USE OF PULSED-FIELD GEL ELECTROPHORESIS TO GENOTYPICALLY CHARACTERIZE SALMONELLAE GROUPED BY SEROTYPE

A Thesis

by

DAMON L. J. DRINNON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2004

Major Subject: Veterinary Microbiology

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Approved as to style and content by:

Charles M. Scanlan (Co-Chair of Committee)	Roger B. Harvey (Co-Chair of Committee)
Russell B. Simpson (Member)	Michael E. Hume (Member)
Ian R. Tizard (Member)	Ann B. Kier (Head of Department)

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ABSTRACT

Use of Pulsed-Field Gel Electrophoresis to Genotypically

Characterize Salmonellae Grouped by Serotype. (May 2004)

Damon L. J. Drinnon, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Charles M. Scanlan
Dr. Roger B. Harvey

The prevention and control of salmonellae in commercial swine operations are becoming increasingly important. The current approach focuses on identifying sources and/or origins of salmonellae contamination before swine are processed for human consumption. The objective of the current study was to assess strain variability among salmonellae grouped by serotype and to determine common origins of contamination (farm or slaughter plant). Salmonellae were previously collected from swine at slaughter, serotyped by the National Veterinary Services Laboratory and stored at - 70°C. Pulsed-field gel electrophoresis (PFGE) was performed to genotypically characterize serotypic isolates using restriction endonuclease *Xba*I. Dendrogram comparisons were also used to assess genotypic similarity when multiple genotypes existed. This study found PFGE to be more discriminatory than serotyping indicating that multiple genotypic strains existed among selected serotypes. On the basis of PFGE results alone, origins of contamination could not be determined in this study. It is suggested by the author, that origins of contamination could be further defined pending future research, in which in-depth longitudinal studies are included. When used as an adjunct to conventional typing methods, PFGE may prove to be a substantial subtyping system in epidemiologic investigations to identify point-of-entry contaminants to the food chain.

DEDICATION

This thesis is dedicated to my loving wife, Kasey. Thank you for your support, encouragement, and above all, your patience. And thank you to my family, especially, to those who encouraged me to pursue my educational goals.

ACKNOWLEDGMENTS

I would like to thank Drs. Harvey and Scanlan for serving as committee Co-Chairs. The author is indebted to committee Co-Chair Dr. R. B. Harvey, who sacrificed time, energy, and effort to help me realize my educational goal. Dr. Harvey graciously allowed me to work under his tutelage at the USDA and his direction was essential in completing this project. I also thank Dr. Harvey for extensively reviewing and editing multiple drafts of my thesis and for the insight he offered. The author is indebted to committee Co-Chair Dr. C. M. Scanlan, who shared with me his knowledge of science and his passion for teaching. Dr. Scanlan's profound work ethic, dedication to educational well-being, and problem solving intellect have been inspirational to my success as a graduate student. I also thank Dr. Scanlan for allowing me to serve as a VTPB 405 T.A., for this has been a unique learning opportunity that has greatly impacted my life. I would like to express my appreciation to committee member Dr. M. E. Hume, who provided assistance and advice during the research of this project. I also thank Dr. Hume for his willingness to review and edit multiple drafts of my thesis and the insight he offered. The author is also grateful to Drs. R. B. Simpson and I. R. Tizard for serving as committee members and for providing advice during the preparation of this thesis. The technical assistance of Charles L. Hernandez is also appreciated. His laboratory skills and competence in molecular biology are second-to-none.

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CHAPTER I

INTRODUCTION

Salmonellae and salmonellosis present significant health concerns for the world's population. Of particular importance is the asymptomatic carriage of salmonellae in food-producing animals. The prevention and control of salmonellae in commercial swine operations are becoming increasingly important. The current approach focuses on identifying sources and/or origins of salmonellae contamination before swine are processed for human consumption. As such, emphasis is directed towards developing intervention strategies to reduce salmonellae prevalence and bacterial load. Typing methods used to identify salmonellae help aid this objective. The present manuscript addresses the use of pulsed-field gel electrophoresis in determining genotypes within serotypes of salmonellae from commercial swine.

This thesis follows the style and format of Journal of Food Protection.

CHAPTER II

REVIEW OF LITERATURE

INTRODUCTION

Foodborne infections are considered some of the most widespread problems of the contemporary world (*24*). The annual patient-related costs of bacterial and parasitic foodborne infections are calculated to be approximately $6.5 billion, excluding compensations for lost wages and/or other social costs (*36*). Several agents of foodborne illness include bacteria, viruses, and parasites and it is estimated that up to 81 million cases of illnesses and up to 9,000 deaths occur annually in the United States (*25*). Many foodborne illnesses are undiagnosed or underreported and it is estimated that bacterial infections account for an overall 30% of cases, 63% of hospitalizations and 72% of deaths (*8*, *25*, *43*). Of the total bacterial foodborne infections accounted for, salmonellae infections result in an estimated 16,000 human hospitalizations and more than 500 deaths annually (*1*).

SALMONELLA

Lignieres coined the name *Salmonella* in 1900 after D. E. Salmon, the bacteriologist who identified *Salmonella choleraesuis* in 1885 (*38*). *Salmonella,* a genus within the family Enterobacteriaceae, is classified as a facultative anaerobic Gram-negative rod, that is motile via peritrichous flagella, usually aerogenic producing gas from glucose, and can utilize citrate as its sole carbon source. The failure to ferment lactose and the ability to produce hydrogen sulfides from sulfur-containing amino acids are features used to identify colonies on primary isolation media (*32*). Because of their

inability to ferment lactose, salmonellae are not part of the coliform group, although frequently they are discussed as if they are part of this group. Salmonellae have an optimal growth temperature in the 35 to 40˚C range, but are capable of growth at higher temperatures (*7*). Salmonellae are ubiquitous pathogens that may be found in humans, livestock, wild mammals, reptiles, birds, and insects (*20, 32*). Salmonellae may survive for long periods in the environment, and it is believed that asymptomatic animal carriage is the major source of infection for both animals and humans.

