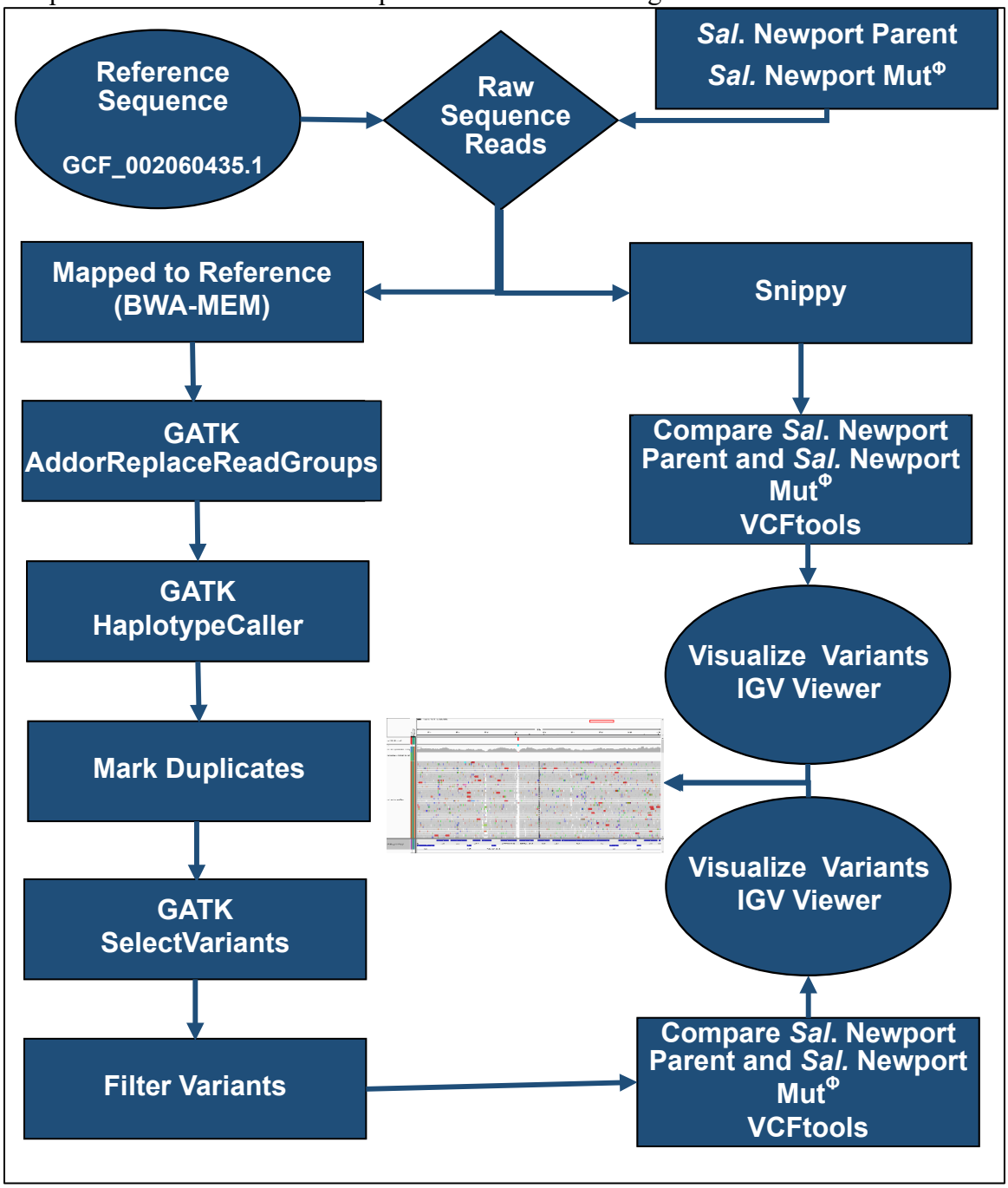
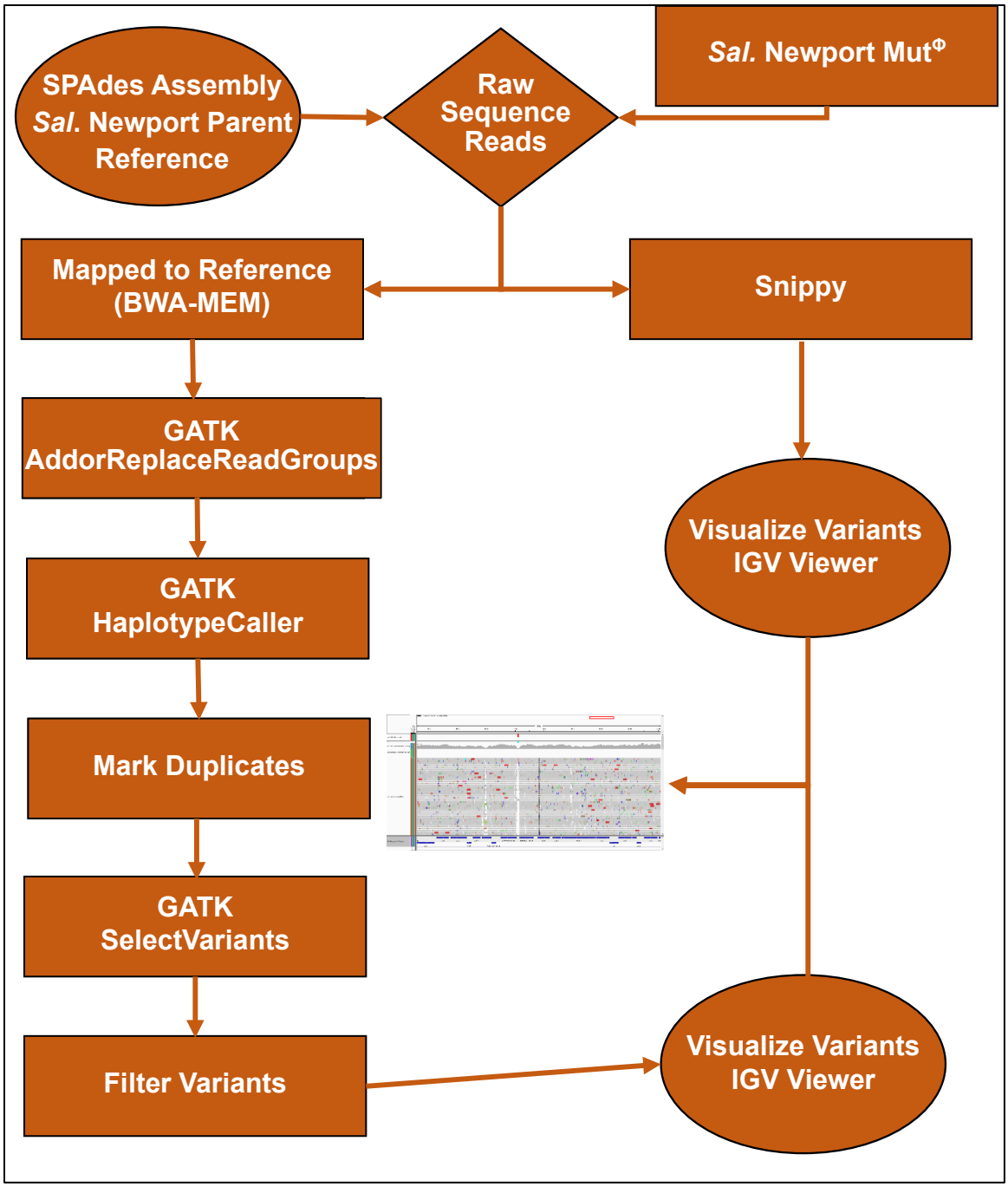


Figure 2.2. Workflows with *Sal.* Newport Parent Assembly as Reference. This flowchart displays the two approaches, GATK Best Practices workflow and Snippy tool, where the reference genome was *Sal*. Newport parent SPAdes assembly. Output vcf files was for the direct variant calling between reference *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$, which were investigated with IGV viewer.



2.3.4 Comparison of Whole Genome Alignments between Short-Read Assemblies and Hybrid (short-read and long-read sequencing) Assemblies for Variant Detection

The *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ fastq files were assembled with SPAdes (v.3.13.0) (Prjibelski et al., 2020). These assemblies (scaffolds.fasta) were aligned to one another by using MUMmer4 (v.4.0.0). MUMmer aligns whole genomes to other whole genomes (Marçais et al., 2018). IGV viewer was used to inspect SNPs detected by MUMmer between *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ (Robinson et al., 2011; Marçais et al., 2018).

Circularized fasta files received from Plasmidsaurus for *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ were compared with MUMmer4 (v.4.0.0). IGV viewer was used to inspect SNPs detected by MUMmer between *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ (Robinson et al., 2011; Marçais et al., 2018).

2.3.5 Characterizing *Sal*. Newport Parent and *Sal*. Newport Mut$^{\Phi}$ with Bacteriophage Adsorption Kinetics, Antisera Agglutination, and Motility.

Adsorption kinetics for S11, S40, S41, S44, and S50 on both parent *S.* Newport and mutant *S.* Newport Mut$^{\Phi}$ was performed to determine if the mutation responsible for bacteriophage resistance affected a potential surface molecule that conferred resistance to bacteriophage. A mid-log phase culture of *Salmonella* was diluted to give 10 mL with an OD_{620} of 0.1–0.2. From the cell suspension, 9 mL was added to a 125 mL flask labeled "A" and 9 ml of Luria-Bertani broth (Difco LB Broth, Miller) containing 1mM magnesium (LBM, Difco) added to a 125 mL flask labeled "C". At time = 0, 1 mL of warmed bacteriophage suspension with a titer of 1 – 3 x 10^5 (PFU/mL) to flask A and 1 mL of bacteriophage to flask C. At one-minute intervals for ten minutes (S11 had additional time for a twenty-minute sample), 0.05 mL aliquots from flask A were added to chilled tubes containing three drops of chloroform. Tubes were vigorously vortexed for 10 seconds before being placed on ice. After ten minutes, 0.05 mL samples from flask C were added to tubes C1 and C2 that contained chloroform. From each sample tube, 0.1 mL was inoculated into molten top agar and poured onto a bottom agar plate using the double agar overlay method as described earlier. The *Salmonella* cell suspension was diluted to the 10^{-6} dilution, and 0.1 mL aliquots from the 10^{-4} to 10^{-6} dilutions were spread onto LB agar plates to determine the concentration (CFU/mL) of the cell suspension. Time = 0 was the average number of plaques on plates from C1 and C2. The adsorption rate constant (k) was calculated from the formula: $k = \frac{2.3}{Bt}\log\frac{P_O}{P}$ where k is the adsorption rate constant, in mL/minute; B is the concentration of bacterial cells; t is the time interval in which the titer falls from P_O (original) to P (final) (Kropinski, 2009). However, if no

difference in absorption kinetics is found, this would suggest that there may be some internal mechanism of resistance.

The *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ LPS O-antigens were serologically tested with the polyvalent *Salmonella* O Antiserum Poly A – I and Vi (BD Difco, Becton, Dickinson, and Company, Sparks, MD), *Salmonella* O Antiserum Poly Group B (groups C_1, C_2, F, G, H) (BD Difco, Becton, Dickinson, and Company, Sparks, MD), and *Salmonella* O Antiserum Group C_2 Factor 6/8 (BD Difco, Becton, Dickinson, and Company, Sparks, MD). The procedure was followed per product label.

Motility was checked by using a modified Luria-Bertani (LB) agar. Agar recipe was typical LB broth (Difco LB Broth, Miller, Becton, Dickinson, and Company, Sparks, MD) with 0.35% Bacto agar (BD, Becton, Dickinson, and Company, Sparks, MD) opposed to the standard 1.5% agar that LB agar, Miller would contain. The 0.35% agar concentration was used because SIM medium uses this percentage of agar to determine motility, but the differentiation of sulfur production and indole formation found in SIM medium was not necessary (BD Diagnostics, 2009). The LB broth, Miller with 0.35% agar (LB agar$^{0.35\%}$) was added to tubes and solidified upright. LB agar$^{0.35\%}$ tube was inoculated by stabbing agar in the center and going down two-thirds of the distance to the butt of the tube with an inoculating needle. Motility would be determined based on observation of diffuse growth outward from the stab line or turbidity throughout the medium. Non-motility bacterial growth would be localized to the line of the stab.

2.4. Results

2.4.1 Variant Detection GATK Best Practices Workflow

The first workflow aligned the *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ fastq files to the reference genome GCF_002060435.1. The *Sal.* Newport parent had 85 filtered variants from reference genome and the *Sal*. Newport Mut$^{\Phi}$ had 69 filtered variants from reference genome. The *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ had twenty-seven unique variants between the two. Ratios of nucleotide bases at sites on the reference genome were viewed on the IGV viewer. All ratios were heterogeneous (**Table 2.1**) variants where the aligned or assembled contigs have different bases at the site location. An example of the heterogeneous ratios as visualized on IGV can be found with **Figure 2.3**.

Table 2.1. GATK Workflow: *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ Unique Variants to Reference GCF_002060435.1.

			Sal. Newport Parent Base Ratios				*Sal*. Newport Mut$^{\Phi}$ Base Ratios			
CHROM	POS1	POS2	A	C	G	T	A	C	G	T
NZ_MXTK01000010.1	.	149679	59%	1%	40%		46%	1%	53%	
NZ_MXTK01000010.1	150125	.		45%		55%		61%		39%
NZ_MXTK01000010.1	150127	.	55%		45%		37%		63%	
NZ_MXTK01000010.1	150128	.	55%	45%			36%	64%		
NZ_MXTK01000010.1	150136	.		44%	1%	55%		58%	1%	41%
NZ_MXTK01000010.1	150187	.	63%	1%	36%		46%		54%	
NZ_MXTK01000010.1	150215	.		40%		60%		54%		46%
NZ_MXTK01000010.1	150216	.		43%	64%	1%		54%	46%	1%
NZ_MXTK01000010.1	150244	.			59%	41%			54%	46%
NZ_MXTK01000010.1	150245	.	42%			58%	45%			55%
NZ_MXTK01000010.1	150250	.	36%	1%	62%		42%	1%	57%	
NZ_MXTK01000010.1	150258	.		63%		37%		57%		43%
NZ_MXTK01000037.1	8371	.	1%	43%	1%	56%	1%	46%		53%
NZ_MXTK01000037.1	.	8645	50%			50%	56%		1%	43%
NZ_MXTK01000037.1	.	9566	60%		40%		44%	1%	55%	
NZ_MXTK01000037.1	.	10392	45%		55%	1%	52%	0%	47%	
NZ_MXTK01000044.1	3075	.	47%		53%		50%		50%	
NZ_MXTK01000044.1	.	3287		34%		66%		48%		52%
NZ_MXTK01000044.1	.	3388	56%		44%		45%		55%	
NZ_MXTK01000044.1	.	3943	51%	49%			53%	47%		
NZ_MXTK01000044.1	22854	.		59%		41%		53%	1%	46%
NZ_MXTK01000044.1	22950	.		62%	38%	1%		48%	51%	2%
NZ_MXTK01000044.1	23087	.	44%	55%	1%		50%	50%		
NZ_MXTK01000005.1	1011	.		52%	48%			46%	54%	
NZ_MXTK01000062.1	3270	.			57%	43%			52%	48%
NZ_MXTK01000062.1	3383	.	44%	1%	54%		48%	1%	51%	
NZ_MXTK01000062.1	3388	.		54%	1%	44%	1%	49%		50%

Figure 2.3. GATK Workflow: IGV Snapshot *Sal*. Newport parent (bottom reads) and *Sal*. Newport Mut$^{\Phi}$ (top reads) Variants to Reference GCF_002060435.1: Called variant from reference genome chromosome NZ_MXTK010000.10.1 at position 149679. Heterogeneous ratios are displayed as split bar colors on coverage line.

The second approach of the GATK Best Practices workflow used the SPAdes assembly of *Sal*. Newport parent as the reference sequence and the *Sal*. Newport Mut$^{\Phi}$ fastq reads were aligned to parent assembly. There were 167 filtered variants called for *Sal*. Newport Mut$^{\Phi}$ from *Sal*. Newport parent assembly. As with the previous approach, many of the variants were heterogeneous variants (**Figure 2.4**); some variants were the result of low coverage (**Figure 2.5 and Figure 2.6**) or found in low coverage and predicted non-coding location (**Figure 2.7**). Out of all the called variants, only one appears to be a real variant (**Figure 2.8**). This variant was called on Node 5, position 249,841 located in the gene oadB_1. This mutation would be a synonymous mutation because it changed from the codon GTG to GTT, which both translate to the amino acid valine.

Figure 2.4. GATK Workflow: IGV Snapshot *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variants from *Sal*. Newport parent assembly is indicated by red to pink bars above the coverage line. Heterogeneous ratios are displayed as split bar colors on coverage line.

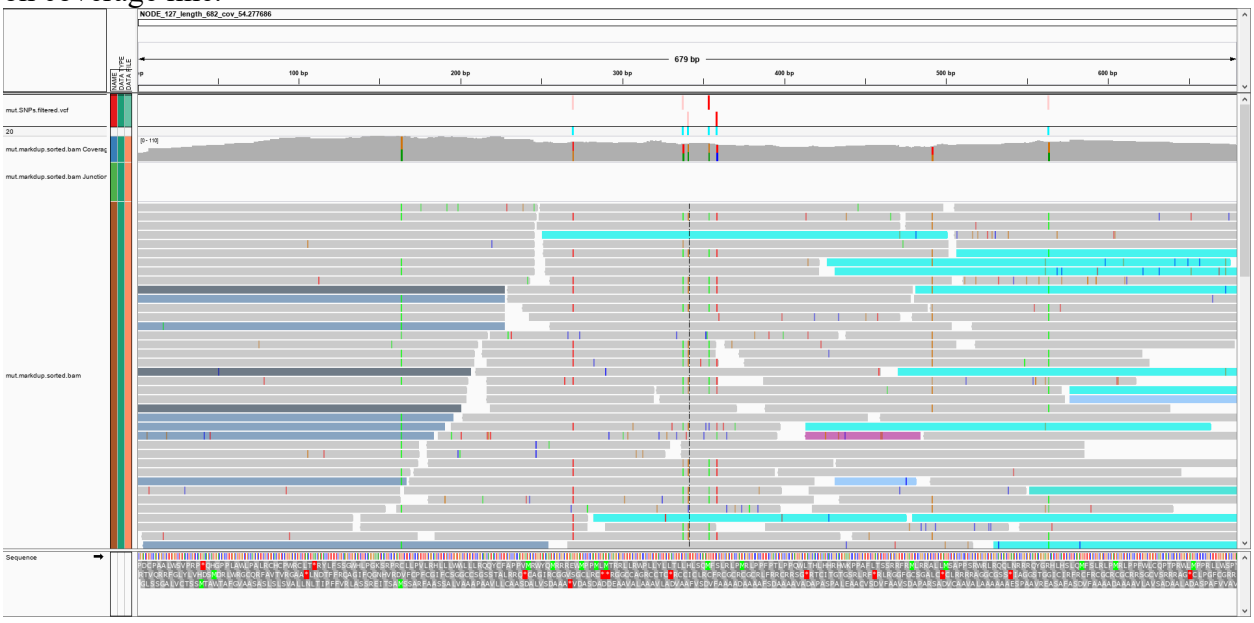


Figure 2.5. GATK Workflow: IGV Snapshot *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variant from *Sal*. Newport parent assembly is indicated by red bar above coverage line. Example of low coverage (this instance depth of 12) near the end of the scaffold at the location.

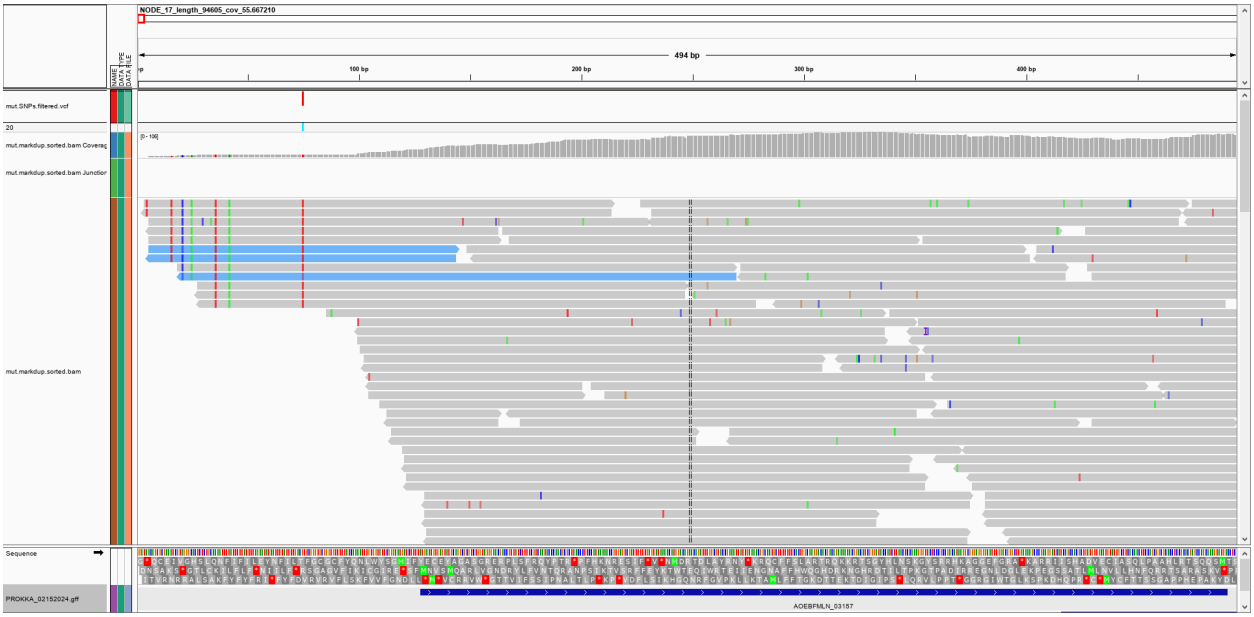


Figure 2.6. GATK Workflow: IGV Snapshot *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variant from *Sal*. Newport parent assembly is indicated by red bar above coverage line. Example of low coverage (this instance depth of 2) with small scaffold of contigs at the location.

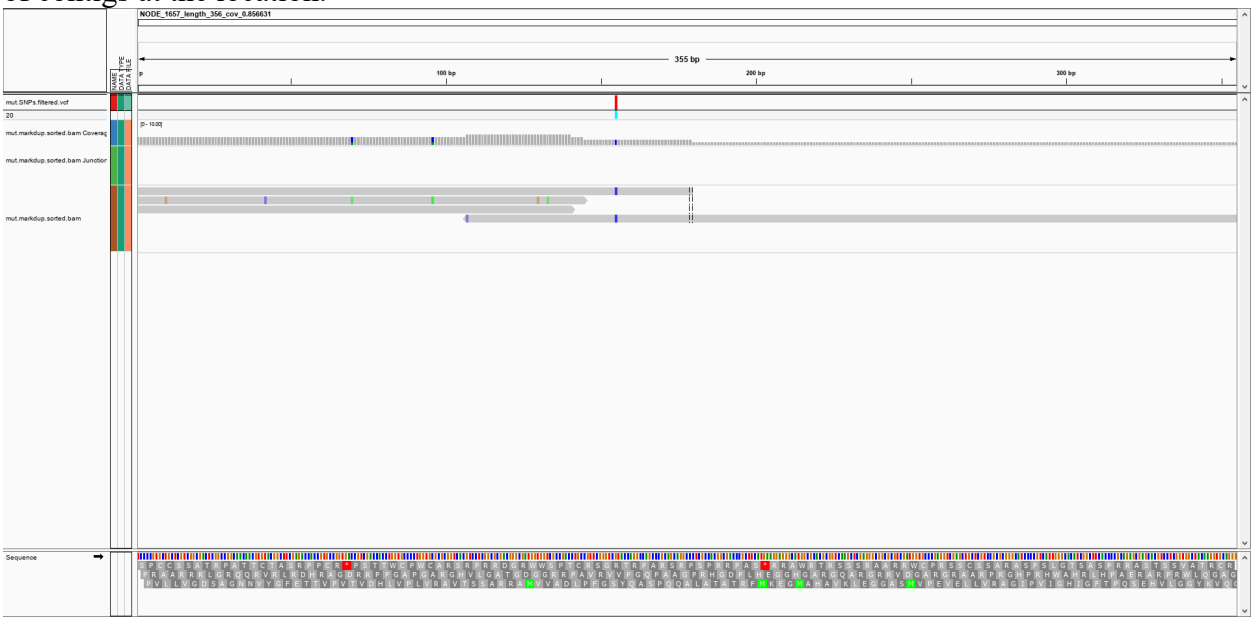


Figure 2.7. GATK Workflow: IGV Snapshot *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variants (4 total) from *Sal*. Newport parent assembly are indicated by red bars above coverage line. Example of low coverage (this instance depths of 4, 4, 5, and 6) and in a predicted non-coding region.

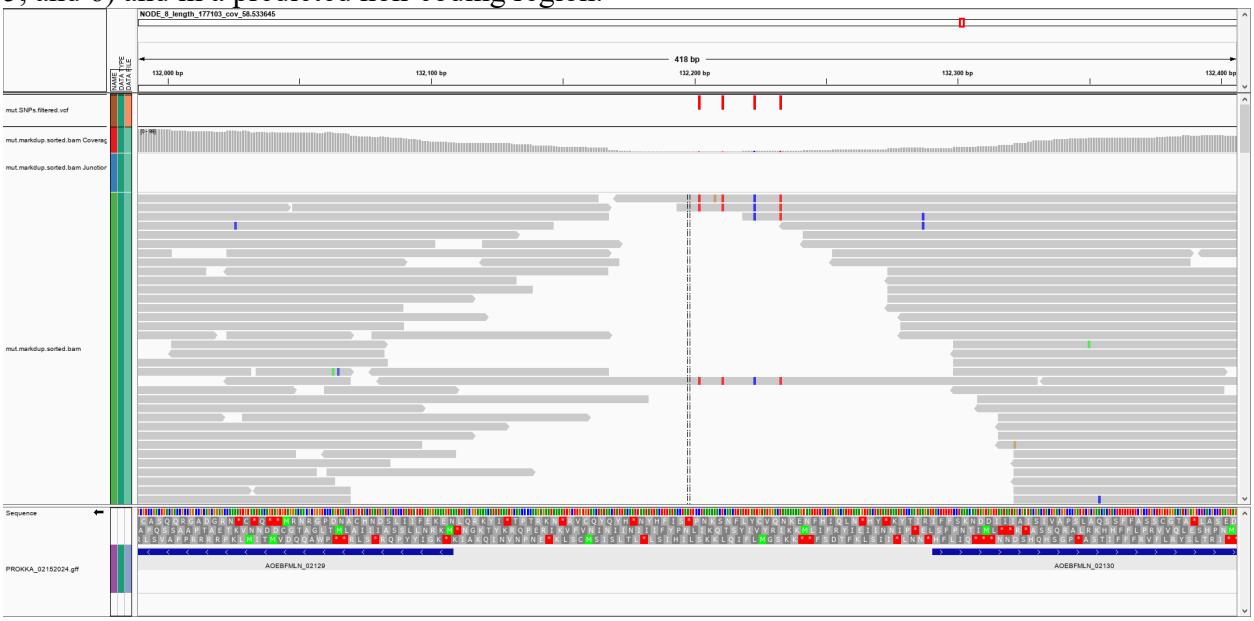
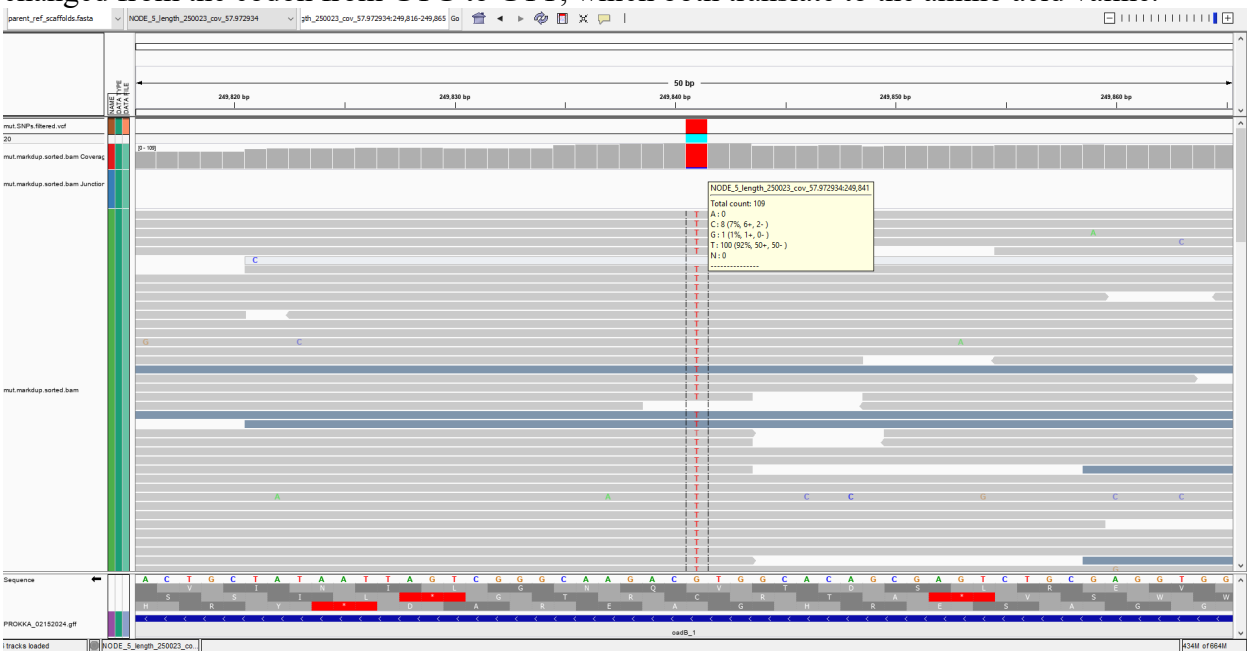


Figure 2.8. GATK Workflow: IGV Snapshot *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variants from *Sal*. Newport parent assembly on Node 5, position 249,841 located in gene oadB_1. This mutation would be a synonymous mutation because it changed from the codon from GTG to GTT, which both translate to the amino acid valine.



2.4.2 Variant Detection Snippy Workflow

The second workflow used the tool Snippy to align the *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ fastq files to the reference genome GCF_002060435.1 and call variants. The *Sal.* Newport parent had 21 filtered variants from reference genome and the *Sal*. Newport Mut$^{\Phi}$ had 23 filtered variants from reference genome. The *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ had 5 unique variants between the two. Ratios of nucleotide bases at sites on the reference genome were viewed on the IGV viewer. All ratios were either heterogenous variants or the result from difference in depth at the position (**Table 2.2**). The called variants with difference in coverage at the sites as visualized on IGV are found in **Figures 2.9**, **2.12**, and **2.13.** Called variants with differences in heterogeneous ratios as visualized on IGV can be found with **Figure 2.10** and **2.11**.

Table 2.2. Snippy Workflow: *Sal*. Newport Parent and *Sal*. Newport Mut$^{\Phi}$ Unique Variants to Reference GCF_002060435.1: Table represents the percentage of nucleotide bases at the called variant locations using Snippy.

			Sal. Newport Parent Base Ratios					*Sal*. Newport Mut$^{\Phi}$ Base Ratios				
CHROM	POS1	POS2	A	C	G	T	Depth	A	C	G	T	Depth
NZ_MXTK01000011.1		144319	100%				5	100%				16
NZ_MXTK01000005.1	57		9%		88%	2%	43	16%	2%	82%		44
NZ_MXTK01000005.1		2110	79%		20%	1%	84	89%		10%	1%	82
NZ_MXTK01000005.1		10629			100%		8			100%		22
NZ_MXTK01000005.1		90402				100%	92				100%	99

Figure 2.9. Snippy Workflow: IGV Snapshot *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ Variants to Reference GCF_002060435.1: Called variant from reference genome chromosome NZ_MXTK01000011.1 at position 144319. Differences in depth at position: *Sal*. Newport parent depth 5 and *Sal*. Newport Mut$^{\Phi}$ depth 16.

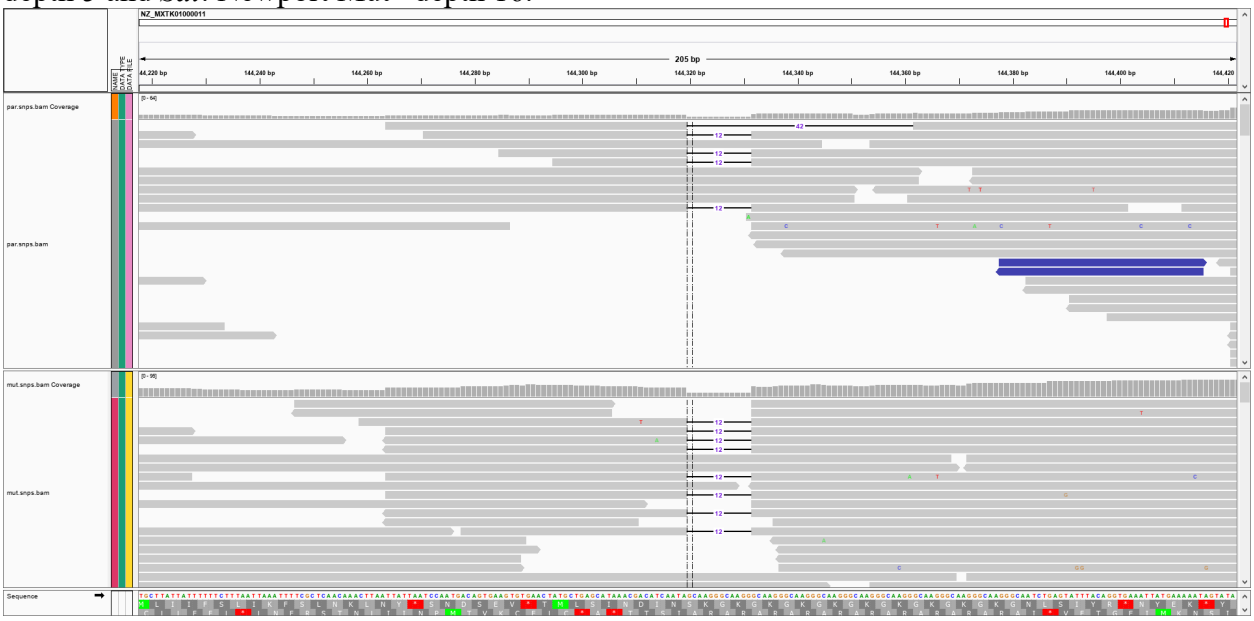


Figure 2.10. Snippy Workflow: IGV Snapshot *Sal*. Newport parent (top reads) and *Sal*. Newport Mut$^{\Phi}$ (bottom reads) Variants to Reference GCF_002060435.1: Called variant from reference genome chromosome NZ_MXTK01000005 at position 57. Differences in heterogeneous base ratios between *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$.

Figure 2.11. Snippy Workflow: IGV Snapshot *Sal*. Newport parent (top reads) and *Sal*. Newport Mut$^{\Phi}$ (bottom reads) Variants to Reference GCF_002060435.1: Called variant from reference genome chromosome NZ_MXTK01000005 at position 2110. Differences in heterogeneous base ratios between *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$.

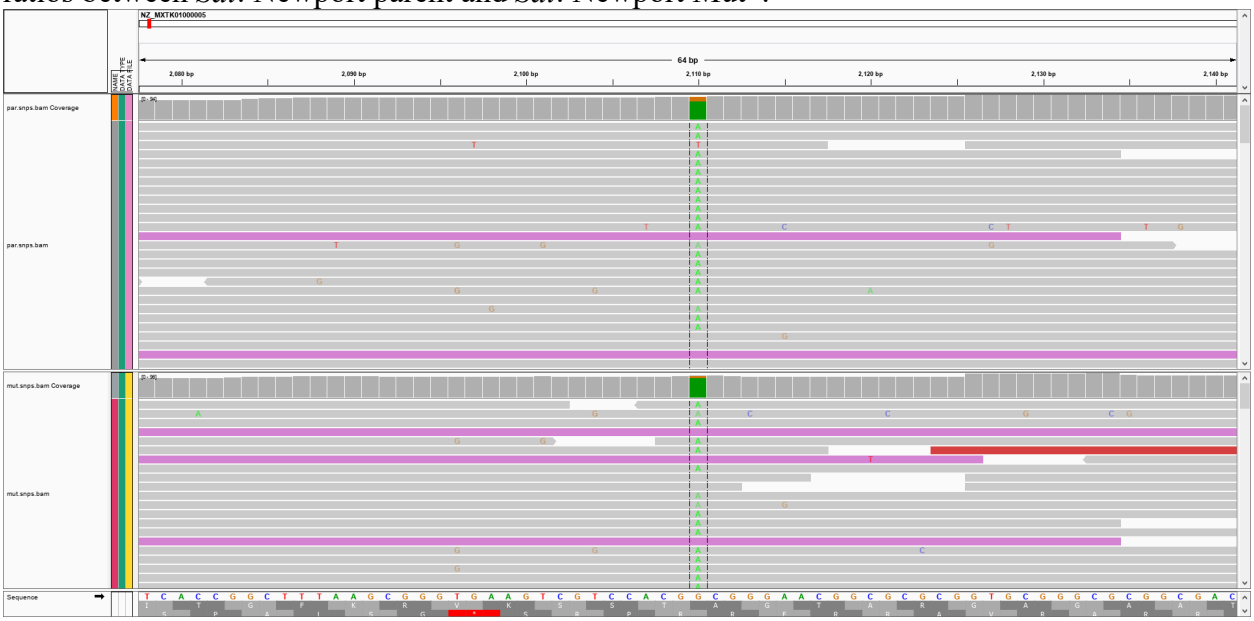


Figure 2.12. Snippy Workflow: IGV Snapshot *Sal*. Newport parent (top reads) and *Sal*. Newport Mut$^{\Phi}$ (bottom reads) Variants to Reference GCF_002060435.1: Called variant from reference genome chromosome NZ_MXTK01000005 at position 10629. Differences in depth at position: *Sal*. Newport parent depth 8 and *Sal*. Newport Mut$^{\Phi}$ depth 22.

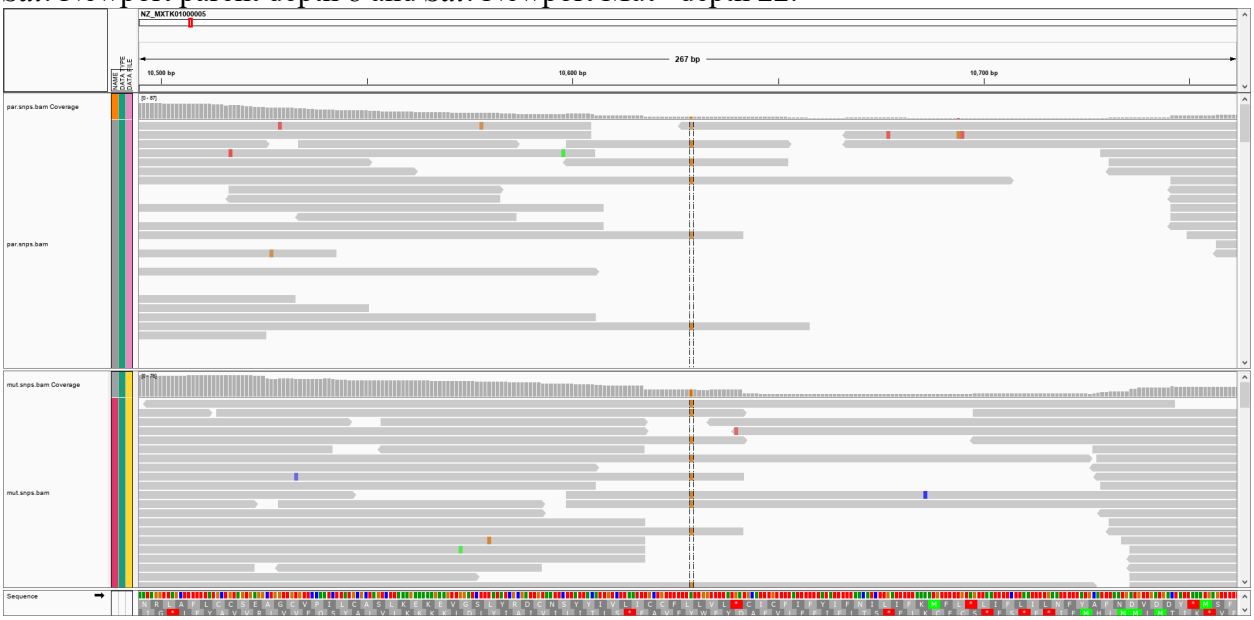


Figure 2.13. Snippy Workflow: IGV Snapshot *Sal*. Newport parent (top reads) and *Sal*. Newport Mut$^{\Phi}$ (bottom reads) Variants to Reference GCF_002060435.1: Called variant from reference genome chromosome NZ_MXTK01000005 at position 90402. Differences in depth at position: *Sal*. Newport parent depth 92 and *Sal*. Newport Mut$^{\Phi}$ depth 99.

The next approach used the tool Snippy to align *Sal*. Newport Mut$^{\Phi}$ fastq files to the SPAdes assembly of *Sal*. Newport parent as the reference genome and call variants. *Sal*. Newport Mut$^{\Phi}$ had 14 filtered variants from *Sal*. Newport parent. The variants called were heterogeneous variants (**Figure 2.14**); some variants were the result of low coverage (**Figure 2.15 and Figure 2.16**) or found in low coverage and predicted non-coding location (**Figure 2.17**). Of all the called variants, only two appear to be real variants. The first variant (**Figure 2.18**) was called on Node 2, position 114,193 in gene AOEBFMLN_00654, which codes for tRNA for tyrosine. The second variant (**Figure 2.19**) was called on Node 5, position 249,841 located in gene oadB_1. This mutation would be a synonymous mutation because it changed the codon from GTG to GTT, which both translate to the amino acid valine.

Figure 2.14. Snippy Workflow: IGV Snapshot of *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variants from *Sal*. Newport parent assembly is indicated by red to pink bars above the coverage line. Hetergeneous ratios are displayed as split bar colors on coverage line.

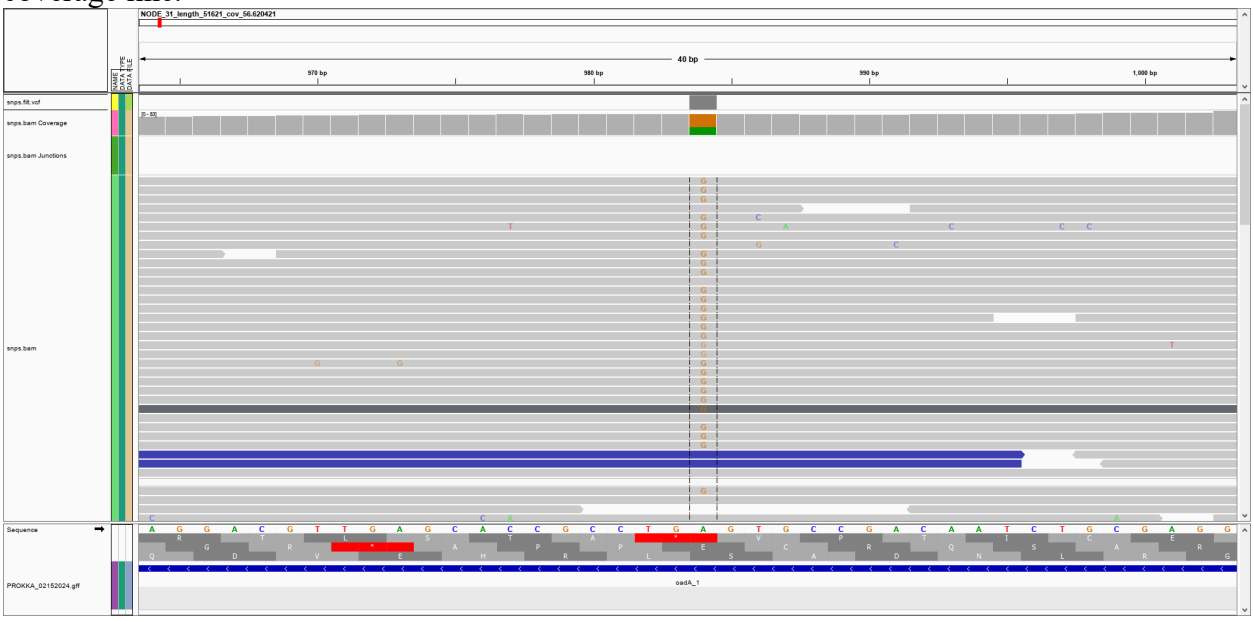


Figure 2.15. Snippy Workflow: IGV Snapshot of *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variant from *Sal*. Newport parent assembly is indicated by grey bar above coverage line. Example of low coverage (this instance depth of 12) near the end of the scaffold at the location.

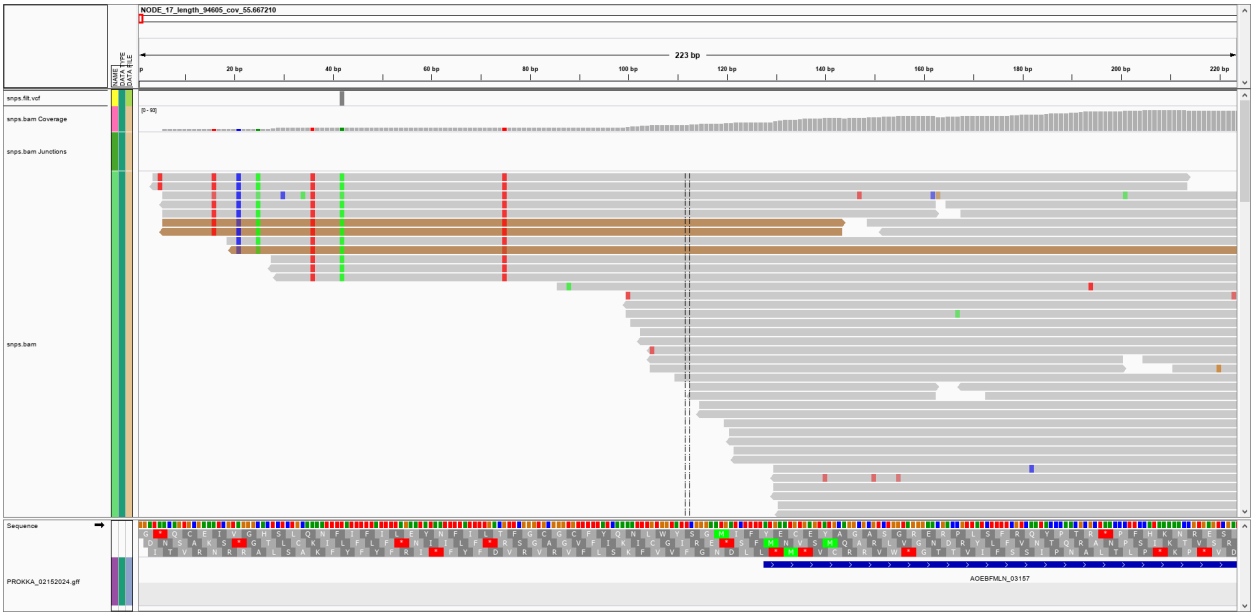


Figure 2.16. Snippy Workflow: IGV Snapshot of *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variants from *Sal*. Newport parent assembly is indicated by grey bars above coverage line. Example of low coverage (this instance depth of 11) found in the middle of the scaffold at both locations.

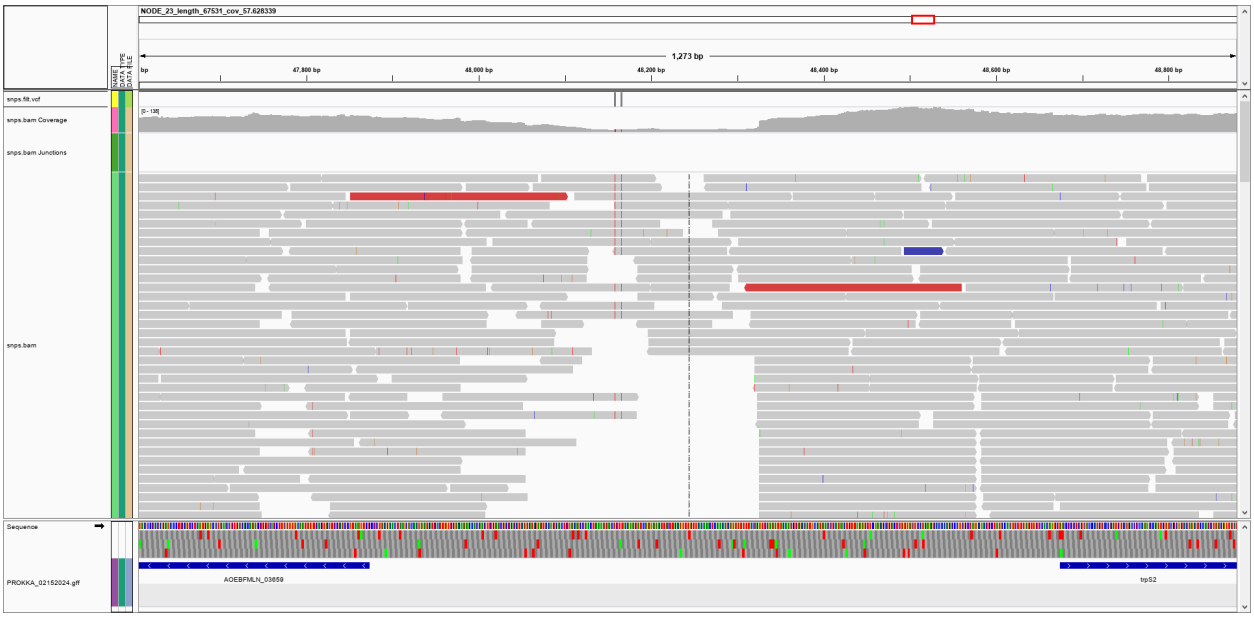


Figure 2.17. Snippy Workflow: IGV Snapshot of *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variant from *Sal*. Newport parent assembly are indicated by grey bar above coverage line. Example of low coverage and in a predicted non-coding region.

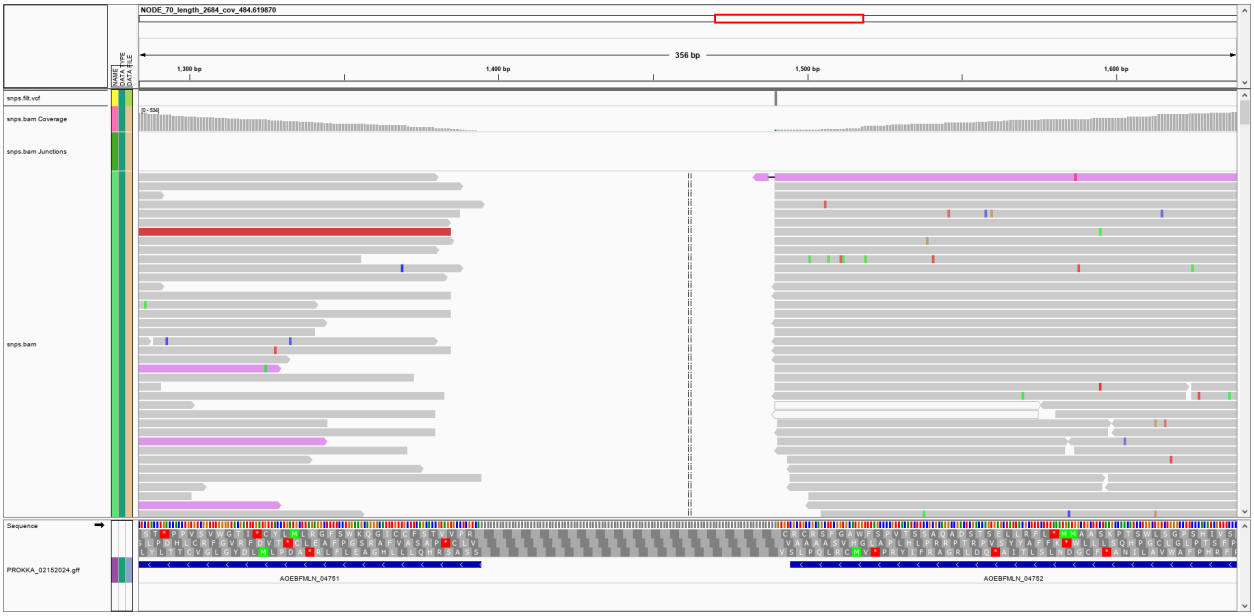


Figure 2.18. Snippy Workflow: IGV Snapshot of *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variants from *Sal*. Newport parent assembly on Node 3, position 114,193 located in gene AOEBFMLN_00654.

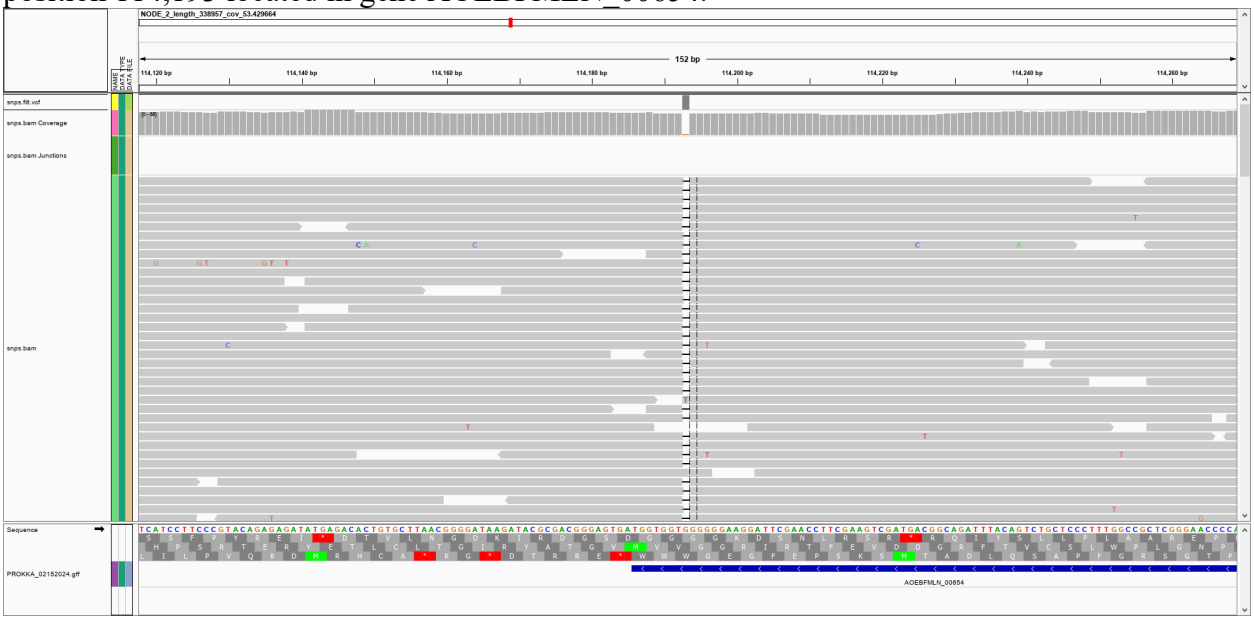
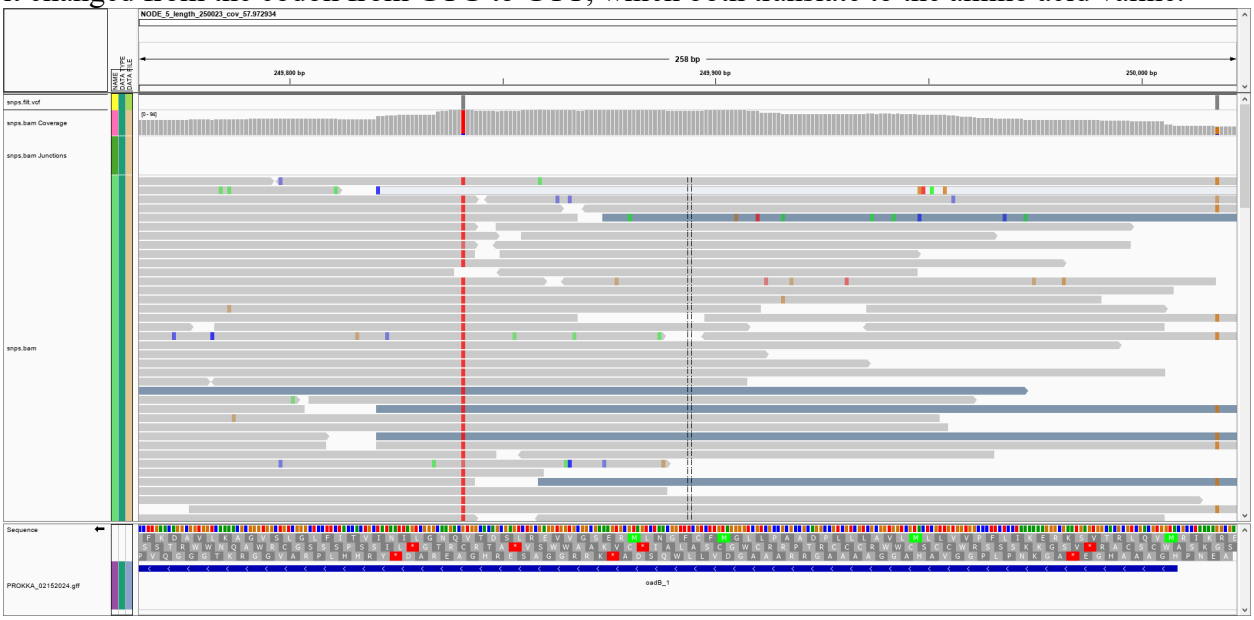


Figure 2.19. Snippy Workflow: IGV Snapshot of *Sal*. Newport Mut$^{\Phi}$ Variants to Reference *Sal*. Newport Parent Assembly: Called variants from *Sal*. Newport parent assembly on Node 5, position 249,841 located in gene oadB_1. This mutation would be a synonymous mutation because it changed from the codon from GTG to GTT, which both translate to the amino acid valine.