SALMONELLOSIS

Salmonellosis, though generally mild and self-limiting, can result in long hospital stays, and in some cases death (*26, 30*). Infections vary in clinical presentation, but diarrhea is the most common clinical manifestation (*1*). The incubation period is typically six to 48 hours and is followed by fever, headache, malaise, abdominal pain, diarrhea, vomiting and muscle aches (*6, 26*). Symptoms usually resolve within a week, but salmonellae are shed in the feces by children less than five years of age for up to 20 weeks and adults for up to eight weeks. It is estimated from volunteer studies that 10^5 to 10^{10} bacteria are required to initiate an infection, but the exact amount needed is variable by strain and by physiological state of the host (*6*). Pathogenic salmonellae ingested in food survive passage through the gastric acid barrier and invade the mucosa of the small and large intestine and produce toxins. Salmonellae's ability to invade epithelial cells stimulates the release of proinflammatory cytokines that induces an inflammatory reaction. The acute inflammatory response causes diarrhea and may lead to ulceration and destruction of the mucosa (*11*).

EPIDEMIOLOGY

The epidemiology of foodborne disease has changed in the last two decades partly because newly recognized pathogens emerge and previously recognized pathogens increase in occurrence or become associated with new food vehicles (*24*). From the late 1800's to 1949, typhoid fever caused by *Salmonella typhi* was the predominant *Salmonella* infection in humans in the United States. The typical clinical illness produced by salmonellae in humans has changed from typhoid fever to gastroenteritis, where the incidence of reported cases of salmonellosis has increased significantly since the mid-1980's (*26*, *38*). The young are most affected, followed by the old, the malnourished, and those living in economically marginal conditions (*45*). FoodNet 1997 reported cases of salmonellosis to be 111/100,000 among children aged less than one year and 9/100,000 for persons 60 years and older (*40*). Most salmonellae produce the same spectrum of human illness, but many salmonellae serovars have different reservoirs and different vehicles of transmission (*8*). For example, salmonellae serovars such as *S. typhi* and *S. pullorum*, have a restricted host range, while most salmonellae serovars, such as *S. typhimurium*, infect a broad range of warm-blooded animals (*6*). Over 2,000 serovars of salmonellae exist, but the majority of confirmed human salmonellosis infections are attributed to a smaller number of serovars. About 95% of the strains causing disease in man comprise fewer than 40 serovars, principally within serogroups A-E (*1*, *24*).

REPORTING OF *SALMONELLA*

To combat the potential threat of salmonellae associated foodborne disease, the Centers for Disease Control (CDC, U.S.), in conjunction with the Association of State and Territorial Epidemiologists, have maintained surveillance of salmonellae infections

since 1962 (*4*). Also, due to the significant epidemiological importance of salmonellae, CDC has launched several new approaches to foodborne disease surveillance, including FoodNet, PulseNet, and The National Antimicrobial Resistance Monitoring System for Enteric Bacteria (*36*). However, surveillance of salmonellosis in the U.S. is primarily passive depending on the reporting of cases by primary physicians and isolates by clinical microbiology laboratories (*4*). Because large foodborne outbreaks tend to attract headlines and focused attention, foodborne infections that occur as individual incidents are usually underreported (*36*). As such, underreporting of cases of human salmonellosis is partly due to people who are ill and yet do not seek medical attention or when physicians fail to order a culture, and when an ordered culture fails to yield salmonellae (*8*). Underreporting of cases makes it impossible to accurately assess the potential benefits of any control program and it is clear that the number of cases of human salmonellosis reported to the CDC each year represents from one-to five-percent of the actual yearly incidence of this infection in the U.S. (*4*). Some researchers have estimated that for every person diagnosed with acute salmonellosis, thirty-seven symptomatic infections went undiagnosed, suggesting the morbidity, and by extension, the mortality due to salmonellae infections, is seriously underestimated. The number of salmonellae infections that go unreported every year may be 20- to100-fold greater than the number of reported infections (*4, 38*).

***SALMONELLA* CARRIAGE**

Undoubtedly, foods of animal origin are a significant source of salmonellae infection in humans and the prevalence of asymptomatic animal carriage of these bacteria has become an increasing concern for the pork industry (*17, 33*). A range of infections is

covered by the term ‘salmonellosis’. The most common type is known as ‘the carrier state’ in which carriage of the organism is not accompanied by symptoms or clinical disease in the host. These carriers are of importance in production animals, because they may serve as reservoirs for further spread of infections by shedding. If their carcasses became contaminated, this could lead to contaminated food products. Salmonellae infections in swine have been responsible for substantial losses in revenue to the swine industry, prompting increased interest in the production of “*Salmonella*-free” feeds and foods in the United States, Europe, and Canada (*9*). Outbreak investigations revealed that between 1973 and 1987, 59 percent of salmonellosis cases could be traced to a specific food vehicle (*41*). Hence, previous increases in human salmonellosis may have been associated with infection in particular types of animals and their entry into the food chain (*24*). In Denmark, human salmonellosis attributed to pork was estimated to be 10-to 15-percent in 1997 and 1998. Likewise, in The Netherlands, it was estimated that approximately 15 percent of human salmonellosis was associated with the ingestion of contaminated pork (*23*). In the United States, salmonellae contamination is being considered as one measure of overall pork quality (*17*). Because of public health concerns, a growing priority is placed upon determining the prevalence of on-farm salmonellae in swine (*12*). It is well documented that carrier pigs may be positive for salmonellae in the mesenteric lymph nodes, tonsils, cecum, and feces (*20*). Thus, an increased emphasis to reduce contamination of meat at slaughter and processing facilities has stimulated interest in identifying means to reduce or eliminate these organisms at the pre-harvest level (*10*).