2.4.3 Comparison of Whole Genome Alignments between Short-Read Assemblies and Hybrid (short-read and long-read sequencing) Assemblies for Variant Detection

A comparison of whole genome alignments from assemblies generated from short-read Illumina sequencing and a whole genome alignment of hybrid assemblies generated from Illumina short-read sequencing and Nanopore long-read sequencing. The short-read SPAdes assembly graphs for the *Sal*. Newport parent (**Figure 2.20**) and *Sal*. Newport Mut$^{\Phi}$ (**Figure 2.21**) have many different branches of the nodes. The assembly graphs from the hybrid assemblies for the *Sal*. Newport parent (**Figure 2.22**) and *Sal*. Newport Mut$^{\Phi}$ (**Figure 2.23**) is much cleaner with individual circles. To simplify an assembly graph, the best graph for a bacterial genome assembly is a single circle (more if there are plasmids). The hybrid assemblies (**Figure 2.22** and **2.23**) are more of a circle when compared to the short-read assemblies (**Figure 2.20** and **2.21**).

Figure 2.20. Bandage (v.0.8.1) viewer of SPAdes assembly graph of short-reads for *Sal*. Newport Parent.

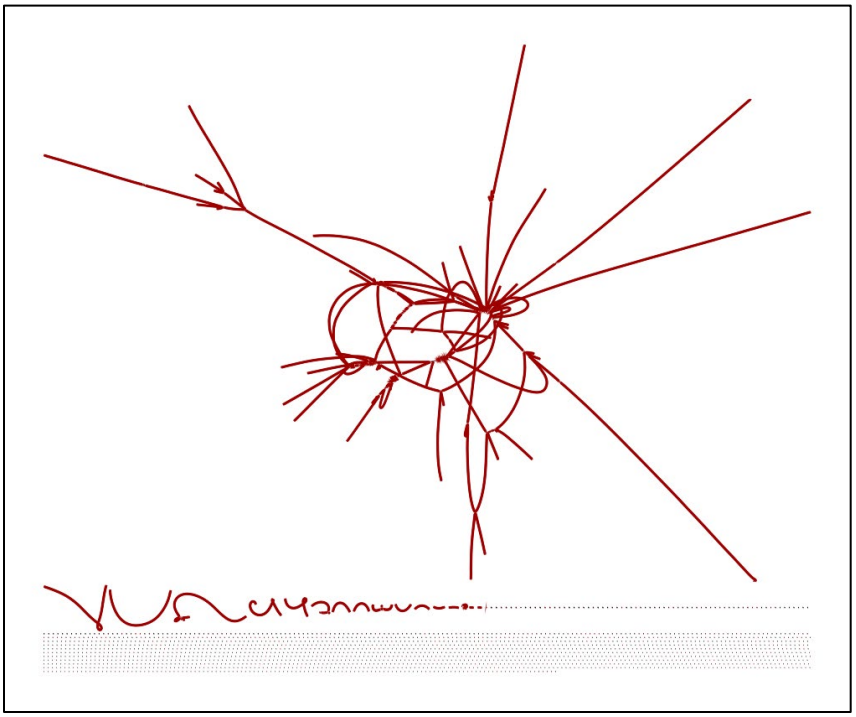


Figure 2.21. Bandage (v.0.8.1) viewer of SPAdes assembly graph of short-reads for *Sal*. Newport Mut$^{\Phi}$.

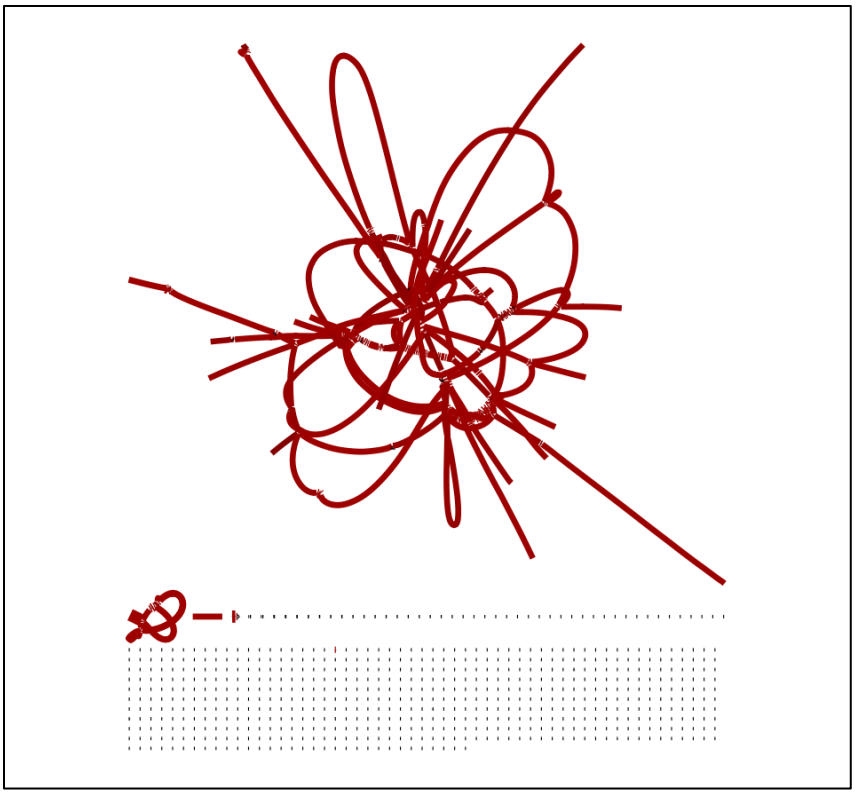


Figure 2.22. Bandage (v.0.8.1) viewer of hybrid assembly graph of short and long-reads for *Sal*. Newport Parent.

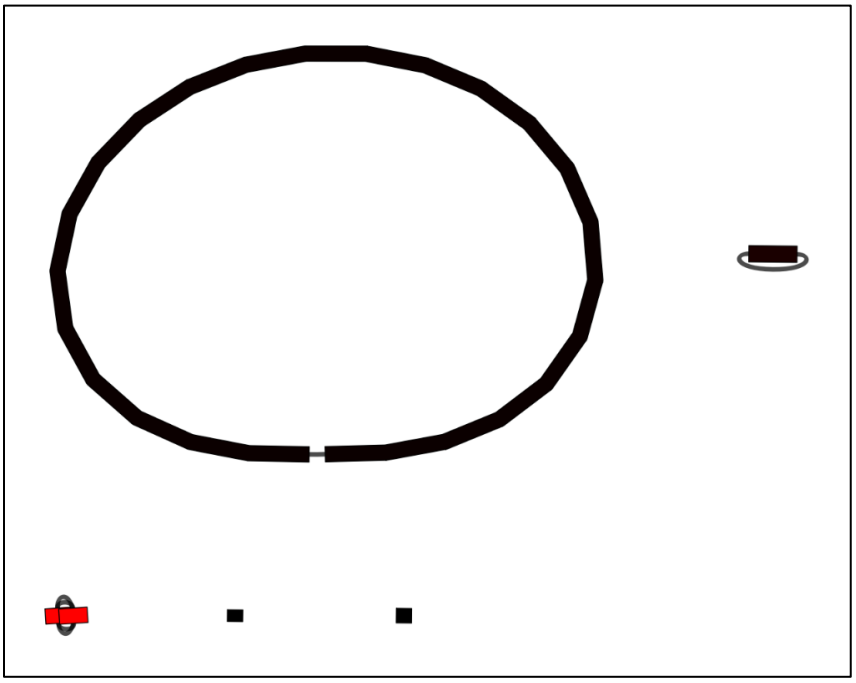
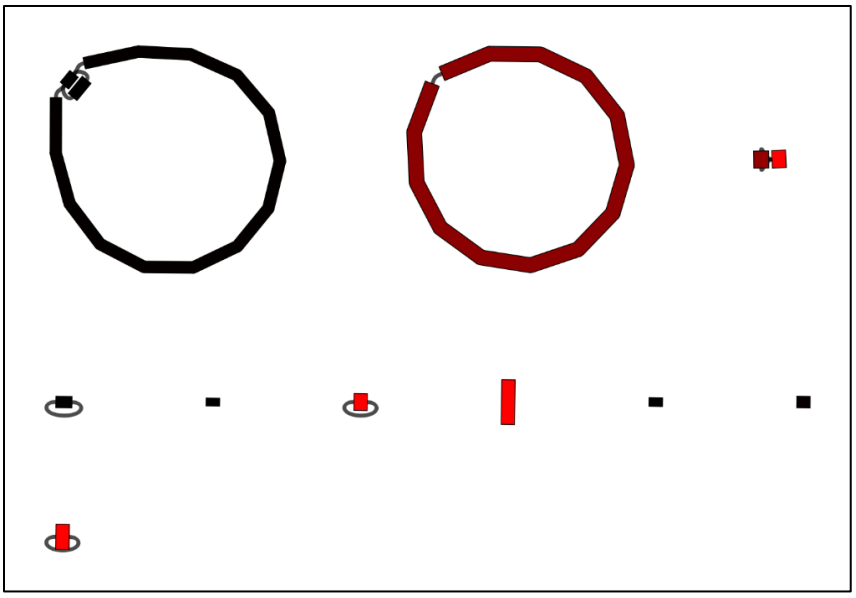


Figure 2.23. Bandage (v.0.8.1) viewer of hybrid assembly graph of short and long-reads for *Sal*. Newport Mut$^{\Phi}$.



The resulting "snps" file from MUMmer4 for the short-read assemblies for the *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ was 384 variants. The resulting "snps" file from MUMmer4 for the hybrid assemblies for the *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ was 4

variants. The variants from the short-read assemblies were an unmanageable number to visually inspect, and therefore, the identification of variants did not continue.

Regarding the whole genome alignment of hybrid assemblies for *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$, the 4 variants are shown in **Table 2.3**. The first SNP was found in a hypothetical protein and this mutation was synonymous because the codon would code for the same amino acid. The second and third SNP was found at locations that theoretically produces a phage tail-collar fiber protein. The second SNP is synonymous and the third SNP is nonsynonymous resulting in a change of amino acid from hydrophobic isoleucine to the hydrophobic methionine. The fourth SNP was found in a the *rfbM* gene, which is involved in LPS biosynthesis ((Shah et al., 2012). This SNP is a nonsynonymous mutation resulting in a change of amino acid from the polar uncharged serine to hydrophobic phenylalanine.

Table 2.3. Hybrid Assembly SNPs. SNPs detected by whole genome alignment between *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ with translated amino acid at position.

***Sal*. Newport Parent**				***Sal*. Newport Mut$^{\Phi}$**				
Contig	**Position**	**Amino Acid**	**Nucleotide**	**Nucleotide**	**Amino Acid**	**Position**	**Contig**	**Gene Product**
contig_1	54992	L	T	C	L	4182826	contig_1	Hypothetical Protein
contig_1	57509	T	G	A	T	4180309	contig_1	Phage tail-collar fibre protein
contig_1	57557	I	T	C	M	4180261	contig_1	Phage tail-collar fibre protein
contig_1	4233813	S	G	A	F	4005	contig_1	rfbM: mannose-1-phosphate guanylyltransferase/ mannose-6-phosphate isomerase

2.4.4 Characterizing Bacteriophage on *Sal.* Newport Parent and *Sal.* Newport Mut$^{\Phi}$ with Adsorption Kinetics, Antisera Agglutination, and Motility.

The results of the EOP (**Table 2.4**) determined that all five phage (S11, S40, S41, S44, S50) had an adsorption rate below 10^{-9} mL/min on the *Sal*. Newport Mut$^{\Phi}$, which would indicate phage-resistance in *Salmonella* as described by Yu *et al.* (Yu et al., 2024). Surprisingly, two of the phage (S11 and S44) also had adsorption rates below 10^{-9} mL/min on the *Sal*. Newport parent. Over 20 minutes, there was a 60.98% decrease in S11 against the *Sal*. Newport parent, compared to a 4.65% increase in S11 against the *Sal*. Newport Mut$^{\Phi}$. Over 5 minutes, there was a 60.18% decrease in S44 against the *Sal*. Newport parent, compared to a 21.23% decrease in S44 against the *Sal*. Newport Mut$^{\Phi}$ over 10 minutes. Over 2 minutes, there was a 65.67% decrease in S40 against the *Sal*. Newport parent, compared to a 7.30% increase in S40 against the *Sal*. Newport Mut$^{\Phi}$ over 8 minutes. Over 4 minutes, there was a 71.96% decrease in S41 against the *Sal*. Newport parent, compared to a 6.54% increase in S41 against the *Sal*. Newport Mut$^{\Phi}$ over 10 minutes. Over

2 minutes, there was a 75.17% decrease in S50 against the *Sal*. Newport parent, compared to a no change in S50 against the *Sal*. Newport Mut$^{\Phi}$ over 10 minutes.

Both *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ agglutinated with all tested *Salmonella* O antiserum agglutinations for Poly A – I and Vi, Poly B, and Group C_2 Factor 6/8. Both *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ appear to have functional motility in LB agar$^{0.35\%}$.

Table 2.4. Efficiency of Plating. Initial starting phage count and final count of phages, S11, S40, S41, S44, and S50, over time with adsorption kinetics against *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$.

		S. Newport Parent			S. Newport Mut$^{\Phi}$		
Phage	Initial Plaque Count	Final Plaque Count	Time to Final Plaque Count	Adsorption rate (mL/min)	Final Plaque Count	Time to Final Plaque Count	Adsorption rate (mL/min)
S11	41	16	20	2.21E-10	43	20	-1.12E-11
S40	233	80	2	2.28E-09	250	8	-4.58E-11
S41	107	30	4	1.35E-09	114	10	-3.30E-11
S44	452	180	5	8.66E-10	356	10	1.04E-10
S50	145	36	2	3.28E-09	145	10	0.00E+00

2.5. Discussion

The purpose of this study was to identify the mutation that contributed to the phage-resistance seen in *Sal*. Newport Mut$^{\Phi}$. The *Sal*. Newport Mut$^{\Phi}$ isolate appeared to be attenuated when inoculated in a calf model. However, when co-inoculated with the *Sal*. Newport parent, both *Sal*. Newport Mut$^{\Phi}$ and *Sal*. Newport parent had an extended *Salmonella* shed (Kitchens, 2016).

There were many lessons learned during the process to identify the SNP in the *Sal*. Newport Mut$^{\Phi}$. Short-read sequencing data gave very inconclusive results. The workflows (both GATK and Snippy) when the *Sal*. Newport parent was assembled and *Sal*. Newport Mut$^{\Phi}$ was aligned to the parent and had the same called variant on Node 5, position 249,841 in gene oadB_1 (**Figure 2.8** and **2.19**). The gene codes for oxaloacetate decarboxylase. The mutation was a synonymous mutation, so there was no change in the amino acid sequence, and therefore, there would be no change in this product. It is possible that if it had been a nonsynonymous mutation, it could have contributed to phage-resistance because oxaloacetate decarboxylase is a membrane-bound enzyme, and it catalyzes the transport of sodium ions through *Salmonella*'s membrane (Wifling and Dimroth, 1989). The workflows (both GATK and Snippy) when the *Sal*. Newport parent was assembled and *Sal*. Newport Mut$^{\Phi}$ was aligned to the parent had many more called variants compared to the *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$ aligned to the reference

GCF_002060435.1. The assembled *Sal*. Newport parent as the reference had 2231 scaffolds or nodes, and some were very short (as low as 78 base scaffold). These small scaffolds had variants called in these locations, but the aligned *Sal*. Newport Mut$^{\Phi}$ might have had one contig aligned to this and if a variant was called, it did not appear to be real. The GATK workflows had more called variants than their Snippy counterparts, but this was probably more of an artifact or differences in variant filtering. Had the GATK filtering been more stringent, it would have called fewer variants.

Detection of a variant that could contribute to phage-resistance was not obtained from the short-read sequencing data alone. The reasoning for this may be that high-throughput short-read sequencing has a major downside in that the reads (150 to 300 base pairs) cannot span repeat elements such as insertion sequences. This forms a fragmented assembly, formed by contigs of unknown order (De Maio et al., 2019; Arredondo-Alonso et al., 2021; Zhang et al., 2021; Hackl S. T. et al., 2022; Lerminiaux et al., 2022; Sereika et al., 2022; Zhao et al., 2023). Illumina has had market dominance, due to its accuracy, data throughput, and cost (Shendure et al., 2017; Hackl S. T. et al., 2022). As of 2020, 82% of all bacterial genomes in the NCBI RefSeq database were sequenced on Illumina's technology (Hackl S. T. et al., 2022). Short-read sequencing was the most common method for assembling bacterial genomes until recently (Breckell and Silander, 2021). ONT platforms have become more popular by allowing researchers to sequence bacterial genomes faster and at a lower cost (Zhang et al., 2021). ONT's long-reads have downsides in low-accuracy base calling, but a complete genome can be obtained by combining this with highly accurate short-read sequencing or hybrid assembly (Zhao et al., 2023). The superiority of using the hybrid assembly compared to the short-read assembly is evident when comparing **Figures 2.20, 2.21, 2.22,** and **2.23**. These findings are almost identical to the findings by Zhang *et al* (2021) when assembling *Haemophilus parasuis* sequences (Zhang et al., 2021).

By using the hybrid assemblies for *Sal*. Newport parent and *Sal*. Newport Mut$^{\Phi}$, a comparison by whole genome alignment indicates that the mutation responsible for the phage-resistant phenotype was in *rfbM* gene. This gene encodes for mannose-1-phosphate guanylyltransferase and mannose-6-phosphate isomerase, which are used for LPS biosynthesis (Schnaitman and Klena, 1993; Shah et al., 2012; Moran, 2014). LPS can play an important role in phage adsorption and infection in gram-negative bacteria and modifications to the LPS structure can lead to phage-resistance (Capparelli et al., 2021). Mutations in the gene that codes for the mannose-1-phosphate guanylyltransferase have conferred phage-resistance in gram-negative

bacteria such as *Hafnia* species, *Dickeya solani*, and *Vibrio cholerae* (Bartnik et al., 2022; Beckman and Waters, 2023; Spencer-Drakes and Sarabia, 2023). Mutations in *rfbM* in *Sal*. Enteritidis has been shown to be attenuating in Caco-2 cell invasion (Shah et al., 2012). Mutations in *rfbM* have also been shown to reduce stress tolerance in *Sal*. Dublin as well as be attenuating in mice (Thomsen et al., 2003). Additionally, a mutation in a gene encoding mannose-1-phosphate guanylyltransferase conferred attenuation of *Brachyspira hampsonii* in mice (Perez et al., 2018). Taken together, this information, the attenuation of *Sal*. Newport Mut$^{\Phi}$ by itself in the calf model and the mutation in *rfbM,* could explain that the reduced virulence and phage-resistance may be due to a defect in LPS biosynthesis.

LPS is a chain of molecules and modification to a gene could have a cascading effect on the chain (Romano and Hung, 2023). Based on the EOP, this mutation conferred phage-resistance to five out of five of the phage cocktail. Even if the phage targeted different O-antigens, a mutation resulting in truncated chains could theoretically result in resistance to many phages that target O-antigens. This study's findings suggest that a mutation to the LPS biosynthesis gene *rfbM* has conferred phage-resistance for S11, S40, S41, S44, and S50. This would indicate that these five phage target LPS as their phage receptor. It is unknown how much or what part of the LPS is targeted by the phage. The *Sal*. Newport Mut$^{\Phi}$ was still able to agglutinate with the *Salmonella* O antiserum for Poly A – I and Vi, Poly Group B, and Group C_2 Factor 6/8. This would suggest that the *Sal*. Newport Mut$^{\Phi}$ still has the O-antigens that the antibodies in these serums can bind to, which means that the *Sal*. Newport Mut$^{\Phi}$ isolate is not a rough isolate. *Salmonella* can have many different O-antigens; this study is not able to predict which O-antigen(s) could be affected by the *rfbM* mutation.

This study indicates that building a phage cocktail containing phage with different molecules for their phage receptors would be important. A study by Yu *et al.* (2024) found that mutations in LPS biosynthesis genes was the most common mutation that confers phage-resistance in *Salmonella*. Yu *et al.* (2024) originally used Illumina Hiseq X Ten platform (150 bp paired-ends) to analyze potential genes associated with reduced susceptibility of *Salmonella* to a phage. Yu *et al.* (2024) then switched to sequencing with Illumina Hiseq X Ten and Nanopore MinION for hybrid assemblies to genomically characterize their 47 phage-resistant isolates. Yu *et al.* (2024) also concluded that it is important to build a cocktail consisting of phage targeting different receptors (Yu et al., 2024).

To build a good phage cocktail, generating phage-resistant mutants may be necessary and then identifying the genes conferring this phage-resistance. When performing the genomic analysis of these mutants, combining short-read and long-read sequencing for a hybrid assembly is critical. GATK and Snippy workflows did generate variants, but with assembled genomes, MUMmer4's whole genome alignment was able to rapidly identify differences in the genomes. Further studies into this subject would involve complementing the mutation with the insertion of another *rfbM* or using CRISPR/Cas9 to genetically edit the mutated *rfbM* gene to return it back to the wild-type genotype. The final step would be determining if this restores phage-sensitivity.

2.6. References

Abedon, S.T., 2014. Phage Therapy: Eco-Physiological Pharmacology. Scientifica (Cairo) 2014, 1–29. doi:10.1155/2014/581639

Arredondo-Alonso, S., Pöntinen, A.K., Cléon, F., Gladstone, R.A., Schürch, A.C., Johnsen, P.J., Samuelsen, Ø., Corander, J., 2021. A High-Throughput Multiplexing and Selection Strategy to Complete Bacterial Genomes. Gigascience 10, 1–13. doi:10.1093/gigascience/giab079

Bartnik, P., Lewtak, K., Fiołka, M., Czaplewska, P., Narajczyk, M., Czajkowski, R., 2022. Resistance of *Dickeya solani* Strain IPO 2222 to Lytic Bacteriophage ΦD5 Results in Fitness Tradeoffs for the Bacterium During Infection. Sci Rep 12, 1–23. doi:10.1038/s41598-022-14956-7

BD Diagnostics, 2009. Difco & BBL Manual: Manual of Microbiological Culture Media, Second. ed, BD Diagnostics - Diagnostic Systems. Becton, Dickinson and Company, Sparks, MD. doi:10.1002/1521-3773(20010316)40:6<9823::AID-ANIE9823>3.3.CO;2-C

Beckman, D.A., Waters, C.M., 2023. *Vibrio cholerae* Phage ICP3 Requires O1 Antigen for Infection. Infect Immun 91, 1–14. doi:10.1128/iai.00026-23

Bicalho, M.L.S., Machado, V.S., Nydam, D. V., Santos, T.M.A., Bicalho, R.C., 2012. Evaluation of Oral Administration of Bacteriophages to Neonatal Calves: Phage Survival and Impact on Fecal *Escherichia coli*. Livest Sci 144, 294–299. doi:10.1016/j.livsci.2011.12.007

Breckell, G.L., Silander, O.K., 2021. Do You Want to Build a Genome? Benchmarking Hybrid Bacterial Genome Assembly Methods. bioRxiv 11, 1–39. doi:https://doi.org/10.1101/2021.11.07.467652

Canning, M., Birhane, M.G., Dewey-Mattia, D., Lawinger, H., Cote, A., Gieraltowski, L., Schwensohn, C., Tagg, K.A., Francois Watkins, L.K., Park Robyn, M., Marshall, K.E., 2023. *Salmonella* Outbreaks Linked to Beef, United States, 2012–2019. J Food Prot 86, 100071. doi:10.1016/j.jfp.2023.100071

Capparelli, R., Cuomo, P., Papaianni, M., Pagano, C., Montone, A.M.I., Ricciardelli, A., Iannelli, D., 2021. Bacteriophage-Resistant *Salmonella* Rissen: An *In vitro* Mitigated Inflammatory Response. Viruses 13, 1–11. doi:10.3390/v13122468

Capparelli, R., Nocerino, N., Lannaccone, M., Ercolini, D., Parlato, M., Chiara, M., Lannelli, D., 2010. Bacteriophage Therapy of *Salmonella enterica*: A Fresh Appraisal of Bacteriophage Therapy. J Infect Dis 201, 52–61. doi:10.1086/648478

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 2011. The Variant Call Format and VCFtools. Bioinformatics 27, 2156–2158. doi:10.1093/bioinformatics/btr330

Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M., 2021. Twelve Years of SAMtools and BCFtools. Gigascience 10, 1–4. doi:10.1093/gigascience/giab008

De Maio, N., Shaw, L.P., Hubbard, A., George, S., Sanderson, N.D., Swann, J., Wick, R., Oun, M.A., Stubberfield, E., Hoosdally, S.J., Crook, D.W., Peto, T.E.A., Sheppard, A.E., Bailey, M.J., Read, D.S., Anjum, M.F., Sarah Walker, A., Stoesser, N., 2019. Comparison of Long-Read Sequencing Technologies in the Hybrid Assembly of Complex Bacterial Genomes. Microb Genom 5, 1–12. doi:10.1099/mgen.0.000294

Delahoy, M.J., Shah, H.J., Weller, D.L., Ray, L.C., Smith, K., McGuire, S., Trevejo, R.T., Scallan Walter, E., Wymore, K., Rissman, T., McMillian, M., Lathrop, S., LaClair, B., Boyle, M.M., Harris, S., Zablotsky-Kufel, J., Houck, K., Devine, C.J., Lau, C.E., Tauxe, R. V., Bruce, B.B., Griffin, P.M., Payne, D.C., 2023. Preliminary Incidence and Trends of Infections Caused by Pathogens Transmitted Commonly Through Food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2022. MMWR Morb Mortal Wkly Rep 72, 701–706. doi:10.15585/mmwr.mm7226a1

Depristo, M.A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J.R., Hartl, C., Philippakis, A.A., Del Angel, G., Rivas, M.A., Hanna, M., McKenna, A., Fennell, T.J., Kernytsky, A.M., Sivachenko, A.Y., Cibulskis, K., Gabriel, S.B., Altshuler, D., Daly, M.J., 2011. A Framework for Variation Discovery and Genotyping Using Next-Generation DNA Sequencing Data. Nat Genet 43, 491–501. doi:10.1038/ng.806

Endersen, L., O'Mahony, J., Hill, C., Ross, R.P., McAuliffe, O., Coffey, A., 2014. Phage Therapy in the Food Industry. Annu Rev Food Sci Technol 5, 327–349. doi:10.1146/annurev-food-030713-092415

Faruque, S.M., Islam, M.J., Ahmad, Q.S., Faruque, A.S.G., Sack, D.A., Nair, G.B., Mekalanos, J.J., 2005a. Self-Limiting Nature of Seasonal Cholera Epidemics: Role of Host-Mediated Amplification of Phage. Proc Natl Acad Sci U S A 102, 6119–6124. doi:10.1073/pnas.0502069102

Faruque, S.M., Naser, I. Bin, Islam, M.J., Faruque, A.S.G., Ghosh, A.N., Nair, G.B., Sack, D.A., Mekalanos, J.J., 2005b. Seasonal Epidemics of Cholera Inversely Correlate with the

Prevalence of Environmental Cholera Phages. Proc Natl Acad Sci U S A 102, 1702–1707. doi:10.1073/pnas.0408992102

FDA, 2015. Veterinary Feed Directive. Fed Regist 80, 31707–31735.

Ford, L., Ellison, Z., Schwensohn, C., Griffin, I., Birhane, M.G., Cote, A., Fortenberry, G.Z., Tecle, S., Higa, J., Spencer, S., Patton, B., Patel, J., Dow, J., Maroufi, A., Robbins, A., Donovan, D., Fitzgerald, C., Burrell, S., Tolar, B., Folster, J.P., Cooley, L.A., Francois Watkins, L.K., 2023. Strain of Multidrug-Resistant *Salmonella* Newport Remains Linked to Travel to Mexico and U.S. Beef Products — United States, 2021–2022. Morbidity and Mortality Weekly Report 72, 1225–1229.

Gupta, A., Fontana, J., Crowe, C., Bolstorff, B., Stout, A., Duyne, S. Van, Hoekstra, M.P., Whichard, J.M., Barrett, T.J., Angulo, F.J., 2003. Emergence of Multidrug-Resistant *Salmonella enterica* Serotype Newport Infections Resistant to Expanded-Spectrum Cephalosporins in the United States 21287, 1707–1716.

Hackl S. T., Harbig T. A., Nieselt K., 2022. Technical Report on Best Practices for Hybrid and Long Read *de novo* Assembly of Bacterial Genomes Utilizing Illumina and Oxford Nanopore Technologies Reads. bioRxiv 10. doi:https://doi.org/10.1101/2022.10.25.513682

Henry, M., Debarbieux, L., 2012. Tools from Viruses: Bacteriophage Successes and Beyond. Virology 434, 151–161. doi:10.1016/j.virol.2012.09.017

Hyman, P., Abedon, S.T., 2010. Bacteriophage Host Range and Bacterial Resistance., in: Advances in Applied Microbiology. Elsevier Inc., San Diego, CA, pp. 217–248. doi:10.1016/S0065-2164(10)70007-1

Kitchens, S.R., 2016. A Study of a Bacteriophage-Resistant *Salmonella* Mutant and Factors Influencing Prevalence of *Salmonella* in a Multi-Species Animal Facility (Master's Thesis). Auburn University.

Kropinski, A.M., 2009. Measurement of the Rate of Attachment of Bacteriophage to Cells, in: Clokie, M.R.J., Kropinski, A.M. (Eds.), Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions. Humana Press, a part of Springer Science+Business Media, New York, NY, pp. 151–156.

Laufer, A.S., Grass, J., Holt, K., Whichard, J.M., Griffin, P.M., Gould, L.H., Service, Intelligence, Development, P., Branch, D.E., Service, Inspection, 2015. Outbreaks of *Salmonella* Infections Attributed to Beef – United States, 1973–2011. Epidemiol Infect 143, 2003–2013. doi:10.1017/S0950268814003112.Outbreaks

León, M., Bastías, R., 2015. Virulence Reduction in Bacteriophage Resistant Bacteria. Front Microbiol 6, 1–7. doi:10.3389/fmicb.2015.00343

Lerminiaux, N., Fakharuddin, K., Mulvey, M.R., Mataseje, L., 2022. Do We Still Need Illumina Sequencing Data?: Evaluating Oxford Nanopore Technologies 2 R10.4.1 Flow Cells and

v14 Library Prep Kits for Gram Negative Bacteria Whole Genome Assemblies. Can J Microbiol 1–23.

Li, H., 2013. Aligning Sequence Reads, Clone Sequences and Assembly Contigs with BWA-MEM 00, 1–3.

Li, H., Durbin, R., 2009. Fast and Accurate Short Read Alignment with Burrows-Wheeler Transform. Bioinformatics 25, 1754–1760. doi:10.1093/bioinformatics/btp324

Luong, T., Salabarria, A.C., Roach, D.R., 2020. Phage Therapy in the Resistance Era: Where Do We Stand and Where Are We Going? Clin Ther 42, 1659–1680. doi:10.1016/j.clinthera.2020.07.014

Mahony, J., McAuliffe, O., Ross, R.P., van Sinderen, D., 2011. Bacteriophages as biocontrol agents of food pathogens. Curr Opin Biotechnol 22, 157–163. doi:10.1016/j.copbio.2010.10.008

Marçais, G., Delcher, A.L., Phillippy, A.M., Coston, R., Salzberg, S.L., Zimin, A., 2018. MUMmer4: A Fast and Versatile Genome Alignment System. PLoS Comput Biol 14, 1–14. doi:10.1371/journal.pcbi.1005944

Marshall, K.E.H., Tewell, M., Tecle, S., Leeper, M., Sinatra, J., Kissler, B., Fung, A., Brown, K., Wagner, D., Trees, E., Hise, K.B., Chaturvedi, V., Schlater, L.K., Morningstar-Shaw, B.R., Whitlock, L., Holt, K., Becker, K., Nichols, M., Williams, I.T., Jhung, M., Wise, M.E., Gieraltowski, L., 2018. Protracted Possible Outbreak of *Salmonella* Newport Infections Linked to Ground Beef: Possible Role of Dairy Cows — 21 States, 2016–2017. MMWR Morb Mortal Wkly Rep 67, 443–446. doi:10.15585/mmwr.mm6715a2

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., DePristo, M.A., 2010. The Genome Analysis Toolkit: A MapReduce Framework for Analyzing Next-Generation DNA Sequencing Data. Genome Res 20, 1297–1303. doi:10.1101/gr.107524.110

Meaden, S., Koskella, B., 2013. Exploring the Risks of Phage Application in the Environment. Front Microbiol 4, 1–8. doi:10.3389/fmicb.2013.00358

Moran, A.P., 2014. Molecular Structure, Biosynthesis, and Pathogenic Roles of Lipopolysaccharides. Helicobacter pylori 81–95. doi:10.1128/9781555818005.ch8

Obeng, N., Pratama, A.A., Elsas, J.D. van, 2016. The Significance of Mutualistic Phages for Bacterial Ecology and Evolution. Trends Microbiol 24, 440–449. doi:10.1016/j.tim.2015.12.009

Perez, J.B.D.S., Fernando, C., Nosach, R. V., Huang, Y., Harding, J.C.S., Hill, J.E., 2018. *In vitro* Attenuation of a Virulent Swine Isolate of *Brachyspira hampsonii*. Pathog Dis 76, 1–10. doi:10.1093/femspd/ftx116

Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A., Korobeynikov, A., 2020. Using SPAdes *de novo* Assembler. Curr Protoc Bioinformatics 70, e102. doi:10.1002/cpbi.102

Rivera-Chávez, F., Bäumler, A.J., 2015. The Pyromaniac Inside You: *Salmonella* Metabolism in the Host Gut. Annu Rev Microbiol 69, 31–48. doi:10.1146/annurev-micro-091014-104108

Robinson, J.T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., Mesirov, J.P., 2011. Integrative Genomics Viewer. Nat Biotechnol 29, 24–26. doi:10.1038/nbt.1754

Rogers, A.W.L., Tsolis, R.M., Bäumler, A.J., 2021. *Salmonella* versus the Microbiome . Microbiology and Molecular Biology Reviews 85. doi:10.1128/mmbr.00027-19

Romano, K.P., Hung, D.T., 2023. Targeting LPS Biosynthesis and Transport in Gram-Negative Bacteria in the Era of Multi-Drug Resistance. Biochim Biophys Acta Mol Cell Res 1870, 119407. doi:10.1016/j.bbamcr.2022.119407

Schnaitman, C.A., Klena, J.D., 1993. Genetics of Lipopolysaccharide Biosynthesis in Enteric Bacteria. Microbiol Rev 57, 655–682. doi:10.1128/mmbr.57.3.655-682.1993

Seemann, T., 2020. Snippy. Github Repository.

Sereika, M., Kirkegaard, R.H., Karst, S.M., Michaelsen, T.Y., Sørensen, E.A., Wollenberg, R.D., Albertsen, M., 2022. Oxford Nanopore R10.4 Long-Read Sequencing Enables the Generation of Near-Finished Bacterial Genomes From Pure Cultures and Metagenomes Without Short-Read or Reference Polishing. Nat Methods 19, 823–826. doi:10.1038/s41592-022-01539-7

Shah, D.H., Zhou, X., Kim, H.Y., Call, D.R., Guard, J., 2012. Transposon Mutagenesis of *Salmonella enterica* Serovar Enteritidis Identifies Genes that Contribute to Invasiveness in Human and Chicken Cells and Survival in Egg Albumen. Infect Immun 80, 4203–4215. doi:10.1128/IAI.00790-12

Shendure, J., Balasubramanian, S., Church, G.M., Gilbert, W., Rogers, J., Schloss, J.A., Waterston, R.H., 2017. DNA Sequencing at 40: Past, Present, and Future. Nature 550, 345–353. doi:10.1038/nature24286

Spencer-Drakes, T.C.J., Sarabia, A., 2023. O-Antigen Biosynthesis Mediates Evolutionary Trade-Offs Within a Simple Community. bioRxiv 4, 88–100.

Sulakvelidze, A., 2013. Using Lytic Bacteriophages to Eliminate or Significantly Reduce Contamination of Food by Foodborne Bacterial Pathogens. J Sci Food Agric 93, 3137–3146. doi:10.1002/jsfa.6222

Thiennimitr, P., Winter, S.E., Bäumler, A.J., Ba, A.J., 2012. *Salmonella*, The Host and Its Microbiota. Curr Opin Microbiol 15, 108–114. doi:10.1016/j.mib.2011.10.002

Thomsen, L.E., Chadfield, M.S., Bispham, J., Wallis, T.S., Olsen, J.E., Ingmer, H., 2003. Reduced Amounts of LPS Affect Both Stress Tolerance and Virulence of *Salmonella enterica*

Serovar Dublin. FEMS Microbiol Lett 228, 225–231. doi:10.1016/S0378-1097(03)00762-6

Varma, J.K., Marcus, R., Stenzel, S.A., Hanna, S.S., Gettner, S., Anderson, B.J., Hayes, T., Shiferaw, B., Crume, T.L., Joyce, K., Fullerton, K.E., Voetsch, A.C., Angulo, F.J., 2006. Highly Resistant *Salmonella* Newport-MDRAmpC Transmitted through the Domestic US Food Supply : A FoodNet Case-Control Study of Sporadic *Salmonella* Newport Infections , 2002 – 2003 30333, 2002–2003.

Wall, S.K., Zhang, J., Rostagno, M.H., Ebner, P.D., 2010. Phage Therapy To Reduce Preprocessing *Salmonella* Infections in Market-Weight Swine. Appl Environ Microbiol 76, 48–53. doi:10.1128/AEM.00785-09

Wifling, K., Dimroth, P., 1989. Isolation and Characterization of Oxaloacetate Decarboxylase of *Salmonella* Typhimurium, a Sodium Ion Pump. Arch Microbiol 152, 584–588. doi:10.1007/BF00425491

Winter, S.E., Thiennimitr, P., Winter, M.G., Butler, B.P., Huseby, D.L., Crawford, R.W., Tsolis, M., Roth, J.R., Ba, A.J., Russell, J.M., Bevins, C.L., Adams, L.G., Tsolis, R.M., Roth, J.R., Bäumler, A.J., 2010. Gut Inflammation Provides a Respiratory Electron Acceptor for *Salmonella*. Nature 467, 426–429. doi:10.1038/nature09415

Wottlin, L.R., Edrington, T.S., Brown, T.R., Zook, C.A., Sulakvelidze, A., Droleskey, R., Genovese, K.J., Nisbet, D.J., 2022. Evaluation of a Preharvest Bacteriophage Therapy for Control of *Salmonella* within Bovine Peripheral Lymph Nodes. J Food Prot 85, 254–260. doi:10.4315/JFP-21-292

Yu, J., Zhang, H., Ju, Z., Huang, J., Lin, C., Wu, J., Wu, Y., Sun, S., Wang, H., Hao, G., Zhang, A., 2024. Increased Mutations in Lipopolysaccharide Biosynthetic Genes Cause Time-Dependent Development of Phage Resistance in *Salmonella*. Antimicrob Agents Chemother 68, 1–17. doi:10.1128/aac.00594-23

Zhang, P., Jiang, D., Wang, Y., Yao, X., Luo, Y., Yang, Z., 2021. Comparison of *De Novo* Assembly Strategies for Bacterial Genomes. Int J Mol Sci 22, 7668. doi:10.3390/ijms22147668

Zhao, W., Zeng, W., Pang, B., Luo, M., Peng, Y., Xu, J., Kan, B., Li, Z., Lu, X., 2023. Oxford Nanopore Long-Read Sequencing Enables the Generation of Complete Bacterial and Plasmid Genomes Without Short-Read Sequencing. Front Microbiol 14, 1–9. doi:10.3389/fmicb.2023.1179966

Chapter 3

Chicken Embryo Lethality Assay with *Salmonella*

3.1. Abstract

Salmonella is a zoonotic enteropathogen that causes significant disease in humans and livestock animals. To combat this problem, viruses of bacteria (bacteriophages) are being viewed as a natural and novel way of reducing foodborne pathogens. It is important to select the best phage when designing a mixture or cocktail bacteriophage. Many *in vitro* methods have been used to select optimum phage. A methodology published by Trotereau and Schouler (2019) examined the use of an avian embryo lethality assay to assess the efficacy of phage against a pathogen (Trotereau and Schouler, 2019). This study examines phage in an avian embryo lethality assay to reduce *Salmonella*. This study also examines the avian embryo lethality assay and virulence in *Salmonella* variants.

3.2. Introduction

Salmonella is a major foodborne pathogen of concern to human health. This pathogen is well known as a poultry-associated species, but also infects other livestock such as beef and dairy cattle as well as swine (Edrington and Brown, 2022). Strict biosafety and hygiene protocols are implemented worldwide in the farming industry, but infectious diseases remain a major challenge. Antimicrobial agents are tools that are necessary to control infectious diseases in animal industries (Boerlin, 2010). In 2015, the Food and Drug Administration (FDA) published in the Federal Register the Veterinary Feed Directive that mandated "medically important to human" antimicrobials could no longer be used to improve animal performance (FDA, 2015). Alternatives to antibiotics to reduce foodborne pathogens to improve animal health and food safety such as vaccination, targeting specific metabolic pathways, probiotics, prebiotics, dietary changes, and management factors One additional alternative to antibiotics is gaining popularity, which is the therapeutic use of bacteriophages (phages)(Klopatek et al., 2021).

Phages are bacterial viruses or viruses that kill bacteria. Because phage kills bacteria, there is an interest in the application of phage to target and kill bacterial pathogens in all stages of the food production chain. These applications include using phage at the farm level for pre-harvest interventions, decontaminating surfaces in food-processing facilities, or post-harvest interventions by direct applications of phage onto harvested foods (Sulakvelidze, 2013). Phage formulations

have already been approved as "Generally Recognized as Safe" (GRAS) by the FDA in foods (Połaska and Sokołowska, 2019; Dhowlaghar and Denes, 2023). Phage can be viewed as a "natural" antimicrobial that has been shown to be effective in the control of *Salmonella* (Wottlin et al., 2022).

It is important when developing phage as a therapeutic or foodborne pathogen intervention, that the right phage has been selected. This includes selecting phages that are highly lytic and that don't have undesirable features such as carrying antibiotic-resistant genes or other unwanted genes (Luong et al., 2020). Selection methods exist for developing phage therapy cocktails (combinations of phage to increase host range and reduce phage-resistant strains), but these methods are *in vitro* methodologies (Haines et al., 2021). Phage effects against pathogens such as *Salmonella* have shown promising results, but more studies need to be conducted to give further insights into their therapeutic effects (Mehmood Khan et al., 2023). Optimizing a phage cocktail with relevant animal models is not always feasible due to the high financial costs and ethical constraints (Thulin et al., 2014; Mukherjee et al., 2022). A methodology proposed by Trotereau and Schouler (2019) utilizes an avian (chicken) embryo (egg) lethality assay to assess the efficacy of phage in an *in vivo* model. Avian embryos are not regulated as animal experiments, have been used widely to study the virulence of pathogens, and are straightforward *in vivo* model to implement (Trotereau and Schouler, 2019). At this time, only one study has been published in a peer-reviewed journal that uses this model to evaluate the efficacy of phage (Nicolas et al., 2023b). The aim of this study is to assess the chicken embryo lethality model as a method to evaluate a phage against *Salmonella*.

3.3. Materials and Methods

3.3.1. *Salmonella* Strains

Bacterial strains included *Sal.* Enteritidis, *Sal*. Infantis, *Sal.* Newport 1351, *Sal.* Newport 3373, *Sal.* Newport 3596, a phage-resistant mutant of *Sal.* Newport 3596 S50.1.3.1 (*S.* Newport Mut$^{\Phi}$), a rough isolate *Sal*. O-:r:1,5, *Sal.* Typhimurium, and the monophasic variant of *Sal.* Typhimurium (*Sal*. 1,4,[5],12:i:-). These isolates were received from the Auburn University College of Veterinary Medicine Diagnostic Bacteriology Laboratory or the Auburn University College of Agriculture Department of Poultry Science. Hyland *et al*. (n.d.) characterized five bacteriophage isolates (S11, S40, S41, S44, S50), originally isolated from *Salmonella*-containing diagnostic cultures (Hyland et al., n.d.). These five phages show lytic activity against *S.* Newport

in vitro and have shown therapeutic effects *in vivo* in an *S.* Newport infection calf model (Hyland et al., n.d.). Two other bacteriophage isolates (S7 and S10), originally isolated from *Salmonella*-containing diagnostic cultures that had been characterized by Shirley (2016), were used in this study (Shirley, 2016). These two phages show lytic activity against *S.* Enteritidis *in vitro* (Shirley, 2016).

3.3.2. Bacteriophage Amplification

To amplify phage to produce high titer stocks, 50mL of log phase *S.* Newport cells growing in Luria-Bertani broth (Difco LB Broth, Miller) containing 1mM magnesium (LBM, Difco) was inoculated with 0.5mL of the purified bacteriophage solution. The lysate was incubated overnight at 37ºC, and then was pelleted at 12,500 xG for 15 minutes. The resulting supernatant was filter sterilized through a 0.2µm filter. To enumerate the phage in each supernatant, a double agar overlay method was used for titration. *S.* Newport cells were cultured to log phase, then diluted to an OD_{620} of 0.8 to 1.0. Serial dilution of each bacteriophage solution was performed, and 0.2mL of the *S.* Newport cells was mixed with 10µL of the bacteriophage solution. The cells were incubated with the bacteriophage for ten minutes before adding 3mL (LBM with 0.7% Bacto agar and 1mM tetrazolium dye) overlay or top agar and pouring the mixture onto an underlay or bottom agar that is Luria-Bertani broth (Difco LB Broth, Miller) containing 1mM magnesium and 1.5% agar (LBM agar). The bacteriophage plaques were enumerated to obtain the plaque forming units per mL (PFU/mL) (Kropinski et al., 2009).

3.3.3. Chicken Egg Embryo Lethality Assay

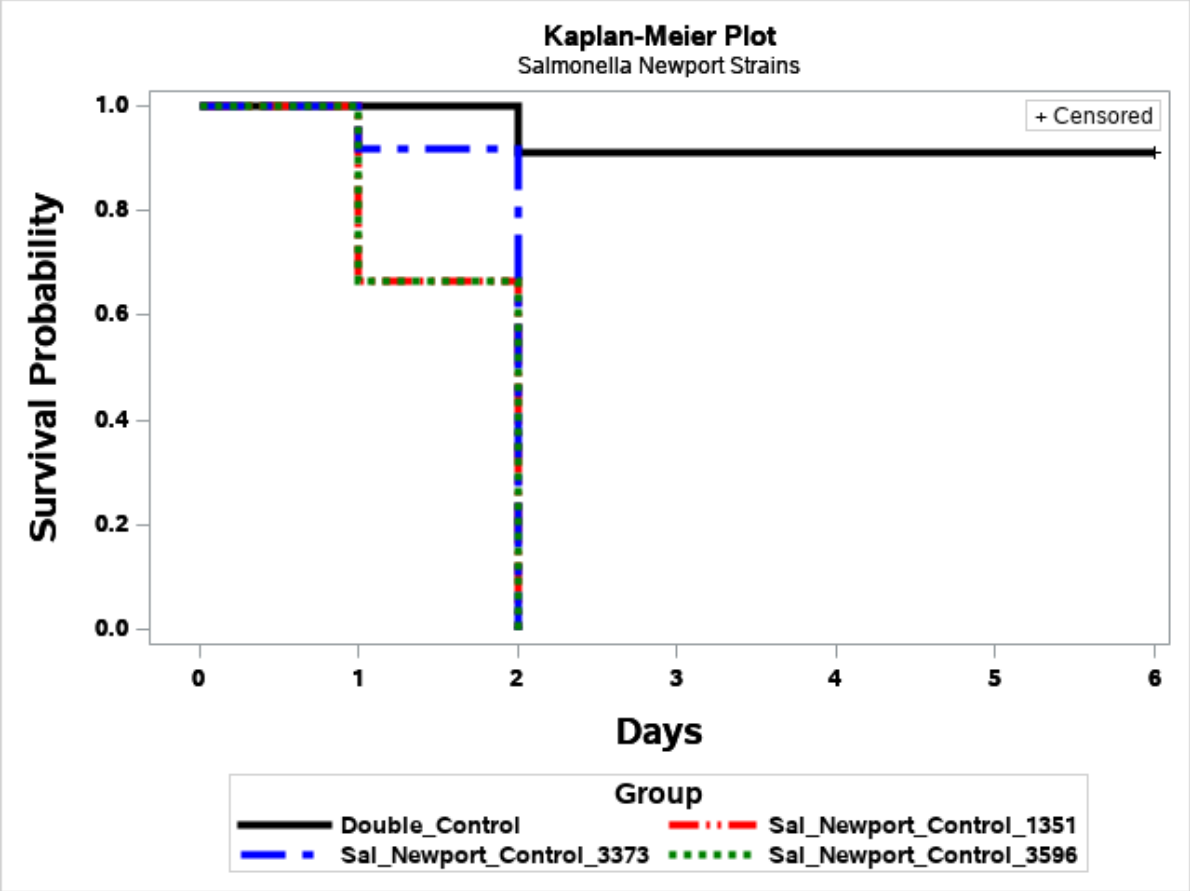
Specific-pathogen free (SPF) eggs (Wayward Acres, Inc and AVS Bio) were incubated until day 11 of development. SPF eggs were inoculated with 10^2 CFU/egg or 10^3 CFU/egg of *Salmonella* in allantoic fluid with a syringe and needle. Two hours after *Salmonella* inoculation, 100 µL of bacteriophage inoculum. The hole in the egg was sealed with wax. Double control groups received Dulbecco's phosphate buffered saline instead of *Salmonella* with inoculation with SM buffer in place of phage treatment. Control groups would be inoculated with *Salmonella* and receive SM buffer in place of phage treatment for assays examining phage treatment. Phage-treated groups are described by multiplicity of infection (MOI), which is an expression of the ratio of plaque-forming units of phage to colony-form units of *Salmonella* at inoculation. An MOI=10^3 would represent a ratio of 10^3 phage to every 1 bacterial cell. Eggs were candled daily to monitor embryo mortality up to day 16 of development. SAS Studio v.3.81 (SAS Institute Inc., Cary, NC, USA), Kaplan–Meier curves and log-rank test were used to analyze data.

3.4. Results

3.4.1. Chicken Embryo Lethality Assay: *Sal.* Newport Control Experiment

SPF eggs were incubated until day 11 of development. SPF eggs were divided into four groups (≈12 eggs per group) and were inoculated with buffer for control group or 10^2 CFU/egg of one of the *Sal.* Newport strains 1351, 3373, or 3596. All three experimental groups had 100% mortality by day 2 (**Figure 3.1**). All three groups were significantly different from the control group ($p \leq 0.001$).

Figure 3.1. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Salmonella* Newport strains 1351, 3373, or 3596 (12 eggs per group).

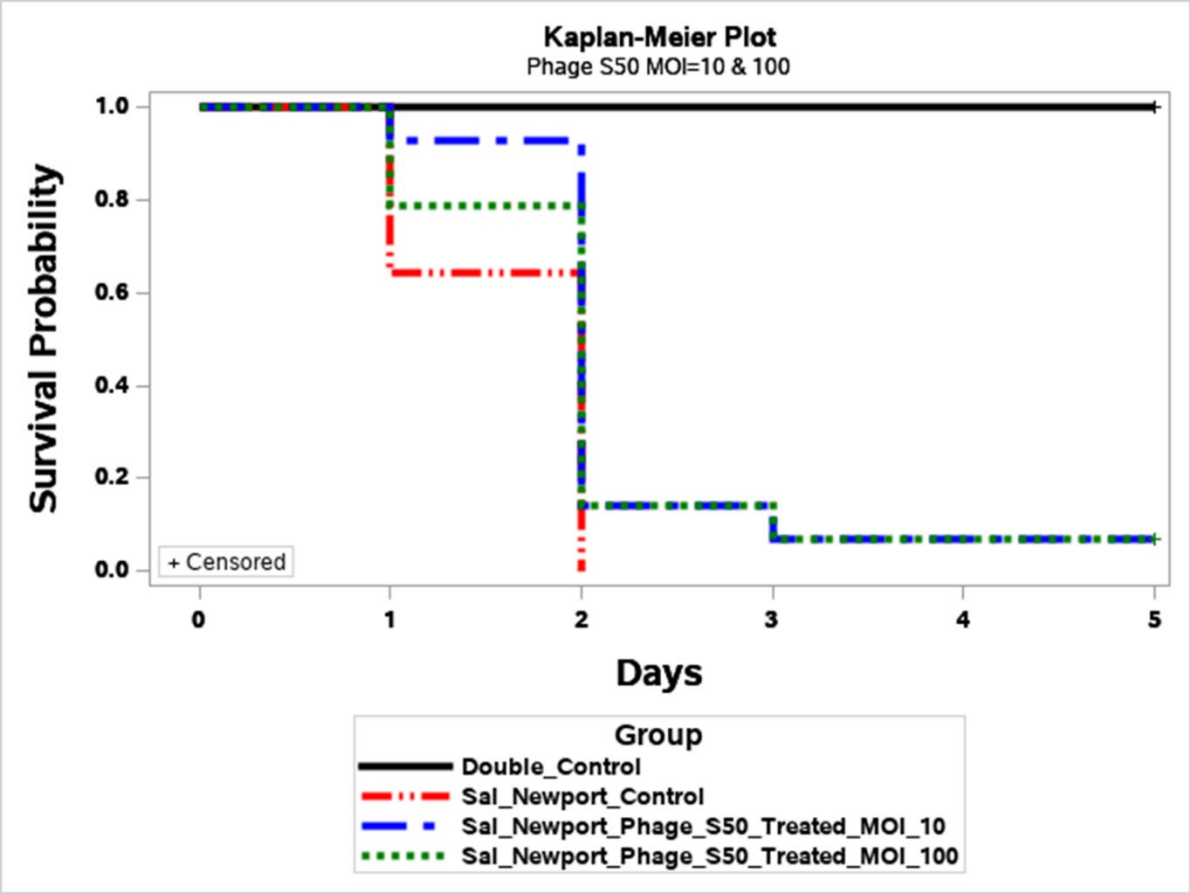


3.4.2. Chicken Embryo Lethality Assay: *Sal.* Newport treated with MOI≈10 or MOI≈100 of bacteriophage S50

SPF eggs were incubated until day 11 of development. SPF eggs were divided into four groups (≈14 eggs per group), with *Salmonella*-challenged groups receiving 10^2 CFU/egg of the *Salmonella* Newport strain 3596. The groups included a double control group, *Sal.* Newport control group, and two experimental phage treated groups: S50 at MOI≈10 or S50 at MOI≈100.

Sal. Newport control group had 100% mortality by day 2 (**Figure 3.2)**. S50 (MOI≈10) and S50 (MOI≈100) had the most mortality by day 2 and additional mortality by day 3, with the remaining eggs surviving for the remainder of the experiment. The control group, S50 (MOI≈10), and S50 (MOI≈100) groups were significantly different from the double control group ($p \leq 0.001$). S50 (MOI≈10), and S50 (MOI≈100) groups were not significantly different from the *Sal*. Newport control group with $p=0.2253$ and $p=0.4087$ respectively.

Figure 3.2. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Salmonella* Newport 3596 and treated with S50 (MOI≈10) and S50 (MOI≈100) (14 eggs per group).

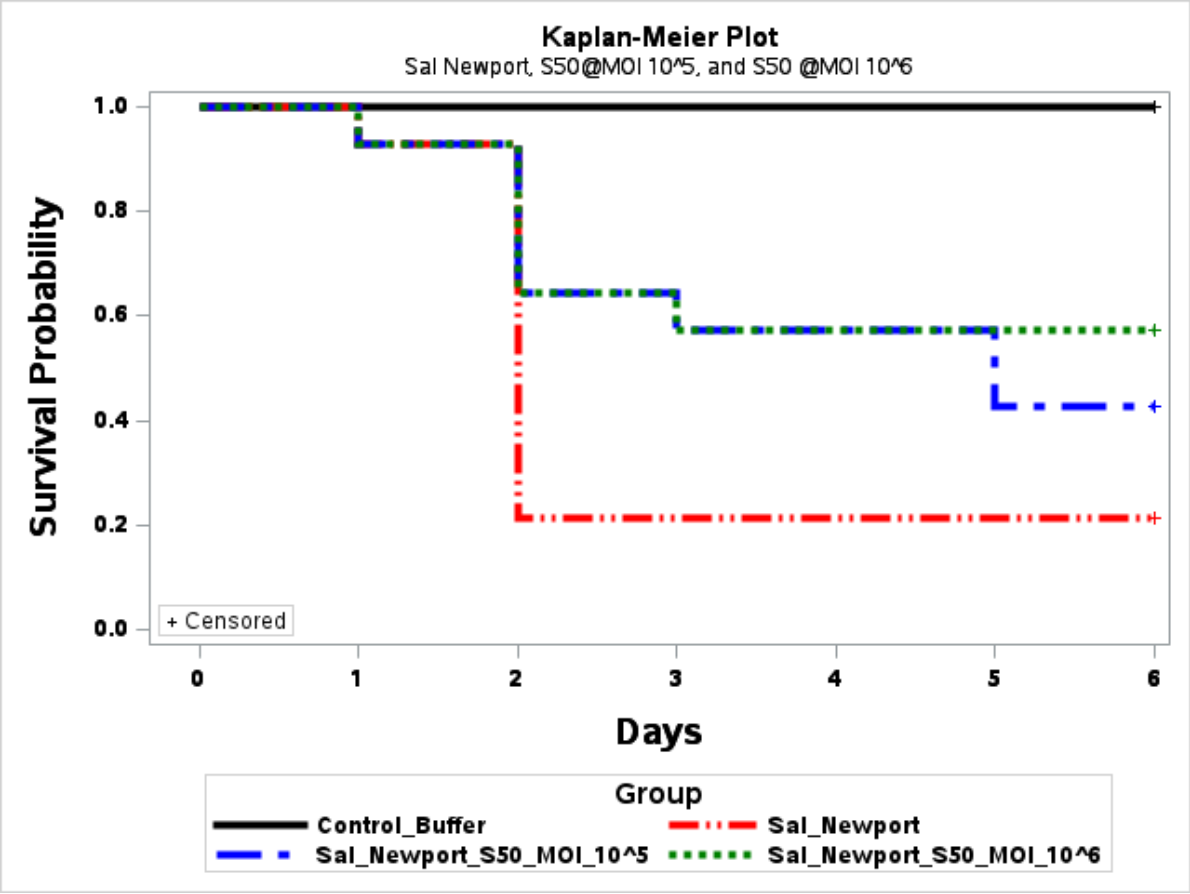


3.4.3. Chicken Embryo Lethality Assay: *Sal*. Newport treated with MOI≈10^5 or MOI≈10^6 of bacteriophage S50

SPF eggs were incubated until day 11 of development. SPF eggs were divided into four groups (≈14 eggs per group), with *Salmonella*-challenged groups receiving 10^2 CFU/egg of the *Sal.* Newport strain 3596. Groups were a double control group, *Sal.* Newport control group, and two experimental phage treated groups: S50 at MOI≈10^5 or S50 at MOI≈10^6. *Sal.* Newport control group had most of the mortality by day 2 (**Figure 3.3)**. S50 (MOI≈10^5) and S50 (MOI≈10^6) had

mortality by day 2 and additional mortality by staggering downward through the duration of the experiment, with S50 (MOI≈10^6) having slightly better survival at the end of the experiment. The double control group was significantly different from the control group ($p \leq 0.001$), S50 (MOI≈10^5) ($p=0.0044$), and S50 (MOI≈10^6) ($p=0.0151$). S50 (MOI≈10^5) was not significantly different from *Sal.* Newport ($p=0.1125$), but S50 (MOI≈10^6) was significantly different from the *Sal*. Newport control group with $p=0.0413$.

Figure 3.3. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Salmonella* Newport 3596 and treated with S50 (MOI≈10) and S50 (MOI≈100) (14 eggs per group).

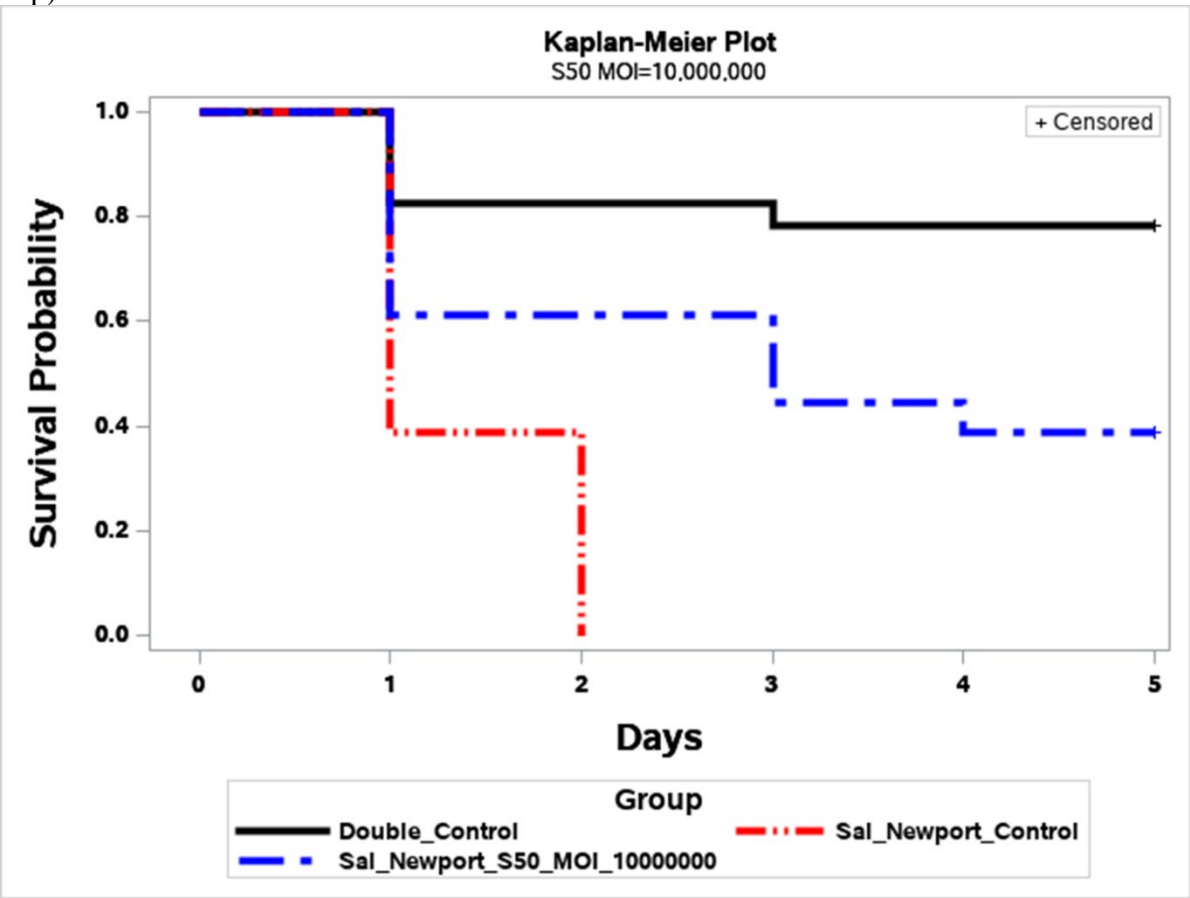


3.4.4. Chicken Embryo Lethality Assay: *Sal*. Newport treated with MOI≈10^7

SPF eggs were incubated until day 11 of development. SPF eggs were divided into three groups (≈18 eggs per group), with *Salmonella*-challenged groups receiving 10^2 CFU/egg of the *Sal.* Newport strain 3596. The groups were a double control group, a *Sal.* Newport control group, and one experimental treated group of S50 at MOI≈10^7. *Sal.* Newport control group had 100% mortality by day 2 (**Figure 3.4**). S50 (MOI≈10^7) had mortality by day 1, day 3, and day 4, with

the remaining eggs surviving till the end of the experiment. The double control group was significantly different from the control group ($p \leq 0.001$) and treated S50 (MOI≈10^7) group ($p=0.0113$). S50 (MOI≈10^7) was significantly different from *Sal.* Newport ($p=0.0067$).

Figure 3.4. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Salmonella* Newport 3596 and treated with S50 at MOI≈10^7 (18 eggs per group).

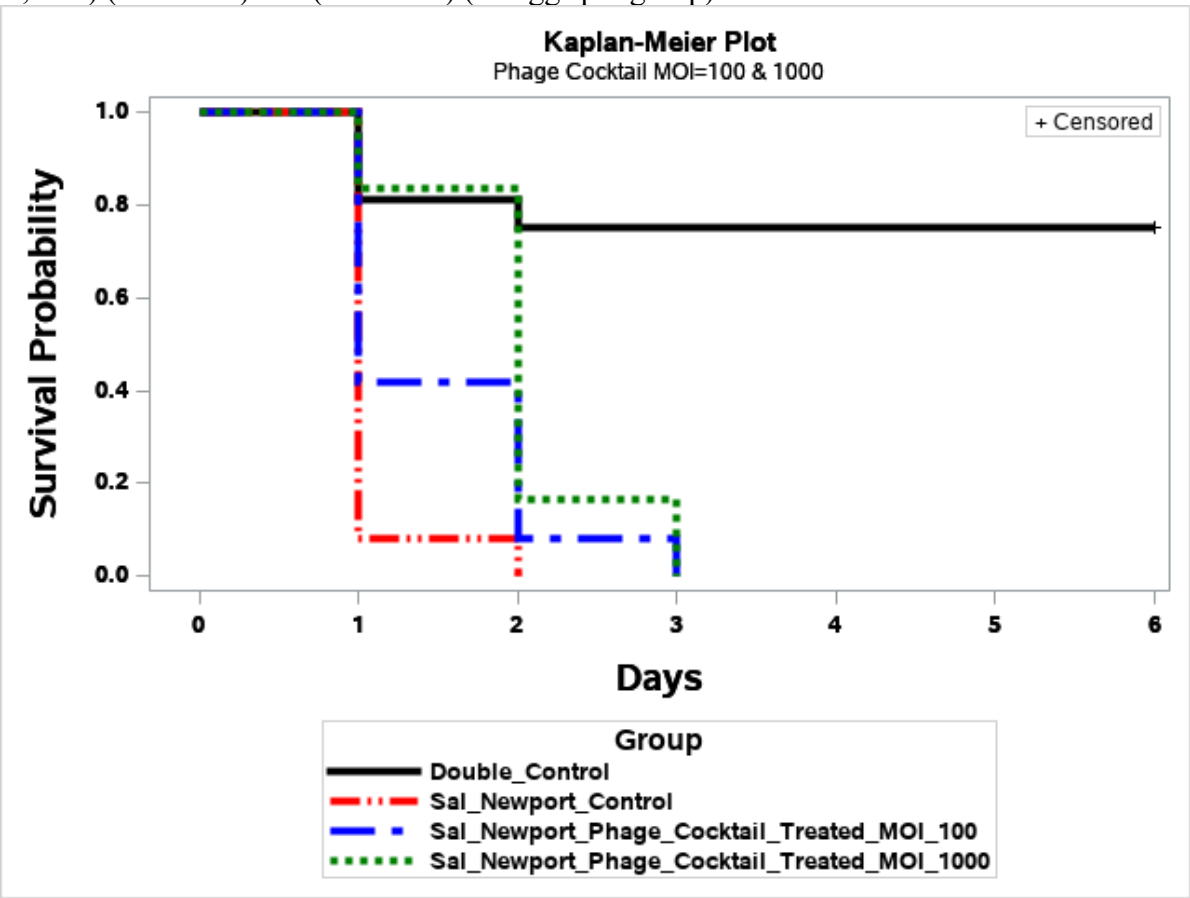


3.4.5. Chicken Embryo Lethality Assay: *Sal.* Newport treated with MOI≈10^2 or MOI≈10^3 of a cocktail of phage (S11, S40, S41, S44, S50).

SPF eggs were incubated until day 11 of development. SPF eggs were divided into four groups (≈12 eggs per group), with *Salmonella*-challenged groups receiving 10^2 CFU/egg of one of the *Salmonella* Newport strain 3596. The groups were a double control group, a *Salmonella* Newport control group, and two experimental treated groups. Both experimental groups consisted of a cocktail of bacteriophages (S11, S40, S41, S44, S50), with one group having an MOI≈10^2 and the second group having an MOI≈10^3. *Salmonella* Newport control group had 100% mortality by day 2 (**Figure 3.5)**. Phage cocktail (MOI≈10^2) and (MOI≈10^3) had 100% mortality by day 3. The control group, phage cocktail (MOI≈10^2), and phage cocktail (MOI≈10^3) groups were significantly

different from the double control group (respectively, $p \leq 0.001$, $p \leq 0.001$, $p = 0.0004$). Phage cocktail (MOI≈10^2) and (MOI≈10^3) groups were not significantly different from the *Sal*. Newport control group with $p = 0.4397$ and $p = 0.1027$ respectively.

Figure 3.5. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Salmonella* Newport 3596 and treated with phage cocktail (S11, S40, S41, S44, S50) (MOI≈10^2) and (MOI≈10^3) (12 eggs per group).

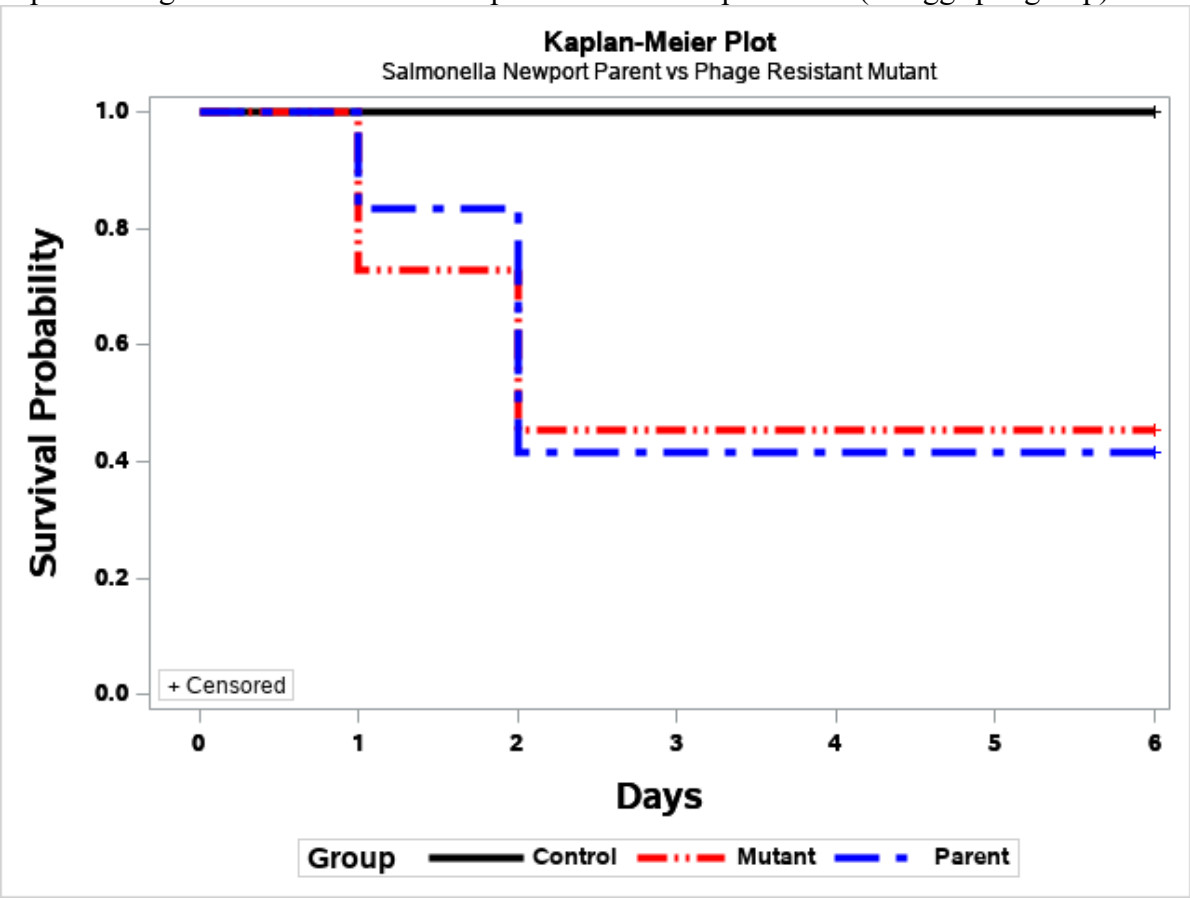


3.4.6. Chicken Embryo Lethality Assay: *Sal*. Newport 3596 and *Sal.* Newport 3596 Mut$^{\Phi}$.

SPF eggs were incubated until day 11 of development. SPF eggs were divided into three groups (≈12 eggs per group), with a negative control group ("control") receiving Dulbecco's phosphate buffered saline, a positive control group ("parent") challenged with 10^2 CFU/egg of *Salmonella* Newport strain 3596 and the experimental group ("mutant") being challenged with 10^2 CFU/egg of a phage-resistant *Salmonella* Newport strain 3596 (*Sal.* Newport Mut$^{\Phi}$). Both *Salmonella*-challenged groups had all mortality observed by day 2 (**Figure 3.6**), with the remaining eggs surviving the remaining duration of the experiment. The positive control parent *Sal.* Newport and experimental *Sal.* Newport Mut$^{\Phi}$ groups were significantly different from the

negative control group with p=0.0058 and p=0.0058 respectively. The positive control parent *Sal.* Newport and experimental *Sal.* Newport Mut$^{\Phi}$ groups were not significantly different from one another with p=0.8990.

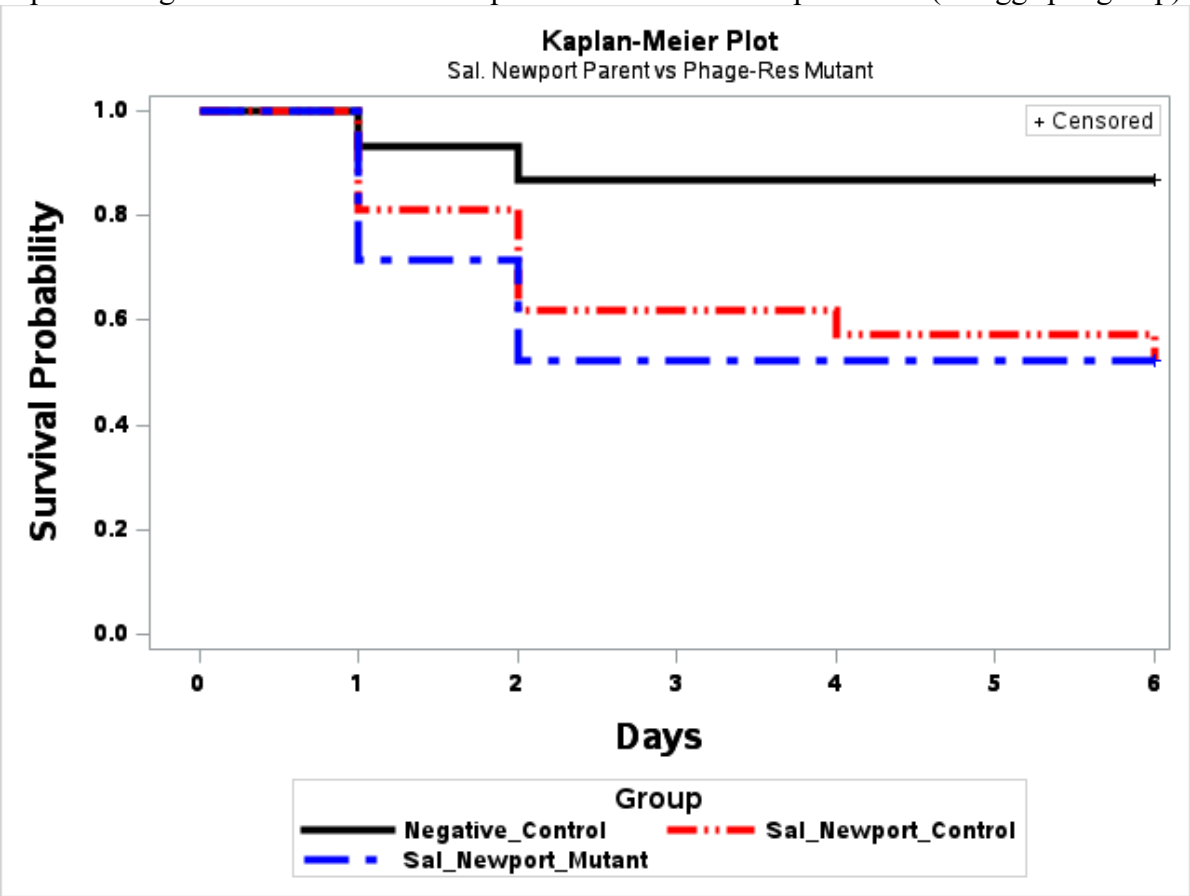
Figure 3.6. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Salmonella* Newport and *Sal.* Newport Mut$^{\Phi}$ (12 eggs per group).



SPF eggs were incubated until day 11 of development. SPF eggs were divided into three groups (≈21 eggs per group), with a negative control group ("Negative_Control") receiving Dulbecco's phosphate buffered saline, a positive control group ("*Sal*_Newport_Control") challenged with 10^2 CFU/egg of *Salmonella* Newport strain 3596 and the experimental group ("*Sal*_Newport_Mutant") being challenged with 10^2 CFU/egg of a *Sal.* Newport Mut$^{\Phi}$. Both *Salmonella*-challenged groups had most mortality observed by day 2 (**Figure 3.7**), the *Sal*_Newport_Control group had an additional mortality by day 4, and both *Salmonella*-challenged groups had the remaining eggs surviving the remaining experiment duration. The positive control *Sal*_Newport_Control was not significantly different from the Negative_Control group with a p=0.0692. The experimental *Sal*_Newport_Mutant was significantly different from the negative

control group with p=0.0471. The positive control *Sal*_Newport_Control and experimental *Sal*_Newport_Mutant groups were not significantly different, with p=0.8959.

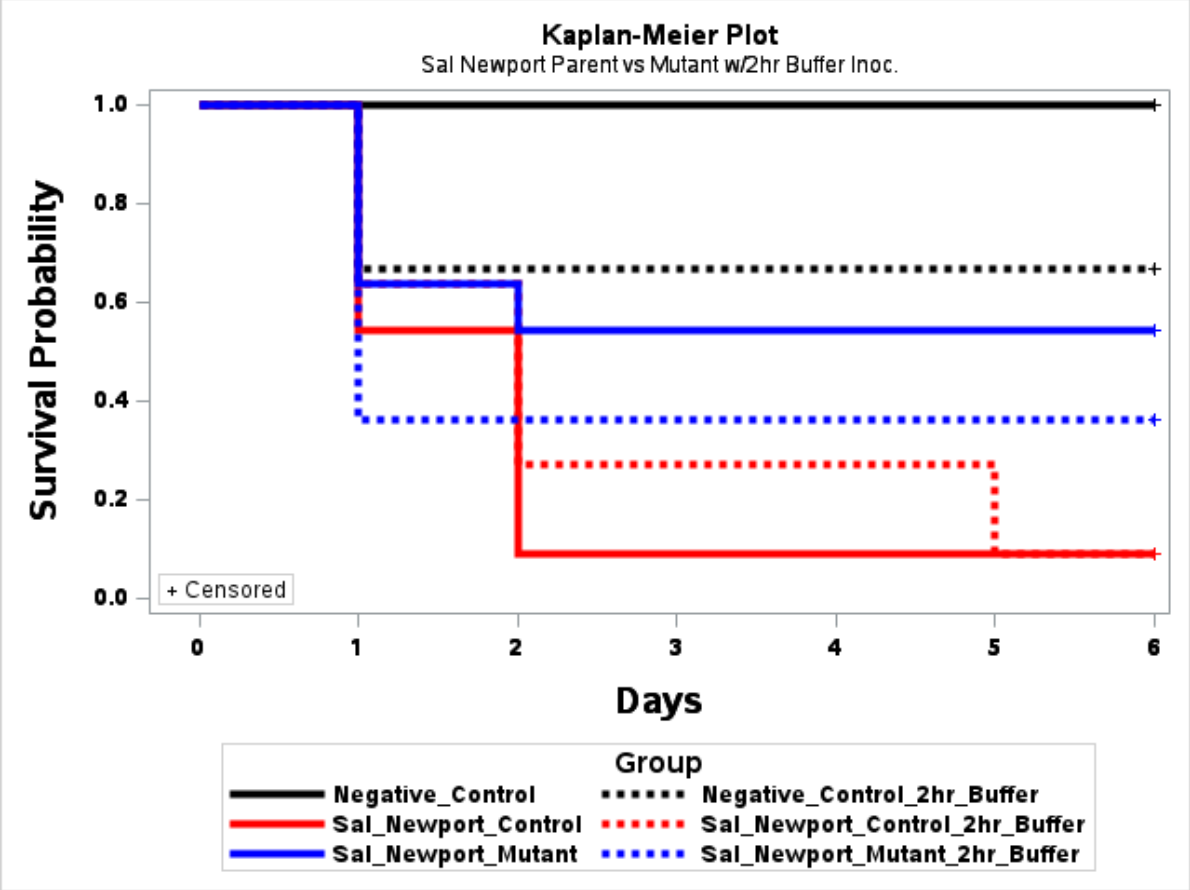
Figure 3.7. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Salmonella* Newport 3596 and *Sal.* Newport Mut$^{\Phi}$ (21 eggs per group).



SPF eggs were incubated until day 11 of development. SPF eggs were divided into six groups (≈11 eggs per group), with a negative control group ("Negative_Control") receiving Dulbecco's phosphate buffered saline, a positive control group ("*Sal*_Newport_Control") challenged with 10^2 CFU/egg of *Sal.* Newport strain 3596 and the experimental group ("*Sal*_Newport_Mutant") being challenged with 10^2 CFU/egg of a *Sal.* Newport Mut$^{\Phi}$. Each of these three groups had a duplicate group that received a SM Buffer inoculation two hours after inoculation with *Salmonella* ("_2hr_Buffer"). The Negative_Control group had no mortality, but the Negative_Control_2hr_Buffer had mortality by day 1 (**Figure 3.8**). The *Sal*_Newport_Control group had the most mortality by day 2. *Sal*_Newport_Control_2hr_Buffer showed additional mortality by day 5. The *Sal*_Newport_Control group had mortality by day 1 and day 2. *Sal*_Newport_Mutant_2hr_Buffer had mortality by day 1 and all other eggs survived the remainder

of the experiment. There was no significant differences when comparing groups to the counterpart "_2hr_Buffer" group *p* values were Negative_Control (p=0.1620), *Sal*_Newport_Control (p=0.8772), and *Sal*_Newport_Mutant (p=0.2955). Significant differences was found with Negative_Control vs Sal_Newport_Control (p=0.0008), Negative_Control vs Sal_Newport_Control_2hr_Buffer (p=0.0018), Negative_Control vs Sal_Newport_Mutant_2hr_Buffer (p=0.0156). The *Sal*_Newport_Control and *Sal*_Newport-_Control_2hr_Buffer did not have any significant differences with either *Sal*_Newport_Mutant (p=0.0510; p=0.0.3374) or *Sal*_Newport_Mutant_2hr_Buffer (p=0.0792; p=0.4347).

Figure 3.8. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Salmonella* Newport 3596 and *Sal.* Newport Mut$^{\Phi}$ as well as a two hour post *Salmonella* inoculated with SM Buffer set of groups. (11 eggs per group).

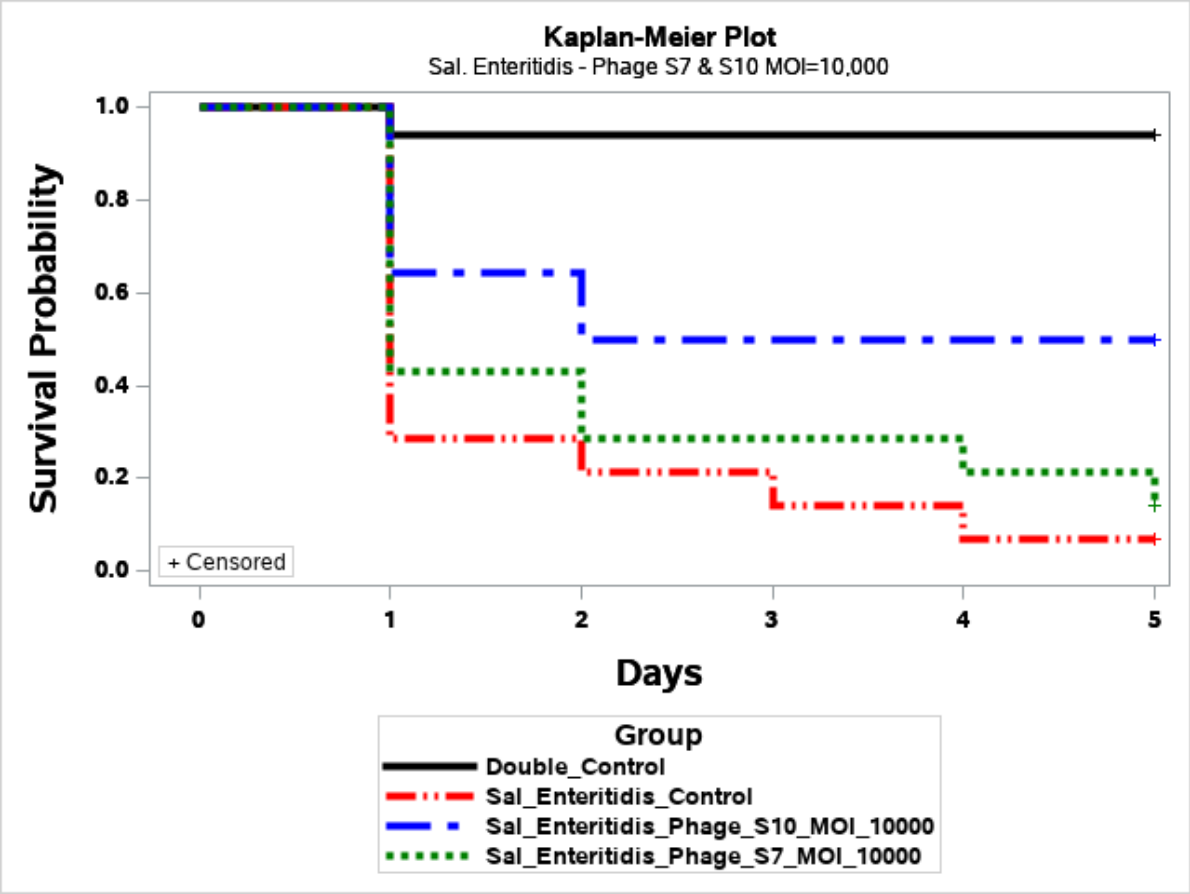


3.4.7. Chicken Embryo Lethality Assay: *Sal.* Enteritidis treated with S7 at MOI≈10^4 or S10 at MOI≈10^4

SPF eggs were incubated until day 11 of development. SPF eggs were divided into four groups (≈14 eggs per group), with *Salmonella*-challenged groups receiving 10^2 CFU/egg of one of the *Salmonella* Enteritidis strains. Groups were a double control group, a positive control *Sal.*

Enteritidis group, and two experimental phage-treated groups: S7 at MOI≈10^4 or S10 at MOI≈10^4. *Sal*_Enteritidis_Control group had most mortality by day 1 (**Figure 3.9)** with additional mortality by days 2, 3, and 4. The Double_Control group was significantly different from *Sal*_Enteritidis_Control group ($p \leq 0.001$), S7 (MOI≈10^4) ($p \leq 0.001$), and S10 (MOI≈10^4) ($p=0.0145$). The *Sal*_Enteritidis_Control group was not significantly different from S7 (MOI≈10^4) ($p=0.6053$), but was significantly different from S10 (MOI≈10^4) ($p=0.0162$). S7 (MOI≈10^4) was not significantly different from S10 (MOI≈10^4) ($p=0.0652$).

Figure 3.9. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Salmonella* Enteritidis and treated with S7 (MOI≈10^4) or S10 (MOI≈10^4) (14 eggs per group).

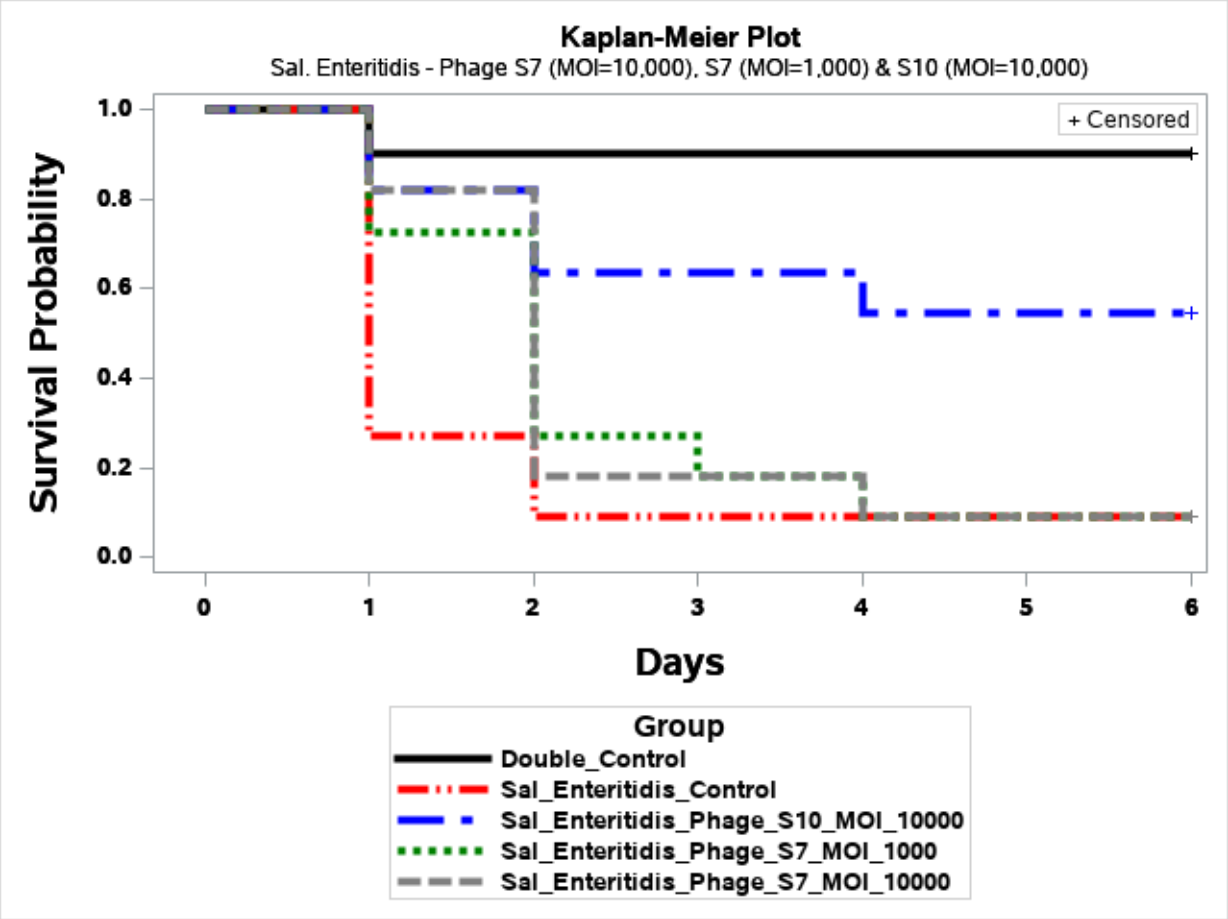


3.4.8. Chicken Embryo Lethality Assay: *Sal*. Enteritidis treated with S7 at MOI≈10^3, S7 at MOI≈10^4, or S10 at MOI≈10^4

SPF eggs were incubated until day 11 of development. SPF eggs were divided into five groups (≈11 eggs per group), with *Salmonella*-challenged groups receiving 10^2 CFU/egg of one of the *Salmonella* Enteritidis. Groups were a double control group, a positive control *Sal.* Enteritidis group, and three experimental treated groups: S7 at MOI≈10^3, S7 at MOI≈10^4, and S10 at

MOI≈10^4. *Sal*_Enteritidis_Control group had the most mortality by day 1 (**Figure 3.10)** with additional mortality by day 2. The Double_Control group was significantly different from *Sal*_Enteritidis_Control group ($p \leq 0.001$), S7 (MOI≈10^3) ($p=0.0012$), and S7 (MOI≈10^4) ($p=0.0020$) but was not significantly different from S10 (MOI≈10^4) ($p=0.2332$). The *Sal*_Enteritidis_Control group was not significantly different from S7 (MOI≈10^3) ($p=0.4028$) and S7 (MOI≈10^4) ($p=0.3358$) but was significantly different from S10 (MOI≈10^4) ($p=0.0031$). S10 (MOI≈10^4) was significantly different from S7 (MOI≈10^3) ($p=0.0496$) but was not significantly different from S7 (MOI≈10^4) ($p=0.0673$). S7 (MOI≈10^3) was not significantly different from S7 (MOI≈10^4) ($p=0.9038$).

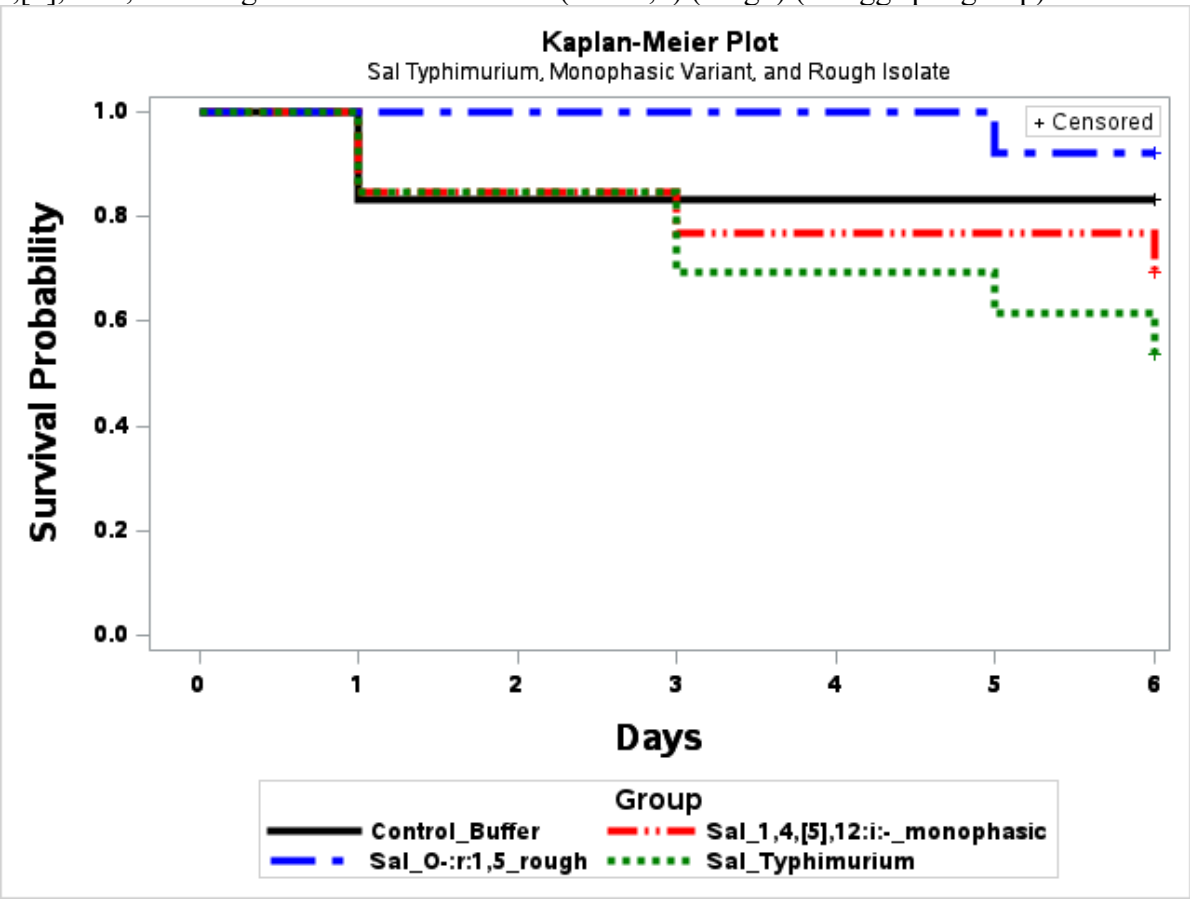
Figure 3.10. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Salmonella* Enteritidis and treated with S7 (MOI≈10^3), S7 (MOI≈10^4), or S10 (MOI≈10^4) (11 eggs per group).



3.4.9. Chicken Embryo Lethality Assay: Comparisons of a rough isolate of *Salmonella* (O-:r:1,5) with *Sal*. Typhimurium, *Sal*. Typhimurium monophasic variant 1,4,[5],12:i-, and a *Sal*. Infantis

SPF eggs were incubated until day 11 of development. SPF eggs were divided into four groups (≈13 eggs per group), with *Salmonella*-challenged groups receiving 10^2 CFU/egg of the *Sal.* Typhimurium, *Sal.* Typhimurium monophasic variant 1,4,[5],12:i-, or a rough isolate of *Salmonella* (O-:r:1,5). The Negative_Control group had mortality like the *Sal*_monophasic (p=0.4541) and *Sal*_Typhimurium (p=0.0979) groups (**Figure 3.11).** The only two groups that had significant differences were between the *Sal*_rough and *Sal*_Typhimurium (p=0.0291).

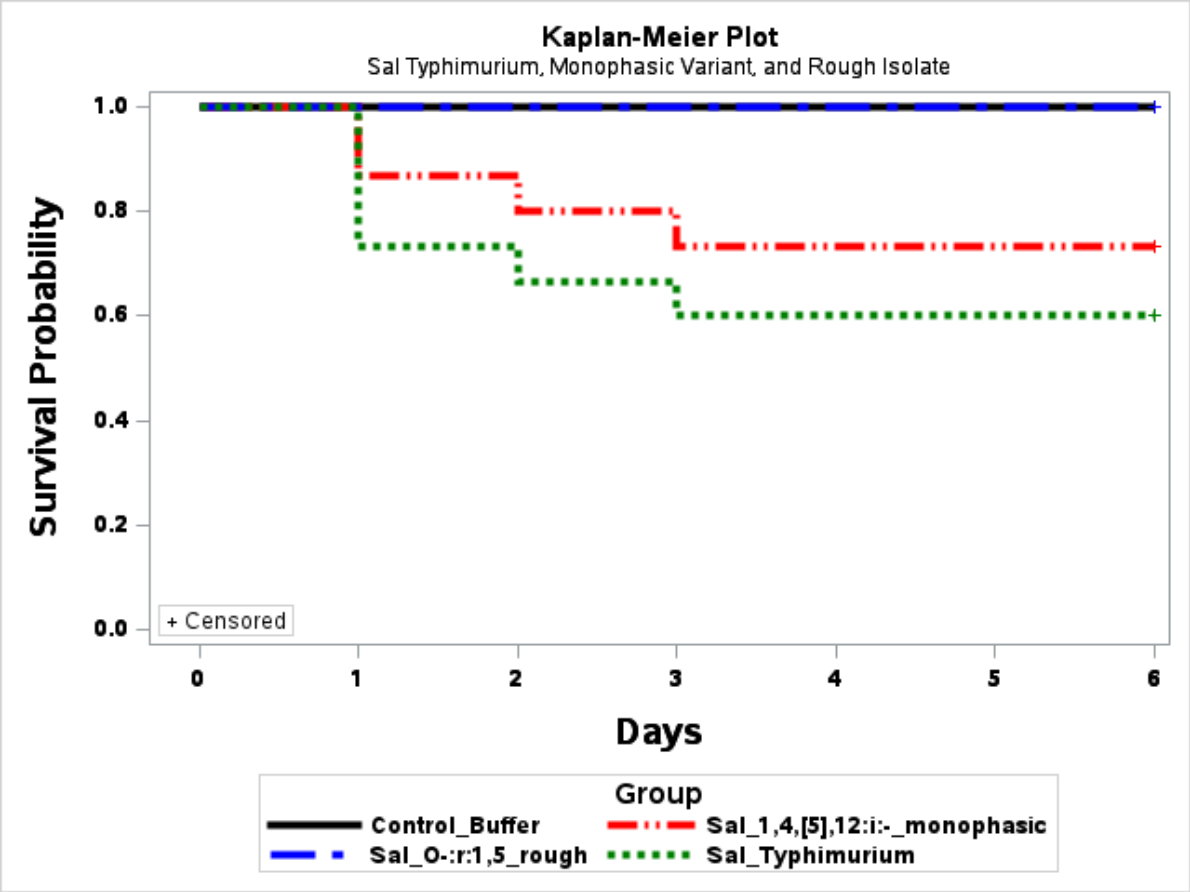
Figure 3.11. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Sal.* Typhimurium, *Sal.* Typhimurium monophasic variant 1,4,[5],12:i-, or a rough isolate of *Salmonella* (O-:r:1,5) (rough) (13 eggs per group).



SPF eggs were incubated until day 11 of development. SPF eggs were divided into four groups (≈13 eggs per group), with *Salmonella*-challenged groups receiving 10^2 CFU/egg of the *Sal.* Typhimurium, *Sal.* Typhimurium monophasic variant 1,4,[5],12:i- (monophasic), or a rough isolate of *Salmonella* (O-:r:1,5) (rough). The *Sal*_Typhimurium had the highest amount of

mortality out of all these groups (**Figure 3.12).** The Negative_Control group only had significant difference with *Sal*_Typhimurium (p=0.0073). The *Sal*_rough was also significantly different from *Sal*_Typhimurium (p=0.0043). The *Sal*_monophasic group was not significantly different from *Sal*_rough (p=0.0692) and *Sal*_Typhimurium (p=0.3061).

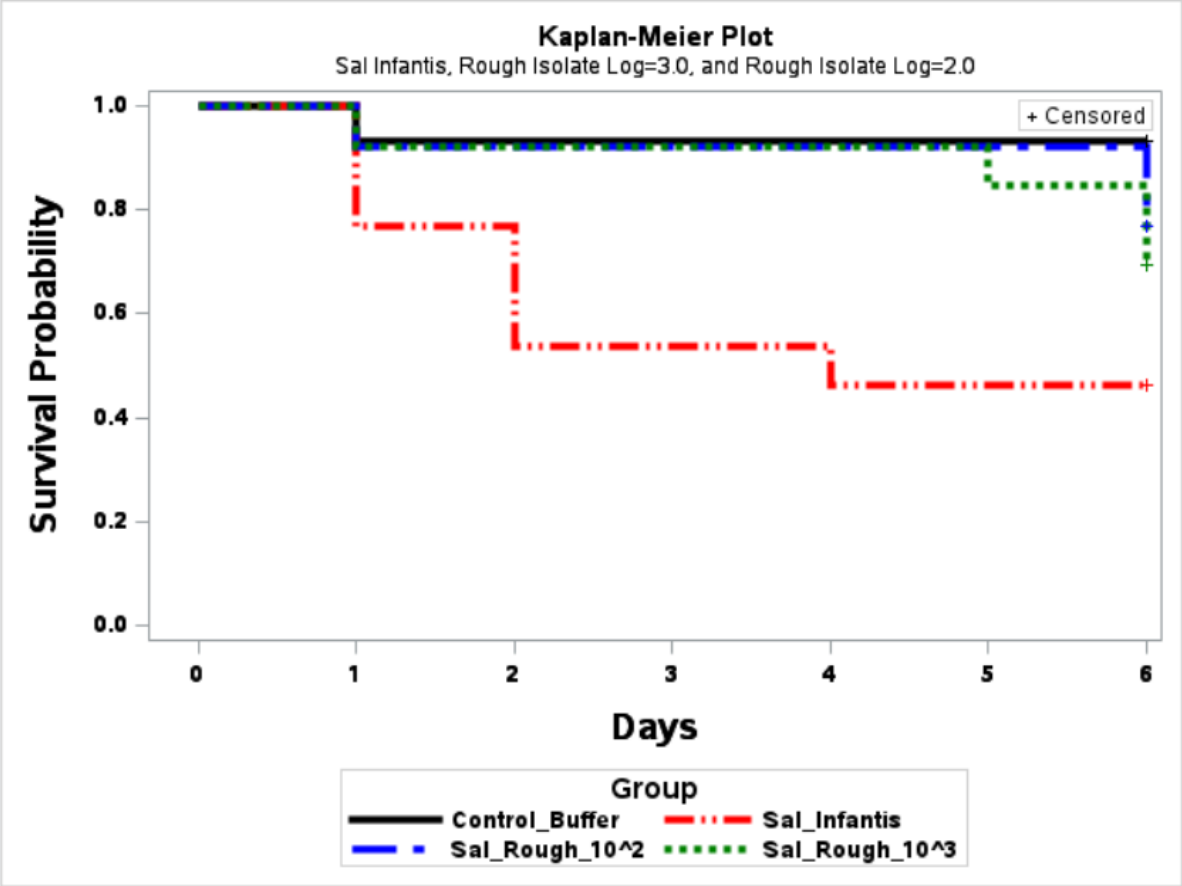
Figure 3.12. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Sal.* Typhimurium, *Sal.* Typhimurium monophasic variant 1,4,[5],12:i-, or a rough isolate of *Salmonella* (O-:r:1,5) (rough) (13 eggs per group).



The rough isolate of *Salmonella* (O-:r:1,5) was sequenced on a Illumina Miniseq. Molecular serotyping was first attempted by using raw fastq reads and SeqSero (v.1.2). Molecular serotyping was not successful due to the predicted antigenic profile being O-antigen O-, H1 antigen r, and H2 antigen 1,5 for the combined prediction of O-:r:1,5. KmerFinder (v3.2) was used to find a closely related reference sequence. The output was NZ_CP093400.1 *Salmonella* enterica subspecies enterica serovar Infantis strain R21.1147. The rough isolate was then compared to a *Salmonella* Infantis in a chicken embryo lethality assay.

The SPF eggs were incubated until day 11 of development. SPF eggs were divided into four groups (≈13 eggs per group), with a group receiving 10^2 CFU/egg of the *Sal.* Infantis, 10^2 CFU/egg of the rough isolate of *Salmonella* (O-:r:1,5), or 10^3 CFU/egg of the rough isolate of *Salmonella* (O-:r:1,5). *Sal.* Infantis was the only group with higher mortality (**Figure 3.13)**. The Negative_Control group only had significant difference with *Sal*_Infantis (p=0.0038). The *Sal*_Infantis group was significantly different from *Sal*_rough_10^2 (p=0.0692) but was not significantly different from *Sal*_rough_10^3 (p=0.0942).

Figure 3.13. Chicken embryo lethality assay: survival analysis Kaplan-Meier curve of experimental groups challenged with *Sal.* Infantis (10^2 CFU/egg), rough isolate of *Salmonella* (O-:r:1,5) (10^2 CFU/egg), or rough isolate of *Salmonella* (O-:r:1,5) (10^3 CFU/egg) (13 eggs per group).



3.5. Discussion

Not all phages are candidates for phage therapy (Luong et al., 2020). Many *in vitro* techniques with phage aim to help with the selection of the best phage candidates, such as the techniques of host range evaluation and efficiency of plating (Haines et al., 2021; Glonti and Pirnay, 2022). Lewis and Hill (2020) state there are disadvantages associated with the use of

phages, but the barrier can be overcome by careful selection of phages (Lewis and Hill, 2020). Selection of the best phages is important before using them in animal models.

Trotereau et al. (2019) describe the chicken embryo lethality model as a straightforward, relevant model to settle the efficacy of phage therapy before controlled clinical trials in targeted animals. Trotereau et al. (2019) references the usefulness of the chicken embryo model's use for *Salmonella enterica* infections and the benefits of discriminating between virulent and avirulent isolates (Trotereau and Schouler, 2019). *Salmonella* serotypes such as *Sal*. Enteritidis and *Sal*. Typhimurium have been used as positive controls in chicken embryo lethality assays (Adam et al., 2002; Rezaee et al., 2021). Adam et al. (2002) had 100% mortality after 42 hours in chicken egg embryos after inoculation with *Salmonella* (Adam et al., 2002).

This study's goal was to develop the chicken embryo lethality assay to select the best phages to target *Sal*. Newport before animal trials in calves. According to the most recent FoodNet report on infections caused by foodborne pathogens, *Sal.* Newport is the third highest serotype responsible for *Salmonella*-associated foodborne disease in humans (Delahoy et al., 2023). *Sal.* Newport is also a serotype commonly linked to cattle and beef (Marshall et al., 2018; Canning et al., 2023; Ford et al., 2023). For this reason, previous research has investigated phage and *Sal*. Newport in a calf model (Hyland et al., n.d.; Kitchens, 2016). Phage S50 was chosen for this study due to its lytic nature in broth, high titer amplification, and plaque clarity with *Sal*. Newport (Kitchens, 2016). This model only showed a significant increase in chicken embryo survival compared to *Sal*. Newport control when S50's MOI was equal to 10^6 (**Figure 3.3**) and 10^7 (**Figure 3.4**), which are very high phage doses per bacterial cell. Both S50 MOIs of 10^6 and 10^7 were significantly different from the control, which means that S50 increased survival at these MOIs but were never equal to survival, as seen in the double control groups.