SALMONELLA TAXONOMY

Salmonella taxonomy is complex, mostly due to the development and use over the years of several different nomenclatures (*45*). Traditionally, salmonellae strains are characterized according to their reaction to sera (serotyping), and for many decades each new serovar was given a new species designation (i.e., *S. typhimurium*, *S. enteritidis*, *S. pullorum*, and *S. dublin*). Today, it is generally accepted that there is only a single species of *Salmonella* (*S. enterica*), rather than the over 2,000 named serovars, although most investigators have continued to write, "*S. typhimurium*", rather than "*S. enterica* serovar Typhimurium" out of convenience and for continuity with the previous literature (*6*). The CDC and clinical laboratories are reporting organisms as serovars, such as *Salmonella* serovar Typhimurium, rather than using the taxonomically correct, but more cumbersome, *Salmonella enterica* subspecies *enterica* serotype Typhimurium (*45*).

SALMONELLA CHARACTERIZATION

Comprehensive typing systems are based on the observation that distinctions can be made between isolates of different species and between isolates of the same species (*35*). The typing method of choice depends on the intended application and commonly used criteria for evaluating typing methods include: cost, speed, ease of use, standardization, reproducibility, automation, and discriminatory ability (*41*). Characteristically, typing systems are defined as either phenotypic or genotypic. Phenotypic systems evaluate constitutive characteristics expressed by an organism, while genotypic systems analyze chromosomal or extrachromosomal DNA (*22*). Several typing methods include, but are not limited to: serotyping, biotyping, antibiotyping, phage typing (PT), plasmid typing, multilocus enzyme electrophoresis (MEE),

ribotyping, polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), DNA sequencing, insertion sequence 200 (IS), and pulsed-field gel electrophoresis (PFGE) (*37*, *36*, *38*, *41*). Phenotypic methods, like serotyping, biotyping, and antibiotyping are capable only of grouping isolates into relatively large categories within a given species, whereby many genotypic methods, like plasmid typing, ribotyping, RAPD, and PFGE are more sensitive/specific and better able to detect subtle differences among strains and/or clones (*20*). The two typing methods used to differentiate salmonellae as characterized according to the current study are described as follows:

Serotyping. Serological examination is performed by antigenic analysis, whereby agglutination reactions are used (*38*). For example, discernible differences in polysaccharide antigens (heat-stable or somatic-O antigens) are identified on the surface of the microorganism using the slide agglutination method, in accordance with the scheme instituted by White and extended by Kauffman (*38, 39, 42*). These surface antigens can be detected by use of antisera representative of all of the heat stable antigens possessed by members of the genus (*38, 41*). Use of specific antisera permits determination of the serogroup to which an isolate belongs. The flagellar-H antigens are determined by selective use of antisera representative of the flagellar antigens possessed by members of the genus. Unlike somatic-O antisera, flagellar-H antisera are used in tube agglutination tests (*38*).

Pulsed-field gel electrophoresis. PFGE characterizes bacteria by banding patterns generated after digestion of bacterial DNA with a specific restriction enzyme (*34*). Restriction enzymes are chosen such that bacterial DNA is cleaved, yielding 8-to

25 DNA bands ranging from 40-to 600 kb (*21*, *29*, *41*). A novel procedural step in PFGE includes combining bacterial isolates with molten agarose and embedding the bacterial suspension into small molds forming agarose plugs (*42*). Specifically, complete bacterial DNA is purified intact and subsequently cut into DNA fragments using restriction enzymes that cut where a specific DNA sequence is present (e.g., restriction enzyme *Xba*I will cut bacterial DNA specifically whenever a sequence of TCTAGA exists). The choice of the restriction enzyme is critical, because each enzyme produces a different number of fragments dependent upon the microbial species analyzed, and is generally based on preliminary experiments to determine the most discriminatory enzyme capable of producing easy-to-interpret reproducible patterns (*41*, *42*). The restriction fragments are resolved in the agarose gel by use of a switching apparatus that changes the direction of the current according to a predetermined pattern (*5*). After staining with ethidium bromide, bands are visualized and photographed (*42*). DNA banding patterns for different bacterial isolates are compared to differentiate distinct bacterial subtypes (*41*). Commercially available software packages (e.g., Molecular Analysis Fingerprinting Software, version 1.69, Bio-Rad Laboratories, Hercules, CA) can provide computerized gel scanning and data analysis that store PFGE patterns for future reference and comparison.

CHAPTER III

GENOTYPIC CHARACTERIZATION OF SALMONELLAE ISOLATED FROM SWINE AT SLAUGHTER

INTRODUCTION

Genotyping systems have been used in foodborne disease outbreak investigations for nearly twenty years, but molecular methods have been applied only more recently in a widespread, coordinated, and standardized fashion (*41*). Previous investigations indicate that PFGE may prove to be potentially valuable in epidemiologic studies and especially where there is need to differentiate disease-causing agents quickly, reliably, and with repeatability. Compared to other genotypic characterization methods, PFGE is more discriminatory and, therefore, is considered the gold standard of molecular typing methods. Simplified laboratory protocols and the advent of a PFGE switching apparatus that ensures quality band resolution, has helped to standardize the technique. For example, the CDC has recently instituted "Pulse-Net", an epidemiologic disease surveillance system designed to track diseases and their agents using a standardized PFGE protocol.

The objective of this study was to test our hypothesis that genotypic characterization (PFGE), was more discriminatory than serotyping of salmonellae. We also hypothesized, that genotypic relatedness could be used to determine common origins of contamination (i.e., farm or slaughter plant). The salmonellae included in this study were part of a previous investigation that examined the recovery of salmonellae in

market-age swine (*13*). The previous investigation sampled ileocecal lymph nodes and cecal contents from market-age swine at slaughter between October 1997 and June 1998. Samples were harvested at a centralized slaughter plant supplied by multiple farms within an integrated Texas swine operation. These samples were collected from approximately 645 market-age swine during 13 visits to the slaughter plant. Four farrow-to-finish farms were sampled three times each (50 pigs per farm), and one gilt-replacement farm (45 pigs per farm) was sampled once (Table 1). Salmonellae-positive swine were identified by methods described by Harvey *et al*. (*13*), and salmonellae lymph node and cecal content isolates were processed in one of two enrichment media (GN Hajna broth or tetrathionate broth) allowing for recovery. Table 2 summarizes the salmonellae used in the present study.

TABLE 1. *Salmonellae collected from swine by collection date and farm source*

Collection Date	Farm[a]	No. Tested	No. Positive	No. Negative
10-Oct-97	EL1	50	10	40
28-Oct-97	CF1	50	12	38
14-Nov-97	EL2	50	14	36
17-Nov-97	CF2	50	36	14
2-Dec-97	CF3	50	31	19
27-Jan-98	FG1	45	5	40
25-Feb-98	EL3	50	7	43
4-Mar-98	BT1	50	35	15
31-Mar-98	BT2	50	37	13
22-Apr-98	EA1	50	30	20
5-May-98	EA2	50	6	44
19-May-98	EA3	50	24	26
9-Jun-98	BT3	50	36	14

[a] Farms are listed chronologically.

TABLE 2. *Salmonellae by farm, origin, and sex*

		Origin		Sex	
Farm[a]	**Isolates**	**Cecal Contents**	**Lymph Nodes**	**Male**	**Female**
BT	137[c]	46	91	79	58
CF	90[c]	15	75	50	40
EA	72[c]	44	28	42	30
EL[b]	35[c]	11	23	15	20
FG	6[d]	5	1	0	6
Total	340	121	218	186	154

[a] Farms are listed alphabetically by designation (i.e., BT, CF, EA, EL, & FG).
[b] Origin data for a salmonellae isolate not available.
[c] Salmonellae collected from a farrow-to-finish farm.
[d] Salmonellae collected from a gilt-replacement farm.

MATERIALS AND METHODS

Salmonellae. Salmonellae included in the current study met three criteria: 1) at least three replicates of each isolate were available, 2) the isolates were viable at the time of the current study, and 3) the isolates were serotyped by National Veterinary Services Laboratory.

PFGE plug procedure. Procedural techniques are described by Hume *et al*., (*15, 16*), and consist of the following modified procedures. Salmonellae (-70ºC) were streaked onto brilliant green agar with 25 µg novobiocin per ml (BGAN), and incubated at 37ºC for 24 h. Single colonies were harvested from BGAN, inoculated into 10 ml tryptic soy broth and incubated at 37ºC for 24 h. Cultured cells were washed three times in cell suspension buffer [CSB, (100 mM TRIS & 100 mM EDTA, pH 8.0) Pulse-Net/CDC] by centrifugation at 8,000 X gravity for 10 min at 25°C and suspended to 2-5 X 10^8 CFU/ml. Equal volumes (1 ml) of suspended cells and 1.6% low-melting ultra

pure agarose (FMC BioProducts, Rockland, MD) in CSB were mixed and suspended in a 45ºC water bath. Mixtures were transferred to plug molds (Bio-Rad Laboratories, Richmond, CA) and stored at 4ºC to polymerize.

PFGE plug wash procedure. In steps designed for cell membrane digestion and cell lysis, plugs samples were incubated for 48 h at 50ºC in 20 ml of lysis buffer [1% sodium lauryl sarcosine; 0.5M EDTA, pH 9-9.3; 0.2 mg/ml proteinase K (Boehringer Mannheim, Indianapolis, IN)]. Plug samples were then washed twice for 0.5 h each in 40 ml of cold (4ºC) Tris-EDTA [10 mM TRIS (USB Specialty Biochemicals, Division of Amersham Life Science, Inc., Cleveland, OH), pH 8.0; 1mM EDTA, pH 9-9.3]. Plugs were washed three times for 1 h each in 40 ml of TE (4ºC) containing 40 µl of phenylmethylsulfonyl fluoride (100 mM PMSF in isopropanol). Additionally, plugs were washed three times for 1 h each in 40 ml of TE (4ºC). Washed plugs were stored in 20 ml of TE at 4ºC.

PFGE digestion and staining procedure. Plugs were sectioned in half and treated with restriction enzyme endonulcease following manufacturer recommendations (*Xba*I, New England BioLabs, Beverly, MA). Digested DNA fragments were resolved in a 1% agarose gel [PFGE Ultra Pure Agarose (Boehringer Mannheim, Indianapolis, IN)] in 150 ml of TRIS/boric-acid/EDTA [TBE (0.089 M TRIS, 0.089 M boric acid, 0.002M EDTA, pH 8.0)] and stained using ethidium bromide. Lambda Ladder [PFGE Marker (New England BioLabs, Beverly, MA)] was used as a reference standard.

PFGE system commands. PFGE was performed using a contour clamped homogenous electrophoresis [CHEF Mapper XA (Bio-Rad Laboratories, Hercules, CA)] system in 2 L of TBE running buffer. CHEF program commands are as follows: initial

switch time, 0.1 s; final switch time, 90.0 s; 6 V/cm; orientation angle, 120º; buffer temperature, 12ºC; and run time 22 h.

Strain characterization. Isolate banding patterns were compared by visual discrimination and assigned a genotype designation. Genotype designations were assigned randomly when banding patterns differed by at least one band. Uppercase values beginning with the first letter of the alphabet were used to distinguish between different banding patterns among serotypic isolates.

Dendrograms. Serotypic salmonellae characterized by genotype (if multiple banding patterns were produced) were subjected to dendrogram analysis [Molecular Analysis Fingerprinting Software (MAFS), version 1.69 (Bio-Rad Laboratories, Hercules, CA)] to assess genetic diversity. MAFS creates a dendrogram and assigns a correlation coefficient [Dice coefficient of similarity, (modification of Jaccard Coefficient); and Unweighted Pair Group Method Using Arithmetic Averages, (UPGMA)] that indicates genetic diversity described as a percent similarity. Percent similarity intervals were arbitrarily assigned as follows: 1) Low, 0% to 50%; 2) Moderate, 51% to 80%; 3) High, 81% to 100%.