The phage cocktail was tested to see if any phages used would influence survival at a reasonable MOI dose. The phage cocktail at an MOI of 10^2 and 10^3 (**Figure 3.5**) was not significantly different from the *Sal*. Newport control, meaning there was no observed effect on the phage in survival.

This isolate 3596 of *Sal*. Newport Mash (v2.3) showed containment of the plasmid p972816 (**Figure 2.22**), which carries multi-drug resistance similar to the multi-drug resistance found in *Sal*. Typhimurium DT104 (Tang et al., 2019). It is known that plasmids carry multi-drug resistance or integrated plasmids such as SGI-1 in *Sal*. Typhimurium DT104 can contain virulence

genes (Tang et al., 2019; Li et al., 2021). It would not be unreasonable to speculate that the *Sal*. Newport strain used in this study could be a hyper-virulent strain, which could explain why the phage-treated egg groups survival never equaled the survival seen in the double control group.

Due to the high MOI necessary to observe differences in survival from *Sal*. Newport control, another serotype and its targeted phage were explored. *Sal*. Enteritidis is a *Salmonella* serotype that is associated with eggs and this serotype is known to contaminate egg contents by vertical transmission from infection of the reproductive organs (Braden, 2006; Gantois et al., 2009). Use of the phages S7 and S10 was explored with *Sal*. Enteritidis in the chicken egg embryo model. The findings (**Figure 3.8** and **Figure 3.9**) showed that an MOI of 10^4 of S10 treated eggs' survival was significantly different from the *Sal*. Enteritidis control group, but S7 treated eggs were not different from the *Sal*. Enteritidis control. However, the S10 MOI of 10^4 was still significantly different from the double control group. This means that S10 did increase survival from the positive control, but still did not have the same survival as the double control eggs. It was found that S7 treatment was not any different from the positive control. Based on these results, S10 provided a therapeutic effect not observed with S7.

Investigating virulence and attenuation with *Salmonella* isolates was explored using the chicken embryo lethality model. A previous study of a phage-resistant mutant of *Sal*. Newport (*Sal.* Newport Mut$^{\Phi}$) was attenuated in a calf model (Kitchens, 2016). The chicken embryo lethality model was used to compare the parent and *Sal.* Newport Mut$^{\Phi}$ (**Figure 3.6 – 3.8**). There was no difference found between the parent *Sal.* Newport and *Sal.* Newport Mut$^{\Phi}$ (**Figures 3.6 and 3.7**). A two-hour inoculation of buffer post-*Salmonella* inoculation was done (**Figure 3.8**) to be consistent with previous phage-treated trials (**Figure 3.3 – 3.5, 3.9, 3.10**). The *Sal.* Newport Mut$^{\Phi}$ groups were not any different from the parent *Sal*. Newport groups. Mortality was observed in the negative groups that received a buffer two hours post-buffer inoculation. Still, no significant differences were observed between this group and the negative control that did not receive a two-hour buffer (p=0.1621). Based on these findings, *Sal.* Newport Mut$^{\Phi}$ does not appear attenuated in the chicken embryo lethality model as seen in our calf model (Kitchens, 2016).

This model was used to examine other variants to see if the chicken embryo lethality model could detect attenuation. Li et al. (2021) identified the insertion of IncQ into the *fliB* gene (phase two flagellar antigen) in the monophasic serotype *Sal*. 1,4,[5],12:i:- that may have resulted in the conversion of *Sal*. Typhimurium (1,4,[5],12:i:1,2) to serotype 1,4,[5],12:i:- (Li et al., 2021). This

change in surface antigen (flagellar) has not attenuated *Sal*. 1,4,[5],12:i:- as *Sal*. 1,4,[5],12:i:- is the sixth highest serotype responsible for *Salmonella-associated* foodborne disease in humans according to the most recent FoodNet report on infections caused by foodborne pathogens (Delahoy et al., 2023). Rough mutants of *Salmonella* lack lipopolysaccharide (LPS) O-antigen, which can affect virulence (Curtiss, 2023). The rough *Salmonella* had a significant increase in survival compared to *Sal*. Typhimurium (**Figure 3.11** and **3.12**). Both the monophasic and the rough *Salmonella* isolates had survival similar to that in the control group (**Figure 3.11** and **3.12**).

WGS data revealed that the rough *Salmonella* (O-:r:1,5) was found to have containment of *Sal*. Infantis (6,7,14:r:1,5). These appear to be the same serotype with the rough isolate missing the 6, 7, and 14 O-antigens. The rough isolate at the standard dose of 10^2 CFU/egg and a ten-fold higher 10^3 CFU/egg dose showed reduced mortality compared to the *Sal*. Infantis at the standard 10^2 CFU/egg dose in the chicken embryo lethality model (**Figure 3.13**). It was observed that there was no difference in survival between rough *Salmonella* doses with the control group, but the *Sal*. Infantis did have significant mortality compared to the control and rough *Salmonella* 10^2 CFU/egg groups. This further strengthens supports that the *Sal.* Infantis rough isolate is attenuated in the chicken embryo lethality assay.

The chicken embryo lethality assay has been used for several bacterial pathogens (Trotereau and Schouler, 2019). Nicolas et al. (2023) found a 90% reduction in chicken embryo mortality with an eight-phage cocktail treatment (MOI=20) against avian pathogenic *Escherichia coli* (Nicolas et al., 2023b). Hao et al. (2024) utilized the methods from Trotereau and Schouler (Trotereau and Schouler, 2019) to test *Sal.* Enteritidis, with the following modifications; the initial dose was dropped 10-fold to $3.2x10^1$ CFUof *Sal*. Enteritidis and $3.2\ x10^3$ PFU of phage () (MOI=10^3). inoculated 1 hour prior to *Sal*. Enteritidis challenge (Hao et al., 2024). This *Salmonella* dosage was avoided in the current study because a dose around 10^1 is challenging to be consistent with dosage. This current study specifically avoided co-inoculation of phage and *Salmonella* or phage treatment before inoculation with the concern of not observing true treatment by phage versus phage lysing bacterial cells before embryo infection.

Staying true to the methods described by Trotereau and Schouler, the chicken embryo lethality assay did not show significant reduction of *Sal.* Newport unless phage treatment doses were very high. There was an observable treatment protection with phage S10 that was not seen with phage S7 against *Sal.* Enteritidis, but S10 protection did not equate to the level of the double

control group. This study was the first to describe the use of the chicken embryo lethality assay to investigate virulence or attenuation of *Salmonella* mutants or variants.

3.6. References

Adam, R., Mussa, S., Lindemann, D., Oelschlaeger, T.A., Deadman, M., Ferguson, D.J.P., Moxon, R., Schroten, H., 2002. The Avian Chorioallantoic Membrane *in ovo* - A Useful Model for Bacterial Invasion Assays. International Journal of Medical Microbiology 292, 267–275. doi:10.1078/1438-4221-00209

Boerlin, P., 2010. Agents As Therapeutics and Growth Promoters in Food Animal Production in Farm Animals and, in: Sabour, P.M., Griffiths, M.W. (Eds.), Bacteriophages in the Control of Food-and Waterborne Pathogens. ASM Press, Washington, DC, pp. 1–10.

Braden, C.R., 2006. *Salmonella enterica* Serotype Enteritidis and Eggs: A National Epidemic in the United States. Clinical Infectious Diseases 43, 512–517. doi:10.1086/505973

Canning, M., Birhane, M.G., Dewey-Mattia, D., Lawinger, H., Cote, A., Gieraltowski, L., Schwensohn, C., Tagg, K.A., Francois Watkins, L.K., Park Robyn, M., Marshall, K.E., 2023. *Salmonella* Outbreaks Linked to Beef, United States, 2012–2019. J Food Prot 86, 100071. doi:10.1016/j.jfp.2023.100071

Curtiss, R., 2023. Vaccines to Control *Salmonella* in Poultry. Avian Dis 67, 427–440. doi:10.1637/aviandiseases-d-23-99988

Delahoy, M.J., Shah, H.J., Weller, D.L., Ray, L.C., Smith, K., McGuire, S., Trevejo, R.T., Scallan Walter, E., Wymore, K., Rissman, T., McMillian, M., Lathrop, S., LaClair, B., Boyle, M.M., Harris, S., Zablotsky-Kufel, J., Houck, K., Devine, C.J., Lau, C.E., Tauxe, R. V., Bruce, B.B., Griffin, P.M., Payne, D.C., 2023. Preliminary Incidence and Trends of Infections Caused by Pathogens Transmitted Commonly Through Food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2022. MMWR Morb Mortal Wkly Rep 72, 701–706. doi:10.15585/mmwr.mm7226a1

Dhowlaghar, N., Denes, T.G., 2023. Control of Residual Phage in the Evaluation of Phage-Based Food Safety Applications. Crit Rev Food Sci Nutr 0, 1–7. doi:10.1080/10408398.2023.2258210

Edrington, T.S., Brown, T.R., 2022. A Commentary on *Salmonella* From a Pre-Harvest Perspective. Frontiers in Animal Science 3, 1–7. doi:10.3389/fanim.2022.877392

FDA, 2015. Veterinary Feed Directive. Fed Regist 80, 31707–31735.

Ford, L., Ellison, Z., Schwensohn, C., Griffin, I., Birhane, M.G., Cote, A., Fortenberry, G.Z., Tecle, S., Higa, J., Spencer, S., Patton, B., Patel, J., Dow, J., Maroufi, A., Robbins, A., Donovan, D., Fitzgerald, C., Burrell, S., Tolar, B., Folster, J.P., Cooley, L.A., Francois Watkins, L.K., 2023. Strain of Multidrug-Resistant *Salmonella* Newport Remains Linked

to Travel to Mexico and U.S. Beef Products — United States, 2021–2022. Morbidity and Mortality Weekly Report 72, 1225–1229.

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T.J., Van Immerseel, F., 2009. Mechanisms of Egg Contamination by *Salmonella* Enteritidis. FEMS Microbiol Rev 33, 718–738. doi:10.1111/j.1574-6976.2008.00161.x

Glonti, T., Pirnay, J., 2022. *In vitro* Measurement Of Phage Characteristics That Are Important for Phage Therapy Outcome. Viruses 14, 1–23.

Haines, M.E.K., Hodges, F.E., Nale, J.Y., Mahony, J., van Sinderen, D., Kaczorowska, J., Alrashid, B., Akter, M., Brown, N., Sauvageau, D., Sicheritz-Pontén, T., Thanki, A.M., Millard, A.D., Galyov, E.E., Clokie, M.R.J., 2021. Analysis of Selection Methods to Develop Novel Phage Therapy Cocktails Against Antimicrobial Resistant Clinical Isolates of Bacteria. Front Microbiol 12, 1–15. doi:10.3389/fmicb.2021.613529

Hao, L., Zhang, Y., Lv, X., Huang, Y., Li, Y., Shi, Y., Wang, F., 2024. Protective Effect of Bacteriophage on Chicken Embryo Damage Caused by *Salmonella* Enteritidis Infection 285–292.

Hyland, J., Kitchens, S., Wright, J., Walz, P., Price, S., n.d. Oral Bacteriophage Treatment to Reduce Fecal Shedding of *Salmonella enterica* Serotype Newport from Calves. Manuscript in Preparation.

Kitchens, S.R., 2016. A Study of a Bacteriophage-Resistant *Salmonella* Mutant and Factors Influencing Prevalence of *Salmonella* in a Multi-Species Animal Facility (Master's Thesis). Auburn University.

Klopatek, S., Callaway, T.R., Wickersham, T., Sheridan, T.G., Nisbet, D.J., 2021. Bacteriophage Utilization in Animal Hygiene, in: Harper, D.R., Abedon, S.T., Burrowes, B.H., McConville, M.L. (Eds.), Bacteriophages: Biology, Technology, Therapy, Volume 2. Springer International Publishing, pp. 891–917. doi:10.1007/978-3-319-41986-2_30/COVER

Kropinski, A.M., Mazzocco, A., Waddell, T.E., Lingohr, E., Johnson, R.P., 2009. Enumeration of Bacteriophages by Double Agar Overlay Plaque Assay, in: Clokie, M.R.J., Kropinski, A.M. (Eds.), Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions. Humana Press, a part of Springer Science+Business Media, New York, NY, pp. 69–76. doi:10.1007/978-1-60327-164-6

Lewis, R., Hill, C., 2020. Overcoming Barriers to Phage Application in Food and Feed. Curr Opin Biotechnol 61, 38–44. doi:10.1016/j.copbio.2019.09.018

Li, C., Tyson, G.H., Hsu, C.H., Harrison, L., Strain, E., Tran, T.T., Tillman, G.E., Dessai, U., McDermott, P.F., Zhao, S., 2021. Long-Read Sequencing Reveals Evolution and Acquisition of Antimicrobial Resistance and Virulence Genes in *Salmonella enterica*. Front Microbiol 12, 1–14. doi:10.3389/fmicb.2021.777817

Luong, T., Salabarria, A.C., Roach, D.R., 2020. Phage Therapy in the Resistance Era: Where Do We Stand and Where Are We Going? Clin Ther 42, 1659–1680. doi:10.1016/j.clinthera.2020.07.014

Marshall, K.E.H., Tewell, M., Tecle, S., Leeper, M., Sinatra, J., Kissler, B., Fung, A., Brown, K., Wagner, D., Trees, E., Hise, K.B., Chaturvedi, V., Schlater, L.K., Morningstar-Shaw, B.R., Whitlock, L., Holt, K., Becker, K., Nichols, M., Williams, I.T., Jhung, M., Wise, M.E., Gieraltowski, L., 2018. Protracted Possible Outbreak of *Salmonella* Newport Infections Linked to Ground Beef: Possible Role of Dairy Cows — 21 States, 2016–2017. MMWR Morb Mortal Wkly Rep 67, 443–446. doi:10.15585/mmwr.mm6715a2

Mehmood Khan, F., Manohar, P., Singh Gondil, V., Mehra, N., Kayode Oyejobi, G., Odiwuor, N., Ahmad, T., Huang, G., 2023. The Applications of Animal Models in Phage Therapy: An Update. Hum Vaccin Immunother 19, 1–23. doi:10.1080/21645515.2023.2175519

Mukherjee, P., Roy, S., Ghosh, D., Nandi, S.K., 2022. Role of Animal Models in Biomedical Research: A Review. Lab Anim Res 38, 1–17. doi:10.1186/s42826-022-00128-1

Nicolas, M., Trotereau, A., Culot, A., Moodley, A., Atterbury, R., Wagemans, J., Lavigne, R., Velge, P., Schouler, C., 2023. Isolation and Characterization of a Novel Phage Collection against Avian-Pathogenic *Escherichia coli* . Microbiol Spectr 11. doi:10.1128/spectrum.04296-22

Połaska, M., Sokołowska, B., 2019. Bacteriophages—A New Hope or a Huge Problem in the Food Industry. AIMS Microbiol 5, 324–347. doi:10.3934/microbiol.2019.4.324

Rezaee, M.S., Liebhart, D., Hess, C., Hess, M., Paudel, S., 2021. Bacterial Infection in Chicken Embryos and Consequences of Yolk Sac Constitution for Embryo Survival. Vet Pathol 58, 71–79. doi:10.1177/0300985820960127

Shirley, J.F., 2016. Development Toward a Bovine *Salmonella* Model and a Bacteriophage Treatment Cocktail to Reduce *Salmonella* Peripheral Lymph Node Carriage in Calves (Master's Thesis). Auburn University.

Sulakvelidze, A., 2013. Using Lytic Bacteriophages to Eliminate or Significantly Reduce Contamination of Food by Foodborne Bacterial Pathogens. J Sci Food Agric 93, 3137–3146. doi:10.1002/jsfa.6222

Tang, L., Zhu, S.L., Fang, X., Li, Y.G., Poppe, C., Johnston, R.N., Liu, G.R., Liu, S.L., 2019. Distinct Evolutionary Origins of Common Multi-Drug Resistance Phenotypes in *Salmonella* Typhimurium DT104: A Convergent Process for Adaptation Under Stress. Molecular Genetics and Genomics 294, 597–605. doi:10.1007/s00438-019-01531-5

Thulin, J.D., Bradfield, J.F., Bergdall, V.K., Conour, L.A., Grady, A.W., Hickman, D.L., Norton, J.N., Wallace, J.M., 2014. The Cost of Self-Imposed Regulatory Burden in Animal Research. FASEB Journal 28, 3297–3300. doi:10.1096/fj.14-254094

Trotereau, A., Schouler, C., 2019a. Use of a Chicken Embryo Lethality Assay to Assess the Efficacy of Phage Therapy, in: Clokie, M.R.J., Kropinski, A., Lavigne, R. (Eds.), Bacteriophages: Methods and Protocols, Volume 4. Hu, New York, NY, pp. 199–205. doi:https://doi.org/10.1007/978-1-4939-8940-9_17

Trotereau, A., Schouler, C., 2019b. Use of a Chicken Embryo Lethality Assay to Assess the Efficacy of Phage Therapy, in: Clokie, M.R.J., Kropinski, A., Lavigne, R. (Eds.), Bacteriophages: Methods and Protocols, Volume 4. Hu, New York, NY, pp. 199–205. doi:https://doi.org/10.1007/978-1-4939-8940-9_17

Wottlin, L.R., Edrington, T.S., Brown, T.R., Zook, C.A., Sulakvelidze, A., Droleskey, R., Genovese, K.J., Nisbet, D.J., 2022. Evaluation of a Preharvest Bacteriophage Therapy for Control of *Salmonella* within Bovine Peripheral Lymph Nodes. J Food Prot 85, 254–260. doi:10.4315/JFP-21-292

Chapter 4

Computational Approaches in a *Salmonella* Outbreak Investigation

4.1. Abstract

Salmonella is an important bacterial pathogen that poses risks to animal and human health. It is important to identify strains of this pathogen and factors that contribute to environmental prevalence when *Salmonella* is found within an animal facility. As technology has advanced, the field of epidemiology is expanding to new computational approaches to solve problems. Machine learning algorithms for determining important factors for environmental prevalence or whole genome sequencing for identification of bacterial strains are newer methods for solving older questions. However, use of these approaches and guidance on how to best use these approaches has been lacking. This study and analysis helps to provide a step that epidemiologists can take into moving in a direction of incorporating these approaches into their research.

4.2. Introduction

Salmonella, a bacterial pathogen of significant concern, is not limited to humans but infects almost all livestock and poultry species (Murray, 2000; Edrington and Brown, 2022). It has been extensively studied in animals and various environmental niches, which are crucial for its survival. *Salmonella* can be disseminated through different water sources, such as effluent discharges, agricultural runoff, excretions by wild animals, and freshwater. Water contaminated with animal waste presents a potential for the proliferation and dissemination of *Salmonella* by wild animals (Murray, 2000). Understanding these environmental niches is not just important but urgent and necessary for effective prevention strategies.

A surveillance study of a veterinary teaching hospital was conducted to delve deeper into the intricate dynamics of *Salmonella* in the environment within a multi-species animal facility (Kitchens, 2016). The challenge of managing *Salmonella* transmission in veterinary settings is not just a task but a complex issue, amplified by the close proximity of diverse species and the pathogen's persistence in the environment. The environment acts as not only a potential source but also a reservoir for *Salmonella* transmission, contributing to many nosocomial outbreaks of salmonellosis (Castor et al., 1989; Amavisit et al., 2001; Schott et al., 2001; Ward et al., 2005; Dunowska et al., 2007; Schaer et al., 2010; Soza-Ossandón et al., 2020; Sebola et al., 2023).

The study of epidemiology has evolved, and a new paradigm is emerging in public health. Artificial intelligence and machine learning are transitioning epidemiology from traditional approaches to modern approaches (Riswantini and Nugraheni, 2022). Traditionally, epidemiology used regression-type modeling to predict a dependent variable given multiple independent variables (Wiemken and Kelley, 2019). Machine learning algorithms use large-scale datasets to extract meaningful patterns (i.e., "learn") and use this "knowledge" to make predictions on other data (Vilne et al., 2019). Machine learning is a new and innovative area in epidemiology, and thus, current research objectives can be considered preliminary due to the latest applications of these methods (Wiemken and Kelley, 2019; Santangelo et al., 2023). These machine learning methods can be used in scientific research to improve existing epidemiology standards (Santangelo et al., 2023).

Animal care providers can easily carry the organism from one horse to another on clothing and hands, and contaminated equipment has also been implicated as a vector in past veterinary hospital outbreaks. Biosafety protocols exist to minimize *Salmonella* environmental contamination and the risk of exposing susceptible animals and people to *Salmonella*. It is critical to monitor environmental *Salmonella* contamination and the incidence of *Salmonella* shedding in at-risk patients to assess the effectiveness of and recognize deficiencies in *Salmonella* control strategies. Clinical surveillance and investigation of nosocomial outbreaks are primarily dependent on determining whether biogeographically related pairs of strains are clonal and originated from the same source or are distinct and would reflect independent transmission events (Salipante et al., 2015). For the past twenty years, the gold standard laboratory method for bacterial relatedness has been pulsed-field gel electrophoresis (PFGE) (Salipante et al., 2015; Carleton and Gerner-smidt, 2016; Tran and Rowlinson, 2019).

The Centers for Disease Control and Prevention (CDC) published a protocol for unified PFGE typing under PulseNet (Neoh et al., 2019). PulseNet is a national laboratory network that connects foodborne illness cases to detect outbreaks. PulseNet's primary subtyping method for detecting clusters of *Salmonella*, *Listeria monocytogenes*, Shiga-toxin producing *E. coli* (STEC), *Vibrio*, *Shigella*, and *Campylobacter* subspecies from clinical, food, veterinary, and environmental sources had been PFGE (Kubota et al., 2019). In 2013, the CDC participated in an interagency collaboration to routinely use whole genome sequencing (WGS) techniques and analyze all clinical and food-related *Listeria monocytogenes* isolates in the United States of America (U.S.) with the

eventual goal of replacing PFGE (Katz et al., 2017). After this trial, PulseNet began transitioning from PFGE to WGS as PulseNet's primary surveillance tool for cluster detection and surveillance (Nadon et al., 2017; Kubota et al., 2019; Neoh et al., 2019; Ribot et al., 2019; Tolar et al., 2019; Tran and Rowlinson, 2019). WGS provides superior discrimination compared with PFGE and allows multiple characterizations of isolates with a single workflow (Besser et al., 2018). In 2019, the United States Department of Agricultural (USDA), the USDA Food Safety and Inspection Service (FSIS), the Association of Public Health Laboratories (APHL), the Food and Drug Administration (FDA), and the CDC discontinued PFGE and moved to WGS as the primary characterization tool (USDA, 2019). This transition from PFGE to WGS also affected the USDA Animal and Plant Health Inspection Service (APHIS) National Veterinary Service Laboratory (NVSL). The NVSL safeguards U.S. animal health and contributes to public health by ensuring a wide variety of information and services centered on diagnosing domestic and foreign animal diseases, supporting disease control and eradication programs, and developing reagents for diagnostic testing, training, and laboratory certification. The NVSL provides diagnostic support for veterinarians, veterinary hospitals, and animal health and disease researchers. Very little research has been published on veterinary hospitals using WGS in epidemiological studies and nosocomial outbreak studies (Leon et al., 2018).

A two-year study of the Auburn University College of Veterinary Medicine (AUCVM) Veterinary Teaching Hospital was conducted to better understand the prevalence of *Salmonella* in a multi-species animal facility (Kitchens, 2016). Pre-harvest interventions can help reduce *Salmonella* in animal feces and on-site control strategies can be successful, but identifying the routes and sources of infection of animals is critical to develop interventions (Humphrey, 2000). Understanding *Salmonella* in livestock animals demonstrates the importance of the "One Health" concept because livestock animals are a source of human and companion animal food products (Walther et al., 2017). We used the Auburn University College of Veterinary Medicine John Thomas Vaughan Large Animal Teaching Hospital to study *Salmonella* in the environment and identify factors of environmental *Salmonella* contamination in the teaching hospital as a model for multi-species animal production facilities. After the initial two-year study, an additional year was conducted to increase the sample number from the first study and evaluate different statistical methods to predict the presence of *Salmonella* more accurately. Other previous studies were conducted to assess *Salmonella* in the on-site dairy herd and its prevalence in the environment of

the Equine Reproduction Center (Michaels et al., 2018; Kitchens et al., 2019). A total of 1395 samples were collected over the different surveillance projects. From those projects, a pilot study of ten isolates were sent to the NVSL for WGS. This study will compare novel statistical methods when investigating factors associated with prevalence and the use of WGS data. In addition, this study is the first study of a pathogen in a veterinary hospital setting that utilizes machine learning methods such as the Bayesian Markov Chain Monte Carlo (MCMC) method and random forest analysis but also uses WGS data for comparing bacterial isolates. And finally, this study examines computational approaches when investigating bacterial pathogens within a multi-species animal facility.

4.3. Materials and Methods

4.3.1. Sampling from Facilities

Samples were collected by a variety of methods. Swab samples were performed by sterile cotton tip applicators or by gauze on structures such as floors, drains, gates, stall walls, and any other structures available for sampling. Before use, each swab was pre-soaked in 0.1% sterile buffered peptone water (Difco). Those swab samples were placed in Whirl-Pak bags (118-ml; Whirl-Pak) for later analysis. Animal fecal samples were collected from the environment and were placed in Whirl-Pak bags (532-ml) before being analyzed. Feed and hay samples were collected by grab sampling. All samplings were collected with clean gloves with changing gloves between samples.

4.3.2. Sampling from Pasture

Methods used are as described with the facility samplings, except for fecal samples. Sterile tongue depressors were used to collect feces from five pat samples pooled together in a sample cup to represent the pasture.

4.3.3. Water Sampling

Using a 60cc catheter-tip syringe to collect small volumes (50-60mL) of water. This was the primary method of water sampling from animal facilities and pastures, which included troughs, bodies of water, and standing puddled water.

4.3.4. Water Sample Culturing

Small-volume water samples from animal housing troughs and standing puddled water were cultured by adding 50mL of sampled water to 50mL of double-concentrated buffered peptone water (2xBPW), which diluted the 2xBPW down to a concentration of 1xBPW. This sample was then cultured and processed as other samples, as described earlier.

4.3.5. *Salmonella* Culture and Detection

The isolation of *Salmonella* from environmental samples method is a modified method from USDA/FSIS/OPHS Microbiology Laboratory Guidebook's "Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Catfish Products and Carcass and Environmental Sponges" (Rose, 2014). Buffered Peptone Water (BPW) was added to the Whirl-Pak bags containing the sample and incubated for 24 hours at 37°C. Pre-enriched samples were divided into two enrichment broths: 0.5 ml into Tetrathionate (Difco) broth tubes (TTh) and 0.1 ml into Rappaport Vassiliadis (Difco) broth (RV) tubes. Tubes were incubated for 24 hours at 41°C. Selective plating was performed by using Xylose Lysine Agar (Difco) supplemented with Tergitol 4 (Difco) (XLT4). Enrichment solutions were streaked and incubated for 24-48 hours at 37°C. Four characteristic colonies, which appear black centered on XLT4 agar, were subcultured onto XLT4 agar and incubated for 24-48 hours at 37°C. One characteristic colony, which appears black centered on XLT4 agar, from each of the four subcultured colonies was biochemically confirmed to be *Salmonella* species based on agar slants of triple sugar iron (TSI) agar (BD Difco), lysine iron (LIA) agar (BD BBL), and urea agar (BD BBL). TSI, LIA, and Urea slants were inoculated in tandem with a single pick from a colony by stabbing the butts and streaking the slants in one operation and incubated for 24 hours at 37°C. A typical positive control on LIA should produce a purple butt with (H_2S-positive) or without (H_2S-negative) blackening of the media. A typical control on TSI should produce a yellow butt and red slant, with (H_2S-positive) or without (H_2S-negative) blackening of the media. A typical control on Urea agar should produce a yellowing of the media. These presumptive positive colonies were serologically confirmed with polyvalent serum A-V for *Salmonella* (Difco).

4.3.6. Data Collection

Samples were collected over three years. Most sampling locations occurred at the AU Large Animal Teaching Hospital. As an operating veterinary hospital, not all stalls, locations, areas, and animals were accessible to collect samples at a given time. All samples were collected out of convenience instead of predetermined specific samples or samples collected randomly. Information was recorded on each sample for future statistical analysis and to assist hospital section head of areas of positive *Salmonella* isolation. The information was recorded on a form designed "In-House" that specialized on characteristics considered important for analysis and to report to clinicians. Information collected on each sample was consolidated for statistical analysis.

Variables observed included recent weather, year, season, sample type, resident animal species, environment, facility, and regional location.

4.3.7. Analysis of Variables

The statistical software R was used for the variable analysis and statistical models. The R programs "epiR" and "epiDisplay" were used to generate mosaic plots where the width bar in the plot denotes the relative proportion of the row variable and the inside bar denotes the distribution of "*Salmonella* Positive" relative proportion as yes or no. For statistical models, samples were split into two data sets: a "train" dataset to train the various models and a "test" dataset to calculate each model's accuracy, sensitivity, and specificity and compare the different models.

A logistic regression model was employed with region and years treated as random effects using the R program "glmmer". Season, species, weather condition, and sample method were the variables included in the model. All variables had to be changed into dummy variables during analysis. A testing set was used to check the accuracy of the model. Prevalence of *Salmonella* was the dependent response (Y), S was season, C was weather condition, M was sample method, and X was species (**Figure 4.1**). After fitting the model, the odds ratios were calculated with reference level within each variable group. In this study, the reference level used for each variable was fall (season), feed/hay (sample method), dry (condition), and wildlife (species). The R program "glht" was used for pairwise comparisons for different levels within categorical variables.

Figure 4.1. Logistic Regression Model with Random Effects. Prevalence of *Salmonella* was the dependent response (Y), S = season, C = weather condition, M =sample method, and X = species

$$ln\left(\frac{p(y=1)}{1-p}\right) = \beta_0 + \beta_1 S + \beta_2 C + \beta_3 M + \beta_4 X + \text{Random Effects}$$

The second model used was the random forest model in R called "randomForest" (v.4.6-12) to determine the variables of most importance. The variables selected to train the model are listed in **Table 4.1**. The model was then tested to determine accuracy, sensitivity, and specificity.

Table 4.1: Variables Selected to Train Model in Random Forest

```
##  [1] "Date"                "Year"                "Season"
##  [4] "Facility"            "Region"              "Sample"
##  [7] "Salmonella_Positive" "Species"             "Condition"
## [10] "Temp"                "Humidity"            "Environment"
## [13] "Sample_Method"       "Water_Source"
```

A Markov-Chain Monte-Carlo (MCMC) model was used as the third model to determine the posterior parameters of the model. This was accomplished with the R program "rJags". The R program "rJags" links JAGS (v.4.3.0) and R for Bayesian modeling. JAGS is a clone of BUGS (Bayesian analysis Using Gibbs Sampling) (Lunn et al., 2009). Season, region, species, condition, and sample method were variables selected for this analysis. Variables were all converted into dummy variables. The accuracy of the model and the importance of the level within each variable were checked. The accuracy, sensitivity, and specificity were determined from the model selected (**Figure 4.2**) in R.

Figure 4.2. MCMC Test Model

$$ln\left(\frac{p(y=1)}{1-p}\right) = \beta_0 + \beta_1 summer + \beta_2 dairy + \beta_3 food.animal + \beta_4 bovine + \beta_5 recent.rainfall + \beta_6 surface.sample + \beta_7 drain.sample + \beta_8 fecal.sample + \beta_9 water.sample$$

4.3.8. Whole-Genome Sequencing

Ten *Salmonella* Muenster isolates from the various Auburn University College of Veterinary Medicine environmental sampling projects or the natural infection projects were selected for WGS. These isolates that ranged from 2012 to 2019 were chosen to give a good snapshot and were from bovine feces/environments or equine environments. WGS was performed on an Illumina MiSeq by the USDA National Veterinary Service Laboratory. Raw sequence paired-end read numbers can be found in **Table 4.2**. USDA NVSL deposited all isolates under Bioproject PRJNA548885. The National Center for Biotechnology submitted GenBank assemblies for these isolates and all assemblies were uploaded to the NCBI Pathogen Detection Database (NCBI PDD) under SNP cluster PDS0000277449.14 (as of 4/24/2024) (alternatively "SNP Tree

Viewer" found on assembly page for SRR10740739 (GCA_015885225.1)). The *Sal.* Muenster Genbank assembly ASM842900v2 was used when a reference was required.

Table 4.2. Auburn University College of Veterinary Medicine *Salmonella* Muenster Isolate SRR Read Numbers and Metadata

SRA_ID	Raw Coverage	Average Read Length	Number of Reads	Sample source	Year Sample Collected
SRR10740739	103.0x	228	1076023	Necropsy Calf	2012
SRR10740740	96.2x	223	1027813	Calf Feces	2017
SRR10740741	82.1x	223	876854	Cow Feces	2016
SRR10740742	90.9x	225	962288	Drain Swab	2019
SRR10740743	90.2x	225	955320	Water on Dairy Road	2016
SRR10740744	95.9x	225	1015015	Water Sample in Equine Barn	2016
SRR10740745	99.7x	226	1051202	Water Sample in Equine Barn	2015
SRR10740746	94.4x	225	999592	Water Sample in Dairy Barn	2015
SRR10740747	94.5x	216	1042044	Water Sample in Food Animal Barn	2014
SRR10740748	98.2x	222	1053590	Hay Sample in Equine Barn	2014

4.3.9. WGS Data Analysis

Unless stated below, sequences were processed and analyzed on the Alabama Supercomputer Authority's High-Performance Computer. All scripts used for analysis can be found at https://github.com/srk0002/Dissertation-Spring-2024. Reads were downloaded with Sequence Read Archive (SRA) Toolkit (v.2.8.1) and reads were checked for quality with FastQC (v.0.10.1). Multiple approaches were taken to analyze the genomes. Some of these approaches required assembled genomes, accomplished with SPAdes (v.3.13.0) using generated contigs.fasta

files (Prjibelski et al., 2020). A quality report of assemblies was generated with QUAST (v.5.2.0) (Gurevich et al., 2013).

To visualize the isolates in relation to other Southeastern U.S. *Salmonella* Muenster isolates, Parsnp (v.1.5.6) was used to create a phylogenetic tree (Edgar, 2004; Bruen et al., 2006; Price et al., 2010; Treangen et al., 2014). From the NCBI PDD, 68 *Salmonella* Muenster assemblies were selected based on "Serotype" and "Location" filters (assembly numbers **Table 4.3**). Using the 68 downloaded assemblies with the 10 AUCVM *Salmonella* Muensters' SPAdes assemblies, Parsnp performed a rapid core-genome alignment and a maximum-likelihood phylogenetic tree.

Table 4.3. Southeastern United States of America *Salmonella* Muenster NCBI Assembly Numbers for Genbank assemblies used along with AUCVM *Salmonella* Muenster isolates for Parsnp phylogenetic tree.

NCBI Assembly Numbers	
GCA_000487035.2_ASM48703v2_genomic.fna	GCA_009165305.1_PDT000195778.2_genomic.fna
GCA_001477695.1_*Salmonella*_enterica_CVM_N29310-SQ_v1.0_genomic.fna	GCA_009240785.1_PDT000209401.2_genomic.fna
GCA_006688485.1_PDT000500582.2_genomic.fna	GCA_009395135.1_PDT000226157.2_genomic.fna
GCA_006745205.1_PDT000413884.2_genomic.fna	GCA_009402195.1_PDT000229839.2_genomic.fna
GCA_007193235.1_PDT000107294.2_genomic.fna	GCA_009539235.1_PDT000258704.2_genomic.fna
GCA_007197415.1_PDT000119965.6_genomic.fna	GCA_010526115.1_PDT000654621.1_genomic.fna
GCA_007372785.1_PDT000417508.1_genomic.fna	GCA_010967155.1_PDT000288628.2_genomic.fna
GCA_007477225.1_PDT000095337.2_genomic.fna	GCA_010984015.1_PDT000300010.2_genomic.fna
GCA_007548235.1_PDT000003977.3_genomic.fna	GCA_010989255.1_PDT000281110.2_genomic.fna
GCA_007762735.1_PDT000121062.4_genomic.fna	GCA_011273085.1_PDT000672584.1_genomic.fna
GCA_008457325.1_PDT000359409.1_genomic.fna	GCA_013732155.1_PDT000762907.1_genomic.fna
GCA_008477705.1_PDT000135073.2_genomic.fna	GCA_014599995.1_PDT000836945.1_genomic.fna
GCA_008484805.1_PDT000205051.2_genomic.fna	GCA_014644855.1_PDT000839010.1_genomic.fna
GCA_008485425.1_PDT000207519.2_genomic.fna	GCA_014783145.1_PDT000849548.1_genomic.fna
GCA_008708975.1_PDT000591820.1_genomic.fna	GCA_014953885.1_PDT000865537.1_genomic.fna
GCA_008831765.1_PDT000112531.2_genomic.fna	GCA_015226695.1_PDT000878304.1_genomic.fna
GCA_008832845.1_PDT000119972.2_genomic.fna	GCA_017104265.1_PDT000974005.1_genomic.fna
GCA_008847025.1_PDT000112532.2_genomic.fna	GCA_019896395.1_PDT001125106.1_genomic.fna
GCA_008868795.1_PDT000112542.2_genomic.fna	GCA_020809325.1_PDT001171756.1_genomic.fna
GCA_008869245.1_PDT000119979.2_genomic.fna	GCA_020891635.1_PDT001175092.1_genomic.fna
GCA_008869705.1_PDT000119969.2_genomic.fna	GCA_023108115.1_PDT001293640.1_genomic.fna
GCA_008869765.1_PDT000119968.2_genomic.fna	GCA_023731055.1_PDT001327703.1_genomic.fna
GCA_008869925.1_PDT000112522.2_genomic.fna	GCA_024597315.1_PDT001380261.1_genomic.fna

GCA_008869965.1_PDT000112525.2_genomic.fna	GCA_024968045.1_PDT001390869.1_genomic.fna
GCA_008869985.1_PDT000112528.2_genomic.fna	GCA_026862175.1_PDT001519678.1_genomic.fna
GCA_008870005.1_PDT000119971.2_genomic.fna	GCA_027754945.1_PDT001566667.1_genomic.fna
GCA_008870025.1_PDT000112538.2_genomic.fna	GCA_029687985.1_PDT001687435.1_genomic.fna
GCA_008870045.1_PDT000112540.2_genomic.fna	GCA_029817135.1_PDT001691441.1_genomic.fna
GCA_008870085.1_PDT000119984.2_genomic.fna	GCA_031059415.1_PDT001869961.1_genomic.fna
GCA_008870145.1_PDT000112523.2_genomic.fna	GCA_031660075.1_PDT001889534.1_genomic.fna
GCA_008924705.1_PDT000138876.2_genomic.fna	GCA_031895455.1_PDT001892960.1_genomic.fna
GCA_009101205.1_PDT000177100.2_genomic.fna	GCA_032498465.1_PDT001917599.1_genomic.fna
GCA_009129865.1_PDT000189080.2_genomic.fna	GCA_034983165.1_PDT002044739.1_genomic.fna
GCA_009161045.1_PDT000187923.2_genomic.fna	GCA_034984245.1_PDT002044696.1_genomic.fna

A second approach was used to examine the phylogeny of only the 10 AUCVM *Sal.* Muensters' SPAdes assemblies (contigs.fasta). This approach used Prokka (v.1.13) for genome annotation of fasta assemblies (Seemann, 2014). Once completed, Roary (3.13.0) was used on the annotated assemblies in gff format to build a pan-genome, identifying the core and accessory genes and building a core genome alignment (Page et al., 2015). FastTree (v.2.1.10) generated an approximately maximum likelihood phylogenetic tree from Roary's output core genome alignment file as a Newick file (Price et al., 2009). A Newick file was generated based on the presence and absence of genes in the accessory genome. The web-based tool Phandango was used to visualize the Newick file with the gene_presence_absence.csv file from Roary (Hadfield et al., 2018).

Two additional approaches utilized two services found on the Center for Genomic Epidemiology webpage. Both of these services used raw fastq files of the 10 AUCVM *Sal.* Muensters. The tool CSI Phylogeny (v.1.4) was first used (Kaas et al., 2014). CSI Phylogeny calls SNPs, filters the SNPs, does site validation, and infers a phylogeny based on the concatenated alignment of the high-quality SNPs. CSI Phylogeny uses FastTree to generate a maximum likelihood tree from the alignment. A heatmap of SNPs between isolates was generated with Microsoft Excel from the SNP matrix file from CSI Phylogeny. The fourth approach used NDtree (v.1.2) (Nucleotide Difference Tree) was used for analysis (Kaas et al., 2014; Leekitcharoenphon et al., 2014). No reference was provided, so NDtree uses the tool KmerFinder to find the closest template as a reference. NDtree infers phylogeny by splitting the raw reads to k-mers and mapping them to a reference genome. An ungapped consensus sequence with the same length as the reference genome is created, and the differences between the consensus sequences are counted and used as the phylogenetic distance (Ahrenfeldt et al., 2017). This approach is different from a

maximum likelihood tree. NDtree generates a Newick file and an SNP matrix. A heatmap of SNPs between isolates was generated with Microsoft Excel from the SNP matrix file.

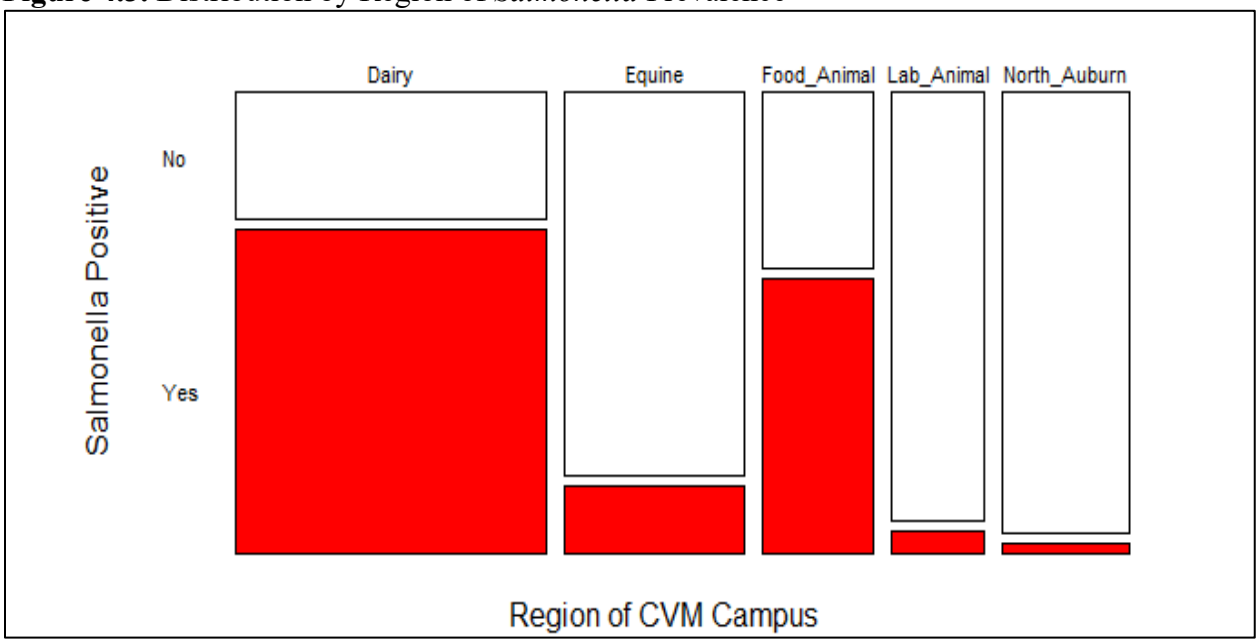
Beautification of phylogenetic tree Newick files were done with MEGA version X, iTol, or Phandango (Roary pipeline only) (Hadfield et al., 2018; Kumar et al., 2018; Letunic and Bork, 2021).

4.4. Results

4.4.1. Analysis of Variables

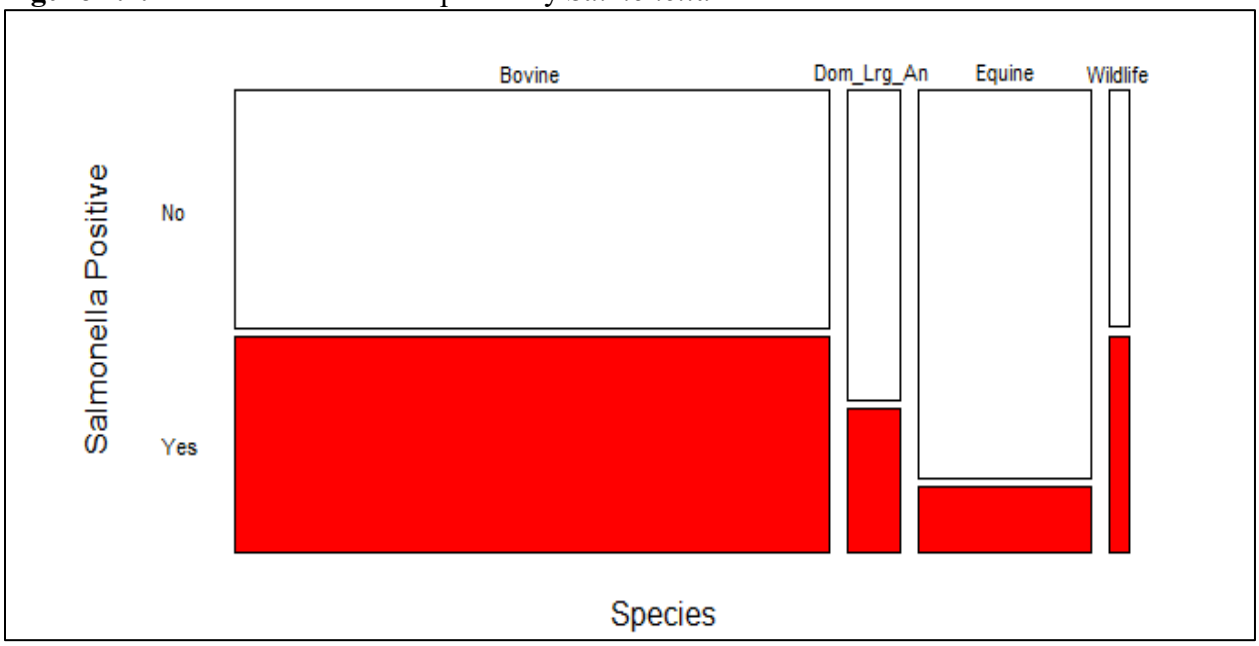
The total number of samples collected over three years was 887 samples. Of 887 samples, 351 (39.57%) were positive for *Salmonella* species. The distribution of the types of samples and prevalence of being *Salmonella* positive are shown in **Figure 4.3 – 4.6**. Amongst the different units, the "Dairy" had the highest prevalence percentage at 71.7% *Salmonella* positive, followed by "Food Animal" (60.8%), "Equine" (14.4%), "Lab Animal Health" (5%), and "North Auburn Beef Herd" (2.2%) (**Figure 4.3**). Amongst the resident animal species, "Wildlife" species had the highest prevalence percentage at 47.6% *Salmonella* positive, followed closely by "Bovine" species (47.4%), "Domestic Large Animal" species (31.6%), and "Equine" species (14.3%) (**Figure 4.4**). Amongst the season samples, the "Summer" samples had the highest prevalence percentage at 47.6% *Salmonella* positive, followed by "Spring" (41.4%), "Fall" (36.2%), and "Winter" (32.1%) (**Figure 4.5**). Amongst the sample types/methods, the "Water" samples had the highest prevalence percentage at 54.3% *Salmonella* positive, followed by "Drain" samples (47.7%), "Fecal" samples (40.8%), "Surface" samples (37.2%), and "Feed or Hay" samples (16.4%) (**Figure 4.6**).

Figure 4.3. Distribution by Region of *Salmonella* Prevalence



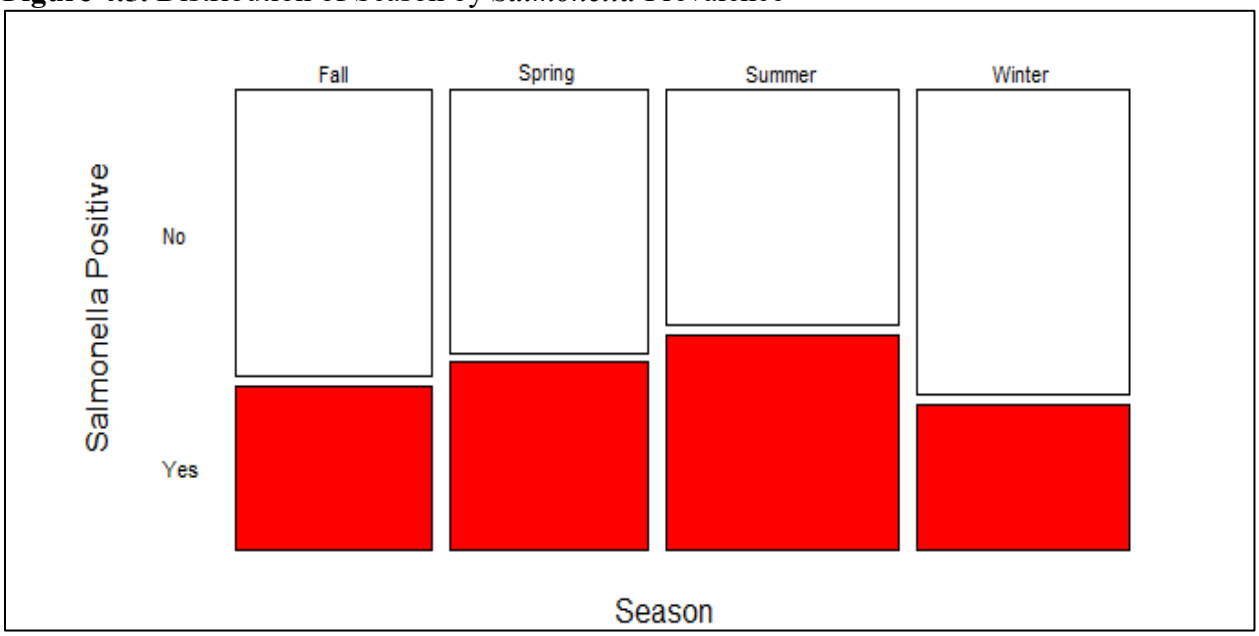
Dairy, Dairy region samples; Equine, Equine region samples; Food_Animal, Food Animal region samples; Lab_Animal, Animal Health region samples; North_Auburn, North Auburn Beef Herd region samples

Figure 4.4. Distribution of Host Species by *Salmonella* Prevalence



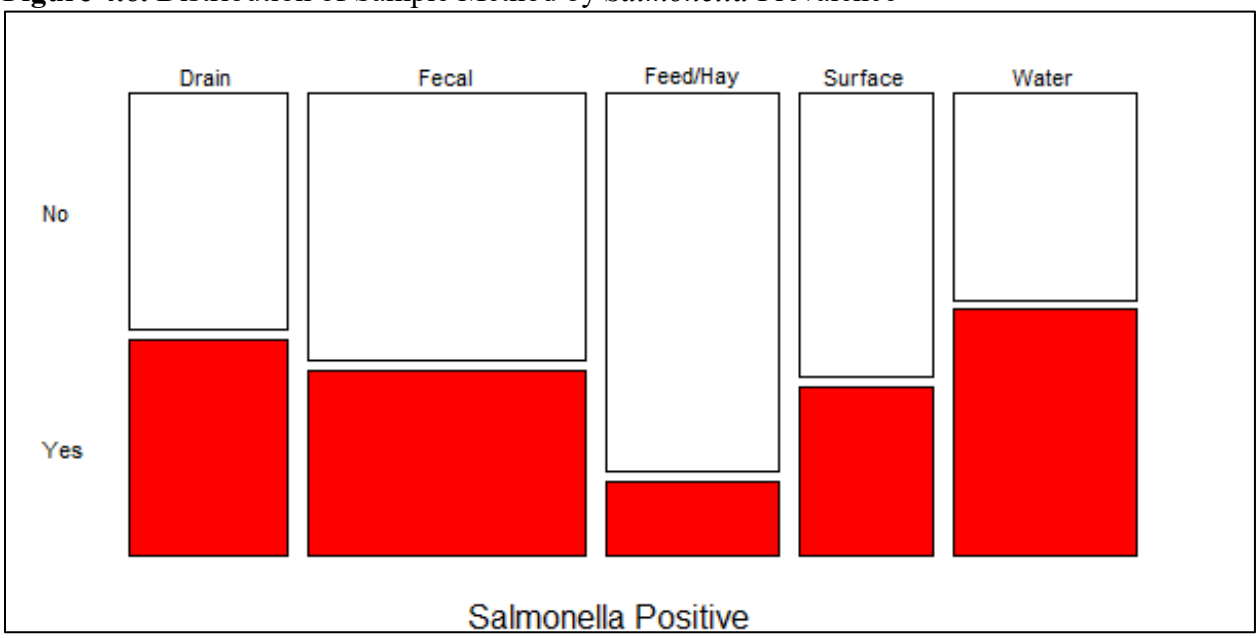
Bovine, Bovine species samples; Dom_Lrg_An, Domestic Large Animal such as Alpacas, Goats, Sheep, Swine species samples; Wildlife, Wildlife species samples from bird or rodents, etc.

Figure 4.5. Distribution of Season by *Salmonella* Prevalence



Fall, Fall season samples; Spring; Spring season samples; Summer, Summer season samples; Winter, Winter season samples

Figure 4.6. Distribution of Sample Method by *Salmonella* Prevalence



Drain, samples collected from drain swabs; Fecal, samples collected from feces; Feed/Hay, grab samples collected from animal feed or hay; Surface, samples collected from swabs of gates, doors, panels, or any other surface; Water, samples collected from water sources

Reference lines used for season was fall, species was wildlife, weather condition was dry weather, and sample type was feed/hay samples. A logistic regression with region and year were treated as a random effect. Significance was found in the odds ratios and ANOVA table (**Table 4.4**). For season, significant differences were found in summer ($p=0.055$) compared to the reference level fall. For species, significant difference was found in bovine ($p=0.010$) compared to the reference level wildlife. For weather condition, we found rain to significantly different ($p=0.031$) compared to reference level dry weather. For sample type, significant differences were found in water ($p<0.001$), drain ($p<0.001$), fecal ($p<0.001$), and surface ($p=0.013$) when compared to the reference level feed/hay. The ANOVA table showed that season, species, weather condition, and sample method were all important to the model. Pairwise comparisons were made for the different levels within categorical variables (**Table 4.5**). Based on this analysis, the variables were ranked (**Table 4.6**). The highest-ranked variables were summer season, bovine species, dry weather, and water sample type. This model had an accuracy of 0.8023, a sensitivity of 0.6747, and a specificity of 0.9247 (**Table 4.7**).

Table 4.4. Logistic Regression with Random Effect: Odds Ratio

	***Salmonella* Positive**		
Predictors	Odds Ratios	CI	p
(Intercept)	0.01	0.00 – 0.07	<0.001
Season			0.127
Fall		Reference	
Spring	1.70	0.90 – 3.19	0.102
Summer	1.82	0.99 – 3.36	0.055
Winter	1.06	0.57 – 1.99	0.855
Species			0.003
Wildlife		Reference	
Bovine	4.64	1.45 – 14.82	0.010
Domestic_large_animal	1.24	0.33 – 4.64	0.748
Equine	4.16	0.45 – 38.60	0.209
Weather Condition			0.031
Dry Weather		Reference	
Rain	0.56	0.33 – 0.95	0.031
Sample Method			2.28e-12
Feed/Hay		Reference	
Drain	12.57	5.60 – 28.22	<0.001
Fecal	8.55	4.19 – 17.47	<0.001
Surface	2.63	1.22 – 5.65	0.013
Water	17.50	7.91 – 38.73	<0.001

Table 4.5. Multiple Comparisons of Means: Tukey Contrasts

Variable	Group		
Species			
Bovine	A		
Equine	A	B	
Wildlife		B	
Domestic Large Animals		B	
Season			
Summer	A		
Spring	A	B	
Winter	A	B	
Fall		B	
Sample Type			
Water	A		
Drain	A		
Fecal	A	B	
Surface		B	
Feed/Hay			C

Table 4.6. Rankings of Variables Based on Multiple Comparisons of Means: Tukey Contrasts

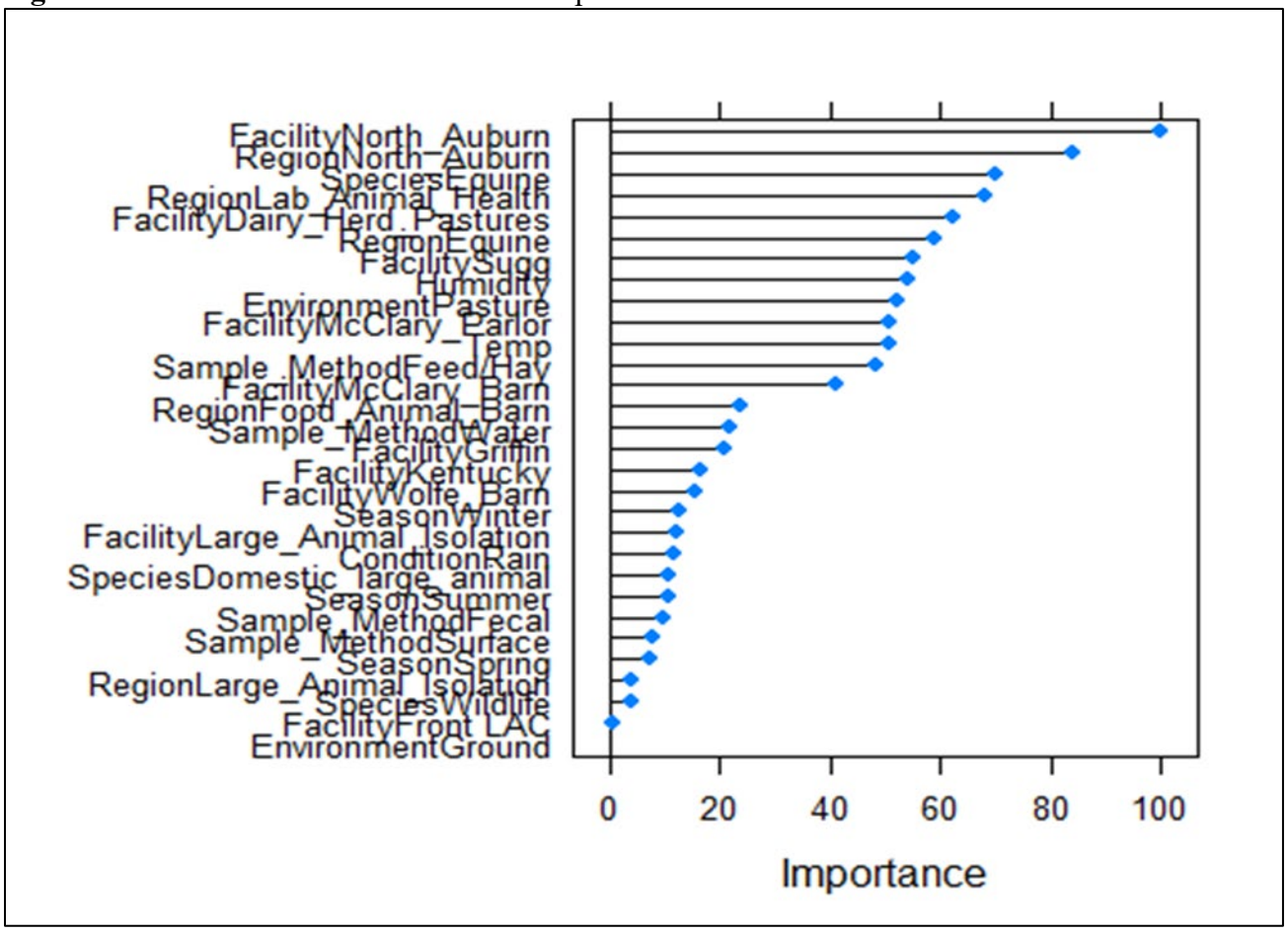
Season	Species	Weather Condition	Sample Type
Summer	Bovine	Dry	Water
Spring	Equine	Rain	Drain
Winter	Wildlife		Fecal
Fall	Domestic Large Animal		Surface
			Feed/Hay

Table 4.7. Accuracy, Sensitivity, and Specificity for Each Model.

Model Type	Accuracy	Sensitivity	Specificity
Logistic Regression	0.8023	0.6747	0.9247
Random Forest	0.8192	0.8429	0.8037
MCMC	0.7910	0.6585	0.9053

Data was divided into train (80% of the total) and test (20% of the total) datasets. We used the train dataset to fit the random forest model. After ten folds, repeated five times, and fitting 10000 trees, the result shows that the system chose mtry=2 as the final model. Variables were ranked by most importance to the model (**Figure 4.7**). Based on this model, the most important variables for predicting *Salmonella* prevalence were the North Auburn Beef Herd facility, the North Auburn region, Equine species, the Lab Animal Health region, and the Dairy Herd Pastures facility. Variables with a high prevalence and importance greater than 50 were the Dairy Herd Pastures facility, Pastures/Outdoor environments, the McClary Dairy Parlor, and the McClary Dairy Barn. A ROC curve was used to determine the best threshold value that could give a better prediction, and the best threshold value was 0.561. The random forest model had an accuracy of 0.8192, a sensitivity of 0.8429, and a specificity of 0.8037 (**Table 4.7**).

Figure 4.7. Random Forests: Variables of Importance



Random Forests ranking of the importance of features within the dataset. By analyzing the impact of each feature on prediction accuracy across all trees, insights into which features drive predictions.

MCMC analysis (**Table 4.1**) variables of significance were rain weather condition, surface samples, drain samples, fecal samples, water samples, summer season, dairy region, food animal region, and bovine species. **Table 4.8** shows the parameter value of the variables and the 95% Confidence Interval (CI) of each parameter. Parameters that don't have a value of zero included in the 95% CI were chosen for the final model from MCMC. These variables were included in the MCMC model, and the model was tested. This model had an accuracy of 0.7910, a sensitivity of 0.6585, and a specificity of 0.9053 (**Table 4.7**).

Table 4.8. MCMC Empirical Mean and Quantiles

Variables of Interest	Mean	2.50%	25%	50%	75%	97.50%
b0	-7.41	-10.57	-8.38	-7.34	-6.33	-4.64
Spring	0.54	-0.09	0.33	0.54	0.75	1.14
Domestic Large Animal Species	0.05	-1.27	-0.42	0.05	0.51	1.33
Equine Barns	2.15	-0.40	1.09	2.05	3.08	5.40
Rain Condition	-0.60	-1.07	-0.76	-0.60	-0.45	-0.15
Surface Sample	1.03	0.24	0.76	1.03	1.30	1.81
Drain Sample	2.56	1.75	2.28	2.56	2.84	3.39
Fecal Sample	2.19	1.49	1.94	2.18	2.44	2.93
Water Sample	2.85	2.05	2.57	2.85	3.13	3.65
Winter	0.13	-0.50	-0.08	0.13	0.35	0.76
Summer	0.67	0.07	0.47	0.67	0.88	1.27
Dairy	5.56	2.96	4.60	5.48	6.53	8.39
Lab Animal Health	0.84	-1.98	-0.17	0.80	1.84	3.87
North Auburn	-0.20	-3.08	-1.24	-0.25	0.82	2.91
Food Animal Barns	4.33	1.72	3.36	4.24	5.31	7.14
Equine Species	1.15	-1.19	0.16	1.13	2.03	3.88
Bovine Species	1.52	0.36	1.13	1.53	1.93	2.64

b0 = y = *Salmonella* positive (yes/no) when training the model.

4.4.2. WGS of *Salmonella* Muenster

All 10 AUCVM *Sal*. Muenster isolates reads where downloaded and split with SRA fastqdump. FastQC analysis of each forward and reverse reads was performed. FastQC Quality Score for SRR10740739 forward reads (**Figure 4.8**) and reverse reads (**Figure 4.9**). All other FastQC Quality Scores were similar to **Figure 4.8** and **Figure 4.9** and thus are not displayed. Trimming was not considered necessary because the graphs suggest that the average quality score falls largely in the "green" quality score range, suggesting that our sequences are high quality. The

QUAST assessment of all 10 AUCVM *Sal*. Muenster isolates (**Figure 4.10**) had an average genome size of 4,806,941.3 base pairs, an average number of contigs of 87.7, an average largest contig of 323,913, and an average N50 of 124,931.5.

Figure 4.8. FastQC Quality Score report for SRR10740739 forward reads.

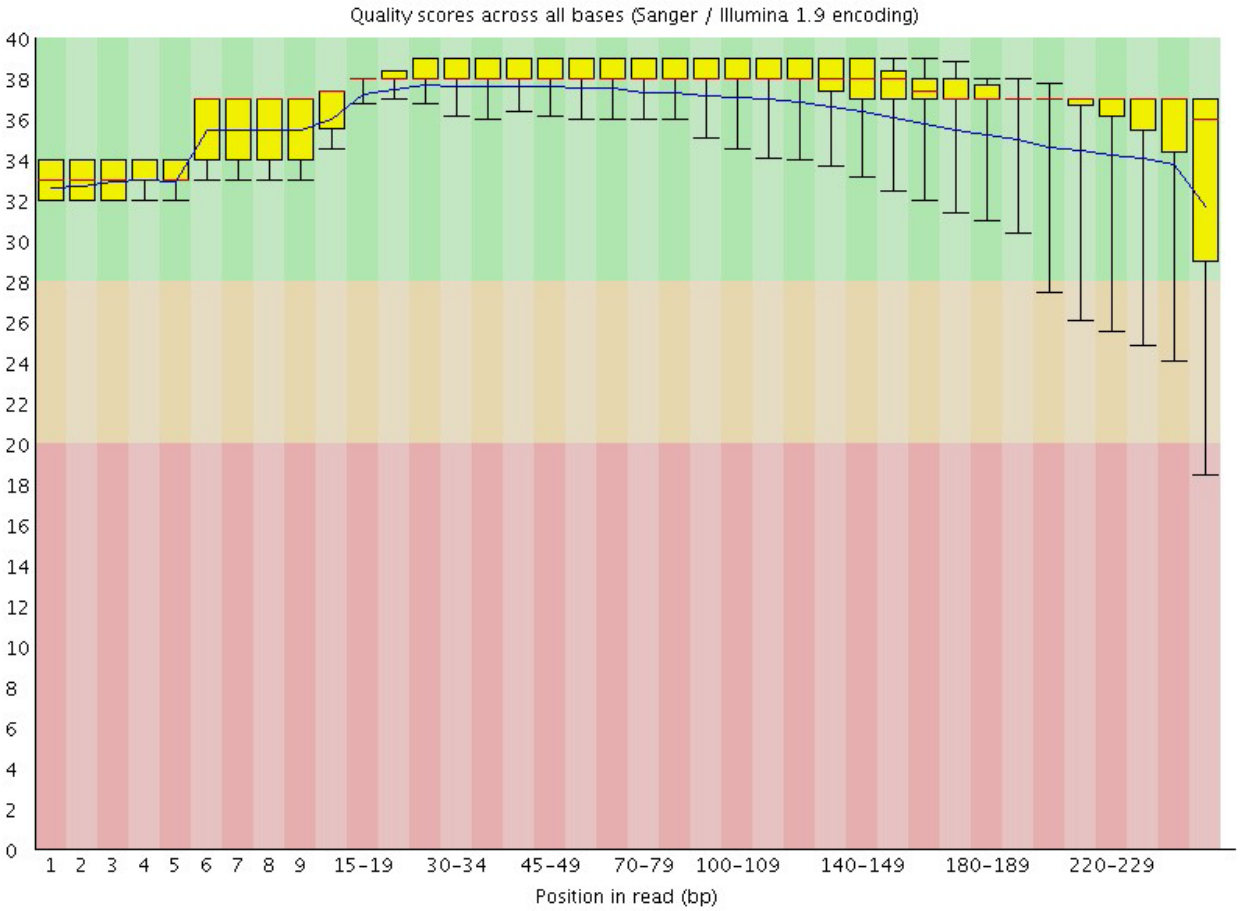


Figure 4.9. FastQC Quality Score report for SRR10740739 reverse reads.

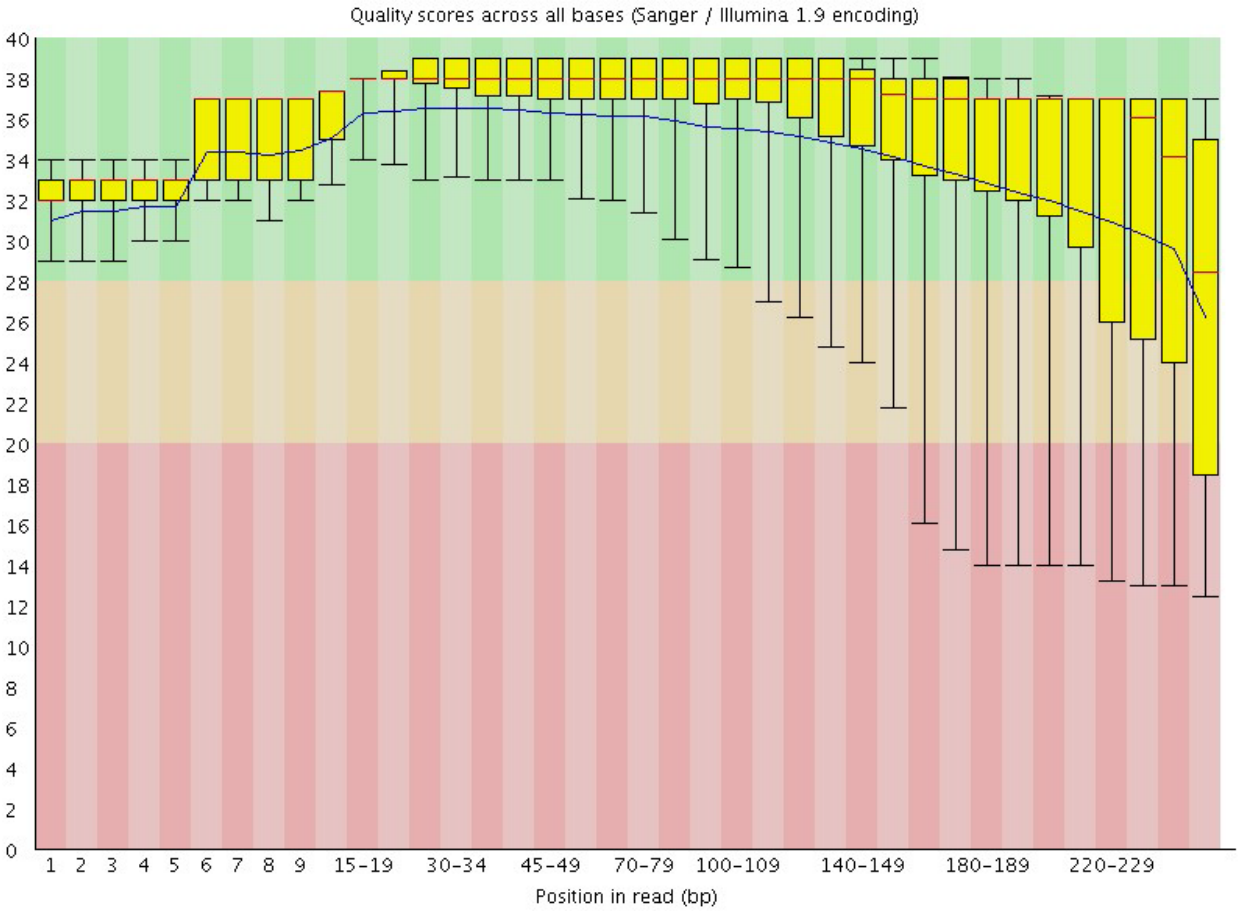


Figure 4.10. Screenshot of Quast Report for all assemblies..

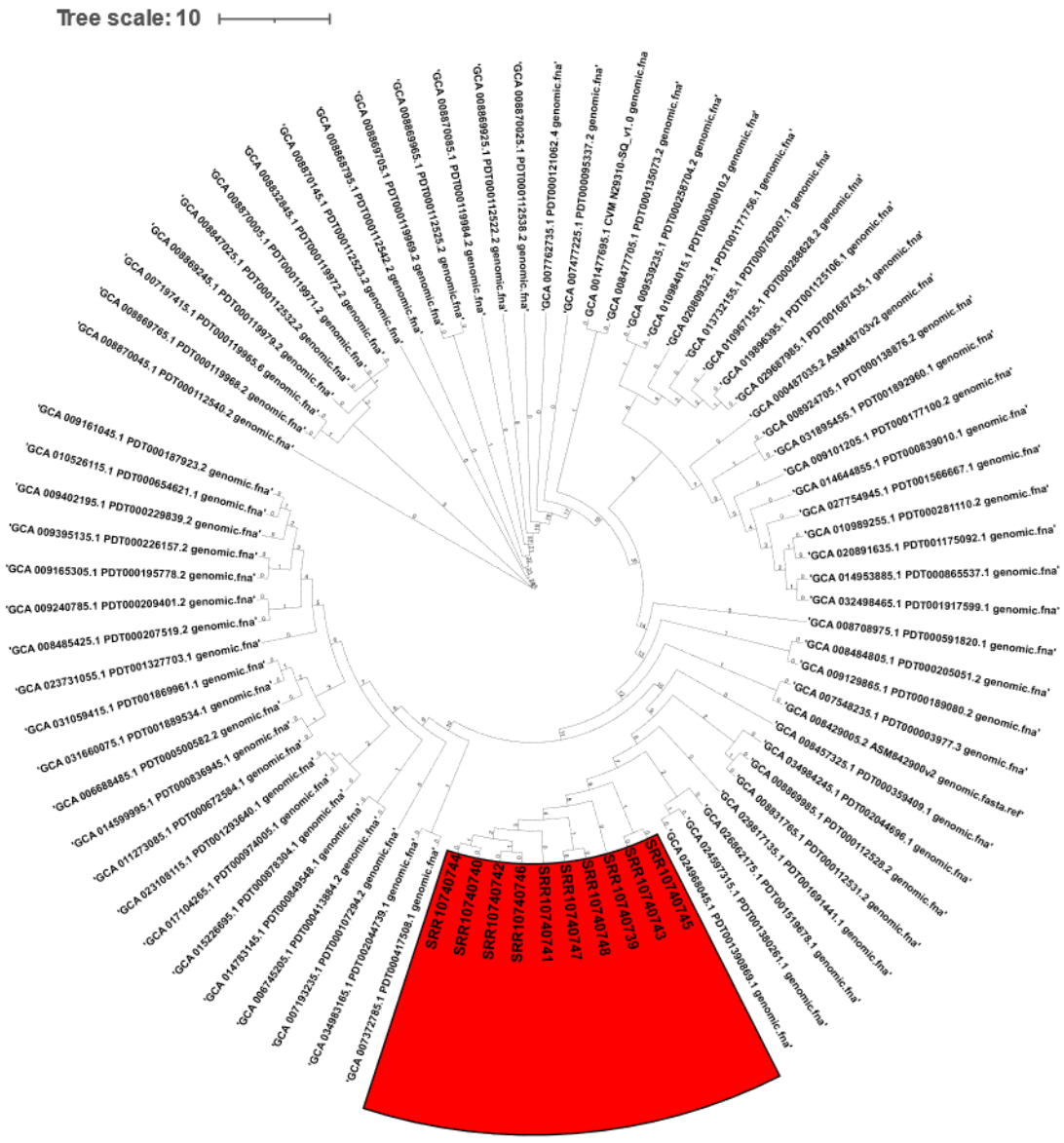
Worst Median Best ☑ Show heatmap

Statistics without reference	SRR10740739	SRR10740740	SRR10740741	SRR10740742	SRR10740743	SRR10740744	SRR10740745	SRR10740746	SRR10740747	SRR10740748
# contigs	80	84	93	59	101	69	96	97	90	108
# contigs (>= 0 bp)	204	262	234	191	323	218	350	247	219	334
# contigs (>= 1000 bp)	70	75	81	52	89	60	82	85	82	95
# contigs (>= 5000 bp)	57	63	66	48	72	50	63	71	63	78
# contigs (>= 10000 bp)	50	54	53	45	62	43	56	66	54	68
# contigs (>= 25000 bp)	41	46	46	42	45	41	44	50	48	49
# contigs (>= 50000 bp)	33	35	35	32	35	33	34	37	37	38
Largest contig	339 053	333 378	353 009	450 580	265 191	425 819	275 057	272 313	291 881	232 849
Total length	4 794 847	4 794 661	4 794 439	4 771 978	4 830 680	4 796 758	4 826 500	4 875 020	4 794 126	4 790 404
Total length (>= 0 bp)	4 821 535	4 844 762	4 827 412	4 807 950	4 877 951	4 837 005	4 884 541	4 906 663	4 824 633	4 840 692
Total length (>= 1000 bp)	4 787 966	4 789 236	4 786 681	4 767 725	4 823 165	4 790 539	4 816 968	4 866 881	4 788 447	4 781 595
Total length (>= 5000 bp)	4 753 165	4 759 438	4 749 950	4 756 651	4 789 151	4 764 458	4 767 071	4 829 336	4 740 015	4 730 276
Total length (>= 10000 bp)	4 705 233	4 692 504	4 654 412	4 736 284	4 718 951	4 719 645	4 720 363	4 794 676	4 678 540	4 656 195
Total length (>= 25000 bp)	4 547 357	4 540 895	4 531 625	4 684 114	4 426 660	4 688 211	4 492 617	4 514 942	4 585 335	4 331 032
Total length (>= 50000 bp)	4 255 866	4 172 914	4 099 664	4 319 169	4 027 451	4 427 823	4 134 582	4 051 451	4 139 288	3 924 317
N50	135 184	117 075	107 984	146 400	133 528	149 628	134 741	102 577	117 105	105 093
N90	45 288	38 094	41 183	58 284	36 562	56 039	38 032	31 906	41 201	25 295
auN	148 302	136 707	149 826	171 868	121 384	176 231	129 713	114 384	127 718	104 260
L50	12	14	13	11	15	11	14	16	14	17
L90	35	39	40	32	43	32	39	46	41	49
GC (%)	52.06	52.06	52.06	52.06	52.06	52.05	52.05	52.07	52.06	52.06
Mismatches										
# N's per 100 kbp	0	0	0	0	0	0	0	0	0	0
# N's	0	0	0	0	0	0	0	0	0	0

Comparing the 10 AUCVM *Sal*. Muenster isolates assemblies with 68 Southeastern U.S. Genbank assemblies (**Figure 4.11)** with Parsnp, the 10 AUCVM *Sal*. Muenster isolates were clustered together as their own branches. This suggests that these isolates are more closely related

when compared to other Southeastern U.S. isolates. Parsnp utilizes core genome SNPs to assess the phylogenetic relationship. The clade of AUCVM *Sal.* Muenster (SRR10740739-SRR10740748) was contained with no other assemblies contained within this clade. There are two main branches, with SRR10740743 and SRR10740745 as one branch and all other AUCVM isolates included within the branch as the earliest isolate assembly of the 2012 SRR10740739.

Figure 4.11. Phylogenetic tree of Southeastern U.S. and AUCVM *Salmonella* Muensters generated by Parsnp.



Based on the Parsnp results of AUCVM isolates being on a contained clade, the 10 AUCVM *Sal*. Muenster isolate assemblies were analyzed as a contained group with no additional assemblies. The pan-genome Prokka-Roary pipeline analysis of the 10 AUCVM *Sal*. Muenster assemblies generated a phylogenetic tree to visualize phylogenetic relationships (**Figure 4.12**). This pipeline displayed three different branches of this group: SRR10740740 and SRR10740744; SRR10740743, SRR10740739, SRR10740747, and SRR10740748; SRR10740746, SRR10740742, SRR10740745, and SRR10740741. The web-based tool Phandango utilizes Roary output files of the Newick tree and the gene presence and absence file to produce an image displaying a phylogenetic tree and a block representing the core and accessory genes present for each assembly (**Figure 4.13**).

Figure 4.12. Phylogenetic of 10 AUCVM *Salmonella* Muensters using Prokka-Roary pipeline.

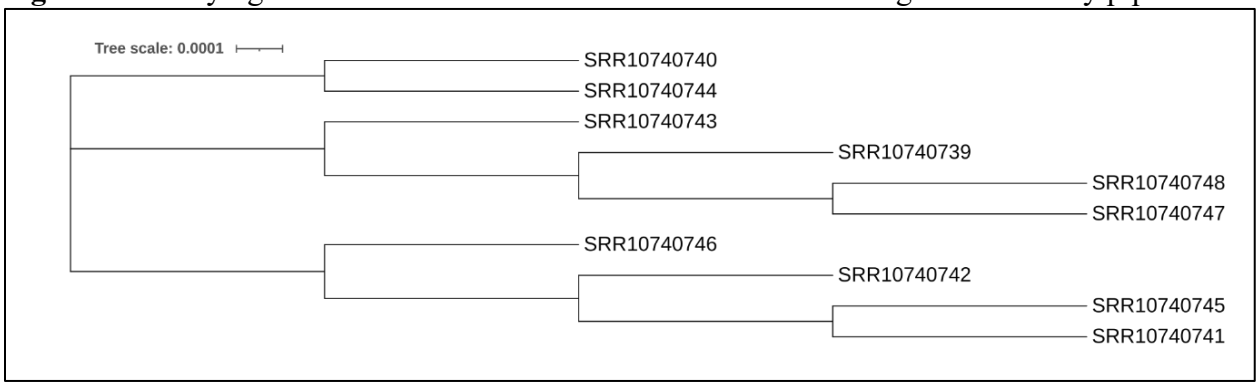
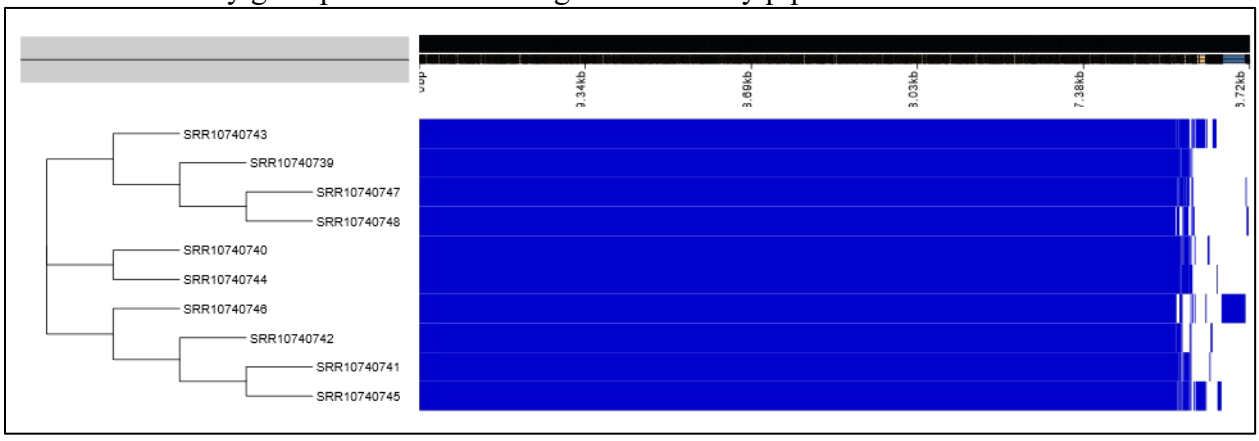


Figure 4.13. Phandango display of phylogenetic tree of 10 AUCVM *Salmonella* Muensters with core and accessory gene presence data using Prokka-Roary pipeline.



The web-based tool CSI Phylogeny was used for SNP detection, and a Newick tree displaying phylogenetic relationships was generated. This tool was most effective with a reference provided (*Sal.* Muenster Genbank assembly ASM842900v2). The 10 AUCVM *Sal.* Muensters fastq files had an average of 96.72% of percent covered of the reference. The resulting phylogenetic tree (**Figure 4.14**) has two main branches, one being SRR10740743 and SRR10740745 and the other containing all other isolates, including the 2012 SRR10740739. The SNP matrix displayed as a heatmap (**Figure 4.15**) shows that the SNPs range between 0 and 36. The number of SNPs between SRR10740743 and SRR10740745 is 4 SNPs. The branch of all other isolates had a SNP range of 0 to 14.

Figure 4.14. Phylogenetic tree of 10 AUCVM *Salmonella* Muensters using CSI Phylogeny.

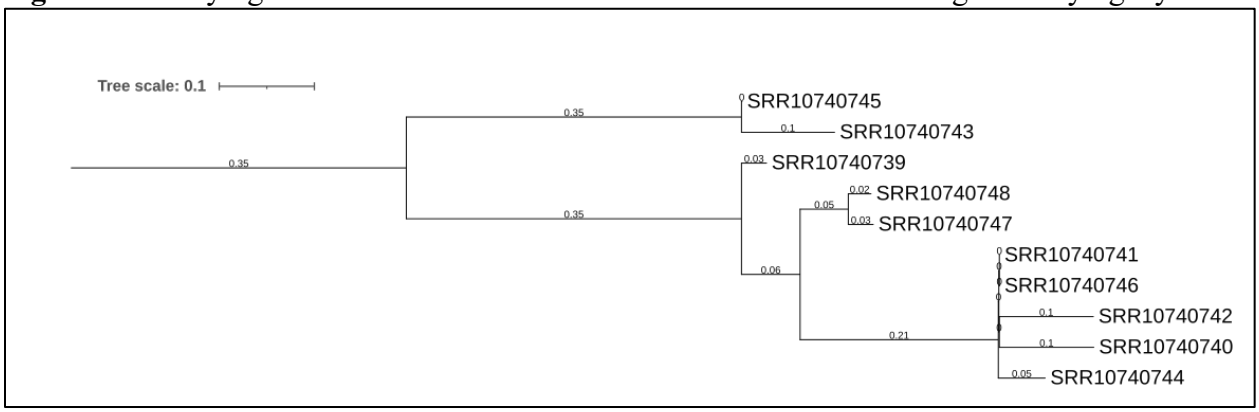
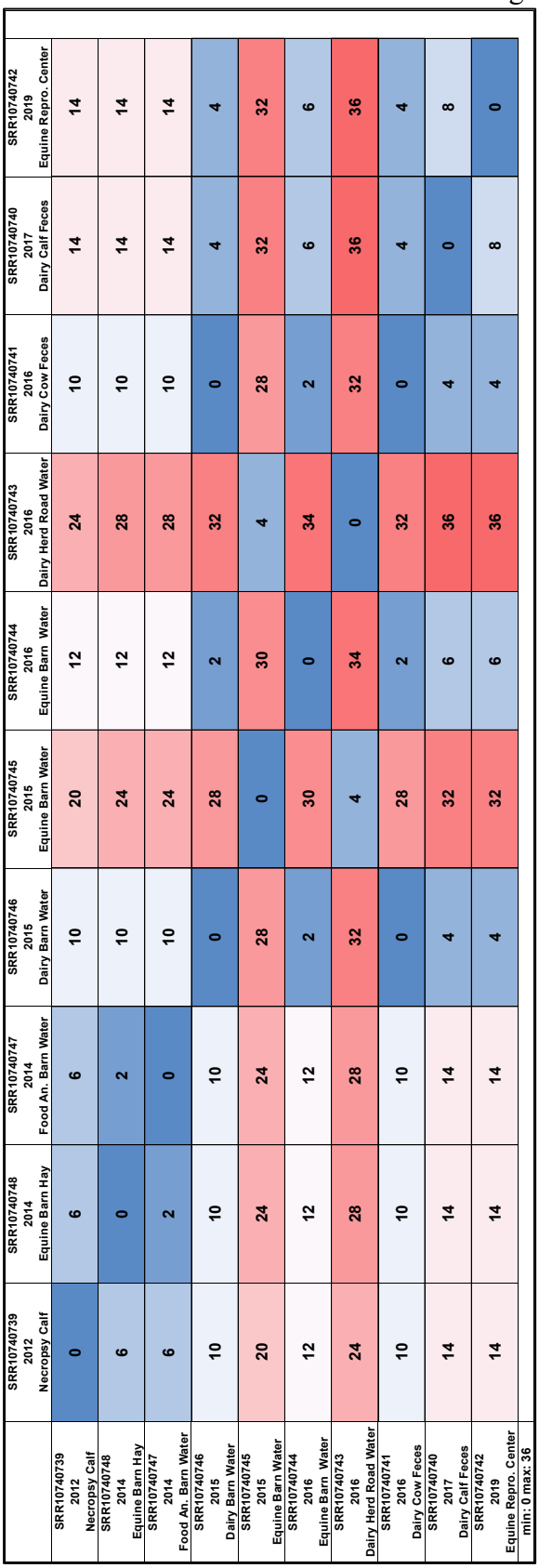


Figure 4.15. SNP Matrix of 10 AUCVM *Salmonella* Muensters using CSI Phylogeny.



	SRR10740739 2012 Necropsy Calf	SRR10740748 2014 Equine Barn Hay	SRR10740747 2014 Food An. Barn Water	SRR10740746 2015 Dairy Barn Water	SRR10740745 2015 Equine Barn Water	SRR10740744 2016 Equine Barn Water	SRR10740743 2016 Dairy Herd Road Water	SRR10740741 2016 Dairy Cow Feces	SRR10740740 2017 Dairy Calf Feces	SRR10740742 2019 Equine Repro. Center
SRR10740739 2012 Necropsy Calf	0	6	6	10	20	12	24	10	14	14
SRR10740748 2014 Equine Barn Hay	6	0	2	10	24	12	28	10	14	14
SRR10740747 2014 Food An. Barn Water	6	2	0	10	24	12	28	10	14	14
SRR10740746 2015 Dairy Barn Water	10	10	10	0	28	2	32	0	4	4
SRR10740745 2015 Equine Barn Water	20	24	24	28	0	30	4	28	32	32
SRR10740744 2016 Equine Barn Water	12	12	12	2	30	0	34	2	6	6
SRR10740743 2016 Dairy Herd Road Water	24	28	28	32	4	34	0	32	36	36
SRR10740741 2016 Dairy Cow Feces	10	10	10	0	28	2	32	0	4	4
SRR10740740 2017 Dairy Calf Feces	14	14	14	4	32	6	36	4	0	8
SRR10740742 2019 Equine Repro. Center	14	14	14	4	32	6	36	4	8	0
min: 0 max: 36										

The web-based tool NDtree also detects SNP and generates a Newick tree representing phylogenetic relationships. This tool was used for comparison to CSI Phylogeny. If no reference is provided, NDtree will use Kmerfinder to a closely related NCBI RefSeq assembly to use a reference. The assembly NC_01194 (*Salmonella* Schwarzengrund CVM19633) was selected as the reference. No statistics were provided from the results to analyze the percentage of reference for each set of fastq reads. The resulting phylogenetic tree (**Figure 4.16**) has two main branches, one being SRR10740743 and SRR10740745 and the other containing all other isolates, including the 2012 SRR10740739. The SNP matrix displayed as a heatmap (**Figure 4.17**) shows that the SNPs range between 0 and 34. The number of SNPs between SRR10740743 and SRR10740745 is 3 SNPs. The branch of all other isolates had a SNP range of 0 to 14.

Figure 4.16. Phylogenetic of 10 AUCVM *Salmonella* Muensters using NDtree.

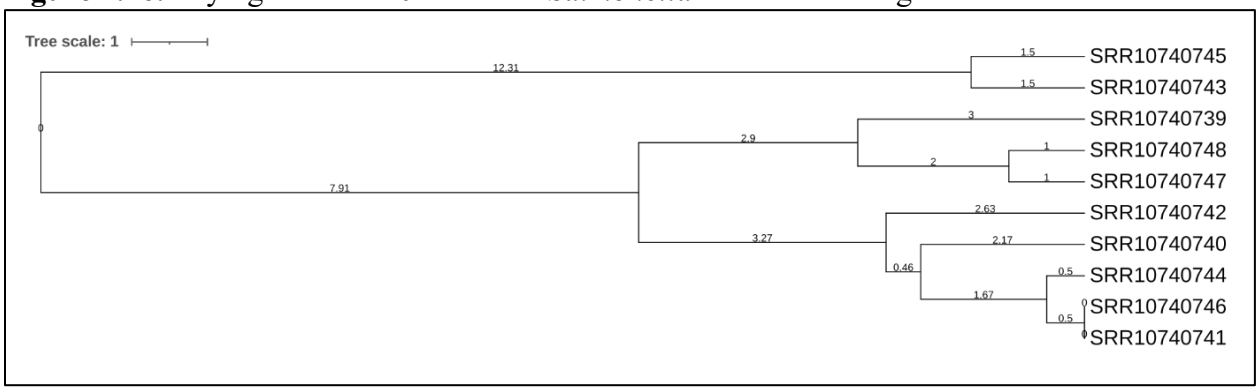
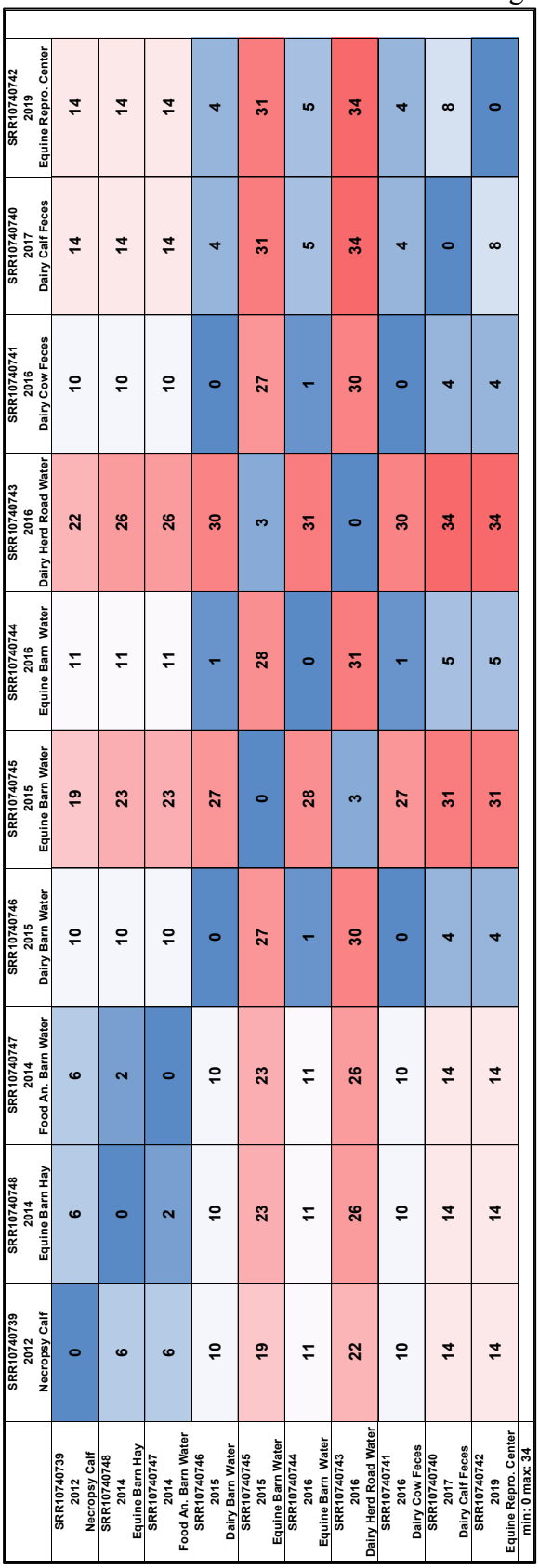


Figure 4.17. SNP Matrix of 10 AUCVM *Salmonella* Muensters using NDtree.



	SRR10740739 2012 Necropsy Calf	SRR10740748 2014 Equine Barn Hay	SRR10740747 2014 Food An. Barn Water	SRR10740746 2015 Dairy Barn Water	SRR10740745 2015 Equine Barn Water	SRR10740744 2016 Equine Barn Water	SRR10740743 2016 Dairy Herd Road Water	SRR10740741 2016 Dairy Cow Feces	SRR10740740 2017 Dairy Calf Feces	SRR10740742 2019 Equine Repro. Center
SRR10740739 2012 Necropsy Calf	0	6	6	10	19	11	22	10	14	14
SRR10740748 2014 Equine Barn Hay	6	0	2	10	23	11	26	10	14	14
SRR10740747 2014 Food An. Barn Water	6	2	0	10	23	11	26	10	14	14
SRR10740746 2015 Dairy Barn Water	10	10	10	0	27	1	30	0	4	4
SRR10740745 2015 Equine Barn Water	19	23	23	27	0	28	3	27	31	31
SRR10740744 2016 Equine Barn Water	11	11	11	1	28	0	31	1	5	5
SRR10740743 2016 Dairy Herd Road Water	22	26	26	30	3	31	0	30	34	34
SRR10740741 2016 Dairy Cow Feces	10	10	10	0	27	1	30	0	4	4
SRR10740740 2017 Dairy Calf Feces	14	14	14	4	31	5	34	4	0	8
SRR10740742 2019 Equine Repro. Center	14	14	14	4	31	5	34	4	8	0
min: 0 max: 34										

4.5. Discussion

The AUCVM J.T. Vaughan Teaching Hospital**,** like many other large animal veterinary teaching hospitals**,** has substantial environmental contamination of *Salmonella* (Schott et al., 2001; Morley, 2002; Cherry et al., 2004; Benedict et al., 2008; Steneroden et al., 2010). This study differs from other studies of *Salmonella* in veterinary teaching hospitals in that it was performed in the absence of an outbreak of clinical disease in university or client animals. We also examined the prevalence of *Salmonella* over three years and analyzed factors that may contribute to the contamination of facilities. This surveillance study of *Salmonella* at a veterinary teaching hospital examined prevalence for a more extended period than most published studies. These findings are similar to previous studies, in which the current study's duration strengthens previous studies' findings. This study was originally designed to serve as a model for environmental prevalence studies of *Salmonella*. The intention was to utilize novel computational approaches such as the use of whole genome sequencing data for strain identification and biostatistical methods to determine factors that may contribute to environmental contamination.

The overall prevalence of *Salmonella-positive* environmental samples at the J.T. Vaughan Teaching Hospital (excluding samples from Animal Health and Research Pastures and the North Auburn off-site beef herd) was 52.77% (n=650). This prevalence appears to be higher than environmental *Salmonella* at other veterinary teaching hospitals. Stenerden et al. (2010) reported that 22.9% of environmental samples collected at the James L. Voss Veterinary Teaching Hospital at Colorado State University were positive for *Salmonella*, with 14.2% of samples containing the outbreak strain, which indicated widespread environmental contamination (Steneroden et al., 2010). An outbreak study at Cornell University found that 0.5% of environmental samples were positive for the outbreak strain (Schott et al., 2001; Cummings et al., 2014). A separate outbreak investigation at Michigan State University found 1.24% of environmental samples cultured and 12% of environmental samples PCR tested for the outbreak strain were positive during facility cleaning and disinfection (Schott et al., 2001). At the New Bolton Center at the University of Pennsylvania School of Veterinary Medicine, 3.3% of environmental samples were positive for *Salmonella* prior to the outbreak. During an outbreak of *Salmonella* Newport within the large animal veterinary teaching hospital, 30% of environmental samples were positive for *Salmonella* Newport, indicating widespread contamination (Schaer et al., 2010). Comparing the prevalence found at our veterinary teaching hospital to these other veterinary teaching hospitals with

environmental contamination issues, it could be concluded that there is widespread contamination of *Salmonella* at the J.T. Vaughan Teaching Hospital at Auburn University.

The sections of highest prevalence (**Figure 4.3**) in our study were the dairy barns/pastures as well as the food animal barns, which had 71.7% and 60.8% positive *Salmonella* prevalence, respectively. Random forest ranked the two dairy facilities and the dairy pastures as important variables for predicting the presence of *Salmonella* (**Figure 4.7**). This is further strengthened by the MCMC analysis, which has the dairy barns/pastures and the food animal barns as variables of significance. Bovine species are the primary patients and resident animals of these facilities and had the highest prevalence of the observed species (**Figure 4.4**). The high level of *Salmonella* prevalence is not surprising because individuals in these units work within both units. It could be presumed that something as simple as individuals moving between these two facilities may be a source of the spread of the *Salmonella*. There are currently no footbaths or any other barriers in place to prevent the movement of infectious disease agents among the facilities, which may increase the likelihood of individuals moving pathogens from one area to another. The sharp decrease in prevalence between food animal barns and equine barns may be attributed to the footbaths located at the front and rear of the equine barns and that individuals working in the equine barns do not work in the food animal or dairy barns.

We found that the environmental prevalence of *Salmonella* was not statistically different ($p > 0.05$) between summer, spring, and winter (**Table 4.4** and **Figure 4.5**), but odds ratios (**Table 4.4**) determined that the summer season was associated with an increased probability of isolation of *Salmonella* compared to the fall season. This finding is similar to previous studies in that the prevalence of *Salmonella* among dairy cattle is higher in the spring, summer, and fall seasons, with peaks typically during the summer months (Fossler et al., 2005; Pangloli et al., 2008; Cummings et al., 2009). Cummings et al. (2009) found fall to be significantly higher for the shedding of *Salmonella* in calves admitted to a veterinary teaching hospital (Cummings et al., 2009). Pangloli et al (2008) found *Salmonella* to have a high prevalence (> 40%) in environmental samples in all seasons with the exception of winter (Pangloli et al., 2008). A study of cattle and environmental sampling factors on *Salmonella* among dairies found that fall, spring, and summer seasons were factors associated with *Salmonella* shedding in cattle (Fossler et al., 2005). We found an increased odds of isolating *Salmonella* during dry weather when compared to recent rainfall, which is

different from previous studies that found the prevalence of *Salmonella* to increase with rainfall (Polo et al., 1999; Haley et al., 2009; Jacobsen and Bech, 2012).

It is not surprising that water (54.3%), drain (47.7%), and fecal (40.8%) sources had the highest prevalence in the environment (**4.6**). Fecal samples and surface (37.2%) were not significantly different (**Table 4.5**), but feed and hay (16.4%) samples were significantly different than all other sample types. It was unsurprising to find water sources with the highest prevalence of *Salmonella* because water can be a source for disseminating enteric pathogens to livestock (Doyle and Erickson, 2006). *Salmonella* was also highly recovered from drain samples, similar to the findings of others who isolated *Salmonella* from floor drains (Castor et al., 1989; Schott et al., 2001; Ward et al., 2005). Others have found that drain surfaces were the most common site of *Salmonella* recovery with a prevalence of 7.3% (Pandya et al., 2009); we also found that the highest prevalence of *Salmonella* was recovered from water and drain sources but drain samples at a much higher level (47.7%) compared to previous studies. The multiple comparisons of means showed that water, drain, and fecal samples are the most important samples to collect when actively surveying for environmental *Salmonella*.

The three different statistical models differed slightly from one another. Still, the important variables indicated were bovine species, summer season, water samples, drain samples, fecal samples, food animal barns, and dairy barns/pastures (**Tables 4.4, 4.6, 4.8, and Figure 4.7**). The random forest model was the most accurate and most sensitive of the three models (**Table 4.7**). Random forest is a non-parametric method, and the variables were ranked according to how well they predict the isolation and non-isolation of *Salmonella* species. This can be useful in determining areas at risk to focus infection control surveillance. The downside is that this does not give information on sources of contamination if an existing outbreak or widespread contamination occurs. The MCMC model provided more narrowed information to assist with the identification of potential sources of contamination. Implementing these algorithms to identify sources of contamination requires causal inferences and commonsense when interpreting the output (Bi et al., 2019; Broadbent and Grote, 2022; Balzer and Westling, 2023).

Machine learning (ML) algorithms are often called a black box because most models are difficult to explain for a single individual (Bi et al., 2019; Wiemken and Kelley, 2019; Hamilton et al., 2021). The use of ML algorithms such as random forest models or MCMC models is lacking, and no standardized approach has been established. This leads to a lack of transparency and

reproducibility challenges compounded by these models being considered a "black box" due to the complexity of the models. These challenges and the expertise of the interdisciplinary components of this research provide additional challenges to using these models. Most research projects encompassing these fields require epidemiologists, clinicians, statisticians, computer scientists, and other personnel such as public health officials, hospital administrators, and researchers to communicate the results and applications (Hamilton et al., 2021).

The use of ML could greatly benefit epidemiologists. Epidemiologists have classically utilized data-focused methods and make various assumptions based on traditional statistical approaches. With traditional hypothesis testing, as more variables are evaluated, the level of statistical error increases substantially (Wiemken and Kelley, 2019). To correct this error, *P*-value adjustments such as Bonferroni corrections are implemented, which can make it difficult to reject the null hypothesis(Rovetta, 2023). ML allows the extraction of patterns from large-scale datasets that normally would be unperceivable (Vilne et al., 2019). ML methods are algorithmic approaches that do not have the issue of being focused on hypothesis testing and can evaluate many variables without increasing statistical error (Wiemken and Kelley, 2019). It is critically important that the training data set is high quality to develop a model to describe the possible patterns and their potential prediction (Wiemken and Kelley, 2019b).

This study also utilizes whole genome sequencing data on bacterial isolates for outbreak detection. The factors associated with prevalence are important, but equally important is providing evidence that a pathogen is related which can be accomplished by analyzing the pathogen's genome. This study looked at four different approaches to investigating bacterial isolate relatedness. Three of the pipelines gave similar results with 10 AUCVM *Sal.* Muenster isolates. Parsnp, CSI Phylogeny, and NDtree all had two major branches, with SRR10740743 and SRR10740745 being on one branch and all others being within the second major branch (**Figures 4.11, 4.14, and 4.16**). These similar findings make sense because all three approaches perform SNP detection on the core genes, which are very stable regions of the bacterial genomes. The findings of two branches would suggest that there are two strains of *Sal.* Muenster located around the AUCVM pastures and facilities. These findings are also supported by looking at the SNP cluster that includes SRR10740739's genome assembly (GCA_015885225.1) (PDS000027449.14 as of 4/24/2024) on the NCBI Pathogen Detection Database. Comparing this study's phylogenetic trees to the NCBI PDD phylogenetic tree (**Figure 4.18**), they all have two major branches, with

the NCBI PDD having the 2012 SRR10740739 placed close to the branch node for all other AUCVM *Sal.* Muenster. The NCBI PDD has 3 additional AUCVM *Sal.* Muenster isolates that were not sequenced by this study; however, they were associated with our 10 AUCVM *Sal.* Muenster isolates.

Figure 4.18. Phylogenetic Tree of NCBI Pathogen Detection Database SNP Cluster PDS000027449.14

The resulting tree of the Prokka-Roary pipeline has three major branches (**Figure 4.13**). This pipeline uses annotated genomes and the presence or absence of genes to determine phylogenetic relationships. This pipeline also did not quantify SNPs or any other type of measure for determining genomic relatedness. Thus, this pipeline would not be recommended when investigating an outbreak.

A downside of the Parsnp analysis was the lack of a generated VCF file or SNP matrix. This result may have been due to the number of additional sequences added to the run because the Parsnp manual describes the generation of a VCF file. CSI Phylogeny and NDtree both generated similar output SNP matrixes. Both used raw fastq files as inputs, but the Kmerfinder selected reference for NDtree was not the same serotype (*Sal.* Schwartzgrund) as the *Sal*. Muensters from the AUCVM. *Sal.* Schwartzgrund is a group B serotype, while *Sal*. Muenster is a group E serotype. I would suspect these serotypes are somewhat different, considering serotypes based on serogrouping. However, *Salmonella* serotypes belong to a subspecies (*enterica*) of a species

(*enterica*) of *Salmonella*, so they are all very closely related. It is still best practice to use a reference as close to queried sequence data as possible. Based on this, the preferred tool to use would be CSI Phylogeny.

Strain determination and SNP cutoffs or thresholds have no widely accepted standard (Octavia et al., 2015; Schürch et al., 2018; Duval et al., 2023; Timsit et al., 2023). Further complicating the subject of SNP cutoffs/thresholds, the SNP cutoffs/thresholds would be dependent on the pathogen of interest and suggested values would be generally applied (Schürch et al., 2018). Octavia (2015) suggests a *Salmonella* SNP cutoff value for whether a case is part of an outbreak as 4 SNPs for an outbreak of one month and a maximum number of 9 SNPs over three months. This is suggested after Octavia (2015) modeled SNP variation over 3 months with different mutation rates (Octavia et al., 2015).

Using the SNP counts provided by CSI Phylogeny, SRR10740743 (Dairy Unit) and SRR10740745 (Equine Unit) only differentiated by 4 SNPs (**Figure 4.15**), and the isolates were isolated 386 days apart. Based on Octavia (2015), these isolates would be considered the same strains, which is intriguing because they were isolated from different units, an outdoor Dairy Herd unit and an Equine barn(Octavia et al., 2015). The isolates, however, appear to be a separate strain from the other set of isolates. The SNP count differences of SRR10740743 and SRR10740745 branch and the other branch ranged from 20 to 36 SNPs (**Figure 4.15**).

Looking at the SNP counts provided by CSI Phylogeny (**Figure 4.15**) for the second major branch, there is a SNP count range between 0 and 14 SNPs over a time period of 2617 days. This second major branch appears to have multiple sub-branches (**Figure 4.14**). The first sub-branch of SRR10740739 (Dairy Unit), SRR10740747 (Food Animal Unit), and SRR10740748 (Equine Unit) SNP count differences ranged between 2 and 6 SNPs over a time period of 909 days. The low end of this range was the 2 SNPs between SRR10740747 and SRR10740748 over a time period of 93 days. The next second sub-branch ranged between 0 and 8 SNPs over a time period of 1493 days. The low end of this range was 0 SNPs between SRR10740741 (Dairy Unit) and SRR10740746 (Dairy Unit) over a time period of 162 days. Within the second sub-branch, the high end of the SNP range was 8 SNPs over a time period of 645 days between SRR10740740 (Dairy Unit) and SRR10740742 (Equine Unit). Between SRR10740740 and SRR10740742, SRR10740742 had the most SNP differences (6 SNPs) with SRR10740744 (Equine Unit), and this was over a 1206-day time period.

With no consensus on a SNP cutoff or threshold or how to calculate SNP cutoff or threshold found in the literature, the threshold of strains over long periods of time could be set at Octavia (2015) suggested 9 SNP cutoff for time periods exceeding 120 days when examining SNPs within the core genes (Octavia et al., 2015). Based on this assumption, there are three strains of *Sal*. Muenster that were isolated from the AUCVM. The strain groups would be as follows: A – SRR10740743 and SRR10740743 (maximum SNPs 4); B – SRR10740739, SRR10740747, and SRR10740748 (maximum SNPs 6); C – SRR10740740, SRR10740741, SRR10740742, SRR10740744, and SRR10740746 (maximum SNPs 8). These three groups fall within the Octavia (2015) SNP cutoff, with time periods being strain A – 386 days, strain B – 909 days, and strain C – 1493 days (Octavia et al., 2015). However, all three strains are closely related, and the SNP analysis with 36 maximum SNPs suggests that these strains are genetically linked and probably all derived from an initial *Sal.* Muenster strain that was introduced into the environment. Sequencing additional isolates could shed further light on this or even uncover additional strains.