RESULTS

Table 3 shows 340 salmonellae and 32 serotypes characterized using PFGE. All serotypic salmonellae produced a genotypic banding pattern (excluding salmonellae in Table 4). Note: serotypes composed of one isolate, one banding pattern, failing to produce a banding pattern, or a combination thereof were not included in the results of the data. Please refer to appendices A and B for further descriptive data.

TABLE 3. *Number of serotypic salmonellae subjected to pulsed-field gel electrophoresis*

No. Isolates	Serotype	Serogroup
71	Schwarzengrund [a]	B
50	Montevideo [a]	C1
39	Agona [a]	B
35	Livingstone [a]	C1
21	Derby [a]	B
20	Anatum [a]	E1
16	Typhimurium [a]	B
14	Javiana [a]	D1
13	Muenster [a]	E1
11	Typhimurium (var. copenhagen) [a]	B
7	Newport [a]	C2
5	Havana [b]	G2
5	Mono 4.5.12:I [b]	NA [c]
4	Heidelberg [b]	B
3	Braenderup [a]	C1
3	Meleagridis [a]	E1
3	Ugnada [a]	E1
2	Infantis [a]	C1
2	Mbandaka [a]	C1
2	Muenchen [b]	C2
2	Orion [b]	E1
2	Thompson [b]	C1
1	Johannesburg [b]	B
1	Mbandaka [b]	NA [c]
1	Menhaden [b]	E3
1	Monophasic [b]	B
1	Multi Serotypes [b]	NA [c]
1	Newbrunswick [b]	E2
1	Tennessee [b]	C1
1	Untypable [b]	E
1	Urbana [b]	N
1	Worthington [b]	G2

[a] Dendrogram analysis of serotypic banding patterns available (serotypic salmonellae producing two or more banding patterns).
[b] No dendrogram analysis available (only one isolate or banding pattern).
[c] Serogroup designation questionable or not available.

TABLE 4. *Serotypic salmonellae failing to produce banding patterns using restriction enzyme Xba*I

		No. Isolates	
Serotype	**Serogroup**	**Banding Pattern**	**No Banding Pattern**
Havana	G2	0	5
Livingstone	C1	33	2
Mbandaka	?	0	1
Muenchen	C2	1	1

***S. schwarzengrund* B.** A total of 71 isolates were collected within this serotype with four banding patterns produced. Sixty-eight (95.8%) of the isolates produced an identical banding pattern designated as genotype A. Three additional banding patterns were produced, each represented by one (1.3%) isolate, and designated as genotypes B, C, and D, respectively. Most serotypic isolates were collected from one farm source (BT, 58/71 or 81.7%), while other isolates were collected from two farm sources (CF, 11/71 or 15.5%; and FG, 2/71 or 2.8%). Out of the 68 genotype A isolates identified, 56 (82.4%) were collected from the same farm source (BT) on three separate collection dates during the months of March and June. Dendrogram analysis indicated an overall composite similarity of 35.2%. The highest percent similarity was 58.2, between genotypic cluster A/B. The percent similarity was 43.4 between genotypic cluster A/B/C (Fig. 1).

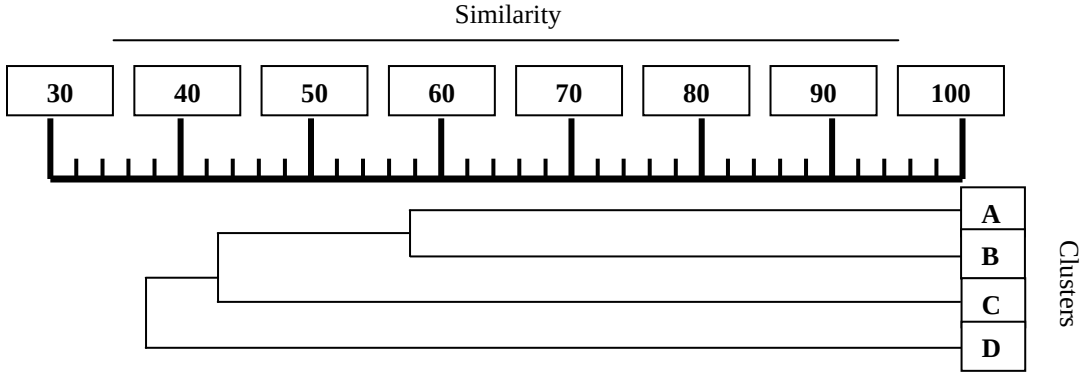


FIGURE 1. *Dendrogram indicating genotypic diversity among* Salmonella schwarzengrund *serogroup B banding patterns.*

***S. montevideo* C1.** A total of 50 isolates were collected within this serotype with five banding patterns being produced. Out of the 50 isolates collected, 37 (74.0%) produced an identical banding pattern designated as genotype B. Four additional banding patterns were produced, each represented by one (2.0%), four (8.0%), six (12.0%), and

two (4.0%) isolates, and were designated as genotypes A, C, D, and E, respectively. Most serotypic isolates were collected from one farm source (CF, 31/50 or 62.0%), while other isolates were collected from four farm sources (BT, 12/50 or 24.0%; EL, 3/50 or 6.0%; EA, 2/50 or 4.0%; and FG, 2/50 or 4.0%). Out of 37 genotype B isolates identified, 30/37 (81.1%) were collected from the same farm source (CF, 30/31 or 96.8%) on two separate collection dates during the months of November and December. Dendrogram analysis indicated an overall composite similarity of 44.4%. The highest percent similarity was 84.6, between genotypic cluster D/E. The percent similarity was 81.5 and 57.9, between genotypic clusters D/E/C and D/E/C/B, respectively (Fig. 2).