Environmental surveillance programs for *Salmonella* have shown a correlation between environmental contamination and infection in animals (Ewart et al., 2001; Burgess et al., 2004; Dunowska et al., 2007; Schaer et al., 2010; Traverse and Aceto, 2015). Identifying the source of environmental contamination or factors associated with contamination is critical for developing interventions to prevent infections in animals. Identifying these factors and showing that isolates are genetically linked is important to develop interventions properly. Hopefully, interventions that interrupt transmission from the environment to animals also help prevent the movement of zoonotic agents into the food chain at the pre-harvest level.

Our results are similar to other studies which suggest that individuals working within the food animal section (dairy barns/pastures and food animal barns) should be more aware of potential risks of nosocomial and zoonotic infections by *Salmonella* and implement intervention strategies to prevent transmission. The food animal section workers should have training in good hygiene, biosecurity, and disease control programs. A good resource that describes the general principles of an infectious disease control program in large animal veterinary hospitals is available (Smith et al., 2004). Currently, at the J.T. Vaughn Large Animal Teaching Hospital, minimal standard operating procedures exist for the monitoring and cleaning practices to contain or prevent *Salmonella* contamination.

No “one size fits all” infection control and prevention program exists. An appropriate infection control plan should be tailored to a facility’s unique operational limits (Dargatz and Traub-Dargatz, 2004; Stockton et al., 2006; Burgess and Morley, 2015). This study found *Salmonella* was associated with dairy barns/pastures and the dairy herd, but this may not be the same at all institutions. *Salmonella* has been documented to move from equine facilities to non-equine patients at Cornell University and the University of Pennsylvania veterinary teaching hospitals (Schaer et al., 2010; Cummings et al., 2014). Analysis of the AUCVM teaching hospital indicates critical control points involving our on-site dairy herd that are unique to the AUCVM. The AUCVM is fortunate to have a model dairy to train students, and analyses from this study should help individuals within these areas to remain vigilant in the prevention of transmission of infectious agents such as *Salmonella*.

This research is the first use of ML algorithms associated with outbreak or prevalence factors within a veterinary hospital setting or animal facility setting. Without the adoption of ML algorithms in hospital or healthcare settings, the real-world applications of ML cannot be realized and further expanded (Hamilton et al., 2021). The continued use of ML is necessary to improve and better utilize ML in epidemiology applications of disease outbreak handling (Riswantini and Nugraheni, 2022). This study is also the first to investigate the genomics of environmental *Salmonella* in an animal facility (veterinary hospital or animal production facility such as a dairy) over a period of time.

4.6. References

Ahrenfeldt, J., Skaarup, C., Hasman, H., Pedersen, A.G., Aarestrup, F.M., Lund, O., 2017. Bacterial Whole Genome-Based Phylogeny: Construction of a New Benchmarking Dataset and Assessment of Some Existing Methods. BMC Genomics 18, 1–13. doi:10.1186/s12864-016-3407-6

Amavisit, P., Markham, P.F., Lightfoot, D., Whithear, K.G., Browning, G.F., 2001. Molecular Epidemiology of *Salmonella* Heidelberg in an Equine Hospital. Vet Microbiol 80, 85–98. doi:10.1016/S0378-1135(00)00373-4

Balzer, L.B., Westling, T., 2023. Demystifying Statistical Inference When Using Machine Learning in Causal Research. Am J Epidemiol 192, 1545–1549. doi:10.1093/aje/kwab200

Benedict, K.M., Morley, P.S., Metre, D.C. Van, 2008. Characteristics of Biosecurity and Infection Control Programs at Veterinary Teaching Hospitals. Journal of American Veterinary Medical Association 233, 767–773.

Besser, J., Carleton, H.A., Gerner-Smidt, P., Lindsey, R.L., Trees, E., 2018. Next-generation sequencing technologies and their application to the study and control of bacterial infections. Clinical Microbiology and Infection 24, 335–341. doi:10.1016/j.cmi.2017.10.013

Bi, Q., Goodman, K.E., Kaminsky, J., Lessler, J., 2019. What is Machine Learning? A Primer for the Epidemiologist. Am J Epidemiol 188, 2222–2239. doi:10.1093/aje/kwz189

Broadbent, A., Grote, T., 2022. Can Robots Do Epidemiology? Machine Learning, Causal Inference, and Predicting the Outcomes of Public Health Interventions. Philos Technol 35, 1–22. doi:10.1007/s13347-022-00509-3

Bruen, T.C., Philippe, H., Bryant, D., 2006. A Simple and Robust Statistical Test for Detecting the Presence of Recombination. Genetics 172, 2665–2681. doi:10.1534/genetics.105.048975

Burgess, B. a, Morley, P.S., Hyatt, D.R., 2004. Environmental Surveillance for *Salmonella enterica* in a Veterinary Teaching Hospital. J Am Vet Med Assoc 225, 1344–1348. doi:10.2460/javma.2004.225.1344

Burgess, B.A., Morley, P.S., 2015. Veterinary Hospital Surveillance Systems. Veterinary Clinics of North America - Small Animal Practice 45, 235–242. doi:10.1016/j.cvsm.2014.11.002

Carleton, H.A., Gerner-smidt, P., 2016. Whole-Genome Sequencing Is Taking over Foodborne Disease Surveillance. Microbe Magazine 11, 311–317. doi:10.1128/microbe.11.311.1

Castor, M., L.Wooley, R.E., Shotts, E.B., Brown, J., Payeur, J.B., 1989. Characteristics of *Salmonella* Isolated From an Outbreak of Equine Salmonellosis in a Veterinary Teaching Hospital. J Equine Vet Sci 9, 236–241.

Cherry, B., Burns, A., Johnson, G.S., Pfeiffer, H., Dumas, N., Barrett, D., Mcdonough, P.L., Eidson, M., 2004. *Salmonella* Typhimurium Outbreak Associated with Veterinary Clinic. Emerg Infect Dis 10, 2249–2251.

Cummings, K.J., Divers, T.J., Mcdonough, P.L., Warnick, L.D., 2009. Fecal Shedding of *Salmonella* spp Among Cattle Admitted to a Veterinary Teaching Hospital. Journal of American Veterinary Medical Association 234, 1578–1585.

Cummings, K.J., Rodriguez-Rivera, L.D., Mitchell, K.J., Hoelzer, K., Wiedmann, M., McDonough, P.L., Altier, C., Warnick, L.D., Perkins, G. a., 2014. *Salmonella enterica* Serovar Oranienburg Outbreak in a Veterinary Medical Teaching Hospital with Evidence of Nosocomial and On-Farm Transmission. Vector-Borne and Zoonotic Diseases 14, 496–502. doi:10.1089/vbz.2013.1467

Dargatz, D. a, Traub-Dargatz, J.L., 2004. Multidrug-Resistant *Salmonella* and Nosocomial Infections. Vet Clin North Am Equine Pract 20, 587–600. doi:10.1016/j.cveq.2004.07.008

Doyle, M.P., Erickson, M.C., 2006. Reducing the Carriage of Foodborne Pathogens in Livestock and Poultry. Poult Sci 85, 960–973.

Dunowska, M., Morley, P.S., Traub-Dargatz, J.L., Davis, M. a, Patterson, G., Frye, J.G., Hyatt, D.R., Dargatz, D. a, 2007. Comparison of *Salmonella enterica* serotype Infantis Isolates from a Veterinary Teaching Hospital. Journal of Ap 102, 1527–1536. doi:10.1111/j.1365-2672.2006.03198.x

Duval, A., Opatowski, L., Brisse, S., 2023. Defining Genomic Epidemiology Thresholds for Common-Source Bacterial Outbreaks: A Modelling Study. Lancet Microbe 4, e349–e357. doi:10.1016/S2666-5247(22)00380-9

Edgar, R.C., 2004. MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput. Nucleic Acids Res 32, 1792–1797. doi:10.1093/nar/gkh340

Ewart, S.L., Schott, H.C., Robison, R.L., Dwyer, R.M., Eberhart, S.W., Walker, R.D., 2001. Identification of Sources of *Salmonella* Organisms in a Veterinary Teaching Hospital and Evaluation of the Effects of Disinfectants on Detection of *Salmonella* Organisms on Surface Materials. J Am Vet Med Assoc 218, 1145–1151. doi:10.2460/javma.2001.218.1145

Fossler, C.P., Wells, S.J., Kaneene, J.B., Ruegg, P.L., Warnick, L.D., Bender, J.B., Eberly, L.E., Godden, S.M., Halbert, L.W., 2005. Herd-level Factors Associated with Isolation of *Salmonella* in a Multi-State Study of Conventional and Organic Dairy Farms I. *Salmonella* Shedding in Cows. Prev Vet Med 70, 257–77. doi:10.1016/j.prevetmed.2005.04.003

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: Quality assessment tool for genome assemblies. Bioinformatics 29, 1072–1075. doi:10.1093/bioinformatics/btt086

Hadfield, J., Croucher, N.J., Goater, R.J., Abudahab, K., Aanensen, D.M., Harris, S.R., 2018. Phandango: An Interactive Viewer for Bacterial Population Genomics. Bioinformatics 34, 292–293. doi:10.1093/bioinformatics/btx610

Haley, B.J., Cole, D.J., Lipp, E.K., 2009. Distribution, Diversity, and Seasonality of Waterborne Salmonellae in a Rural Watershed. Appl Environ Microbiol 75, 1248–1255. doi:10.1128/AEM.01648-08

Hamilton, A.J., Strauss, A.T., Martinez, D.A., Hinson, J.S., Levin, S., Lin, G., Klein, E.Y., 2021. Machine Learning and Artificial Intelligence: Applications in Healthcare Epidemiology. Antimicrobial Stewardship and Healthcare Epidemiology 1, 1–6. doi:10.1017/ash.2021.192

Humphrey, T., 2000. Public-health Aspects of *Salmonella* Infection, in: Wray, C., Wray, A. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, New York, NY, pp. 245–264.

Jacobsen, C.S., Bech, T.B., 2012. Soil Survival of *Salmonella* and Transfer to Freshwater and Fresh Produce. Food Research International 45, 557–566. doi:10.1016/j.foodres.2011.07.026

Kaas, R.S., Leekitcharoenphon, P., Aarestrup, F.M., Lund, O., 2014. Solving the Problem of Comparing Whole Bacterial Genomes Across Different Sequencing Platforms. PLoS One 9, 1–8. doi:10.1371/journal.pone.0104984

Kitchens, S.R., 2016. A Study of a Bacteriophage-Resistant *Salmonella* Mutant and Factors Influencing Prevalence of *Salmonella* in a Multi-Species Animal Facility. Auburn University.

Kitchens, S.R., Michaels, M.T., Price, S.B., 2019. Movement and Persistence of *Salmonella* in a Veterinary Teaching Hospital, in: 2019 Phi Zeta Research Day Forum. Auburn, AL, p. 20.

Kubota, K.A., Wolfgang, W.J., Baker, D.J., Boxrud, D., Turner, L., Trees, E., Carleton, H.A., Gerner-Smidt, P., 2019. PulseNet and the Changing Paradigm of Laboratory-Based Surveillance for Foodborne Diseases. Public Health Reports 134, 22S-28S. doi:10.1177/0033354919881650

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35, 1547–1549. doi:10.1093/molbev/msy096

Leekitcharoenphon, P., Nielsen, E.M., Kaas, R.S., Lund, O., Aarestrup, F.M., 2014. Evaluation of Whole Genome Sequencing for Outbreak Detection of *Salmonella enterica*. PLoS One 9. doi:10.1371/journal.pone.0087991

Leon, I.M., Lawhon, S.D., Norman, K.N., Threadgill, D.S., Ohta, N., Vinasco, J., Scott, H.M., 2018. Serotype Diversity and Antimicrobial Resistance Among *Salmonella enterica* Isolates from Patients at an Equine Referral Hospital. Appl Environ Microbiol 84. doi:10.1128/AEM.02829-17

Letunic, I., Bork, P., 2021. Interactive Tree of Life (iTOL) v5: An Online Tool for Phylogenetic Tree Display and Annotation. Nucleic Acids Res 49, W293–W296. doi:10.1093/nar/gkab301

Lunn, D., Spiegelhalter, D., Thomas, A., Best, N., 2009. The BUGS project: Evolution, Critique and Future Directions. Stat Med 28, 3049–30.67. doi:10.1002/sim.3680

Michaels, M.T., Kitchens, S.R., Price, S.B., 2018. Environmental Sampling for *Salmonella* in an Equine Reproduction Center, in: 2018 Phi Zeta Research Day Forum. Auburn, AL, p. 20.

Morley, P.S., 2002. Biosecurity of Veterinary Practices. Veterinary Clinics of North America: Food Animal Practice 18, 133–155. doi:10.1016/S0749-0720(02)00009-9

Nadon, C., Walle, I. Van, Gerner-smidt, P., Campos, J., Chinen, I., Concepcion-acevedo, J., 2017. PulseNet International: Vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance. Eurosurveillance 22, 1–12. doi:https://doi.org/10.2807/1560-7917.ES.2017.22.23.30544

Neoh, H. min, Tan, X.E., Sapri, H.F., Tan, T.L., 2019. Pulsed-field gel electrophoresis (PFGE): A review of the "gold standard" for bacteria typing and current alternatives. Infection, Genetics and Evolution 74, 103935. doi:10.1016/j.meegid.2019.103935

Octavia, S., Wang, Q., Tanaka, M.M., Kaur, S., Sintchenko, V., Lan, R., 2015. Delineating Community Outbreaks of *Salmonella enterica* Serovar Typhimurium by Use of Whole-Genome Sequencing: Insights into Genomic Variability within an Outbreak. J Clin Microbiol 53, 1063–1071. doi:10.1128/JCM.03235-14

Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T.G., Fookes, M., Falush, D., Keane, J.A., Parkhill, J., 2015. Roary: Rapid large-scale prokaryote pan genome analysis. Bioinformatics 31, 3691–3693. doi:10.1093/bioinformatics/btv421

Pandya, M., Wittum, T., Tadesse, D.A., Gebreyes, W., Hoet, A., 2009. Environmental *Salmonella* Surveillance in The Ohio State University Veterinary Teaching Hospital. Vector-Borne and Zoonotic Diseases 9, 649–654. doi:10.1089/vbz.2008.0120

Pangloli, P., Dje, Y., Ahmed, O., Doane, C.A., Oliver, S.P., Draughon, F.A., 2008. Seasonal Incidence and Molecular Characterization of *Salmonella* from Dairy Cows, Calves, and Farm Environment. Foodborne Pathog Dis 5, 87–96. doi:10.1089/fpd.2008.0048

Polo, F., Figueras, M.J., Inza, I., Sala, J., Fleisher, J.M., Guarro, J., 1999. Prevalence of *Salmonella* Serotypes in Environmental Waters and Their Relationships with Indicator Organisms. Antonie Van Leeuwenhoek 75, 285–92. doi:10.1023/A:1001817305779

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 - Approximately Maximum-Likelihood Trees for Large Alignments. PLoS One 5. doi:10.1371/journal.pone.0009490

Price, M.N., Dehal, P.S., Arkin, A.P., 2009. Fasttree: Computing Large Minimum Evolution Trees with Profiles Instead of a Distance Matrix. Mol Biol Evol 26, 1641–1650. doi:10.1093/molbev/msp077

Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A., Korobeynikov, A., 2020. Using SPAdes *de novo* Assembler. Curr Protoc Bioinformatics 70, e102. doi:10.1002/cpbi.102

Ribot, E.M., Freeman, M., Hise, K.B., Gerner-Smidt, P., 2019. PulseNet: Entering the Age of Next-Generation Sequencing. Foodborne Pathog Dis 16, 451–456. doi:10.1089/fpd.2019.2634

Riswantini, D., Nugraheni, E., 2022. Machine Learning in Handling Disease Outbreaks: A Comprehensive Review. Bulletin of Electrical Engineering and Informatics 11, 2169–2186. doi:10.11591/eei.v11i4.3612

Rose, B.E., 2014. Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Catfish Products and Carcass and Environmental Sponges, in: Dey, B.P., Lattuada, C.P. (Eds.), USDA/FSIS/OPHS Microbiology Laboratory Guidebook. Washington, D.C., p. MLG 4.08.

Rovetta, A., 2023. Common Statistical Errors in Scientific Investigations: A Simple Guide to Avoid Unfounded Decisions. Cureus 15, 1–9. doi:10.7759/cureus.33351

Salipante, S.J., SenGupta, D.J., Cummings, L.A., Land, T.A., Hoogestraat, D.R., Cookson, B.T., 2015. Application of Whole-Genome Sequencing for Bacterial Strain Typing in Molecular Epidemiology. J Clin Microbiol 53, 1072–1079. doi:10.1128/JCM.03385-14

Santangelo, O.E., Gentile, V., Pizzo, S., Giordano, D., Cedrone, F., 2023. Machine Learning and Prediction of Infectious Diseases: A Systematic Review. Mach Learn Knowl Extr 5, 175–198. doi:10.3390/make5010013

Schaer, B.L.D., Aceto, H., Rankin, S.C., 2010. Outbreak of Salmonellosis Caused by *Salmonella enterica* Serovar Newport MDR-AmpC in a Large Animal Veterinary Teaching Hospital. J Vet Intern Med 24, 1138–1146.

Schott, H.C., Ewart, S.L., Walker, R.D., Dwyer, R.M., Dietrich, S., Eberhart, S.W., Kusey, J., Stick, J. a, Derksen, F.J., 2001. An Outbreak of Salmonellosis Among Horses at a Veterinary Teaching Hospital. J Am Vet Med Assoc 218, 1152–1159. doi:10.2460/javma.2001.218.1152

Schürch, A.C., Arredondo-Alonso, S., Willems, R.J.L., Goering, R. V., 2018. Whole Genome Sequencing Options for Bacterial Strain Typing and Epidemiologic Analysis Based on Single Nucleotide Polymorphism Versus Gene-by-Gene–Based Approaches. Clinical Microbiology and Infection 24, 350–354. doi:10.1016/j.cmi.2017.12.016

Sebola, D.C., Oguttu, J.W., Kock, M.M., Qekwana, D.N., 2023. Hospital-Acquired and Zoonotic Bacteria from a Veterinary Hospital and Their Associated Antimicrobial-Susceptibility Profiles: A Systematic Review. Front Vet Sci 9. doi:10.3389/fvets.2022.1087052

Seemann, T., 2014. Prokka: Rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069. doi:10.1093/bioinformatics/btu153

Smith, B.P., House, J.K., Magdesian, K.G., Jang, S.S., Cabral, R.L., Madigan, J.E., Herthel, W.E., 2004. Principles of an Infectious Disease Control Program for Preventing Nosocomial Gastrointestinal and Respiratory Tract Diseases in Large Animal Veterinary hospitals. J Am Vet Med Assoc 225, 1186–1195. doi:10.2460/javma.2004.225.1186

Soza-Ossandón, P., Rivera, D., Tardone, R., Riquelme-Neira, R., García, P., Hamilton-West, C., Adell, A.D., González-Rocha, G., Moreno-Switt, A.I., 2020. Widespread Environmental Presence of Multidrug-Resistant *Salmonella* in an Equine Veterinary Hospital That Received Local and International Horses. Front Vet Sci 7, 1–9. doi:10.3389/fvets.2020.00346

Steneroden, K.K., Metre, D.C. Van, Jackson, C., Morley, P.S., 2010. Detection and Control of a Nosocomial Outbreak Caused by *Salmonella* Newport at a Large Animal Hospital. J Vet Intern Med 24, 606–616.

Stockton, K. a, Morley, P.S., Hyatt, D.R., Burgess, B. a, Patterson, G., Dunowska, M., Lee, D.E., 2006. Evaluation of the Effects of Footwear Hygiene Protocols on Nonspecific Bacterial Contamination of Floor Surfaces in an Equine Hospital. J Am Vet Med Assoc 228, 1068–1073. doi:10.2460/javma.228.7.1068

Timsit, S., Armand-Lefèvre, L., Le Goff, J., Salmona, M., 2023. The Clinical and Epidemiological Impacts of Whole Genomic Sequencing on Bacterial and Virological Agents. Infect Dis Now 54, 104844. doi:10.1016/j.idnow.2023.104844

Tolar, B., Joseph, L.A., Schroeder, M.N., Stroika, S., Ribot, E.M., Hise, K.B., Gerner-Smidt, P., 2019. An Overview of PulseNet USA Databases. Foodborne Pathog Dis 16, 457–462. doi:10.1089/fpd.2019.2637

Tran, A., Rowlinson, M.-C., 2019. Application of Next-Generation Sequencing in Public Health Epidemiology and Outbreak Investigation. Advances in Molecular Pathology 2, 89–97. doi:10.1016/j.yamp.2019.07.007

Traverse, M., Aceto, H., 2015. Environmental Cleaning and Disinfection. Veterinary Clinics of North America - Small Animal Practice 45, 299–330. doi:10.1016/j.cvsm.2014.11.011

Treangen, T.J., Ondov, B.D., Koren, S., Phillippy, A.M., 2014. The Harvest Suite for Rapid Core-Genome Alignment and Visualization of Thousands of Intraspecific Microbial Genomes. Genome Biol 15, 1–15. doi:10.1186/s13059-014-0524-x

USDA, 2019. Delay in FSIS' Plans to Discontinue PFGE for *E. coli* and Move to WGS as the Primary Characterization Tool FSIS. FSIS Constituent Update 22, 1–3.

Vilne, B., Meistere, I., Grantiņa-Ieviņa, L., Ķibilds, J., 2019. Machine Learning Approaches for Epidemiological Investigations of Food-Borne Disease Outbreaks. Front Microbiol 10. doi:10.3389/fmicb.2019.01722

Walther, B., Tedin, K., Lübke-Becker, A., 2017. Multidrug-Resistant Opportunistic Pathogens Challenging Veterinary Infection Control. Vet Microbiol 200, 71–78. doi:10.1016/j.vetmic.2016.05.017

Ward, M.P., Brady, T.H., Coue, L.L., Liljebjelke, K., Maurer, J.J., Ching, C., 2005. Investigation and Control of an Outbreak of Salmonellosis Caused by Multidrug-Resistant *Salmonella* Typhimurium in a Population of Hospitalized Horses. Vet Microbiol 107, 233–240. doi:10.1016/j.vetmic.2005.01.019

Wiemken, T.L., Kelley, R.R., 2019. Machine Learning in Epidemiology and Health Outcomes Research. Annu Rev Public Health 41, 21–36. doi:10.1146/annurev-publhealth-040119-094437

Chapter 5

Discussion

5.1 *Salmonella* Introduction

The work described in this dissertation examines different fields of the microbiology of *Salmonella*. The first area dealt with genomic analysis of a bacteriophage-resistant mutant of *Salmonella* to identify a mutation that would confer the phenotype of phage-resistance and potentially explain fecal shedding from the calf model from my master's thesis (Kitchens, 2016). The second area dealt with using a chicken embryo lethality assay and potential uses as a model to transition from *Salmonella in vitro* experiments to *Salmonella in vivo* experiments. The third area dealt with utilizing novel statistical methods to determine factors that are associated with environmental *Salmonella* prevalence and an outbreak *Salmonella* strain detection. This dissertation encompasses bioinformatics of *Salmonella*, *Salmonella* and phage therapy *in ovo* modeling, and microbial epidemiology.

5.2 Genomic Analysis of a Bacteriophage-Resistant Mutant of *Salmonella*

Bacteriophage (phage) therapy is a growing area of interest, and the unintended consequence of generating phage-resistant mutants of pathogens of interest should be explored. The phage-resistant mutant of *Sal*. Newport attenuation in a calf model has been explored (Kitchens, 2016). The next step would be to identify the mutation that could confer the phage-resistance and attenuation.

It was previously reported that the phage-resistant mutant of *Sal*. Newport (*Sal.* Newport Mut$^{\Phi}$) was resistant to four (S11, S40, S41, S50) out of five (S44) of the phages from a phage cocktail designed to treat *Sal*. Newport infected calves. This conclusion was made based on spot lysis of the phage lysates onto a double agar lawn of *Sal*. Newport, as only S44 could still cause lysis. However, the spot lysis was opaque as opposed to the complete, clear lysis, as seen on the *Sal*. Newport parent. Also, S44 was able to cause lysis in broth culture, but not as dramatic as S44 lysis in broth with the *Sal*. Newport parent (Kitchens, 2016). The current study calculated the adsorption kinetics of the phage to the *Sal*. Newport parent and the *Sal.* Newport Mut$^{\Phi}$ (**Table 2.4**). The adsorption kinetic rates of S11, S40, and S41 were all negative values due to the formula used for calculation and the endpoint phage titer (count) being slightly higher than the initial titer. The endpoint titers were within a 10% difference from the initial titer, which is an acceptable

percentage of error when counting plaques. The titration over time bounced around the initial titer, so the adsorption kinetics rate is no different than the initial titer. There was a slight decrease in the adsorption kinetics rate for S44 on *Sal.* Newport parent compared to *Sal.* Newport Mut$^{\Phi}$. One interesting finding is that the adsorption kinetics rate of S11 and S44 on the *Sal*. Newport parent ($2.21x10^{-10}$ mL/min and $8.66x10^{-10}$ mL/min, respectively) was much lower than S40, S41, and S50 on the *Sal*. Newport parent ($2.28x10^{-9}$ mL/min, $1.35x10^{-9}$ mL/min, and $3.28x10^{-9}$ mL/min, respectively). A study by Yu *et al*. (2024) described phage-resistance as an EOP below 10^{-9}. By this standard, the *Sal*. Newport parent was resistant to both S11 and S44 phage before any selection. It appears that the EOP value of 10^{-9} is an arbitrary cutoff that Yu *et al*. choose as opposed to using a reference to support that value (Yu et al., 2024).

Short-read sequencing alone was not able to identify any variant that would confer resistance to the phage, regardless of whether a reference was used for the GATK pipeline or Snippy. The same was true for the draft assembly of the *Sal*. Newport parent and using the GATK pipeline or Snippy against the *Sal.* Newport Mut$^{\Phi}$. The short-read and long-read sequencing with the hybrid assembly, however, did identify a variant that could potentially confer phage-resistance.

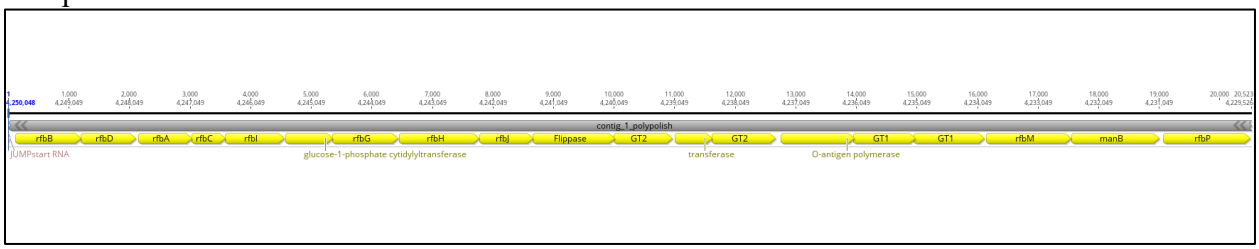
Whole-genome alignment of the *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ detected four SNPs. Two were synonymous mutations, while the other two were nonsynonymous mutations. The nonsynonymous mutations are mutations that cause changes in the amino acid sequence and, thus, are the only mutations of concern. The two nonsynonymous mutations were in a gene for phage tail-collar protein and in the *rfbM* gene, which is important for the formation of the O-antigen. The nonsynonymous mutation in the *rfbM* gene is more likely responsible for the phage-resistance phenotype found in the *Sal.* Newport Mut$^{\Phi}$.

A quick blast search on NCBI with the top 100 multiple sequence alignments (*Sal*. Newport parent – **Supplemental Figure 1** and *Sal.* Newport Mut$^{\Phi}$ – **Supplemental Figure 2**) shows the amino acid change at position 29. The top 100 hits of the *Sal*. Newport parent show that all 100 matches have the same amino acid (serine) at position 29. Regarding the *Sal.* Newport Mut$^{\Phi}$, the first hit was 100% match and had phenylalanine at position 29. The remaining 99 hits all have a serine at position 29. So, it appears that this position is highly conserved, and it would be suspected the reasoning would be that this position is important for the confirmation of the protein structure.

Visualization of the *rfb* gene cluster with Geneious Prime (**Figure 5.1**) gives a layout of this gene cluster that is responsible for the O-antigen biosynthesis of both the *Sal*. Newport parent

and *Sal.* Newport Mut$^{\Phi}$. Each *rfb* gene encodes an enzyme that is responsible for the production of a sugar molecule. The *rfbM* gene is two genes from the end of the gene cluster. The O-antigen is composed of repeating units of three sugars and can range up to 40 units (Hong et al., 2023). Theoretically, the mutation in *rfbM* could either hinder the production of the third sugar or stop the chain from the elongating period. The effect of this mutation at this point would be entirely speculative. We did show that some portion of the O-antigen is present in the *Sal.* Newport Mut$^{\Phi}$. This is based on the results using the *Salmonella* O antiserum. The *Sal.* Newport Mut$^{\Phi}$ was able to agglutinate with all three antisera (PolyA – I and Vi; Poly B; Group C_2 Factor 6/8), which are polyclonal and specific for certain serogroup O antigens.

Figure 5.1: Geneious Prime image of the *rfb* gene cluster found in *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$.

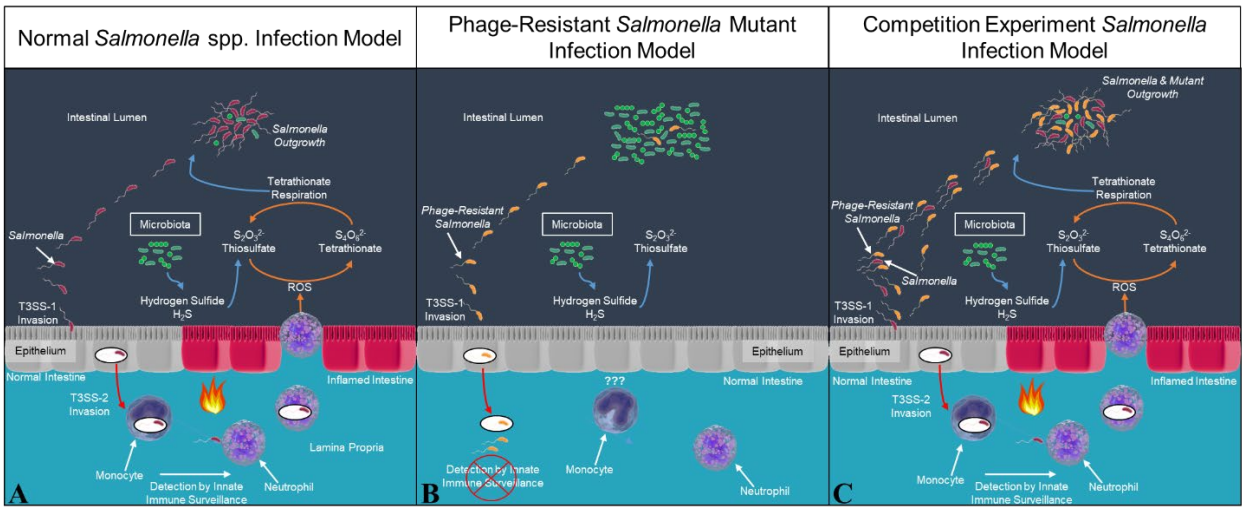


Based on the assumption that the *rfbM* mutation is responsible for the phage-resistance phenotype, the model explains the unusual mutant shedding found in the competition experiment between the *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ in calves. (Kitchens, 2016). A review of this model can be found in Chapter 1.7.2. It was originally proposed that the mutation interfered with the expression of the T3SS-1 (or less likely T3SS-2) and, therefore, the *Sal.* Newport Mut$^{\Phi}$ could not invade the gut epithelium, which would never lead to the cascade where *Salmonella* stimulates the immune system. Sense there is no inflammation, there is no neutrophil recruitment and no reactive oxygen species are produced. This would prevent thiosulfate ($S_2O_3^{2-}$) from being oxidized to tetrathionate (S4O62–) and the *Sal.* Newport Mut$^{\Phi}$ could not outcompete the normal gut microbiota. However, during the competition experiment, the *Sal*. Newport parent could invade and the tetrathionate ($S_4O_6^{2-}$) cascade occurs, which allows the *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ to outcompete the normal gut microbiota.

The updated model based on this study's findings can be found in **Figure 5.2**. This model suggests that the *rfbM* mutation led to a truncated O-antigen being formed. The O-antigen being

truncated versus not being present at all might allow the *Sal*. Newport to still be resistant to complement-mediated lysis (Bjanes and Nizet, 2021; Han et al., 2024). However, the truncated O-antigen could make it difficult for complement C3a and C5a opsonization and chemoattraction, which are both important as one marks the cell for phagocytosis and the other being an important proinflammatory chemoattractant, respectively (Krzyżewska-Dudek et al., 2022). A truncated O-antigen could protect the bacterium from serum-mediated lysis and make the cell resist to phagocytosis or the C3 opsonization, which both lead to inflammation. A study by Murray *et al*. (2006) found that mutations that produce truncated O-antigens can have resistance to complement-mediated lysis if four or more repeat sugar units are present, but the O-antigen needs more than fifteen sugar repeat units for complement activation. So, a *Salmonella* bacterium with an O-antigen greater than four and less than fifteen can have the benefit of resistance to complement-mediated lysis and resistance to complement ((Murray et al., 2006). This change to the model would allow the *Sal.* Newport Mut$^{\Phi}$ to invade the epithelium, but the bacterium does not stimulate the immune system because the decreased or lack of complement C3a opsonization and reduced macrophage phagocytosis. This would decrease the inflammation associated with salmonellosis, and therefore, the neutrophils are not recruited to the same extent and prevent thiosulfate ($S_2O_3^{2-}$) from being oxidized to tetrathionate ($S_4O_6^{2-}$). However, in the competition experiment, the *Sal*. Newport parent has normal infection dynamics, including, inflammation, neutrophil recruitment, production of tetrathionate ($S_4O_6^{2-}$), and therefore *Sal*. Newport parent and *Sal.* Newport Mut$^{\Phi}$ both can outcompete the normal gut microbiota. This updated model would also explain that in the experiment where calves received ten times the normal dose, those calves had elevated body temperature and abbreviated diarrhea, which was characterized as mild salmonellosis (Kitchens, 2016). The updated model provides the assumption from Murray *et al*. (2006) that the complement resistance is more of a reduced likelihood of the complement binding because the O-antigen is shorter but does not necessarily eliminate the possibility (Murray et al., 2006). Using this assumption, the ten times dose could lead to an increased chance of complement binding, and the *Salmonella*-induced inflammation occurs, but at a much lower amount. This could be responsible for mild salmonellosis.

Figure 5.2: Updated hypothetical model of dynamics in calf experimental infections.



5.3 Chicken Embryo Lethality Assay with *Salmonella*

This study was inspired by the methods publication "Use of a Chicken Embryo Lethality Assay to Assess the Efficacy of Phage Therapy" by Trotereau et al. (2006). This methods publication discussed using the chicken embryo lethality assay to screen phage for phage therapy against avian pathogenic *Escherichia coli* (APEC). It was suggested to be a relevant, straightforward model before conducting controlled clinical trials (targeted animals or humans). A phage treatment dose of $2x10^3$ PFU/egg was suggested. Trotereau *et al*. (2019) used an example of their phage ESC05 to show a 100% survival of chicken embryos infected with APEC and phage treated, compared to only a 30% survival rate for control chicken embryos infected with APEC with no treatment. (Trotereau and Schouler, 2019). Our question was, could this method be used for screening *Salmonella* Newport phage for survival protection prior to experimental trials in our calf model?

The results of this study were not as clear regarding phage treatment in *Salmonella*-infected chicken embryos as Trotereau *et al*. (2019) found in the method with APEC. We initially started with our most active phage (S50) against *Sal*. Newport. We found only a significant difference for S50 (MOI≈10^5) (p=0.0044) compared to the *Sal*. Newport control group. At the MOI≈10^5 to MOI≈10^7 of S50, survival rates ranged from between 40% to 60%. This was noticeably less than

the findings by Trotereau *et al*. (2006). Based on the very high MOIs of S50 to have any treatment effect, we moved to testing different *Salmonella* serovars.

Due to the food safety concern of *Sal*. Enteritidis vertical transmission from hen to egg and that *Sal*. Enteritidis foodborne infections are associated with the consumption of eggs, *Sal*. Enteritidis was chosen as the next serovar tested with phage therapy in the chicken embryo lethality assay (Howard et al., 2012). No significant differences were found between them compared to *Sal*. Enteritidis control until an MOI≈10^4 of S10 (p=0.0162 and p=0.0031) and no significant difference was found for S7 (MOI≈10^4; p=0.6053 and p=0.3358). Based on these findings, there were differences in the treatment effect of different phages against *Salmonella*, so the purpose of the model to compare different phages is a possibility. The downside was that it was not as noticeable as the example by Trotereau *et al.* (2019).

The chicken embryo lethality assay was beneficial when examining virulence of mutants of *Salmonella*. This was observed in the experiment with the rough *Salmonella* (O-:r:1,5) at the normal phage dose and a ten-fold higher dose compared to a *Sal*. Infantis control (p=0.0479 and p=0.1216, respectively). There was significant difference found between the *Sal*. Infantis control and the normal dose of *Salmonella* (O-:r:1,5) (p=0.0479). Therefore, the chicken embryo lethality assay could be used to test for attenuation.

Currently (3/11/2024) only two manuscripts using the chicken embryo lethality assay and phage have cited Trotereau *et al*. (2019), with one being Nicolas *et al*. (2023) and a preprint manuscript by Hao *et al*. (2023) (Nicolas et al., 2023a; Hao et al., 2024). Nicolas *et al*. (2023) and Trotereau *et al*. (2019) both have Catherine Schouler as the corresponding author and both use the chicken embryo lethality assay for APEC and phage (Trotereau and Schouler, 2019; Nicolas et al., 2023a). Hao *et al*. (2024) is a preprint manuscript using the chicken embryo lethality assay to evaluate phage and *Salmonella* Enteritidis. Hao *et al*. (2024) modified Trotereau *et al*. (2019) methods by reducing the bacterial inoculum by twenty-fold and inoculating the eggs with phage one hour prior to the bacterial challenge as opposed to the two hours after challenge by Trotereau *et al*. (2019) (Nicolas et al., 2023a; Hao et al., 2024). Hao *et al*. had a 53% survival rate in phage-treated groups and a 33% survival rate in *Sal*. Enteritidis control groups.

Our study was faithful to the methodology by Trotereau *et* al. (2019) apart from increasing the MOI of phage treatments (Trotereau and Schouler, 2019). Our study contrasts Hao *et al*. (2024) by the bacterial inoculum dose and the timing for phage treatment. We considered decreasing the

bacterial inoculum, but at a ten-fold decrease, the inoculum is not consistent per egg due to such low CFUs per mL. We also avoided treating the chicken embryos with phage prior to or at time of bacterial challenge (Hao et al., 2024). The concern was that Hao et al.'s findings were based on the immediate infection and lysis of cells as opposed to allowing *Salmonella* to begin infection, which we considered a more representative "treatment" of infection.

There was a difference in obtaining significant results when comparing experiments involving phage protection against *Sal*. Enteritidis in the chicken embryo lethality assay versus using the chicken embryo lethality assay to examine the virulence of mutants in *Salmonella*. It appears that in using this model, one must temper their expectations when examining *Salmonella*. This may be because *Salmonella* is a virulent pathogen that does well infecting and amplifying in a chicken embryo. *Salmonella* has been used in chicken embryo lethality models but is typically a positive control for egg lethality when examining other types of bacterial organisms and mutants of those bacterial organisms (Adam et al., 2002; Rezaee et al., 2021). Thus, th chicken embryo lethality model for *Salmonella* can be useful as long as researchers are aware of the high mortality *Salmonella* causes in this model at relatively low doses

5.4 Computational Approaches in *Salmonella* Outbreak Investigation

Originally, a two-year study of the environmental prevalence of *Salmonella* was undertaken at the Auburn University College of Veterinary Medicine (AUCVM) John Thomas Vaughan Large Animal Teaching Hospital. The original study used risk ratios to determine factors that contribute to the risk of *Salmonella* prevalence in the environment. Summer season, water samples, drain swabs, building or indoor samples, dairy barns and pastures, and food animal barns were associated with the isolation of *Salmonella* (Kitchens, 2016). This current study was different based on the addition of a third year of sampling and looking at novel statistical methods for evaluating factors contributing to *Salmonella*'s prevalence. This study also examined sequence data from *Sal*. Muenster isolates from different units at the AUCVM.

The three data analysis methods were logistic regression with random effects, random forest analysis, and Markov Chain Monte Carlo (MCMC). All three models are supervised machine learning algorithms and require that data be split into two groups: train data to train each model and test data to calculate accuracy, sensitivity, and specificity. Each approach had its own pros and cons and its own way of determining factors important to *Salmonella*'s environmental prevalence.

The logistic regression's output was based on odds ratios. The variable with the lowest prevalence attributed to a group of variables was used as the reference. A multiple comparison of means (Tukey Contrasts) allowed us to group variables that were not significantly different from one another (**Table 4.5**). Based on the groupings, we were able to rank variables and determine variables with higher odds of association with the variable contributing to environmental *Salmonella* (**Table 4.6**). The benefit of this model is that it is one of the most widely used statistical methods when analyzing multivariate data (Vaessen et al., 1998; Muñoz-Cuevas et al., 2012; Mughini-Gras et al., 2014; Denagamage et al., 2015; Williams et al., 2016; Burgess and Morley, 2018, 2019; Rivera et al., 2021). The variables considered most associated with *Salmonella* in the environment were bovine species, equine species, summer season, spring season, winter season, water samples, drain samples, and fecal samples. No facilities, units, or regions were analyzed in this model due to the inclusion of random effects and trying to mitigate the effect of certain facilities and units increased positive samples. This way, we could determine the other factors uniformly.

With the random forest analysis, variables were ranked as the most important for predicting *Salmonella* prevalence (**Table 4.7**). One downside of this analysis is that it includes variables ranked most important for predicting *Salmonella* prevalence or being positive and negative for *Salmonella*. It is important to understand that negatively associated variables are ranked amongst the positive variables. For this reason, the North Auburn facility and region are ranked highest because there was only one positive sample for the entire duration of the three years. If we look at just the variables that had a high prevalence and importance greater than 50, we find samples collected from dairy herd pastures, pastures and outdoor samples, samples collected from the McClary Dairy Parlor, and samples collected from the McClary Dairy Barn as the most important for the presence of *Salmonella*.

The final analysis was the MCMC analysis. This analysis generated parameter values of the variables and a 95% Confidence Interval of each parameter. Any parameter (variable) that does not have a zero value in the 95% Confidence Interval was selected for the final model from the MCMC. These parameters are what are determined to be important for the prevalence of *Salmonella* (**Table 4.8)**. The values that are below zero in the 95% Confidence Interval are considered important for not isolating *Salmonella*, which was only in rainy weather conditions. The variables contributing to *Salmonella*'s presence were surface samples, drain samples, fecal

samples, water samples, summer season samples, samples from the dairy barns and facilities, samples from the food animal barns, and samples from bovine species.

These machine learning algorithms and models aim for high predictive accuracy as opposed to a *p*-value. Each model uses the test data to evaluate the model's accuracy (measure correctly labeled data instances over total number of instances), sensitivity (model to correctly identify true cases), and specificity (model to directly identify negative cases). This information is summarized in **Table 4.7**. The Random Forest model was the most accurate and sensitive but was the least specific. The logistic regression was the most specific. Based on these findings, the Random Forest analysis appears to be the superior model by being the most accurate and determining true cases. This model has the highest chance of false positives by being the least specific.

The Random Forest model appears to be the best model for determining variables associated with the prevalence of *Salmonella* in the environment. This model allows for individual barns, buildings, and pastures to be included in the model as opposed to the other two models needing these to be consolidated into units or regions. The downside of the model is the ranking of variables associated with isolation and non-isolation of *Salmonella*. The logistic regression had the best specificity, but the ranking of variables and that most variables were considered not different from one another can be problematic for determining important variables. The MCMC was not the best in any of the categories of accuracy, sensitivity, and specificity; however, it seemed to be the more balanced model. The model is easy to understand once you understand that the parameters or variables to include in the model are the ones that the Confidence Interval is entirely above or below zero. With this knowledge, the variables associated with isolating *Salmonella* are easy to understand. Based on all the models, the factors most associated with the prevalence of *Salmonella* would be samples collected from bovine species, samples collected during the summer season, and samples collected from any of the dairy barns and the pastures used by the dairy section.

Only one publication has examined risk factors for veterinary hospital environmental contamination with *Salmonella* (Burgess and Morley, 2018). Burgess *et al*. (2018) evaluated 5273 environmental samples from routine surveillance for *Salmonella* at the Colorado State University Veterinary Teaching Hospital. Data regarding variables of interest were obtained from an electronic medical records database. Burgess *et al*. (2018) used logistic regression with a selection

of variables based on univariable associations, variable cluster analysis, and variable loading on principal components. Factors describing contamination risk include hospital type (livestock hospital), species, number of days patients are shedding *Salmonella*, and caseload. The probability of detecting *Salmonella* in the environment increased as demand for personnel increased. Principle components analysis was the most beneficial for understanding the correlation in variance structure related to similar variables. Burgess *et al*. (2018) determined that livestock caseload, patient disease severity, presence of patients shedding *Salmonella*, and locations of samples collected are important risk factors. Burgess *et al*. (2018) recommend that veterinarians and personnel remain vigilant during times of high caseloads and be aware of patient groups prone to shedding *Salmonella* (Burgess and Morley, 2018).

There is no standardized approach to model building in epidemiology, which can contribute to a lack of transparency and hamper reproducibility (Hamilton et al., 2021). ML algorithms can also suffer from data drift. This is when there is a mismatch between conditions to train the model and the real-world application of the model. This can occur when the ML model was initially trained, but either the training data was skewed or changes in the environment or time affect the model's performance. In these cases, retraining the data is necessary (Sahiner et al., 2023). Using AI tools such as ChatGPT, you get a slightly different result every time you ask a question (Bothra et al., 2023). The same occurrence happens when using these ML algorithms. Each time the ML algorithm is used, a different set of randomly selected data may be used for training the model, or the model learns differently. This can cause variations in the overall model and slightly affect the accuracy, specificity, and sensitivity. In addition, these models are so complex, they are "black box" models. The "black box" is explained as data goes in and decisions come out, but the process between the input and output cannot be visualized (Russo and Bonassi, 2015; Bi et al., 2019; Wiemken and Kelley, 2019; Hamilton et al., 2021; Becker et al., 2022; Broadbent and Grote, 2022; Sahiner et al., 2023). At the same time, this lack of understanding of the complex path that leads to a decision is what makes these tools so powerful. ML algorithms allow for a deeper level of interpretation of complex association patterns, which would otherwise be undetectable to the model (Becker et al., 2022). There are limitations in ML algorithms like a user's potential lack of interpretability of the output, maybe it is the computational intensity, the nuances of building the model, data drift, reproducibility, the model's inability to help the present patients or outbreaks, model retraining, or lack of expertise in computer science, computational biology, or data science

by epidemiologists (Porta and Bolúmar, 2016; Hamilton et al., 2021; Dharma et al., 2023; Sahiner et al., 2023). This study was the first veterinary hospital epidemiological study of *Salmonella* to employ these methods. Machine learning is novel, which also means that improvements to the implementation of these methods are necessary. Each additional study furthers the next researcher as more is built on the previous. Epidemiologists should remain open-minded to this rapidly advancing area of data analysis.

The aspect of whole-genome sequencing of isolates collected from a veterinary hospital is something that has yet to be reported. The most advanced strain detection methodology for environmental contamination or outbreak detection at a veterinary hospital would be the use of pulse-field gel electrophoresis (Schaer et al., 2010; Steneroden et al., 2010; Cummings et al., 2014; Soza-Ossandón et al., 2020). The current study examined the use of short-read sequencing to generate phylogenetic trees to determine the relatedness of bacterial isolates. When this project was initiated, the literature to work from was limited, and utilized simple tools that someone with little experience in bioinformatics could use when examining WGS data for strain identification.

One initial concern is the obsession with phylogenetic trees. Phylogenetic trees are useless without context. Phylogenetic trees can span Domains of species all the way down to individual strains or subspecies. The phylogenetic tree is a representation of a type of data analysis. The Prokka-Roary pipeline use is not appropriate for answering the question on strains. Roary is a pan-genome analysis tool that compares the presence or absence of core and accessory genes between isolates. When isolates are closely related (SNP differences versus whole genes), Roary may have problems determining differences. Another complication is with using draft assemblies from the short-reads sequencing. The possibility of misassembly could make it appear that genes are present or absent, which leads to problems with Roary.

The other three tools (Parsnp, CSI Phylogeny, NDtree) were all very similar. All three perform a SNP analysis of core genes. These core genes are common among an organism and relatively stable in the genome. CSI Phylogeny and NDtree both output a SNP matrix to compare SNPs between isolates. The minor differences (<2 SNPs per isolate comparison) between CSI Phylogeny and NDtree makes these two almost identical. The differences may have come down to the SNP filtering between these tools. Parsnp did not generate a SNP matrix, so there could not be a comparison.

The best comparison for these findings can be done with the NCBI Pathogen Detection Project (https://www.ncbi.nlm.nih.gov/pathogens/). The NCBI Pathogen Detection Project is a daily monitored (almost real-time) worldwide database thats purpose is to perform a SNP analysis and cluster any bacterial raw sequence files uploaded to the short-read archive (SRA) at NCBI. This database uncovered international outbreaks that would not have been detected otherwise (Timme et al., 2019; Perestrelo et al., 2023; Zaghen et al., 2023; Sayers et al., 2024). All the *Sal*. Muenster sequences were uploaded to NCBI and can be found as a part of SNP cluster PDS000027449.12 on the NCBI Pathogen Detection Browser. Based on the findings from the NCBI Pathogen Detection Browser, the *Sal*. Muenster cluster had a range of 1 to 40 SNPs. This was close to the CSI Phylogeny's (0 to 36 SNPs) and NDtree's (0 to 34 SNPs) findings.

Using CSI Phylogeny's SNP output as a conservative route of higher number of SNPs, it is interesting to visualize two lineages of the AUCVM *Sal*. Muenster. If we use Ocatavia *et al*. (2015) cutoff of 9 SNPs, then there are three strains (Octavia et al., 2015). Octavia *et al*. (2015) calculated this cutoff for time periods of 120 days, but this value was theoretically based on the mutation rates of *Salmonella*. However, our real-world analysis indicates that this value could be extended beyond 120 days. We found only 8 SNP differences with a cluster of isolates that spanned 1493 days.

A concern regarding our findings is the limited number of isolates that were sequenced and that only one serotype (Muenster) was sequenced. We chose specific isolates to give a snapshot of the AUCVM *Sal*. Muenster over time and across different facilities. Maybe additional sequencing of isolates might give new insights into the dynamics of strain deviation over time. Another concern is that the analysis used for the SNP analysis was based on core genes' SNPs as opposed to SNPs across the whole genome. This makes sense from an epidemiological perspective because it could be problematic to look at hypervariable regions such as mobile genetic elements (prophage, transposons, plasmids) or sites prone to recombination (Wilson et al., 2011). CSI Phylogeny and NDtree are reliable web services offered by the Center for Genomic Epidemiology (CGE) (https://genomicepidemiology.org/). Even the CDC utilizes web services from CGE for antimicrobial resistance, point mutations that cause resistance, and plasmid identification (Ford et al., 2023).

This study details how newer, novel computational approaches can assist with veterinary epidemiology and the study of *Salmonella* found in veterinary hospital environments.

5.5 Conclusions

The current studies all feature methods that are newer to *Salmonella* research. Retrospectively, some things would have changed. Short-read and long-read sequencing (as performed in Chapter 2) of the *Sal*. Muenster isolates from the AUCVM would have been good to examine. Maybe hybrid assemblies would have different results or a different number of SNPs. I was unaware of the complexity of finding variants with short-read sequence data with the *Sal*. Newport parent and phage-resistant mutant. Oxford Nanopore Technologies (ONT) long-read sequencing has improved over the last few years and utilization of ONT long-read sequencing is increasing (Sereika et al., 2022; Zhao et al., 2023). I'm unsure if hybrid assembly would have been successful early on due to the recent advancements of ONT long-read sequencing. Regarding the chicken embryo lethality assay, I would have just lowered my expectations of major increases in the survival of the embryos with phage. The assay aims to screen phage in a model to transition selected phage from flask to animal. The benefit of the chicken embryo model is that lethality occurred with the phage treatment, but this is still better to attempt as opposed to the utilization of costly (both financially and ethically) animal models. We were able to visualize differences in survival with phage S7 compared to S10 against *Sal*. Enteritidis infection in the chicken embryo lethality assay.

These projects were all unique methods to our program. Like all new methods, optimization of the methods is necessary. There were important findings in these studies, such as the potential point mutation that conferred phage-resistance and attenuation in calves for *Sal*. Newport. The chicken embryo lethality assay can be useful for identifying attenuated strains of *Salmonella* and screening phage prior to inclusion in phage cocktails for phage therapy. ML algorithms and WGS can be useful for identifying sources of environmental contamination. The SNP cutoffs for individual *Salmonella* strains proposed by Octavia *et al*. (2015) could be extended beyond the 120-day time period as we showed this cutoff was applicable up to 1493 days.

These studies all provide foundations for future work. This could include complementing the mutation found in the phage-resistant *Sal.* Newport, testing additional phage with the chicken embryo lethality assay, including hospital data as variables in the ML algorithms, and sequencing additional *Sal.* Muenster isolates, just to name a few examples. Hopefully, this work will provide roadmaps for future researchers interested in these novel approaches to *Salmonella* research.

5.6 References

Adam, R., Mussa, S., Lindemann, D., Oelschlaeger, T.A., Deadman, M., Ferguson, D.J.P., Moxon, R., Schroten, H., 2002. The Avian Chorioallantoic Membrane *in ovo* - A Useful Model for Bacterial Invasion Assays. International Journal of Medical Microbiology 292, 267–275. doi:10.1078/1438-4221-00209

Becker, A.K., Ittermann, T., Dörr, M., Felix, S.B., Nauck, M., Teumer, A., Völker, U., Völzke, H., Kaderali, L., Nath, N., 2022. Analysis of Epidemiological Association Patterns of Serum Thyrotropin by Combining Random Forests and Bayesian Networks. PLoS One 17, 1–17. doi:10.1371/journal.pone.0271610

Bi, Q., Goodman, K.E., Kaminsky, J., Lessler, J., 2019. What is Machine Learning? A Primer for the Epidemiologist. Am J Epidemiol 188, 2222–2239. doi:10.1093/aje/kwz189

Bjanes, E., Nizet, V., 2021. More than a Pore: Nonlytic Antimicrobial Functions of Complement and Bacterial Strategies for Evasion. Microbiology and Molecular Biology Reviews 85. doi:10.1128/mmbr.00177-20

Bothra, A., Cao, Y., Černý, J., Arora, G., 2023. The Epidemiology of Infectious Diseases Meets AI: A Match Made in Heaven. Pathogens 12, 12–14. doi:10.3390/pathogens12020317

Broadbent, A., Grote, T., 2022. Can Robots Do Epidemiology? Machine Learning, Causal Inference, and Predicting the Outcomes of Public Health Interventions. Philos Technol 35, 1–22. doi:10.1007/s13347-022-00509-3

Burgess, B.A., Morley, P.S., 2019. Risk Factors for Shedding of *Salmonella enterica* Among Hospitalized Large Animals Over a 10-year Period in a Veterinary Teaching Hospital. J Vet Intern Med 33, 2239–2248. doi:10.1111/jvim.15579

Burgess, B.A., Morley, P.S., 2018. Risk Factors for Veterinary Hospital Environmental Contamination with *Salmonella enterica*. Epidemiol Infect 146, 1282–1292. doi:10.1017/S0950268818001164

Cummings, K.J., Rodriguez-rivera, L.D., Mitchell, K.J., Hoelzer, K., Wiedmann, M., Mcdonough, P.L., Altier, C., Warnick, L.D., Perkins, G.A., 2014. *Salmonella enterica* Serovar Oranienburg Outbreak in a Veterinary Medical Teaching Hospital with Evidence of Nosocomial and On-Farm Transmission. Vector-Borne and Zoonotic Diseases 14, 496–502. doi:10.1089/vbz.2013.1467

Denagamage, T., Jayarao, B., Patterson, P., Wallner-Pendleton, E., Kariyawasam, S., 2015. Risk Factors Associated With *Salmonella* in Laying Hen Farms: Systematic Review of Observational Studies. Avian Dis 59, 291–302.