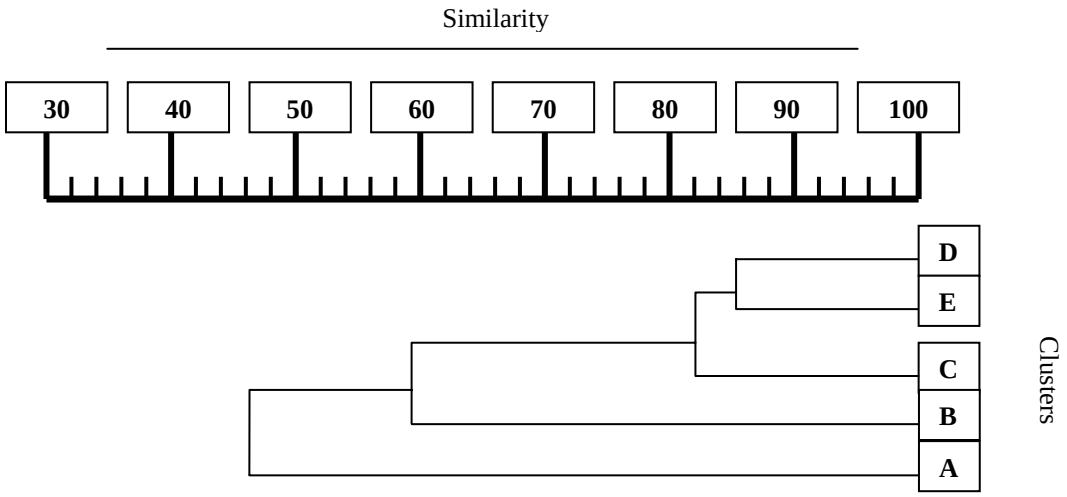


FIGURE 2. *Dendrogram indicating genotypic diversity among* Salmonella montevideo *serogroup C1 banding patterns.*

***S. agona* B.** A total of 39 isolates were collected within this serotype, with seven banding patterns produced. Out of the 39 isolates collected, 18 (46.2%) produced an identical banding pattern designated as genotype A. Six additional banding patterns were produced, each represented by 11 (28.2%), four (10.3%), one (2.6%), three (7.7%), one

(2.6%), and one (2.6%) isolates, and designated as genotypes B, C, D, E, F, and G, respectively. Most serotypic isolates were collected from one farm source (BT, 26/39 or 66.7%), with the remaining isolates collected from three farm sources (CF, 6/39 or 15.4%; EL, 5/39 or 12.8%; and EA, 2/39 or 5.1%). Out of 18 genotype A isolates identified, 14 (77.8%) were collected from the same farm source (BT, 14/26 or 53.8%) on two separate collection dates during the months of March and June. Dendrogram analysis indicated an overall composite similarity of 49.8%. The highest % similarity was 89.5 and 89.1 between genotypic clusters F/G and A/B, respectively. The % similarity were 74.4, 69.4 and 74.6 between genotypic clusters F/G/E, F/G/E/D and A/B/C, respectively (Fig. 3).

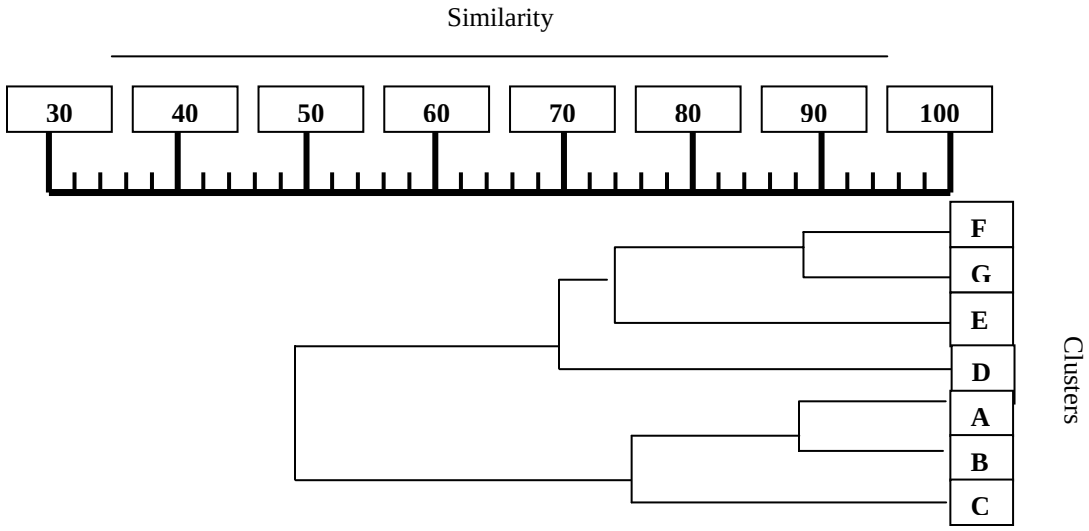


FIGURE 3. *Dendrogram indicating genotypic diversity among* Salmonella agona *serogroup B banding patterns.*

***S. livingstone* C1.** A total of 35 isolates were collected within this serotype with two banding patterns produced. Note: two serotypic isolates did not produce a banding pattern. Out of the 35 isolates collected, 32 (91.4%) produced an identical banding

pattern designated as genotype A. Another banding pattern was produced, represented by one (2.9%) isolate, and was designated as genotype B. Most serotypic isolates were collected from one farm source (CF, 19/35 or 54.3%), while other isolates were collected from three farm sources (BT, 3/35 or 8.6%; EL, 1/35 or 2.9%; and EA, 12/35 or 34.3%). Out of 32 genotype A isolates identified, 17 (53.1%) were collected from the same farm source (CF, 17/19 or 89.5%) on two separate collection dates during the months of November and December. Dendrogram analysis indicated an overall composite similarity of 61.3% (Fig. 4).