Dharma, C., Fu, R., Chaiton, M., 2023. Table 2 Fallacy in Descriptive Epidemiology: Bringing Machine Learning to the Table. Int J Environ Res Public Health 20. doi:10.3390/ijerph20136194

Ford, L., Ellison, Z., Schwensohn, C., Griffin, I., Birhane, M.G., Cote, A., Fortenberry, G.Z., Tecle, S., Higa, J., Spencer, S., Patton, B., Patel, J., Dow, J., Maroufi, A., Robbins, A., Donovan, D., Fitzgerald, C., Burrell, S., Tolar, B., Folster, J.P., Cooley, L.A., Francois Watkins, L.K., 2023. Strain of Multidrug-Resistant *Salmonella* Newport Remains Linked to Travel to Mexico and U.S. Beef Products — United States, 2021–2022. Morbidity and Mortality Weekly Report 72, 1225–1229.

Hamilton, A.J., Strauss, A.T., Martinez, D.A., Hinson, J.S., Levin, S., Lin, G., Klein, E.Y., 2021. Machine Learning and Artificial Intelligence: Applications in Healthcare Epidemiology. Antimicrobial Stewardship and Healthcare Epidemiology 1, 1–6. doi:10.1017/ash.2021.192

Han, J., Aljahdali, N., Zhao, S., Tang, H., Harbottle, H., Hoffmann, M., Frye, J.G., Foley, S.L., 2024. Infection Biology of *Salmonella enterica*. EcoSal Plus 0, 1–54. doi:10.1128/ecosalplus.esp-0001-2023

Hao, L., Zhang, Y., Lv, X., Huang, Y., Li, Y., Shi, Y., Wang, F., 2024. Protective Effect of Bacteriophage on Chicken Embryo Damage Caused by *Salmonella* Enteritidis Infection 285–292.

Hong, Y., Hu, D., Verderosa, A.D., Qin, J., Totsika, M., Reeves, P.R., 2023. Repeat-Unit Elongations To Produce Bacterial Complex Long Polysaccharide Chains, an O-Antigen Perspective. EcoSal Plus 11. doi:10.1128/ecosalplus.esp-0020-2022

Howard, Z.R., Bryan, C.A.O., Crandall, P.G., Ricke, S.C., 2012. *Salmonella* Enteritidis in Shell Eggs: Current Issues and Prospects for Control. Food Research International 45, 755–764. doi:10.1016/j.foodres.2011.04.030

Kitchens, S.R., 2016. A Study of a Bacteriophage-Resistant *Salmonella* Mutant and Factors Influencing Prevalence of *Salmonella* in a Multi-Species Animal Facility (Master's Thesis). Auburn University.

Krzyżewska-Dudek, E., Kotimaa, J., Kapczyńska, K., Rybka, J., Meri, S., 2022. Lipopolysaccharides and Outer Membrane Proteins as Main Structures Involved in Complement Evasion Strategies of Non-Typhoidal *Salmonella* Strains. Mol Immunol 150, 67–77. doi:10.1016/j.molimm.2022.08.009

Mughini-Gras, L., Enserink, R., Friesema, I., Heck, M., van Duynhoven, Y., van Pelt, W., 2014. Risk Factors for Human Salmonellosis Originating from Pigs, Cattle, Broiler Chickens and Egg Laying Hens: A Combined Case-Control and Source Attribution Analysis. PLoS One 9, e87933. doi:10.1371/journal.pone.0087933

Muñoz-Cuevas, M., Metris, A., Baranyi, J., 2012. Predictive Modelling of *Salmonella*: From Cell Cycle Measurements to E-Models. Food Research International 45, 852–862. doi:10.1016/j.foodres.2011.04.033

Murray, G.L., Attridge, S.R., Morona, R., 2006. Altering the Length of the Lipopolysaccharide O antigen has an Impact on the Interaction of *Salmonella enterica* Serovar typhimurium with

Macrophages and Complement. J Bacteriol 188, 2735–2739. doi:10.1128/JB.188.7.2735-2739.2006

Nicolas, M., Faurie, A., Girault, M., Lavillatte, S., Menanteau, P., Chaumeil, T., Riou, M., Velge, P., Schouler, C., 2023. *In ovo* Administration of a Phage Cocktail Partially Prevents Colibacillosis in Chicks. Poult Sci 102, 1–9. doi:10.1016/j.psj.2023.102967

Octavia, S., Wang, Q., Tanaka, M.M., Kaur, S., Sintchenko, V., Lan, R., 2015. Delineating Community Outbreaks of *Salmonella enterica* Serovar Typhimurium by Use of Whole-Genome Sequencing: Insights into Genomic Variability within an Outbreak. J Clin Microbiol 53, 1063–1071. doi:10.1128/JCM.03235-14

Perestrelo, S., Amaro, A., Brouwer, M.S.M., Clemente, L., Ribeiro Duarte, A.S., Kaesbohrer, A., Karpíšková, R., Lopez-Chavarrias, V., Morris, D., Prendergast, D., Pista, A., Silveira, L., Skarżyńska, M., Slowey, R., Veldman, K.T., Zając, M., Burgess, C., Alvarez, J., 2023. Building an International One Health Strain Level Database to Characterise the Epidemiology of AMR Threats: ESBL—AmpC Producing *E. coli* as An Example—Challenges and Perspectives. Antibiotics 12, 1–18. doi:10.3390/antibiotics12030552

Porta, M., Bolúmar, F., 2016. Caution: Work in Progress: While the Methodological "Revolution" Deserves In-Depth Study, Clinical Researchers and Senior Epidemiologists Should Not Be Disenfranchised. Eur J Epidemiol 31, 535–539. doi:10.1007/s10654-016-0181-3

Rezaee, M.S., Liebhart, D., Hess, C., Hess, M., Paudel, S., 2021. Bacterial Infection in Chicken Embryos and Consequences of Yolk Sac Constitution for Embryo Survival. Vet Pathol 58, 71–79. doi:10.1177/0300985820960127

Rivera, D., Allel, K., Dueñas, F., Tardone, R., Soza, P., Hamilton-west, C., Moreno-switt, A.I., 2021. Screening the Presence of Non-Typhoidal *Salmonella* in Different Animal Systems and the Assessment of Antimicrobial Resistance. Animals 11, 1–15. doi:10.3390/ani11061532

Russo, S., Bonassi, S., 2015. Prospects and Pitfalls of Machine Learning in Nutritional Epidemiology. Encyclopedia of Food and Health 14, 1–17. doi:10.1016/B978-0-12-384947-2.00494-3

Sahiner, B., Chen, W., Samala, R.K., Petrick, N., 2023. Data Drift in Medical Machine Learning: Implications and Potential Remedies. British Journal of Radiology 96, 1.

Sayers, E.W., Beck, J., Bolton, E.E., Brister, J.R., Chan, J., Comeau, D.C., Connor, R., DiCuccio, M., Farrell, C.M., Feldgarden, M., Fine, A.M., Funk, K., Hatcher, E., Hoeppner, M., Kane, M., Kannan, S., Katz, K.S., Kelly, C., Klimke, W., Kim, S., Kimchi, A., Landrum, M., Lathrop, S., Lu, Z., Malheiro, A., Marchler-Bauer, A., Murphy, T.D., Phan, L., Prasad, A.B., Pujar, S., Sawyer, A., Schmieder, E., Schneider, V.A., Schoch, C.L., Sharma, S., Thibaud-Nissen, F., Trawick, B.W., Venkatapathi, T., Wang, J., Pruitt, K.D., Sherry, S.T., 2024. Database Resources of the National Center for Biotechnology Information. Nucleic Acids Res 52, D33–D43. doi:10.1093/nar/gkad1044

Schaer, B.L.D., Aceto, H., Rankin, S.C., 2010. Outbreak of Salmonellosis Caused by *Salmonella enterica* Serovar Newport MDR-AmpC in a Large Animal Veterinary Teaching Hospital. J Vet Intern Med 24, 1138–1146.

Sereika, M., Kirkegaard, R.H., Karst, S.M., Michaelsen, T.Y., Sørensen, E.A., Wollenberg, R.D., Albertsen, M., 2022. Oxford Nanopore R10.4 Long-Read Sequencing Enables the Generation of Near-Finished Bacterial Genomes From Pure Cultures and Metagenomes Without Short-Read or Reference Polishing. Nat Methods 19, 823–826. doi:10.1038/s41592-022-01539-7

Soza-Ossandón, P., Rivera, D., Tardone, R., Riquelme-Neira, R., García, P., Hamilton-West, C., Adell, A.D., González-Rocha, G., Moreno-Switt, A.I., 2020. Widespread Environmental Presence of Multidrug-Resistant *Salmonella* in an Equine Veterinary Hospital That Received Local and International Horses. Front Vet Sci 7, 1–9. doi:10.3389/fvets.2020.00346

Steneroden, K.K., Metre, D.C. Van, Jackson, C., Morley, P.S., Van Metre, D.C., Jackson, C., Morley, P.S., 2010. Detection and Control of a Nosocomial Outbreak Caused by *Salmonella* Newport at a Large Animal Hospital. J Vet Intern Med 24, 606–616. doi:https://doi.org/10.1111/j.1939-1676.2010.0484.x

Timme, R.E., Leon, M.S., Allard, M.W., 2019. Utilizing the Public GenomeTrakr Database for Foodborne Pathogen Traceback, in: Bridier, A. (Ed.), Foodborn Bacterial Pathogens: Methods and Protocols, Methods in Molecular Biology. pp. 449–481. doi:10.1525/9780520959743-017

Trotereau, A., Schouler, C., 2019. Use of a Chicken Embryo Lethality Assay to Assess the Efficacy of Phage Therapy, in: Clokie, M.R.J., Kropinski, A., Lavigne, R. (Eds.), Bacteriophages: Methods and Protocols, Volume 4. Hu, New York, NY, pp. 199–205. doi:https://doi.org/10.1007/978-1-4939-8940-9_17

Vaessen, M.A., Veling, J., Frankena, K., Graat, E.A.M., Klunder, T., 1998. Risk Factors for *Salmonella* Dublin Infection on Dairy Farms. Veterinary Quarterly 20, 97–99. doi:10.1080/01652176.1998.9694848

Wiemken, T.L., Kelley, R.R., 2019. Machine Learning in Epidemiology and Health Outcomes Research. Annu Rev Public Health 41, 21–36. doi:10.1146/annurev-publhealth-040119-094437

Williams, S., Markey, P., Harlock, M., Binns, P., Gaggin, J., Patel, M., 2016. Individual and Household-Level Risk Factors for Sporadic Salmonellosis in Children. Journal of Infection 72, 36–44. doi:10.1016/j.jinf.2015.09.014

Wilson, B.A., Salyers, A.A., Whitt, D.D., Winkler, M.E., 2011. Mechanisms of Genetic Modification and Exchange: Role in Pathogen Evolution, in: Bacterial Pathogenesis: A Molecular Approach. ASM Press, Washington, DC, pp. 111–129.

Yu, J., Zhang, H., Ju, Z., Huang, J., Lin, C., Wu, J., Wu, Y., Sun, S., Wang, H., Hao, G., Zhang, A., 2024. Increased Mutations in Lipopolysaccharide Biosynthetic Genes Cause Time-Dependent Development of Phage Resistance in *Salmonella*. Antimicrob Agents Chemother 68, 1–17. doi:10.1128/aac.00594-23

Zaghen, F., Sora, V.M., Meroni, G., Laterza, G., Martino, P.A., Soggiu, A., Bonizzi, L., Zecconi, A., 2023. Epidemiology of Antimicrobial Resistance Genes in *Staphylococcus aureus* Isolates from a Public Database in a One Health Perspective—Sample Characteristics and Isolates' Sources. Antibiotics 12, 1225. doi:10.3390/antibiotics12071225

Zhao, W., Zeng, W., Pang, B., Luo, M., Peng, Y., Xu, J., Kan, B., Li, Z., Lu, X., 2023. Oxford Nanopore Long-Read Sequencing Enables the Generation of Complete Bacterial and Plasmid Genomes Without Short-Read Sequencing. Front Microbiol 14, 1–9. doi:10.3389/fmicb.2023.1179966

Chapter 6

References

Aawar, M. Al, Srivastava, A., 2022. Random Forest of Epidemiological Models for Influenza Forecasting, arXiv preprint. Association for Computing Machinery. doi:https://doi.org/10.48550/arXiv.2206.08967

Abatcha, M.G., Zakaria, Z., Kaur, D.G., Thong, K.L., 2014. Typing of *Salmonella* Species: A Mini-Review. Journal of Natural Sciences Research 4, 13–17.

Abedon, S., Thomas-Abedon, C., 2010. Phage Therapy Pharmacology. Curr Pharm Biotechnol 11, 28–47. doi:10.2174/138920110790725410

Abedon, S.T., 2014. Phage Therapy: Eco-Physiological Pharmacology. Scientifica (Cairo) 2014, 1–29. doi:10.1155/2014/581639

Abedon, S.T., 2010. The 'Nuts and Bolts' of Phage Therapy. Curr Pharm Biotechnol 11, 1.

Achtman, M., Wain, J., Weill, F.X., Nair, S., Zhou, Z., Sangal, V., Krauland, M.G., Hale, J.L., Harbottle, H., Uesbeck, A., Dougan, G., Harrison, L.H., Brisse, S., 2012. Multilocus Sequence Typing as a Replacement for Serotyping in *Salmonella enterica*. PLoS Pathog 8. doi:10.1371/journal.ppat.1002776

Adam, R., Mussa, S., Lindemann, D., Oelschlaeger, T.A., Deadman, M., Ferguson, D.J.P., Moxon, R., Schroten, H., 2002. The Avian Chorioallantoic Membrane *in ovo* - A Useful Model for Bacterial Invasion Assays. International Journal of Medical Microbiology 292, 267–275. doi:10.1078/1438-4221-00209

Agbaje, M., Begum, R.H., Oyekunle, M.A., Ojo, O.E., Adenubi, O.T., 2011. Evolution of *Salmonella* nomenclature : a critical note 497–503. doi:10.1007/s12223-011-0075-4

Ahrenfeldt, J., Skaarup, C., Hasman, H., Pedersen, A.G., Aarestrup, F.M., Lund, O., 2017. Bacterial Whole Genome-Based Phylogeny: Construction of a New Benchmarking Dataset and Assessment of Some Existing Methods. BMC Genomics 18, 1–13. doi:10.1186/s12864-016-3407-6

Albert, A., 2017. Biostatistics: Facing the Interpretation of 2 × 2 Tables. J Belg Soc Radiol 101, 1–5. doi:10.5334/jbr-btr.1399

Al-Hamadany, W.S., 2021. *Salmonella* and the Immune System, in: Lamas, A., Regal, P., Franco, C. (Eds.), *Salmonella* Spp. - A Global Challenge. IntechOpen, p. 13. doi:DOI: 10.5772/intechopen.95673

Alyousef, M.K., Al-Sayed, A.A., Al Afif, A., Alamoudi, U., Leblanc, J.M., Leblanc, R., 2020. A Pain in the Neck: *Salmonella* spp. as an Unusual Cause of a Thyroid Abscess. A Case Report and Review of the Literature. BMC Infect Dis 20, 1–8. doi:10.1186/s12879-020-05161-w

Amavisit, P., Markham, P.F., Lightfoot, D., Whithear, K.G., Browning, G.F., 2001. Molecular Epidemiology of *Salmonella* Heidelberg in an Equine Hospital. Vet Microbiol 80, 85–98. doi:10.1016/S0378-1135(00)00373-4

Anderson, M.E.C., 2015. Contact Precautions and Hand Hygiene in Veterinary Clinics. Veterinary Clinics of NA: Small Animal Practice 45, 343–360. doi:10.1016/j.cvsm.2014.11.003

Andrews-Polymenis, H.L., Bäumler, A.J., Beth, A., Fang, F.C., 2010. Taming the Elephant: *Salmonella* Biology, Pathogenesis, and Prevention. Infect Immun 78, 2356–2369. doi:10.1128/IAI.00096-10

Arredondo-Alonso, S., Pöntinen, A.K., Cléon, F., Gladstone, R.A., Schürch, A.C., Johnsen, P.J., Samuelsen, Ø., Corander, J., 2021. A High-Throughput Multiplexing and Selection Strategy to Complete Bacterial Genomes. Gigascience 10, 1–13. doi:10.1093/gigascience/giab079

Azam, A.H., Tanji, Y., 2019. Bacteriophage-Host Arm Race: An Update on the Mechanism of Phage Resistance in Bacteria and Revenge of the Phage with the Perspective for Phage Therapy. Appl Microbiol Biotechnol 103, 2121–2131. doi:10.1007/s00253-019-09629-x

Balzer, L.B., Westling, T., 2023. Demystifying Statistical Inference When Using Machine Learning in Causal Research. Am J Epidemiol 192, 1545–1549. doi:10.1093/aje/kwab200

Barapatre, H., Jangir, J., Bajpai, S., Chawla, B., Keswani, G., 2023. Analytics of Epidemiological Data Using Machine Learning Models. International Journal of Next-Generation Computing 14, 255–262.

Bardina, C., Spricigo, D.A., Cortés, P., Llagostera, M., 2012. Significance of the Bacteriophage Treatment Schedule in Reducing *Salmonella* Colonization of Poultry. Appl Environ Microbiol 78, 6600–6607. doi:10.1128/aem.01257-12

Bartnik, P., Lewtak, K., Fiołka, M., Czaplewska, P., Narajczyk, M., Czajkowski, R., 2022. Resistance of *Dickeya solani* Strain IPO 2222 to Lytic Bacteriophage ΦD5 Results in Fitness Tradeoffs for the Bacterium During Infection. Sci Rep 12, 1–23. doi:10.1038/s41598-022-14956-7

Bäumler, A.J., Winter, S.E., Thiennimitr, P., Casadesús, J., 2011. Intestinal and Chronic Infections: *Salmonella* Lifestyles. Environ Microbiol 3, 508–517. doi:10.1111/j.1758-2229.2011.00242.x

BD Diagnostics, 2009. Difco & BBL Manual: Manual of Microbiological Culture Media, Second. ed, BD Diagnostics - Diagnostic Systems. Becton, Dickinson and Company, Sparks, MD. doi:10.1002/1521-3773(20010316)40:6<9823::AID-ANIE9823>3.3.CO;2-C

Becker, A.K., Ittermann, T., Dörr, M., Felix, S.B., Nauck, M., Teumer, A., Völker, U., Völzke, H., Kaderali, L., Nath, N., 2022. Analysis of Epidemiological Association Patterns of Serum Thyrotropin by Combining Random Forests and Bayesian Networks. PLoS One 17, 1–17. doi:10.1371/journal.pone.0271610

Beckman, D.A., Waters, C.M., 2023. *Vibrio cholerae* Phage ICP3 Requires O1 Antigen for Infection. Infect Immun 91, 1–14. doi:10.1128/iai.00026-23

Benedict, K.M., Morley, P.S., Metre, D.C. Van, 2008. Characteristics of Biosecurity and Infection Control Programs at Veterinary Teaching Hospitals. Journal of American Veterinary Medical Association 233, 767–773.

Besser, J., Carleton, H.A., Gerner-Smidt, P., Lindsey, R.L., Trees, E., 2018. Next-generation sequencing technologies and their application to the study and control of bacterial infections. Clinical Microbiology and Infection 24, 335–341. doi:10.1016/j.cmi.2017.10.013

Bi, Q., Goodman, K.E., Kaminsky, J., Lessler, J., 2019. What is Machine Learning? A Primer for the Epidemiologist. Am J Epidemiol 188, 2222–2239. doi:10.1093/aje/kwz189

Bicalho, M.L.S., Machado, V.S., Nydam, D. V., Santos, T.M.A., Bicalho, R.C., 2012. Evaluation of Oral Administration of Bacteriophages to Neonatal Calves: Phage Survival and Impact on Fecal *Escherichia coli*. Livest Sci 144, 294–299. doi:10.1016/j.livsci.2011.12.007

Bjanes, E., Nizet, V., 2021. More than a Pore: Nonlytic Antimicrobial Functions of Complement and Bacterial Strategies for Evasion. Microbiology and Molecular Biology Reviews 85. doi:10.1128/mmbr.00177-20

Bleriot, I., Pacios, O., Blasco, L., Fernández-García, L., López, M., Ortiz-Cartagena, C., Barrio-Pujante, A., García-Contreras, R., Pirnay, J.P., Wood, T.K., Tomás, M., 2024. Improving Phage Therapy by Evasion of Phage Resistance Mechanisms. JAC Antimicrob Resist 6, 1–16. doi:10.1093/jacamr/dlae017

Blower, T.R., Pei, X.Y., Short, F.L., Fineran, P.C., Humphreys, D.P., Luisi, B.F., Salmond, G.P.C., 2011. A Processed Noncoding RNA Regulates an Altruistic Bacterial Antiviral System. Nat Struct Mol Biol 18, 185–191. doi:10.1038/nsmb.1981

Boerlin, P., 2010. Agents As Therapeutics and Growth Promoters in Food Animal Production in Farm Animals and, in: Sabour, P.M., Griffiths, M.W. (Eds.), Bacteriophages in the Control of Food-and Waterborne Pathogens. ASM Press, Washington, DC, pp. 1–10.

Bothra, A., Cao, Y., Černý, J., Arora, G., 2023. The Epidemiology of Infectious Diseases Meets AI: A Match Made in Heaven. Pathogens 12, 12–14. doi:10.3390/pathogens12020317

Braden, C.R., 2006. *Salmonella enterica* Serotype Enteritidis and Eggs: A National Epidemic in the United States. Clinical Infectious Diseases 43, 512–517. doi:10.1086/505973

Breckell, G.L., Silander, O.K., 2021. Do You Want to Build a Genome? Benchmarking Hybrid Bacterial Genome Assembly Methods. bioRxiv 11, 1–39. doi:https://doi.org/10.1101/2021.11.07.467652

Broadbent, A., Grote, T., 2022. Can Robots Do Epidemiology? Machine Learning, Causal Inference, and Predicting the Outcomes of Public Health Interventions. Philos Technol 35, 1–22. doi:10.1007/s13347-022-00509-3

Brown, E.W., Bell, R., Zhang, G., Timme, R., Zheng, J., Hammack, T.S., Allard, M.W., 2021. *Salmonella* Genomics in Public Health and Food Safety. EcoSal Plus 9. doi:10.1128/ecosalplus.esp-0008-2020

Bruen, T.C., Philippe, H., Bryant, D., 2006. A Simple and Robust Statistical Test for Detecting the Presence of Recombination. Genetics 172, 2665–2681. doi:10.1534/genetics.105.048975

Burgess, B. a, Morley, P.S., Hyatt, D.R., 2004. Environmental Surveillance for *Salmonella enterica* in a Veterinary Teaching Hospital. J Am Vet Med Assoc 225, 1344–1348. doi:10.2460/javma.2004.225.1344

Burgess, B.A., 2023. *Salmonella* in Horses. Veterinary Clinics of North America - Equine Practice 39, 25–35. doi:10.1016/j.cveq.2022.11.005

Burgess, B.A., Morley, P.S., 2019. Risk Factors for Shedding of *Salmonella enterica* Among Hospitalized Large Animals Over a 10-year Period in a Veterinary Teaching Hospital. J Vet Intern Med 33, 2239–2248. doi:10.1111/jvim.15579

Burgess, B.A., Morley, P.S., 2018. Risk Factors for Veterinary Hospital Environmental Contamination with *Salmonella enterica*. Epidemiol Infect 146, 1282–1292. doi:10.1017/S0950268818001164

Burgess, B.A., Morley, P.S., 2015. Veterinary Hospital Surveillance Systems. Veterinary Clinics of North America - Small Animal Practice 45, 235–242. doi:10.1016/j.cvsm.2014.11.002

Burgess, B.A., Morley, P.S., 2014. Managing *Salmonella* in Equine Populations. Veterinary Clinics of NA: Equine Practice 30, 623–640. doi:10.1016/j.cveq.2014.08.005

Burgess, B.A., Weese, J.S., 2022. Prevention of Infectious Diseases in Hospital Environments, Fifth Edit. ed, Greene's Infectious Diseases of the Dog and Cat, Fifth Edition. Elsevier Inc., St. Louis, Missouri. doi:10.1016/B978-0-323-50934-3.00015-X

Canning, M., Birhane, M.G., Dewey-Mattia, D., Lawinger, H., Cote, A., Gieraltowski, L., Schwensohn, C., Tagg, K.A., Francois Watkins, L.K., Park Robyn, M., Marshall, K.E., 2023. *Salmonella* Outbreaks Linked to Beef, United States, 2012–2019. J Food Prot 86, 100071. doi:10.1016/j.jfp.2023.100071

Capparelli, R., Cuomo, P., Papaianni, M., Pagano, C., Montone, A.M.I., Ricciardelli, A., Iannelli, D., 2021. Bacteriophage-Resistant *Salmonella* Rissen: An *In vitro* Mitigated Inflammatory Response. Viruses 13, 1–11. doi:10.3390/v13122468

Capparelli, R., Nocerino, N., Lannaccone, M., Ercolini, D., Parlato, M., Chiara, M., Lannelli, D., 2010a. Bacteriophage Therapy of *Salmonella enterica*: A Fresh Appraisal of Bacteriophage Therapy. J Infect Dis 201, 52–61. doi:10.1086/648478

Capparelli, R., Nocerino, N., Lanzetta, R., Silipo, A., Amoresano, A., Giangrande, C., Becker, K., Blaiotta, G., Evidente, A., Cimmino, A., Iannaccone, M., Parlato, M., Medaglia, C., Roperto, S., Roperto, F., Ramunno, L., Iannelli, D., 2010b. Bacteriophage-Resistant *Staphylococcus*

aureus Mutant Confers Broad Immunity Against Staphylococcal Infection in Mice. PLoS One 5, 1–13. doi:10.1371/journal.pone.0011720

Carleton, H.A., Gerner-smidt, P., 2016. Whole-Genome Sequencing Is Taking over Foodborne Disease Surveillance. Microbe Magazine 11, 311–317. doi:10.1128/microbe.11.311.1

Carpena, N., Manning, K.A., Dokland, T., Marina, A., Penadés, J.R., 2016. Convergent Evolution of Pathogenicity Islands in Helper *cos* Phage Interference. Philosophical Transactions of the Royal Society B: Biological Sciences 371. doi:10.1098/rstb.2015.0505

Castor, M., L.Wooley, R.E., Shotts, E.B., Brown, J., Payeur, J.B., 1989. Characteristics of *Salmonella* Isolated From an Outbreak of Equine Salmonellosis in a Veterinary Teaching Hospital. J Equine Vet Sci 9, 236–241.

CDC, C. for D.C. and P., 2018. National Enteric Disease Surveillance: *Salmonella* Annual Report, 2016. US Department of Health and Human Services, Centers for Disease Control and Prevention: Atlanta, GA, USA http://www.cdc.gov/ncezid/dfwed/pdfs/*Salmonella*-an.

Chattaway, M.A., Dallman, T.J., Larkin, L., Nair, S., McCormick, J., Mikhail, A., Hartman, H., Godbole, G., Powell, D., Day, M., Smith, R., Grant, K., 2019. The Transformation of Reference Microbiology Methods and Surveillance for *Salmonella* With the Use of Whole Genome Sequencing in England and Wales. Front Public Health 7, 1–12. doi:10.3389/fpubh.2019.00317

Chattaway, M.A., Langridge, G.C., Wain, J., 2021. *Salmonella* Nomenclature in the Genomic Era: a Time for Change. Sci Rep 11, 1–8. doi:10.1038/s41598-021-86243-w

Chen, Z., Meng, J., 2022. Critical Assessment of Short-Read Assemblers for the Metagenomic Identification of Foodborne and Waterborne Pathogens Using Simulated Bacterial Communities. Microorganisms 10. doi:10.3390/microorganisms10122416

Cherry, B., Burns, A., Johnson, G.S., Pfeiffer, H., Dumas, N., Barrett, D., Mcdonough, P.L., Eidson, M., 2004. *Salmonella* Typhimurium Outbreak Associated with Veterinary Clinic. Emerg Infect Dis 10, 2249–2251.

Cobo-Simón, M., Hart, R., Ochman, H., 2023. Gene Flow and Species Boundaries of the Genus *Salmonella*. mSystems 8, 1–12. doi:10.1128/msystems.00292-23

Coburn, B., Grassl, G.A., Finlay, B.B., 2007. *Salmonella*, the Host and Disease: a Brief Review. Immunol Cell Biol 85, 112–118. doi:10.1038/sj.icb.7100007

Costa, L.F., Paixão, T.A., Tsolis, R.M., Bäumler, A.J., Santos, R.L., 2012. Salmonellosis in Cattle: Advantages of Being an Experimental Model. Res Vet Sci 93, 1–6. doi:10.1016/j.rvsc.2012.03.002

Cummings, K.J., Divers, T.J., Mcdonough, P.L., Warnick, L.D., 2009. Fecal Shedding of *Salmonella* spp Among Cattle Admitted to a Veterinary Teaching Hospital. Journal of American Veterinary Medical Association 234, 1578–1585.

Cummings, K.J., Rodriguez-Rivera, L.D., Mitchell, K.J., Hoelzer, K., Wiedmann, M., Mcdonough, P.L., Altier, C., Warnick, L.D., Perkins, G.A., 2014. *Salmonella enterica* Serovar Oranienburg Outbreak in a Veterinary Medical Teaching Hospital with Evidence of Nosocomial and On-Farm Transmission. Vector-Borne and Zoonotic Diseases 14, 496–502. doi:10.1089/vbz.2013.1467

Curtiss, R., 2023. Vaccines to Control *Salmonella* in Poultry. Avian Dis 67, 427–440. doi:10.1637/aviandiseases-d-23-99988

Dalmasso, M., Strain, R., Neve, H., Franz, C.M.A.P.A.P., Cousin, F.J., Ross, R.P., Hill, C., Cousin, J., Ross, R.P., Hill, C., 2016. Three New *Escherichia coli* Phages from the Human Gut Show Promising Potential for Phage Therapy. PLoS One 11, 1–16. doi:10.1371/journal.pone.0156773

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 2011. The Variant Call Format and VCFtools. Bioinformatics 27, 2156–2158. doi:10.1093/bioinformatics/btr330

Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M., 2021. Twelve Years of SAMtools and BCFtools. Gigascience 10, 1–4. doi:10.1093/gigascience/giab008

Dargatz, D. a, Traub-Dargatz, J.L., 2004. Multidrug-Resistant *Salmonella* and Nosocomial Infections. Vet Clin North Am Equine Pract 20, 587–600. doi:10.1016/j.cveq.2004.07.008

De Lucia, A., Rabie, A., Smith, R.P., Davies, R., Ostanello, F., Ajayi, D., Petrovska, L., Martelli, F., 2018. Role of Wild Birds and Environmental Contamination in the Epidemiology of *Salmonella* Infection in an Outdoor Pig Farm. Vet Microbiol 227, 148–154. doi:10.1016/j.vetmic.2018.11.003

De Maio, N., Shaw, L.P., Hubbard, A., George, S., Sanderson, N.D., Swann, J., Wick, R., Oun, M.A., Stubberfield, E., Hoosdally, S.J., Crook, D.W., Peto, T.E.A., Sheppard, A.E., Bailey, M.J., Read, D.S., Anjum, M.F., Sarah Walker, A., Stoesser, N., 2019. Comparison of Long-Read Sequencing Technologies in the Hybrid Assembly of Complex Bacterial Genomes. Microb Genom 5, 1–12. doi:10.1099/mgen.0.000294

Delahoy, M.J., Shah, H.J., Weller, D.L., Ray, L.C., Smith, K., McGuire, S., Trevejo, R.T., Scallan Walter, E., Wymore, K., Rissman, T., McMillian, M., Lathrop, S., LaClair, B., Boyle, M.M., Harris, S., Zablotsky-Kufel, J., Houck, K., Devine, C.J., Lau, C.E., Tauxe, R. V., Bruce, B.B., Griffin, P.M., Payne, D.C., 2023. Preliminary Incidence and Trends of Infections Caused by Pathogens Transmitted Commonly Through Food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2022. MMWR Morb Mortal Wkly Rep 72, 701–706. doi:10.15585/mmwr.mm7226a1

Denagamage, T., Jayarao, B., Patterson, P., Wallner-Pendleton, E., Kariyawasam, S., 2015. Risk Factors Associated With *Salmonella* in Laying Hen Farms: Systematic Review of Observational Studies. Avian Dis 59, 291–302.

Depristo, M.A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J.R., Hartl, C., Philippakis, A.A., Del Angel, G., Rivas, M.A., Hanna, M., McKenna, A., Fennell, T.J., Kernytsky, A.M., Sivachenko, A.Y., Cibulskis, K., Gabriel, S.B., Altshuler, D., Daly, M.J., 2011. A Framework for Variation Discovery and Genotyping Using Next-Generation DNA Sequencing Data. Nat Genet 43, 491–501. doi:10.1038/ng.806

Dharma, C., Fu, R., Chaiton, M., 2023. Table 2 Fallacy in Descriptive Epidemiology: Bringing Machine Learning to the Table. Int J Environ Res Public Health 20. doi:10.3390/ijerph20136194

Dhowlaghar, N., Denes, T.G., 2023. Control of Residual Phage in the Evaluation of Phage-Based Food Safety Applications. Crit Rev Food Sci Nutr 0, 1–7. doi:10.1080/10408398.2023.2258210

Doyle, M.P., Erickson, M.C., 2006. Reducing the Carriage of Foodborne Pathogens in Livestock and Poultry. Poult Sci 85, 960–973.

Drulis-Kawa, Z., Majkowska-Skrobek, G., Maciejewska, B., 2015. Bacteriophages and Phage-Derived Proteins – Application Approaches. Curr Med Chem 22, 1757–1773. doi:10.2174/0929867322666150209152851

Dunowska, M., Morley, P.S., Traub-Dargatz, J.L., Davis, M. a, Patterson, G., Frye, J.G., Hyatt, D.R., Dargatz, D. a, 2007. Comparison of *Salmonella enterica* serotype Infantis Isolates from a Veterinary Teaching Hospital. Journal of Ap 102, 1527–1536. doi:10.1111/j.1365-2672.2006.03198.x

Duval, A., Opatowski, L., Brisse, S., 2023. Defining Genomic Epidemiology Thresholds for Common-Source Bacterial Outbreaks: A Modelling Study. Lancet Microbe 4, e349–e357. doi:10.1016/S2666-5247(22)00380-9

Eagle, S.H.C., Robertson, J., Bastedo, D.P., Liu, K., Nash, J.H.E., 2023. Evaluation of Five Commercial DNA Extraction Kits Using *Salmonella* as a Model for Implementation of Rapid Nanopore Sequencing in Routine Diagnostic Laboratories. Access Microbiol 5, 1–20. doi:10.1099/acmi.0.000468.v3

Edgar, R.C., 2004. MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput. Nucleic Acids Res 32, 1792–1797. doi:10.1093/nar/gkh340

Edrington, T.S., Brown, T.R., 2022. A Commentary on *Salmonella* From a Pre-Harvest Perspective. Frontiers in Animal Science 3, 1–7. doi:10.3389/fanim.2022.877392

Egido, J.E., Costa, A.R., Aparicio-Maldonado, C., Haas, P.J., Brouns, S.J.J., 2022. Mechanisms and Clinical Importance of Bacteriophage Resistance. FEMS Microbiol Rev 46, 1–16. doi:10.1093/femsre/fuab048

Endersen, L., O'Mahony, J., Hill, C., Ross, R.P., McAuliffe, O., Coffey, A., 2014. Phage Therapy in the Food Industry. Annu Rev Food Sci Technol 5, 327–349. doi:10.1146/annurev-food-030713-092415

Evangelopoulou, G., Kritas, S., Govaris, A., Burriel, A.R., 2013. Animal Salmonelloses: a Brief Review of "Host Adaptation and Host Specificity" of *Salmonella* spp. Vet World 6, 703–708. doi:10.14202/vetworld.2013.703-708

Ewart, S.L., Schott, H.C., Robison, R.L., Dwyer, R.M., Eberhart, S.W., Walker, R.D., 2001. Identification of Sources of *Salmonella* Organisms in a Veterinary Teaching Hospital and Evaluation of the Effects of Disinfectants on Detection of *Salmonella* Organisms on Surface Materials. J Am Vet Med Assoc 218, 1145–1151. doi:10.2460/javma.2001.218.1145

Faruque, S.M., Islam, M.J., Ahmad, Q.S., Faruque, A.S.G., Sack, D.A., Nair, G.B., Mekalanos, J.J., 2005a. Self-Limiting Nature of Seasonal Cholera Epidemics: Role of Host-Mediated Amplification of Phage. Proc Natl Acad Sci U S A 102, 6119–6124. doi:10.1073/pnas.0502069102

Faruque, S.M., Naser, I. Bin, Islam, M.J., Faruque, A.S.G., Ghosh, A.N., Nair, G.B., Sack, D.A., Mekalanos, J.J., 2005b. Seasonal Epidemics of Cholera Inversely Correlate with the Prevalence of Environmental Cholera Phages. Proc Natl Acad Sci U S A 102, 1702–1707. doi:10.1073/pnas.0408992102

FDA, 2015. Veterinary Feed Directive. Fed Regist 80, 31707–31735.

Feng, Y., Lin, E., Zou, S., Chen, C.L., Chiu, C.H., 2019. Complete Genome Sequence of *Salmonella enterica* Serovar Sendai Shows H Antigen Convergence with S. Miami and Recent Divergence From *S*. Paratyphi A. BMC Genomics 20, 1–10. doi:10.1186/s12864-019-5798-7

Fierer, J., 2022. Invasive Non-typhoidal *Salmonella* (iNTS) Infections. Clinical Infectious Diseases 75, 732–738.

Figueira, R., Holden, D.W., 2012. Functions of the *Salmonella* Pathogenicity Island 2 (SPI-2) Type III Secretion System Effectors. Microbiology (N Y) 158, 1147–1161. doi:10.1099/mic.0.058115-0

Fineran, P.C., Salmond, G.P.C., Blower, T.R., Lilley, K.S., Humphreys, D.P., Foulds, I.J., 2009. The Phage Abortive Infection System, ToxIN, Functions as a Protein–RNA Toxin–Antitoxin Pair. Proceedings of the National Academy of Sciences 106, 894–899. doi:10.1073/pnas.0808832106

Fiorentin, L., Vieira, N.D., Barioni Jr, W., 2005. Oral Treatment with Bacteriophages Reduces the Concentration of *Salmonella* Enteritidis PT4 in Caecal Contents of Broilers. Avian Dis 34, 258–263. doi:10.1080/01445340500112157

Fitzgerald, C., Collins, M., Duyne, S. Van, Mikoleit, M., Brown, T., Fields, P., 2007. Multiplex , Bead-Based Suspension Array for Molecular Determination of Common *Salmonella* Serogroups. J Clin Microbiol 45, 3323–3334. doi:10.1128/JCM.00025-07

Flyg, C., Kenne, K., Boman, H.G., 1980. Insect Pathogenic Properties of *Serratia marcescens*: Phage-Resistant Mutants with a Decreased Resistance to *Cecropia* Immunity and a Decreased Virulence to *Drosophila*. J Gen Microbiol 120, 173–181. doi:10.1099/00221287-120-1-173

Ford, L., Ellison, Z., Schwensohn, C., Griffin, I., Birhane, M.G., Cote, A., Fortenberry, G.Z., Tecle, S., Higa, J., Spencer, S., Patton, B., Patel, J., Dow, J., Maroufi, A., Robbins, A., Donovan, D., Fitzgerald, C., Burrell, S., Tolar, B., Folster, J.P., Cooley, L.A., Francois Watkins, L.K., 2023. Strain of Multidrug-Resistant *Salmonella* Newport Remains Linked to Travel to Mexico and U.S. Beef Products — United States, 2021–2022. Morbidity and Mortality Weekly Report 72, 1225–1229.

Fossler, C.P., Wells, S.J., Kaneene, J.B., Ruegg, P.L., Warnick, L.D., Bender, J.B., Eberly, L.E., Godden, S.M., Halbert, L.W., 2005. Herd-level Factors Associated with Isolation of *Salmonella* in a Multi-State Study of Conventional and Organic Dairy Farms I. *Salmonella* Shedding in Cows. Prev Vet Med 70, 257–77. doi:10.1016/j.prevetmed.2005.04.003

Foster, N., Tang, Y., Berchieri, A., Geng, S., Jiao, X., Barrow, P., 2021. Revisiting Persistent *Salmonella* Infection and the Carrier State: What Do We Know? Pathogens 10, 1–21. doi:10.3390/pathogens10101299

Galán-Relaño, Á., Valero Díaz, A., Huerta Lorenzo, B., Gómez-Gascón, L., Mena Rodríguez, M.ª. Á., Carrasco Jiménez, E., Pérez Rodríguez, F., Astorga Márquez, R.J., 2023. *Salmonella* and Salmonellosis: An Update on Public Health Implications and Control Strategies. Animals 13, 3–22. doi:10.3390/ani13233666

Gal-Mor, O., 2019. Persistent Infection and Long-Term Carriage of Typhoidal and Nontyphoidal Salmonellae. Clin Microbiol Rev 32, 1–31.

Gal-Mor, O., Boyle, E.C., Grassl, G.A., 2014. Same Species, Different Diseases: How and Why Typhoidal and Non-Typhoidal *Salmonella enterica* Serovars Differ. Front Microbiol 5, 1–10. doi:10.3389/fmicb.2014.00391

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T.J., Van Immerseel, F., 2009. Mechanisms of Egg Contamination by *Salmonella* Enteritidis. FEMS Microbiol Rev 33, 718–738. doi:10.1111/j.1574-6976.2008.00161.x

Garaizar, J., Laorden, L., 2023. Bacterial Genomics and Epidemiology. Microorganisms 11, 10–11. doi:10.3390/microorganisms11061428

Ge, H., Fu, S., Guo, H., Hu, M., Xu, Z., Zhou, X., Chen, X., Jiao, X., 2022. Application and Challenge of Bacteriophage in the Food Protection. Int J Food Microbiol 380, 109872. doi:10.1016/j.ijfoodmicro.2022.109872

Giani, A.M., Gallo, G.R., Gianfranceschi, L., Formenti, G., 2020. Long Walk to Genomics: History and Current Approaches to Genome Sequencing and Assembly. Comput Struct Biotechnol J 18, 9–19. doi:10.1016/j.csbj.2019.11.002

Glonti, T., Pirnay, J., 2022. *In vitro* Measurement Of Phage Characteristics That Are Important for Phage Therapy Outcome. Viruses 14, 1–23.

Grant, A.J., Morgan, F.J.E., McKinley, T.J., Foster, G.L., Maskell, D.J., Mastroeni, P., 2012. Attenuated *Salmonella* Typhimurium Lacking the Pathogenicity Island-2 Type 3 Secretion System Grow to High Bacterial Numbers inside Phagocytes in Mice. PLoS Pathog 8. doi:10.1371/journal.ppat.1003070

Grimont, P.A., Weill, F.-X., 2007. Antigenic Formulae of the *Salmonella* Serovars, 9th ed, WHO Collaborating Centre for Reference and Research on *Salmonella*.

Gunn, J.S., Marshall, J.M., Baker, S., Dongol, S., Charles, R.C., Ryan, E.T., 2014. *Salmonella* Chronic Carriage: Epidemiology, Diagnosis, and Gallbladder Persistence. Trends Microbiol 22, 648–655. doi:10.1016/j.tim.2014.06.007

Gupta, A., Fontana, J., Crowe, C., Bolstorff, B., Stout, A., Duyne, S. Van, Hoekstra, M.P., Whichard, J.M., Barrett, T.J., Angulo, F.J., 2003. Emergence of Multidrug-Resistant *Salmonella enterica* Serotype Newport Infections Resistant to Expanded-Spectrum Cephalosporins in the United States 21287, 1707–1716.

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: Quality assessment tool for genome assemblies. Bioinformatics 29, 1072–1075. doi:10.1093/bioinformatics/btt086

Hackl S. T., Harbig T. A., Nieselt K., 2022. Technical Report on Best Practices for Hybrid and Long Read *de novo* Assembly of Bacterial Genomes Utilizing Illumina and Oxford Nanopore Technologies Reads. bioRxiv 10. doi:https://doi.org/10.1101/2022.10.25.513682

Hadfield, J., Croucher, N.J., Goater, R.J., Abudahab, K., Aanensen, D.M., Harris, S.R., 2018. Phandango: An Interactive Viewer for Bacterial Population Genomics. Bioinformatics 34, 292–293. doi:10.1093/bioinformatics/btx610

Haines, M.E.K., Hodges, F.E., Nale, J.Y., Mahony, J., van Sinderen, D., Kaczorowska, J., Alrashid, B., Akter, M., Brown, N., Sauvageau, D., Sicheritz-Pontén, T., Thanki, A.M., Millard, A.D., Galyov, E.E., Clokie, M.R.J., 2021. Analysis of Selection Methods to Develop Novel Phage Therapy Cocktails Against Antimicrobial Resistant Clinical Isolates of Bacteria. Front Microbiol 12, 1–15. doi:10.3389/fmicb.2021.613529

Haley, B.J., Cole, D.J., Lipp, E.K., 2009. Distribution, Diversity, and Seasonality of Waterborne Salmonellae in a Rural Watershed. Appl Environ Microbiol 75, 1248–1255. doi:10.1128/AEM.01648-08

Hallstrom, K., McCormick, B.A., 2011. *Salmonella* Interaction With and Passage Through the Intestinal Mucosa: Through the Lens of the Organism. Front Microbiol 2, 1–10. doi:10.3389/fmicb.2011.00088

Hamilton, A.J., Strauss, A.T., Martinez, D.A., Hinson, J.S., Levin, S., Lin, G., Klein, E.Y., 2021. Machine Learning and Artificial Intelligence: Applications in Healthcare Epidemiology. Antimicrobial Stewardship and Healthcare Epidemiology 1, 1–6. doi:10.1017/ash.2021.192

Han, J., Aljahdali, N., Zhao, S., Tang, H., Harbottle, H., Hoffmann, M., Frye, J.G., Foley, S.L., 2024. Infection Biology of *Salmonella enterica*. EcoSal Plus 0, 1–54. doi:10.1128/ecosalplus.esp-0001-2023

Hao, L., Zhang, Y., Lv, X., Huang, Y., Li, Y., Shi, Y., Wang, F., 2024. Protective Effect of Bacteriophage on Chicken Embryo Damage Caused by *Salmonella* Enteritidis Infection 285–292.

Heierson, A., Siden, I., Kivaisi, A., Boman, H.G., 1986. Bacteriophage-Resistant Mutants of *Bacillus thuringiensis* with Decreased Virulence in Pupae of *Hyalophora cecropia*. J Bacteriol 167, 18–24. doi:10.1128/jb.167.1.18-24.1986

Henry, M., Debarbieux, L., 2012. Tools from Viruses: Bacteriophage Successes and Beyond. Virology 434, 151–161. doi:10.1016/j.virol.2012.09.017

Hensel, M., Hinsley, A.P., Sawers, G., Berks, B.C., 1999. The Genetic Basis of Tetrathionate Respiration in *Salmonella* Typhimurium. Mol Microbiol 32, 275–287.

Higginson, E.E., Simon, R., Tennant, S.M., 2016. Animal Models for Salmonellosis: Applications in Vaccine Research. Clinical and Vaccine Immunology 23, 746–756. doi:10.1128/CVI.00258-16.Editor

Hong, Y., Hu, D., Verderosa, A.D., Qin, J., Totsika, M., Reeves, P.R., 2023. Repeat-Unit Elongations To Produce Bacterial Complex Long Polysaccharide Chains, an O-Antigen Perspective. EcoSal Plus 11. doi:10.1128/ecosalplus.esp-0020-2022

Howard, Z.R., Bryan, C.A.O., Crandall, P.G., Ricke, S.C., 2012. *Salmonella* Enteritidis in Shell Eggs: Current Issues and Prospects for Control. Food Research International 45, 755–764. doi:10.1016/j.foodres.2011.04.030

Hu, T., Chitnis, N., Monos, D., Dinh, A., 2021. Next-Generation Sequencing Technologies: An Overview. Hum Immunol 82, 801–811. doi:10.1016/j.humimm.2021.02.012

Humphrey, T., 2000. Public-health Aspects of *Salmonella* Infection, in: Wray, C., Wray, A. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, New York, NY, pp. 245–264.

Hwang, D., Rothrock, M.J., Pang, H., Dev Kumar, G., Mishra, A., 2020. Farm Management Practices that Affect the Prevalence of *Salmonella* in Pastured Poultry Farms. LWT- Food Science and Technology 127, 109423. doi:10.1016/j.lwt.2020.109423

Hyland, J., Kitchens, S., Wright, J., Walz, P., Price, S., n.d. Oral Bacteriophage Treatment to Reduce Fecal Shedding of *Salmonella enterica* Serotype Newport from Calves. Manuscript in Preparation.

Hyman, P., Abedon, S.T., 2010a. Bacteriophage Host Range and Bacterial Resistance., in: Advances in Applied Microbiology. Elsevier Inc., San Diego, CA, pp. 217–248. doi:10.1016/S0065-2164(10)70007-1

Hyman, P., Abedon, S.T., 2010b. Bacteriophage Host Range and Bacterial Resistance., in: Advances in Applied Microbiology. Elsevier Inc., San Diego, CA, pp. 217–248. doi:10.1016/S0065-2164(10)70007-1

Jacobsen, C.S., Bech, T.B., 2012. Soil Survival of *Salmonella* and Transfer to Freshwater and Fresh Produce. Food Research International 45, 557–566. doi:10.1016/j.foodres.2011.07.026

Kaas, R.S., Leekitcharoenphon, P., Aarestrup, F.M., Lund, O., 2014. Solving the Problem of Comparing Whole Bacterial Genomes Across Different Sequencing Platforms. PLoS One 9, 1–8. doi:10.1371/journal.pone.0104984

Kitchens, S.R., 2016. A Study of a Bacteriophage-Resistant *Salmonella* Mutant and Factors Influencing Prevalence of *Salmonella* in a Multi-Species Animal Facility (Master's Thesis). Auburn University.

Kitchens, S.R., Michaels, M.T., Price, S.B., 2019. Movement and Persistence of *Salmonella* in a Veterinary Teaching Hospital, in: 2019 Phi Zeta Research Day Forum. Auburn, AL, p. 20.

Klopatek, S., Callaway, T.R., Wickersham, T., Sheridan, T.G., Nisbet, D.J., 2021. Bacteriophage Utilization in Animal Hygiene, in: Harper, D.R., Abedon, S.T., Burrowes, B.H., McConville, M.L. (Eds.), Bacteriophages: Biology, Technology, Therapy, Volume 2. Springer International Publishing, pp. 891–917. doi:10.1007/978-3-319-41986-2_30/COVER

Kong, Q., Yang, J., Liu, Q., Alamuri, P., Roland, K.L., Curtiss, R., 2011. Effect of Deletion of Genes Involved in Lipopolysaccharide Core and O-Antigen Synthesis on Virulence and Immunogenicity of *Salmonella enterica* Serovar Typhimurium. Infect Immun 79, 4227–4239. doi:10.1128/IAI.05398-11

Kortright, K.E., Done, R.E., Chan, B.K., Souza, V., Turner, P.E., 2022. Selection for Phage Resistance Reduces Virulence of *Shigella flexneri*. Appl Environ Microbiol 88, 1–15. doi:10.1128/AEM.01514-21

Kropinski, A.M., 2009. Measurement of the Rate of Attachment of Bacteriophage to Cells, in: Clokie, M.R.J., Kropinski, A.M. (Eds.), Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions. Humana Press, a part of Springer Science+Business Media, New York, NY, pp. 151–156.

Kropinski, A.M., Mazzocco, A., Waddell, T.E., Lingohr, E., Johnson, R.P., 2009. Enumeration of Bacteriophages by Double Agar Overlay Plaque Assay, in: Clokie, M.R.J., Kropinski, A.M. (Eds.), Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions. Humana Press, a part of Springer Science+Business Media, New York, NY, pp. 69–76. doi:10.1007/978-1-60327-164-6

Krzyżewska-Dudek, E., Kotimaa, J., Kapczyńska, K., Rybka, J., Meri, S., 2022. Lipopolysaccharides and Outer Membrane Proteins as Main Structures Involved in Complement Evasion Strategies of Non-Typhoidal *Salmonella* Strains. Mol Immunol 150, 67–77. doi:10.1016/j.molimm.2022.08.009

Kubota, K.A., Wolfgang, W.J., Baker, D.J., Boxrud, D., Turner, L., Trees, E., Carleton, H.A., Gerner-Smidt, P., 2019. PulseNet and the Changing Paradigm of Laboratory-Based Surveillance for Foodborne Diseases. Public Health Reports 134, 22S-28S. doi:10.1177/0033354919881650

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35, 1547–1549. doi:10.1093/molbev/msy096

Laanto, E., Bamford, J.K.H., Laakso, J., Sundberg, L.R., 2012. Phage-Driven Loss of Virulence in a Fish Pathogenic Bacterium. PLoS One 7. doi:10.1371/journal.pone.0053157

Labrie, S.J., Samson, J.E., Moineau, S., 2010. Bacteriophage Resistance Mechanisms. Nat Rev Microbiol 8, 317–327. doi:10.1038/nrmicro2315

Laufer, A.S., Grass, J., Holt, K., Whichard, J.M., Griffin, P.M., Gould, L.H., Service, Intelligence, Development, P., Branch, D.E., Service, Inspection, 2015. Outbreaks of *Salmonella* Infections Attributed to Beef – United States, 1973–2011. Epidemiol Infect 143, 2003–2013. doi:10.1017/S0950268814003112.Outbreaks

Leekitcharoenphon, P., Nielsen, E.M., Kaas, R.S., Lund, O., Aarestrup, F.M., 2014. Evaluation of Whole Genome Sequencing for Outbreak Detection of *Salmonella enterica*. PLoS One 9. doi:10.1371/journal.pone.0087991

Leon, I.M., Lawhon, S.D., Norman, K.N., Threadgill, D.S., Ohta, N., Vinasco, J., Scott, H.M., 2018. Serotype Diversity and Antimicrobial Resistance Among *Salmonella enterica* Isolates from Patients at an Equine Referral Hospital. Appl Environ Microbiol 84. doi:10.1128/AEM.02829-17

León, M., Bastías, R., 2015. Virulence Reduction in Bacteriophage Resistant Bacteria. Front Microbiol 6, 1–7. doi:10.3389/fmicb.2015.00343

Lerminiaux, N., Fakharuddin, K., Mulvey, M.R., Mataseje, L., 2022. Do We Still Need Illumina Sequencing Data?: Evaluating Oxford Nanopore Technologies 2 R10.4.1 Flow Cells and v14 Library Prep Kits for Gram Negative Bacteria Whole Genome Assemblies. Can J Microbiol 1–23.