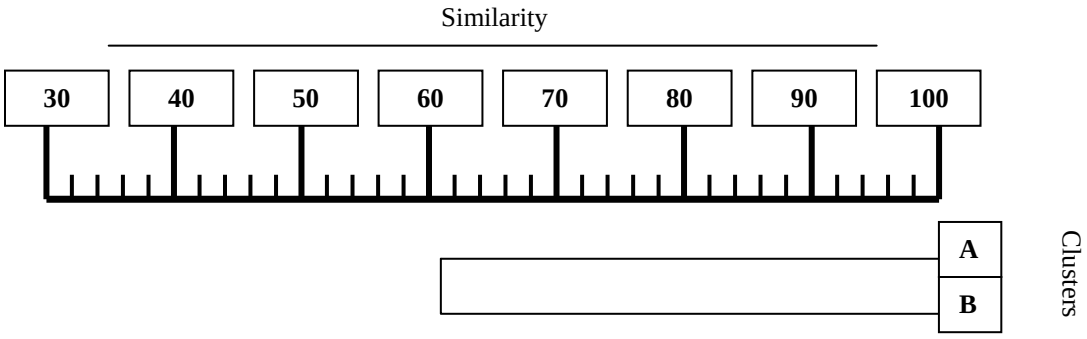


FIGURE 4. *Dendrogram indicating genotypic diversity among* Salmonella livingstone *serogroup C1 banding patterns.*

***S. derby* B.** A total of 21 isolates were collected within this serotype with three banding patterns produced. Out of the 21 isolates collected, 16 (76.2%) produced an identical banding pattern designated as genotype B. Two other banding patterns were produced, each represented by two (9.5%) and three (14.3%) isolates designated as genotypes A and C, respectively. Most serotypic isolates were collected from one farm source (BT, 10/21 or 47.6%), while other isolates were collected from four farm sources (CF, 1/21 or 4.8%; EL, 6/21 or 28.6%; EA, 3/21 or 14.3%; and FG, 1/21 or 4.8%). Out

of 16 genotype B isolates identified, nine (56.3%) were collected from the same farm source (BT, 9/10 or 90.0%) on one collection date during the month of June. Dendrogram analysis indicated an overall composite similarity of 81.1%. The highest % similarity was 91.1, between genotypic cluster A/B (Fig. 5).

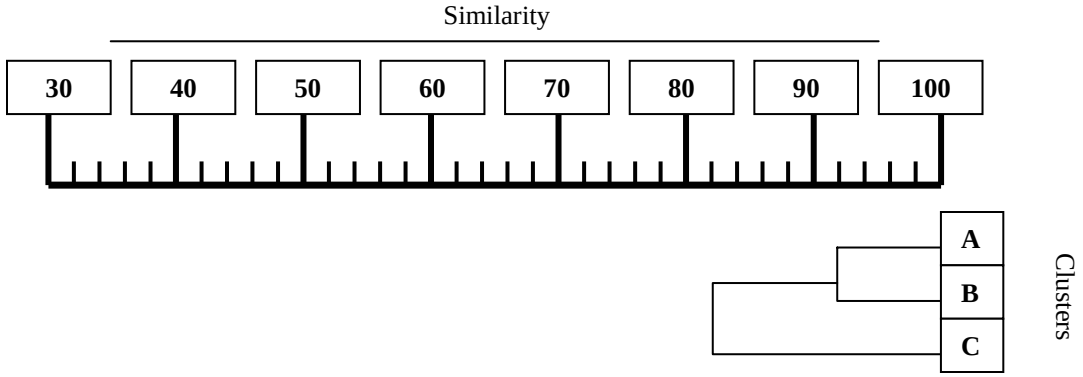


FIGURE 5. *Dendrogram indicating genotypic diversity among* Salmonella derby *serogroup B banding patterns*.

***S. anatum* E1.** A total of 20 isolates were collected within this serotype with seven banding patterns produced. Out of the 20 isolates collected, 7 (35.0%) and 5 (25.0%) produced different banding patterns, respectively, and were designated as genotypes A and B. Five additional banding patterns were produced, each represented by one (5.0%), one (5.0%), four (20.0%), one (5.0%), and one (5.0%) isolates, designated as genotypes C, D, E, F, and G, respectively. Most serotypic isolates were collected from one farm source (BT, 9/20 or 45.0%), while other isolates were collected from three farm sources (CF, 4/20 or 20.0%; EL, 1/20 or 5.0%; EA, 6/20 or 30.0%). Out of seven genotype A isolates identified, four (57.1%) were collected from the same farm source (CF, 4/4 or 100.0%) on three separate collection dates during the months of October, November, and December. Out of five genotype B isolates identified, four (80.0%) were

collected from the same farm source (EA, 4/6 or 66.7%) on two separate collection dates during the month of May. Dendrogram analysis indicated an overall composite similarity of 48.3%. The highest % similarity were 84.2 and 79.1, between genotypic clusters A/B and A/B/C, respectively. The % similarity were 74.9, 65.7 and 62.7, between genotypic clusters E/F, E/F/A/B/C, and D/G, respectively (Fig. 6).