Letunic, I., Bork, P., 2021. Interactive Tree of Life (iTOL) v5: An Online Tool for Phylogenetic Tree Display and Annotation. Nucleic Acids Res 49, W293–W296. doi:10.1093/nar/gkab301

Lewis, R., Hill, C., 2020. Overcoming Barriers to Phage Application in Food and Feed. Curr Opin Biotechnol 61, 38–44. doi:10.1016/j.copbio.2019.09.018

Li, C., Tyson, G.H., Hsu, C.H., Harrison, L., Strain, E., Tran, T.T., Tillman, G.E., Dessai, U., McDermott, P.F., Zhao, S., 2021. Long-Read Sequencing Reveals Evolution and Acquisition of Antimicrobial Resistance and Virulence Genes in *Salmonella enterica*. Front Microbiol 12, 1–14. doi:10.3389/fmicb.2021.777817

Li, H., 2013. Aligning Sequence Reads, Clone Sequences and Assembly Contigs with BWA-MEM 00, 1–3.

Li, H., Durbin, R., 2009. Fast and Accurate Short Read Alignment with Burrows-Wheeler Transform. Bioinformatics 25, 1754–1760. doi:10.1093/bioinformatics/btp324

Liang, D.W., Lu, J.H., Wu, Q., Ke, B.X., Jiang, C.H., Long, J., Fang, Y.P., Lin, L.J., Zeng, N.Y., 2016. Comparing the Ability of Luminex xMAP *Salmonella* Serotyping Assay and Traditional Serotyping Method for Serotyping *Salmonella* Isolated from Southern Chinese Population. J Appl Microbiol 120, 1668–1676. doi:10.1111/jam.13106

Lilienfeld, A.M., 1980. Advances in Quantitative Methods in Epidemiology. Public Health Reports 95, 462–469.

Lin, B., Hui, J., Mao, H., 2021. Nanopore Technology and Its Applications in Gene Sequencing. Biosensors (Basel) 11. doi:10.3390/bios11070214

Liu, B., Knirel, Y.A., Feng, L., Perepelov, A. V, Senchenkova, S.N., Reeves, P.R., Wang, L., 2014. Structural Diversity in *Salmonella* O Antigens and Its Genetic Basis. FEMS Microbiol Rev 38, 56–89. doi:10.1111/1574-6976.12034

Liu, D., Van Belleghem, J.D., de Vries, C.R., Burgener, E., Chen, Q., Manasherob, R., Aronson, J.R., Amanatullah, D.F., Tamma, P.D., Suh, G.A., 2021. The Safety and Toxicity of Phage Therapy: A Review of Animal and Clinical Studies. Viruses 13, 1–20. doi:10.3390/v13071268

Lope, D.J., Demirhan, H., 2024. Spatial and Spatio-Temporal Epidemiology JAGS Model Specification for Spatiotemporal Epidemiological Modelling. Spat Spatiotemporal Epidemiol 49, 100645. doi:10.1016/j.sste.2024.100645

López, F.E., De, M., Pescaretti, M., Morero, R., Delgado, M.A., 2012. *Salmonella* Typhimurium General Virulence Factors: A Battle of David Against Goliath? Food Research International 45, 842–851. doi:10.1016/j.foodres.2011.08.009

Lunn, D., Spiegelhalter, D., Thomas, A., Best, N., 2009. The BUGS project: Evolution, Critique and Future Directions. Stat Med 28, 3049–30.67. doi:10.1002/sim.3680

Luong, T., Salabarria, A.C., Roach, D.R., 2020. Phage Therapy in the Resistance Era: Where Do We Stand and Where Are We Going? Clin Ther 42, 1659–1680. doi:10.1016/j.clinthera.2020.07.014

Mahony, J., McAuliffe, O., Ross, R.P., van Sinderen, D., 2011. Bacteriophages as biocontrol agents of food pathogens. Curr Opin Biotechnol 22, 157–163. doi:10.1016/j.copbio.2010.10.008

Marçais, G., Delcher, A.L., Phillippy, A.M., Coston, R., Salzberg, S.L., Zimin, A., 2018. MUMmer4: A Fast and Versatile Genome Alignment System. PLoS Comput Biol 14, 1–14. doi:10.1371/journal.pcbi.1005944

Marchello, C.S., Birkhold, M., Crump, J.A., Martin, L.B., Ansah, M.O., Breghi, G., Canals, R., Fiorino, F., Gordon, M.A., Kim, J.H., Hamaluba, M., Hanumunthadu, B., Jacobs, J., Kariuki, S., Malvolti, S., Mantel, C., Marks, F., Medaglini, D., Mogasale, V., Msefula, C.L., Muthumbi, E., Niyrenda, T.S., Onsare, R., Owusu-Dabo, E., Pettini, E., Ramasamy, M.N., Soura, B.A., Spadafina, T., Tack, B., 2022. Complications and Mortality of Non-Typhoidal *Salmonella* Invasive Disease: A Global Systematic Review and Meta-Analysis. Lancet Infect Dis 22, 692–705. doi:10.1016/S1473-3099(21)00615-0

Marshall, K.E.H., Tewell, M., Tecle, S., Leeper, M., Sinatra, J., Kissler, B., Fung, A., Brown, K., Wagner, D., Trees, E., Hise, K.B., Chaturvedi, V., Schlater, L.K., Morningstar-Shaw, B.R., Whitlock, L., Holt, K., Becker, K., Nichols, M., Williams, I.T., Jhung, M., Wise, M.E., Gieraltowski, L., 2018. Protracted Possible Outbreak of *Salmonella* Newport Infections Linked to Ground Beef: Possible Role of Dairy Cows — 21 States, 2016–2017. MMWR Morb Mortal Wkly Rep 67, 443–446. doi:10.15585/mmwr.mm6715a2

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., DePristo, M.A., 2010. The Genome Analysis Toolkit: A MapReduce Framework for Analyzing Next-Generation DNA Sequencing Data. Genome Res 20, 1297–1303. doi:10.1101/gr.107524.110

McQuiston, J.R., Parrenas, R., Gheesling, L., Brenner, F., Fields, P.I., 2004. Sequencing and Comparative Analysis of Flagellin Genes fliC, fljB, and flpA from *Salmonella*. J Clin Microbiol 42, 1923–1932. doi:10.1128/JCM.42.5.1923

Mcquiston, J.R., Waters, R.J., Dinsmore, B.A., Mikoleit, M.L., Fields, P.I., 2011. Molecular Determination of H Antigens of *Salmonella* by Use of a Microsphere-Based Liquid Array. J Clin Microbiol 49, 565–573. doi:10.1128/JCM.01323-10

Meaden, S., Koskella, B., 2013. Exploring the Risks of Phage Application in the Environment. Front Microbiol 4, 1–8. doi:10.3389/fmicb.2013.00358

Mehmood Khan, F., Manohar, P., Singh Gondil, V., Mehra, N., Kayode Oyejobi, G., Odiwuor, N., Ahmad, T., Huang, G., 2023. The Applications of Animal Models in Phage Therapy: An Update. Hum Vaccin Immunother 19, 1–23. doi:10.1080/21645515.2023.2175519

Menanteau, P., Kempf, F., Trotereau, J., Virlogeux-Payant, I., Gitton, E., Dalifard, J., Gabriel, I., Rychlik, I., Velge, P., 2018. Role of Systemic Infection, Cross Contaminations and Super-Shedders in *Salmonella* Carrier State in Chicken. Environ Microbiol 20, 3246–3260. doi:10.1111/1462-2920.14294

Michaels, M.T., Kitchens, S.R., Price, S.B., 2018. Environmental Sampling for *Salmonella* in an Equine Reproduction Center, in: 2018 Phi Zeta Research Day Forum. Auburn, AL, p. 20.

Mokraoui, N.R., Ganim, R., Paez, A., 2023. Chest Wall Abscess and Costochondritis Due to *Salmonella enterica* Serotype Choleraesuis: a Case Report. J Med Case Rep 17, 1–7. doi:10.1186/s13256-023-04229-w

Monack, D.M., 2012. *Salmonella* Persistence and Transmission Strategies. Curr Opin Microbiol 15, 100–107. doi:10.1016/j.mib.2011.10.013

Monk, A.B., Rees, C.D., Barrow, P., Hagens, S., Harper, D.R., 2010. Bacteriophage applications: where are we now? Lett Appl Microbiol 51, 363–369. doi:10.1111/j.1472-765X.2010.02916.x

Moran, A.P., 2014. Molecular Structure, Biosynthesis, and Pathogenic Roles of Lipopolysaccharides. Helicobacter pylori 81–95. doi:10.1128/9781555818005.ch8

Morley, P.S., 2002. Biosecurity of Veterinary Practices. Veterinary Clinics of North America: Food Animal Practice 18, 133–155. doi:10.1016/S0749-0720(02)00009-9

Morley, P.S., Weese, J.S., 2015. Biosecurity and Infection Control for Large Animal Practices, in: Smith, B.P. (Ed.), Large Animal Internal Medicine. Elsevier, New York, NY, pp. 1407–1431.

Mughini-Gras, L., Enserink, R., Friesema, I., Heck, M., van Duynhoven, Y., van Pelt, W., 2014. Risk Factors for Human Salmonellosis Originating from Pigs, Cattle, Broiler Chickens and Egg Laying Hens: A Combined Case-Control and Source Attribution Analysis. PLoS One 9, e87933. doi:10.1371/journal.pone.0087933

Mukherjee, P., Roy, S., Ghosh, D., Nandi, S.K., 2022. Role of Animal Models in Biomedical Research: A Review. Lab Anim Res 38, 1–17. doi:10.1186/s42826-022-00128-1

Muñoz-Cuevas, M., Metris, A., Baranyi, J., 2012. Predictive Modelling of *Salmonella*: From Cell Cycle Measurements to E-Models. Food Research International 45, 852–862. doi:10.1016/j.foodres.2011.04.033

Murray, C.J., 2000. Environmental Aspects of *Salmonella*, in: *Salmonella* in Domestic Animals. pp. 265–283.

Murray, G.L., Attridge, S.R., Morona, R., 2006. Altering the Length of the Lipopolysaccharide O antigen has an Impact on the Interaction of *Salmonella enterica* Serovar typhimurium with Macrophages and Complement. J Bacteriol 188, 2735–2739. doi:10.1128/JB.188.7.2735-2739.2006

Nadon, C., Walle, I. Van, Gerner-smidt, P., Campos, J., Chinen, I., Concepcion-acevedo, J., 2017. PulseNet International: Vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance. Eurosurveillance 22, 1–12. doi:https://doi.org/10.2807/1560-7917.ES.2017.22.23.30544

Naushad, S., Ogunremi, D., Huang, H., 2023. *Salmonella*: A Brief Review, in: Huang, H., Naushad, S. (Eds.), *Salmonella* - Perspectives for Low-Cost Prevention, Control and Treatment. IntechOpen, pp. 1–22. doi:DOI: 10.5772/intechopen.112948

Neoh, H. min, Tan, X.E., Sapri, H.F., Tan, T.L., 2019. Pulsed-field gel electrophoresis (PFGE): A review of the "gold standard" for bacteria typing and current alternatives. Infection, Genetics and Evolution 74, 103935. doi:10.1016/j.meegid.2019.103935

Nicolas, M., Faurie, A., Girault, M., Lavillatte, S., Menanteau, P., Chaumeil, T., Riou, M., Velge, P., Schouler, C., 2023a. *In ovo* Administration of a Phage Cocktail Partially Prevents Colibacillosis in Chicks. Poult Sci 102, 1–9. doi:10.1016/j.psj.2023.102967

Nicolas, M., Trotereau, A., Culot, A., Moodley, A., Atterbury, R., Wagemans, J., Lavigne, R., Velge, P., Schouler, C., 2023b. Isolation and Characterization of a Novel Phage Collection against Avian-Pathogenic *Escherichia coli* . Microbiol Spectr 11. doi:10.1128/spectrum.04296-22

Nieto, P.A., Pardo-roa, C., Salazar-echegarai, F.J., Tobar, H.E., Coronado-arr, I., Riedel, C.A., Kalergis, A.M., Bueno, S.M., 2016. New Insights About Excisable Pathogenicity Islands in *Salmonella* and Their Contribution to Virulence. Microbes Infect 18, 302–309. doi:10.1016/j.micinf.2016.02.001

Obeng, N., Pratama, A.A., Elsas, J.D. van, 2016. The Significance of Mutualistic Phages for Bacterial Ecology and Evolution. Trends Microbiol 24, 440–449. doi:10.1016/j.tim.2015.12.009

Octavia, S., Wang, Q., Tanaka, M.M., Kaur, S., Sintchenko, V., Lan, R., 2015. Delineating Community Outbreaks of *Salmonella enterica* Serovar Typhimurium by Use of Whole-Genome Sequencing: Insights into Genomic Variability within an Outbreak. J Clin Microbiol 53, 1063–1071. doi:10.1128/JCM.03235-14

Oechslin, F., 2018. Resistance Development to Bacteriophages Occurring During Bacteriophage Therapy. Viruses 10, 1–23. doi:10.3390/v10070351

Olsen, J., Sudan, M., Arah, O.A., Kheifets, L., 2017. The Methodological 'Revolution': Caution Accepted. Eur J Epidemiol 32, 165–166. doi:10.1007/s10654-016-0194-y

Oludairo, O.O., Kwaga, J.K.P., Kabir, J., Abdu, P.A., Gitanjali, A., Perrets, A., Cibin, V., Lettini, A.A., Aiyedun, J.O., 2023. Ecology and Epidemiology of *Salmonella* spp. Isolated From the Environment and the Roles Played by Wild Animals in Their Maintenance. Int J One Health 9, 1–9. doi:10.14202/IJOH.2023.1-9

Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T.G., Fookes, M., Falush, D., Keane, J.A., Parkhill, J., 2015. Roary: Rapid large-scale prokaryote pan genome analysis. Bioinformatics 31, 3691–3693. doi:10.1093/bioinformatics/btv421

Panda, A., Tatarov, I., Masek, B.J., Hardick, J., Crusan, A., Wakefield, T., Carroll, K., Yang, S., Hsieh, Y.H., Lipsky, M.M., McLeod, C.G., Levine, M.M., Rothman, R.E., Gaydos, C.A., DeTolla, L.J., 2014. A Rabbit Model of Non-Typhoidal *Salmonella* Bacteremia. Comp Immunol Microbiol Infect Dis 37, 211–220. doi:10.1016/j.cimid.2014.05.004

Pandya, M., Wittum, T., Tadesse, D.A., Gebreyes, W., Hoet, A., 2009. Environmental *Salmonella* Surveillance in The Ohio State University Veterinary Teaching Hospital. Vector-Borne and Zoonotic Diseases 9, 649–654. doi:10.1089/vbz.2008.0120

Pangloli, P., Dje, Y., Ahmed, O., Doane, C.A., Oliver, S.P., Draughon, F.A., 2008. Seasonal Incidence and Molecular Characterization of *Salmonella* from Dairy Cows, Calves, and Farm Environment. Foodborne Pathog Dis 5, 87–96. doi:10.1089/fpd.2008.0048

Penziner, S., Schooley, R.T., Pride, D.T., 2021. Animal Models of Phage Therapy. Front Microbiol 12, 1–13. doi:10.3389/fmicb.2021.631794

Perestrelo, S., Amaro, A., Brouwer, M.S.M., Clemente, L., Ribeiro Duarte, A.S., Kaesbohrer, A., Karpíšková, R., Lopez-Chavarrias, V., Morris, D., Prendergast, D., Pista, A., Silveira, L., Skarżyńska, M., Slowey, R., Veldman, K.T., Zając, M., Burgess, C., Alvarez, J., 2023. Building an International One Health Strain Level Database to Characterise the Epidemiology of AMR Threats: ESBL—AmpC Producing *E. coli* as An Example—Challenges and Perspectives. Antibiotics 12, 1–18. doi:10.3390/antibiotics12030552

Perez, J.B.D.S., Fernando, C., Nosach, R. V., Huang, Y., Harding, J.C.S., Hill, J.E., 2018. *In vitro* Attenuation of a Virulent Swine Isolate of *Brachyspira hampsonii*. Pathog Dis 76, 1–10. doi:10.1093/femspd/ftx116

Podlacha, M., Grabowski, Ł., Kosznik-Kawśnicka, K., Zdrojewska, K., Stasiłojć, M., Węgrzyn, G., Węgrzyn, A., 2021. Interactions of Bacteriophages with Animal and Human Organisms—Safety Issues in the Light of Phage Therapy. Int J Mol Sci 22, 1–40. doi:10.3390/ijms22168937

Połaska, M., Sokołowska, B., 2019. Bacteriophages—A New Hope or a Huge Problem in the Food Industry. AIMS Microbiol 5, 324–347. doi:10.3934/microbiol.2019.4.324

Polo, F., Figueras, M.J., Inza, I., Sala, J., Fleisher, J.M., Guarro, J., 1999. Prevalence of *Salmonella* Serotypes in Environmental Waters and Their Relationships with Indicator Organisms. Antonie Van Leeuwenhoek 75, 285–92. doi:10.1023/A:1001817305779

Porta, M., Bolúmar, F., 2016. Caution: Work in Progress: While the Methodological "Revolution" Deserves In-Depth Study, Clinical Researchers and Senior Epidemiologists Should Not Be Disenfranchised. Eur J Epidemiol 31, 535–539. doi:10.1007/s10654-016-0181-3

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2 - Approximately Maximum-Likelihood Trees for Large Alignments. PLoS One 5. doi:10.1371/journal.pone.0009490

Price, M.N., Dehal, P.S., Arkin, A.P., 2009. Fasttree: Computing Large Minimum Evolution Trees with Profiles Instead of a Distance Matrix. Mol Biol Evol 26, 1641–1650. doi:10.1093/molbev/msp077

Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A., Korobeynikov, A., 2020. Using SPAdes *de novo* Assembler. Curr Protoc Bioinformatics 70, e102. doi:10.1002/cpbi.102

Que, F., Wu, S., Huang, R., 2013. *Salmonella* Pathogenicity Island 1 (SPI-1) at Work. Curr Opin Microbiol 66, 582–587. doi:10.1007/s00284-013-0307-8

Raetz, C.R.H., Whitfield, C., 2002. Lipopolysaccharide Endotoxins. Annu Rev Biochem 71, 635–700. doi:10.1146/annurev.biochem.71.110601.135414

Ragione, R.M. La, Metcalfe, H.J., Villarreal-Ramos, B., Werling, D., 2013. *Salmonella* Infections in Cattle, in: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, Boston, MA, pp. 233–262.

Ranveer, S.A., Dasriya, V., Ahmad, M.F., Dhillon, H.S., Samtiya, M., Shama, E., Anand, T., Dhewa, T., Chaudhary, V., Chaudhary, P., Behare, P., Ram, C., Puniya, D.V., Khedkar, G.D., Raposo, A., Han, H., Puniya, A.K., 2024. Positive and Negative Aspects of Bacteriophages and Their Immense Role in the Food Chain. NPJ Sci Food 8. doi:10.1038/s41538-023-00245-8

Rezaee, M.S., Liebhart, D., Hess, C., Hess, M., Paudel, S., 2021. Bacterial Infection in Chicken Embryos and Consequences of Yolk Sac Constitution for Embryo Survival. Vet Pathol 58, 71–79. doi:10.1177/0300985820960127

Ribot, E.M., Freeman, M., Hise, K.B., Gerner-Smidt, P., 2019. PulseNet: Entering the Age of Next-Generation Sequencing. Foodborne Pathog Dis 16, 451–456. doi:10.1089/fpd.2019.2634

Riswantini, D., Nugraheni, E., 2022. Machine Learning in Handling Disease Outbreaks: A Comprehensive Review. Bulletin of Electrical Engineering and Informatics 11, 2169–2186. doi:10.11591/eei.v11i4.3612

Rivera, D., Allel, K., Dueñas, F., Tardone, R., Soza, P., Hamilton-west, C., Moreno-switt, A.I., 2021. Screening the Presence of Non-Typhoidal *Salmonella* in Different Animal Systems and the Assessment of Antimicrobial Resistance. Animals 11, 1–15. doi:10.3390/ani11061532

Rivera-Chávez, F., Bäumler, A.J., 2015. The Pyromaniac Inside You : *Salmonella* Metabolism in the Host Gut. Annu Rev Microbiol 69, 31–48. doi:10.1146/annurev-micro-091014-104108

Robinson, J.T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., Mesirov, J.P., 2011. Integrative Genomics Viewer. Nat Biotechnol 29, 24–26. doi:10.1038/nbt.1754

Rogers, A.W.L., Tsolis, R.M., Bäumler, A.J., 2021. *Salmonella* versus the Microbiome . Microbiology and Molecular Biology Reviews 85. doi:10.1128/mmbr.00027-19

Romano, K.P., Hung, D.T., 2023. Targeting LPS biosynthesis and transport in gram-negative bacteria in the era of multi-drug resistance. Biochim Biophys Acta Mol Cell Res 1870, 119407. doi:10.1016/j.bbamcr.2022.119407

Rose, B.E., 2014. Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Catfish Products and Carcass and Environmental Sponges, in: Dey, B.P., Lattuada, C.P. (Eds.), USDA/FSIS/OPHS Microbiology Laboratory Guidebook. Washington, D.C., p. MLG 4.08.

Rossi, G., Shih, B.B.J., Egbe, N.F., Motta, P., Duchatel, F., Kelly, R.F., Ndip, L., Sander, M., Tanya, V.N., Lycett, S.J., Bronsvoort, B.M., Muwonge, A., 2023. Unraveling the Epidemiology of *Mycobacterium bovis* Using Whole-Genome Sequencing Combined with Environmental and Demographic Data. Front Vet Sci 10. doi:10.3389/fvets.2023.1086001

Rovetta, A., 2023. Common Statistical Errors in Scientific Investigations: A Simple Guide to Avoid Unfounded Decisions. Cureus 15, 1–9. doi:10.7759/cureus.33351

Russo, S., Bonassi, S., 2015. Prospects and Pitfalls of Machine Learning in Nutritional Epidemiology. Encyclopedia of Food and Health 14, 1–17. doi:10.1016/B978-0-12-384947-2.00494-3

Sahiner, B., Chen, W., Samala, R.K., Petrick, N., 2023. Data Drift in Medical Machine Learning: Implications and Potential Remedies. British Journal of Radiology 96, 1.

Salipante, S.J., SenGupta, D.J., Cummings, L.A., Land, T.A., Hoogestraat, D.R., Cookson, B.T., 2015. Application of Whole-Genome Sequencing for Bacterial Strain Typing in Molecular Epidemiology. J Clin Microbiol 53, 1072–1079. doi:10.1128/JCM.03385-14

Salmon, D.E., Smith, T., 1885. Report on Swine Plague, in: Second Annual Report of the Bureau of Animal Industry for the Year. House document (United States. Congress. House), Washington, D.C., pp. 184–246.

Salmond, G.P.C., Fineran, P.C., 2015. A century of the phage: past, present and future. Nat Rev Microbiol 13, 777–786. doi:10.1038/nrmicro3564

Sande, C., Whitfield, C., 2021. Capsules and Extracellular Polysaccharides in *Escherichia coli* and *Salmonella*. EcoSal Plus 9. doi:10.1128/ecosalplus.esp-0033-2020

Santangelo, O.E., Gentile, V., Pizzo, S., Giordano, D., Cedrone, F., 2023. Machine Learning and Prediction of Infectious Diseases: A Systematic Review. Mach Learn Knowl Extr 5, 175–198. doi:10.3390/make5010013

Santos, R.L., Raffatellu, M., Bevins, C.L., Adams, L.G., Tükel, Ç., Tsolis, M., Bäumler, A.J., 2009. Life in the Inflamed Intestine, *Salmonella* Style. Trends Microbiol 17, 498–506. doi:10.1016/j.tim.2009.08.008

Santos, R.L., Zhang, S., Tsolis, R.M., Kingsley, R.A., 2001. Animal Models of *Salmonella* Infections: Enteritis Versus Typhoid Fever. Microbes Infect 3, 1335–1344.

Sarhan, W.A., Azzazy, H.M.E., 2015. Phage Approved in Food, Why Not as a Therapeutic? Expert Rev Anti Infect Ther 13, 91–101. doi:10.1586/14787210.2015.990383

Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., Thakare, R.P., Banday, S., Mishra, A.K., Das, G., Malonia, S.K., 2023. Next-Generation Sequencing Technology: Current Trends and Advancements. Biology (Basel) 12, 1–25. doi:10.3390/biology12070997

Sayers, E.W., Beck, J., Bolton, E.E., Brister, J.R., Chan, J., Comeau, D.C., Connor, R., DiCuccio, M., Farrell, C.M., Feldgarden, M., Fine, A.M., Funk, K., Hatcher, E., Hoeppner, M., Kane, M., Kannan, S., Katz, K.S., Kelly, C., Klimke, W., Kim, S., Kimchi, A., Landrum, M., Lathrop, S., Lu, Z., Malheiro, A., Marchler-Bauer, A., Murphy, T.D., Phan, L., Prasad, A.B., Pujar, S., Sawyer, A., Schmieder, E., Schneider, V.A., Schoch, C.L., Sharma, S., Thibaud-Nissen, F., Trawick, B.W., Venkatapathi, T., Wang, J., Pruitt, K.D., Sherry, S.T., 2024. Database Resources of the National Center for Biotechnology Information. Nucleic Acids Res 52, D33–D43. doi:10.1093/nar/gkad1044

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R. V, Widdowson, M., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne Illness Acquired in the United States — Major Pathogens 1–21. doi:10.3201/eid1701.P11101

Schaer, B.L.D., Aceto, H., Rankin, S.C., 2010. Outbreak of Salmonellosis Caused by *Salmonella enterica* Serovar Newport MDR-AmpC in a Large Animal Veterinary Teaching Hospital. J Vet Intern Med 24, 1138–1146.

Schnaitman, C.A., Klena, J.D., 1993. Genetics of lipopolysaccharide biosynthesis in enteric bacteria. Microbiol Rev 57, 655–682. doi:10.1128/mmbr.57.3.655-682.1993

Schott, H.C., Ewart, S.L., Walker, R.D., Dwyer, R.M., Dietrich, S., Eberhart, S.W., Kusey, J., Stick, J. a, Derksen, F.J., 2001. An Outbreak of Salmonellosis Among Horses at a Veterinary Teaching Hospital. J Am Vet Med Assoc 218, 1152–1159. doi:10.2460/javma.2001.218.1152

Schultz, B.M., Melo-Gonzalez, F., Salazar, G.A., Porto, B.N., Riedel, C.A., Kalergis, A.M., Bueno, S.M., 2021. New Insights on the Early Interaction Between Typhoid and Non-typhoid *Salmonella* Serovars and the Host Cells. Front Microbiol 12, 1–20. doi:10.3389/fmicb.2021.647044

Schürch, A.C., Arredondo-Alonso, S., Willems, R.J.L., Goering, R. V., 2018. Whole Genome Sequencing Options for Bacterial Strain Typing and Epidemiologic Analysis Based on Single Nucleotide Polymorphism Versus Gene-by-Gene–Based Approaches. Clinical Microbiology and Infection 24, 350–354. doi:10.1016/j.cmi.2017.12.016

Sebola, D.C., Oguttu, J.W., Kock, M.M., Qekwana, D.N., 2023. Hospital-Acquired and Zoonotic Bacteria from a Veterinary Hospital and Their Associated Antimicrobial-Susceptibility Profiles: A Systematic Review. Front Vet Sci 9. doi:10.3389/fvets.2022.1087052

Seemann, T., 2020. snippy. Github Repository.

Seemann, T., 2014. Prokka: Rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069. doi:10.1093/bioinformatics/btu153

Segall, A.M., Roach, D.R., Strathdee, S.A., 2019. Stronger Together? Perspectives on Phage-Antibiotic Synergy in Clinical Applications of Phage Therapy. Curr Opin Microbiol 51, 46–50. doi:10.1016/j.mib.2019.03.005

Sereika, M., Kirkegaard, R.H., Karst, S.M., Michaelsen, T.Y., Sørensen, E.A., Wollenberg, R.D., Albertsen, M., 2022. Oxford Nanopore R10.4 Long-Read Sequencing Enables the Generation of Near-Finished Bacterial Genomes From Pure Cultures and Metagenomes Without Short-Read or Reference Polishing. Nat Methods 19, 823–826. doi:10.1038/s41592-022-01539-7

Serghiou, S., Rough, K., 2023. Deep Learning for Epidemiologists: An Introduction to Neural Networks. Am J Epidemiol 192, 1904–1916. doi:10.1093/aje/kwad107

Seth-smith, H.M.B., 2008. SPI-7: *Salmonella*'s Vi-Encoding Pathogenicity Island. J Infect Dev Ctries 2, 267–271.

Seth-smith, H.M.B., Fookes, M.C., Okoro, C.K., Baker, S., Harris, S.R., Scott, P., Pickard, D., Quail, M.A., Churcher, C., Sanders, M., Harmse, J., Dougan, G., Parkhill, J., Thomson, N.R., 2012. Structure, Diversity, and Mobility of the *Salmonella* Pathogenicity Island 7 Family of Integrative and Conjugative Elements within *Enterobacteriaceae*. J Bacteriol 194, 1494–1504. doi:10.1128/JB.06403-11

Shabbir, M.A.B., Hao, H., Shabbir, M.Z., Wu, Q., Sattar, A., Yuan, Z., 2016. Bacteria vs. Bacteriophages: Parallel Evolution of Immune Arsenals. Front Microbiol 7, 1–8. doi:10.3389/fmicb.2016.01292

Shah, D.H., Zhou, X., Kim, H.Y., Call, D.R., Guard, J., 2012. Transposon Mutagenesis of *Salmonella enterica* Serovar Enteritidis Identifies Genes that Contribute to Invasiveness in Human and Chicken Cells and Survival in Egg Albumen. Infect Immun 80, 4203–4215. doi:10.1128/IAI.00790-12

Shamim Hasan Zahid, M., Nashir Udden, S.M., Faruque, A.S.G., Calderwood, S.B., Mekalanos, J.J., Faruque, S.M., 2008. Effect of Phage on the Infectivity of *Vibrio cholerae* and Emergence of Genetic Variants. Infect Immun 76, 5266–5273. doi:10.1128/IAI.00578-08

Shendure, J., Balasubramanian, S., Church, G.M., Gilbert, W., Rogers, J., Schloss, J.A., Waterston, R.H., 2017. DNA Sequencing at 40: Past, Present, and Future. Nature 550, 345–353. doi:10.1038/nature24286

Shirley, J.F., 2016. Development Toward a Bovine *Salmonella* Model and a Bacteriophage Treatment Cocktail to Reduce *Salmonella* Peripheral Lymph Node Carriage in Calves (Master's Thesis). Auburn University.

Shivaprasad, H.L., Methner, U., Barrow, P.A., 2013. *Salmonella* Infections in the Domestic Fowl, in: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, Boston, MA, p. 192.

Simon, R., Tennant, S.M., Galen, J.E., Levine, M.M., 2011. Mouse Models to Assess the Efficacy of Non-Typhoidal *Salmonella* Vaccines: Revisiting the Role of Host Innate Susceptibility and Routes of Challenge. Vaccine 29, 5094–5106. doi:10.1016/j.vaccine.2011.05.022

Skurnik, M., Strauch, E., 2006. Phage therapy: Facts and fiction. International Journal of Medical Microbiology 296, 5–14. doi:10.1016/j.ijmm.2005.09.002

Smith, B.P., 2004. Evolution of Equine Infection Control Programs. Veterinary Clinics Equine Practice 20, 521–530. doi:10.1016/j.cveq.2004.07.002

Smith, B.P., House, J.K., Magdesian, K.G., Jang, S.S., Cabral, R.L., Madigan, J.E., Herthel, W.E., 2004. Principles of an Infectious Disease Control Program for Preventing Nosocomial Gastrointestinal and Respiratory Tract Diseases in Large Animal Veterinary hospitals. J Am Vet Med Assoc 225, 1186–1195. doi:10.2460/javma.2004.225.1186

Smith, R.P., Andres, V., Cheney, T.E., Martelli, F., Gosling, R., Marier, E., Rabie, A., Gilson, D., Davies, R.H., 2018. How Do Pig Farms Maintain Low *Salmonella* Prevalence: A Case-Control Study. Epidemiol Infect 146, 1909–1915. doi:10.1017/S0950268818002248

Smith, T., 1894. The Hog-Cholera Group of Bacteria. U.S. Department of Agriculture. Bureau of Animal Industry Bulletin 6, 6–40.

Soza-Ossandón, P., Rivera, D., Tardone, R., Riquelme-Neira, R., García, P., Hamilton-West, C., Adell, A.D., González-Rocha, G., Moreno-Switt, A.I., 2020. Widespread Environmental Presence of Multidrug-Resistant *Salmonella* in an Equine Veterinary Hospital That Received Local and International Horses. Front Vet Sci 7, 1–9. doi:10.3389/fvets.2020.00346

Spencer-Drakes, T.C.J., Sarabia, A., 2023. O-Antigen Biosynthesis Mediates Evolutionary Trade-Offs Within a Simple Community. bioRxiv 4, 88–100.

Štefek, M., Švejda, D., Zvárová, J., Tomečková, M., 1997. Epidemiology Tools Program and Selected Medical Applications. Int J Med Inform 45, 35–41.

Steneroden, K.K., Metre, D.C. Van, Jackson, C., Morley, P.S., 2010. Detection and Control of a Nosocomial Outbreak Caused by *Salmonella* Newport at a Large Animal Hospital. J Vet Intern Med 24, 606–616. doi:https://doi.org/10.1111/j.1939-1676.2010.0484.x

Sterzenbach, T., Crawford, R.W., Winter, S.E., Bäumler, A.J., 2013. *Salmonella* Virulence Mechanisms and their Genetic Basis, in: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, Boston, MA, pp. 80–103.

Stevens, M.P., Gray, J.T., 2013. *Salmonella* Infections in Pigs, in: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, Boston, MA, pp. 263–294.

Stevens, M.P., Kingsley, R.A., 2021. *Salmonella* Pathogenesis and Host-Adaptation in Farmed Animals. Curr Opin Microbiol 63, 52–58. doi:10.1016/j.mib.2021.05.013

Stockton, K. a, Morley, P.S., Hyatt, D.R., Burgess, B. a, Patterson, G., Dunowska, M., Lee, D.E., 2006. Evaluation of the Effects of Footwear Hygiene Protocols on Nonspecific Bacterial Contamination of Floor Surfaces in an Equine Hospital. J Am Vet Med Assoc 228, 1068–1073. doi:10.2460/javma.228.7.1068

Sulakvelidze, A., 2013. Using Lytic Bacteriophages to Eliminate or Significantly Reduce Contamination of Food by Foodborne Bacterial Pathogens. J Sci Food Agric 93, 3137–3146. doi:10.1002/jsfa.6222

Sulakvelidze, A., Kutter, E., 2005. Bacteriophage Therapy in Humans, in: Kutter, E., Sulakvelidze, A. (Eds.), Bacteriophages: Biology and Applications. CRC Press, Boca Raton, Florida, pp. 381–436.

Sumrall, E.T., Shen, Y., Keller, A.P., Rismondo, J., Pavlou, M., Eugster, M.R., Boulos, S., Disson, O., Thouvenot, P., Kilcher, S., Wollscheid, B., Cabanes, D., Lecuit, M., Gründling, A., Loessner, M.J., 2019. Phage Resistance at the Cost of Virulence: *Listeria monocytogenes* Serovar 4b Requires Galactosylated Teichoic Acids for InlB-Mediated Invasion. PLoS Pathog 15, 1–29. doi:10.1371/journal.ppat.1008032

Tang, J., Gu, Y., Wang, X., Luo, Y., Zhang, F., Zheng, J., Wang, Y., Shen, X., Xu, L., 2024. *Salmonella* T3SS-Elicited Inflammatory Innate Immune Response Inhibits Type I IFN Response in Macrophages. Vet Microbiol 289, 109970. doi:10.1016/j.vetmic.2023.109970

Tang, L., Zhu, S.L., Fang, X., Li, Y.G., Poppe, C., Johnston, R.N., Liu, G.R., Liu, S.L., 2019. Distinct Evolutionary Origins of Common Multi-Drug Resistance Phenotypes in *Salmonella* Typhimurium DT104: A Convergent Process for Adaptation Under Stress. Molecular Genetics and Genomics 294, 597–605. doi:10.1007/s00438-019-01531-5

Tang, M., Huang, Z., Zhang, X., Kong, J., Zhou, B., Han, Y., Zhang, Y., Chen, L., Zhou, T., 2023. Phage Resistance Formation and Fitness Costs of Hypervirulent *Klebsiella pneumoniae* Mediated by K2 Capsule-Specific Phage and the Corresponding Mechanisms. Front Microbiol 14, 1–18. doi:10.3389/fmicb.2023.1156292

Tennant, S.M., Maclennan, C.A., Simon, R., Martin, L.B., Khan, M.I., 2016. Nontyphoidal *Salmonella* Disease: Current Status of Vaccine Research and Development. Vaccine 34, 2907–2910. doi:10.1016/j.vaccine.2016.03.072

Thiennimitr, P., Winter, S.E., Bäumler, A.J., Ba, A.J., 2012. *Salmonella*, The Host and Its Microbiota. Curr Opin Microbiol 15, 108–114. doi:10.1016/j.mib.2011.10.002

Thomas, C., Methner, U., Marz, M., Linde, J., 2023. Oxford Nanopore Technologies—A Valuable Tool to Generate Whole-Genome Sequencing Data for in silico Serotyping and the Detection of Genetic Markers in *Salmonella*. Front Vet Sci 10, 1–11. doi:10.3389/fvets.2023.1178922

Thomsen, L.E., Chadfield, M.S., Bispham, J., Wallis, T.S., Olsen, J.E., Ingmer, H., 2003. Reduced Amounts of LPS Affect Both Stress Tolerance and Virulence of *Salmonella enterica* Serovar Dublin. FEMS Microbiol Lett 228, 225–231. doi:10.1016/S0378-1097(03)00762-6

Thulin, J.D., Bradfield, J.F., Bergdall, V.K., Conour, L.A., Grady, A.W., Hickman, D.L., Norton, J.N., Wallace, J.M., 2014. The Cost of Self-Imposed Regulatory Burden in Animal Research. FASEB Journal 28, 3297–3300. doi:10.1096/fj.14-254094

Tillotson, K., Savage, C.J., Salman, M.D., Gentry-Weeks, C., Rice, D., Fedorka-Cray, P.J., Hendricksen, D.A., Jones, R.L., Nelson, A.W., Traub-Dargatz, J.L., 1997. Outbreak of *Salmonella* Infantis Infection in a Large Animal Veterinary Teachin Hospital. Journal of American Veterinary Medical Association 211, 1554–1557.

Timme, R.E., Leon, M.S., Allard, M.W., 2019. Utilizing the Public GenomeTrakr Database for Foodborne Pathogen Traceback, in: Bridier, A. (Ed.), Foodborn Bacterial Pathogens: Methods and Protocols, Methods in Molecular Biology. pp. 449–481. doi:10.1525/9780520959743-017

Timoney, J., 2013. *Salmonella* Infections in Horses, in: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in Domestic Animals. CABI Publishing, Boston, MA, pp. 305–317.

Timsit, S., Armand-Lefèvre, L., Le Goff, J., Salmona, M., 2023. The Clinical and Epidemiological Impacts of Whole Genomic Sequencing on Bacterial and Virological Agents. Infect Dis Now 54, 104844. doi:10.1016/j.idnow.2023.104844

Tolar, B., Joseph, L.A., Schroeder, M.N., Stroika, S., Ribot, E.M., Hise, K.B., Gerner-Smidt, P., 2019. An Overview of PulseNet USA Databases. Foodborne Pathog Dis 16, 457–462. doi:10.1089/fpd.2019.2637

Toro, H., Price, S.B., McKee, S., Hoerr, F.J., Krehling, J., Perdue, M., Bauermeister, L., 2005. Use of Bacteriophages in Combination with Competitive Exclusion to Reduce *Salmonella* from Infected Chickens. Avian Dis 49, 118–124. doi:10.1637/7286-100404R

Tran, A., Rowlinson, M.-C., 2019. Application of Next-Generation Sequencing in Public Health Epidemiology and Outbreak Investigation. Advances in Molecular Pathology 2, 89–97. doi:10.1016/j.yamp.2019.07.007

Traverse, M., Aceto, H., 2015. Environmental Cleaning and Disinfection. Veterinary Clinics of North America - Small Animal Practice 45, 299–330. doi:10.1016/j.cvsm.2014.11.011

Treangen, T.J., Ondov, B.D., Koren, S., Phillippy, A.M., 2014. The Harvest Suite for Rapid Core-Genome Alignment and Visualization of Thousands of Intraspecific Microbial Genomes. Genome Biol 15, 1–15. doi:10.1186/s13059-014-0524-x

Trotereau, A., Schouler, C., 2019. Use of a Chicken Embryo Lethality Assay to Assess the Efficacy of Phage Therapy, in: Clokie, M.R.J., Kropinski, A., Lavigne, R. (Eds.), Bacteriophages: Methods and Protocols, Volume 4. Hu, New York, NY, pp. 199–205. doi:https://doi.org/10.1007/978-1-4939-8940-9_17

Tsolis, E.M., Adams, L.G., Ficht, T.A., Ba, A.J., 1999. Contribution of *Salmonella* Typhimurium Virulence Factors to Diarrheal Disease in Calves. Infect Immun 67, 4879–4885.

Uelze, L., Grützke, J., Borowiak, M., Hammerl, J.A., Juraschek, K., Deneke, C., Tausch, S.H., Malorny, B., 2020. Typing Methods Based on Whole Genome Sequencing Data. One Health Outlook 2, 1–19. doi:10.1186/s42522-020-0010-1

US HHS, U.D. of H. and H.S., 2023. Healthy People 2030: Foodborne Illness [WWW Document]. US Department of Health and Human Services, Centers for Disease Control and Prevention: Atlanta, GA, USA. URL https://health.gov/healthypeople/objectives-and-data/browse-objectives/foodborne-illness/reduce-infections-caused-*Salmonella*-fs-04 (accessed 3.3.24).

USDA, 2019. Delay in FSIS' Plans to Discontinue PFGE for *E. coli* and Move to WGS as the Primary Characterization Tool FSIS. FSIS Constituent Update 22, 1–3.

Uzzau, S., Brown, D.J., Wallis, T., Rubino, S., Leori, G., Bernard, S., Casadesús, J., Platt, D.J., Olsen, J.E., 2000. Host Adapted Serotypes of *Salmonella enterica*. Epidemiol Infect 125, 229–255. doi:10.1017/S0950268899004379

Vaessen, M.A., Veling, J., Frankena, K., Graat, E.A.M., Klunder, T., 1998. Risk Factors for *Salmonella* Dublin Infection on Dairy Farms. Veterinary Quarterly 20, 97–99. doi:10.1080/01652176.1998.9694848

van der Sar, A.M., Musters, R.J.P., van Eeden, F.J.M., Appelmelk, B.J., Vandenbroucke-Grauls, C.M.J.E., Bitter, W., 2003. Zebrafish Embryos as a Model Host for the Real Time Analysis of *Salmonella* Typhimurium Infections. Cell Microbiol 5, 601–611. doi:10.1046/j.1462-5822.2003.00303.x

Van Immerseel, F., De Buck, J., Pasmans, F., Bohez, L., Boyen, F., Haesebrouck, F., Ducatelle, R., Immerseel, F. Van, Buck, J. De, Pasmans, F., Bohez, L., Boyen, F., Haesebrouck, F., Ducatelle, R., 2004. Intermittent Long-Term Shedding and Induction of Carrier Birds after Infection of Chickens Early Posthatch with a Low or High Dose of *Salmonella* Enteritidis. Poult Sci 83, 1911–1916. doi:10.1093/ps/83.11.1911

Varma, J.K., Marcus, R., Stenzel, S.A., Hanna, S.S., Gettner, S., Anderson, B.J., Hayes, T., Shiferaw, B., Crume, T.L., Joyce, K., Fullerton, K.E., Voetsch, A.C., Angulo, F.J., 2006. Highly Resistant *Salmonella* Newport-MDRAmpC Transmitted through the Domestic US Food Supply : A FoodNet Case-Control Study of Sporadic *Salmonella* Newport Infections , 2002 – 2003 30333, 2002–2003.

Vilne, B., Meistere, I., Grantiņa-Ieviņa, L., Ķibilds, J., 2019. Machine Learning Approaches for Epidemiological Investigations of Food-Borne Disease Outbreaks. Front Microbiol 10. doi:10.3389/fmicb.2019.01722

Wagner, S., Grin, I., Malmsheimer, S., Singh, N., Torres-Vargas, C.E., Westerhausen, S., 2018. Bacterial type III Secretion Systems: A Complex Device for the Delivery of Bacterial Effector Proteins into Eukaryotic Host Cells. FEMS Microbiol Lett 365, 1–13. doi:10.1093/femsle/fny201

Wall, S.K., Zhang, J., Rostagno, M.H., Ebner, P.D., 2010. Phage Therapy To Reduce Preprocessing *Salmonella* Infections in Market-Weight Swine. Appl Environ Microbiol 76, 48–53. doi:10.1128/AEM.00785-09

Walther, B., Tedin, K., Lübke-Becker, A., 2017. Multidrug-Resistant Opportunistic Pathogens Challenging Veterinary Infection Control. Vet Microbiol 200, 71–78. doi:10.1016/j.vetmic.2016.05.017

Wang, C., Nie, T., Lin, F., Connerton, I.F., Lu, Z., Zhou, S., Hang, H., 2019. Resistance Mechanisms Adopted by a *Salmonella* Typhimurium Mutant Against Bacteriophage. Virus Res 273, 197759. doi:10.1016/j.virusres.2019.197759

Ward, M.P., Brady, T.H., Coue, L.L., Liljebjelke, K., Maurer, J.J., Ching, C., 2005. Investigation and Control of an Outbreak of Salmonellosis Caused by Multidrug-Resistant *Salmonella* Typhimurium in a Population of Hospitalized Horses. Vet Microbiol 107, 233–240. doi:10.1016/j.vetmic.2005.01.019

Wick, R.R., Judd, L.M., Holt, K.E., 2023. Assembling the Perfect Bacterial Genome using Oxford Nanopore and Illumina sequencing. PLoS Comput Biol 19, 1–12. doi:10.1371/journal.pcbi.1010905

Wiemken, T.L., Kelley, R.R., 2019. Machine Learning in Epidemiology and Health Outcomes Research. Annu Rev Public Health 41, 21–36. doi:10.1146/annurev-publhealth-040119-094437

Wifling, K., Dimroth, P., 1989. Isolation and Characterization of Oxaloacetate Decarboxylase of *Salmonella* Typhimurium, a Sodium Ion Pump. Arch Microbiol 152, 584–588. doi:10.1007/BF00425491

Williams, S., Markey, P., Harlock, M., Binns, P., Gaggin, J., Patel, M., 2016. Individual and Household-Level Risk Factors for Sporadic Salmonellosis in Children. Journal of Infection 72, 36–44. doi:10.1016/j.jinf.2015.09.014

Wilson, B.A., Salyers, A.A., Whitt, D.D., Winkler, M.E., 2011. Mechanisms of Genetic Modification and Exchange: Role in Pathogen Evolution, in: Bacterial Pathogenesis: A Molecular Approach. ASM Press, Washington, DC, pp. 111–129.

Winter, S.E., Bäumler, A.J., 2011. A Breathtaking Feat: To Compete with the Gut Microbiota, *Salmonella* Drives its Host to Provide a Respiratory Electron Acceptor. Gut Microbes 2, 58–60. doi:10.4161/gmic.2.1.14911

Winter, S.E., Thiennimitr, P., Winter, M.G., Butler, B.P., Huseby, D.L., Crawford, R.W., Tsolis, M., Roth, J.R., Ba, A.J., Russell, J.M., Bevins, C.L., Adams, L.G., Tsolis, R.M., Roth, J.R., Bäumler, A.J., 2010. Gut Inflammation Provides a Respiratory Electron Acceptor for *Salmonella*. Nature 467, 426–429. doi:10.1038/nature09415

Wottlin, L.R., Edrington, T.S., Brown, T.R., Zook, C.A., Sulakvelidze, A., Droleskey, R., Genovese, K.J., Nisbet, D.J., 2022. Evaluation of a Preharvest Bacteriophage Therapy for Control of *Salmonella* within Bovine Peripheral Lymph Nodes. J Food Prot 85, 254–260. doi:10.4315/JFP-21-292

Wray, C., Todd, N., Mclaren, I., Beedell, Y., Rowe, B., 1990. The Epidemiology of *Salmonella* Infection of Calves: The Role of Dealers. Epidemiol Infect 105, 295–305.

Wright, J.G., Tengelsen, L.A., Smith, K.E., Bender, J.B., Frank, R.K., Grendon, J.H., Rice, D.H., Thiessen, A.M.B., Gilbertson, C.J., Sivapalasingam, S., Barrett, T.J., Besser, T.E., Hancock, D.D., Angulo, F.J., 2005. *Salmonella* Typhimurium in Four Animal Facilities. Emerg Infect Dis 11, 1235–1241.

Wu, X., Luo, H., Ge, C., Xu, F., Deng, X., Wiedmann, M., Baker, R.C., Stevenson, A.E., Zhang, G., Tang, S., 2023. Evaluation of Multiplex Nanopore Sequencing for *Salmonella* Serotype Prediction and Antimicrobial Resistance Gene and Virulence Gene Detection. Front Microbiol 13, 1–14. doi:10.3389/fmicb.2022.1073057

Yosef, I., Manor, M., Kiro, R., Qimron, U., 2015. Temperate and Lytic Bacteriophages Programmed to Sensitize and Kill Antibiotic-Resistant Bacteria. Proceedings of the National Academy of Sciences 112, 7267–7272. doi:10.1073/pnas.1500107112

Yoshida, C.E., Kruczkiewicz, P., Laing, C.R., Lingohr, E.J., Gannon, V.P.J., Nash, J.H.E., Taboada, E.N., 2016. The *Salmonella* In Silico Typing Resource (SISTR): An Open Web-Accessible Tool for Rapidly Typing and Subtyping Draft *Salmonella* Genome Assemblies. PLoS One 11, 1–17. doi:10.1371/journal.pone.0147101

Yu, J., Zhang, H., Ju, Z., Huang, J., Lin, C., Wu, J., Wu, Y., Sun, S., Wang, H., Hao, G., Zhang, A., 2024. Increased Mutations in Lipopolysaccharide Biosynthetic Genes Cause Time-Dependent Development of Phage Resistance in *Salmonella*. Antimicrob Agents Chemother 68, 1–17. doi:10.1128/aac.00594-23

Zaghen, F., Sora, V.M., Meroni, G., Laterza, G., Martino, P.A., Soggiu, A., Bonizzi, L., Zecconi, A., 2023. Epidemiology of Antimicrobial Resistance Genes in *Staphyloccocus aureus* Isolates from a Public Database in a One Health Perspective—Sample Characteristics and Isolates' Sources. Antibiotics 12, 1225. doi:10.3390/antibiotics12071225

Zhang, F., Cheng, W., 2022. The Mechanism of Bacterial Resistance and Potential Bacteriostatic Strategies. Antibiotics 11. doi:10.3390/antibiotics11091215

Zhang, P., Jiang, D., Wang, Y., Yao, X., Luo, Y., Yang, Z., 2021. Comparison of *De novo* Assembly Strategies for Bacterial Genomes. Int J Mol Sci 22, 7668. doi:10.3390/ijms22147668

Zhang, S., den Bakker, H.C., Li, S., Chen, J., Dinsmore, B.A., Lane, C., Lauer, A.C., Fields, P.I., Deng, X., 2019. SeqSero2: Rapid and Improved *Salmonella* Serotype Determination Using Whole-Genome Sequencing Data. Appl Environ Microbiol 85. doi:10.1128/AEM.01746-19

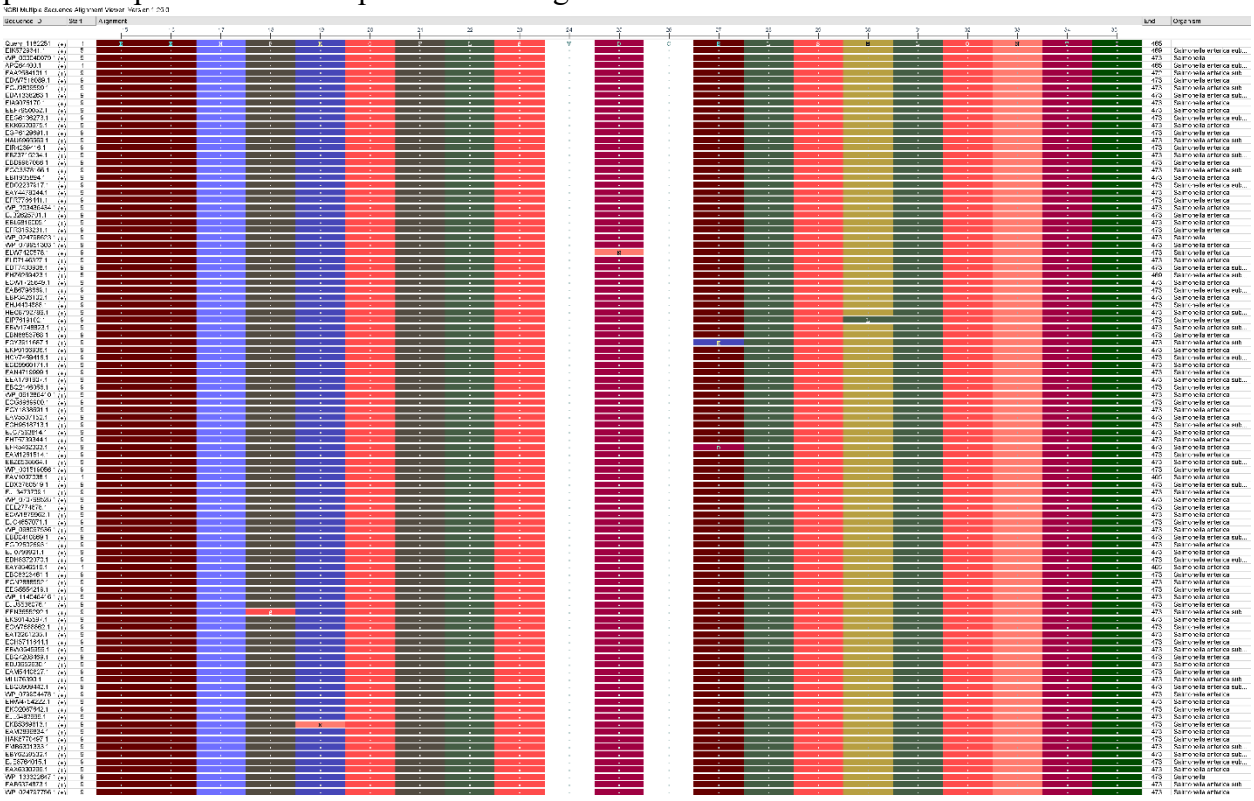
Zhang, S., Yin, Y., Jones, M.B., Zhang, Z., Kaiser, B.L.D., Dinsmore, B.A., Fitzgerald, C., Fields, P.I., Deng, X., 2015. *Salmonella* Serotype Determination Utilizing High-Throughput Genome Sequencing Data. J Clin Microbiol 53, 1685–1692. doi:10.1128/JCM.00323-15

Zhao, W., Zeng, W., Pang, B., Luo, M., Peng, Y., Xu, J., Kan, B., Li, Z., Lu, X., 2023. Oxford Nanopore Long-Read Sequencing Enables the Generation of Complete Bacterial and Plasmid Genomes Without Short-Read Sequencing. Front Microbiol 14, 1–9. doi:10.3389/fmicb.2023.1179966

Zocchetti, C., Consonni, D., Bertazzi, P.A., 1997. Relationship Between Prevalence Rate Ratios and Odds Ratios in Cross-Sectional Studies. Int J Epidemiol 26, 220–223. doi:10.1093/ije/26.1.220

Appendice. Supplemental Data

Supplemental Figure 1: NCBI Protein Blast of *rfbM* amino acid sequence of *Sal*. Newport parent. Top 100 hits with top four hits being 100% matches.



Supplemental Figure 2: NCBI Protein Blast of *rfbM* amino acid sequence of *Sal*. Newport mutant. Top 100 hits with top hit being 100% match.