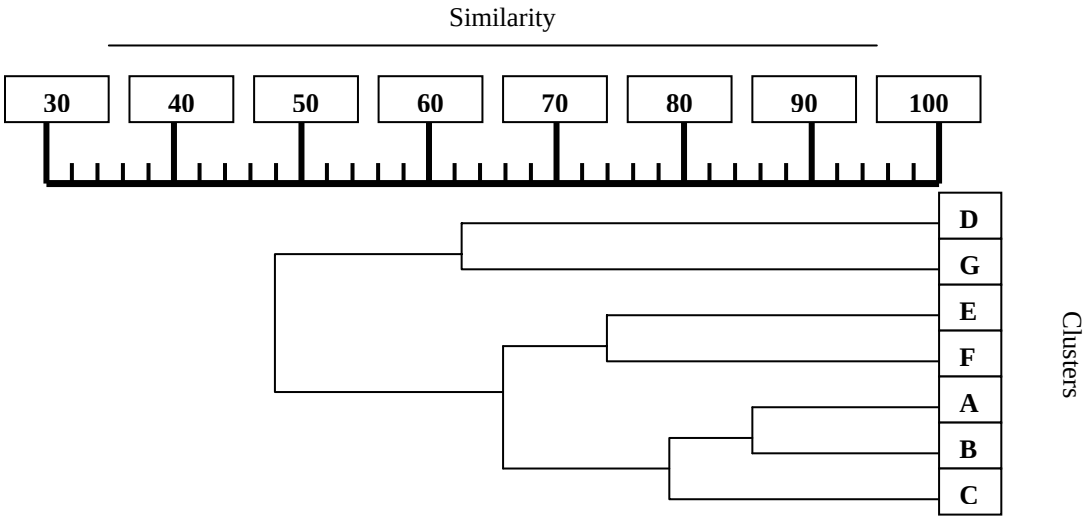


FIGURE 6. *Dendrogram indicating genotypic diversity among* Salmonella anatum *serogroup E1 banding patterns*.

***S. typhimurium* B.** A total of 16 isolates were collected within this serotype, with five banding patterns produced. Out of the 16 isolates collected, nine (56.3%) produced an identical banding pattern designated as genotype D. Four other banding patterns were produced, each represented by two (12.5%), two (12.5%), two (12.5%), and one (6.3%) isolates, designated as genotypes A, B, C, and E, respectively. Most serotypic isolates were collected from farm EA (12/16 or 75.0%), while other isolates were collected from farm CF (4/16 or 25.0%). Out of nine genotype D isolates identified, nine (100.0%) were collected from the same farm source (EA, 9/ 12 or 75.0%) on two separate collection

dates during the months of April and May. Dendrogram analysis indicated an overall composite similarity of 55.1%. The highest % similarity was 84.4, between genotypic cluster A/B. The % similarity were 75.8 and 66.2 between genotypic clusters C/D and C/D/A/B, respectively (Fig. 7).

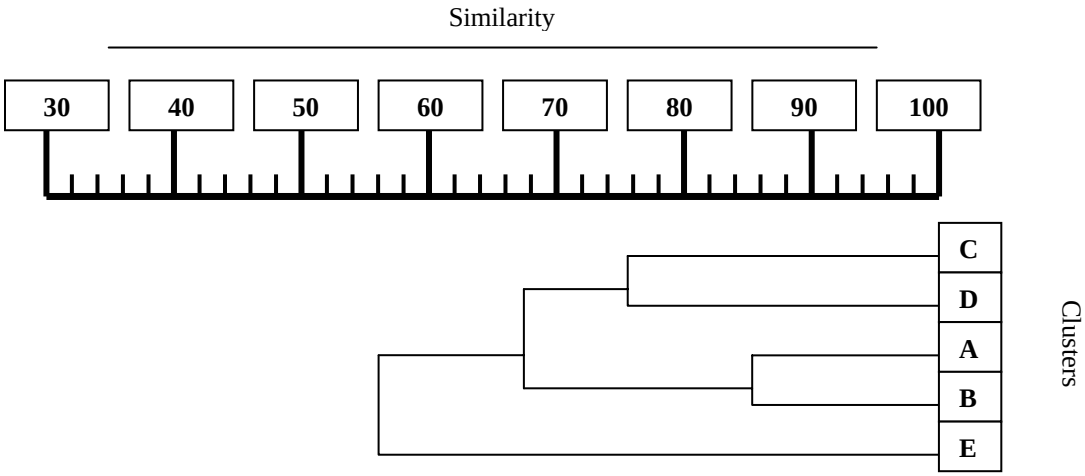


FIGURE 7. *Dendrogram indicating genotypic diversity among* Salmonella typhimurium *serogroup B banding patterns.*

***S. javiana* D1.** A total of 14 isolates were collected within this serotype with two banding patterns produced. Out of the 14 isolates collected, 13 (92.9%) produced an identical banding pattern designated as genotype A. Another banding pattern was produced represented by one (7.1%) isolate, and designated as genotype B. All serotypic isolates were collected from one farm source (EA, 14/14 or 100.0%) on one collection date during the month of May. Dendrogram analysis indicated an overall composite similarity of 35.2% (Fig. 8).

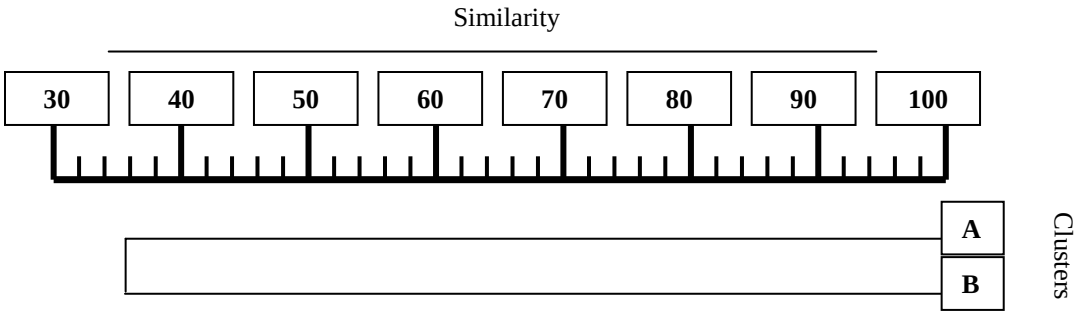


FIGURE 8. *Dendrogram indicating genotypic diversity among* Salmonella javiana *serogroup D1 banding patterns.*

***S. muenster* E1.** A total of 13 isolates were collected within this serotype with two banding patterns produced. Out of the 13 isolates collected, 12 (92.3%) produced an identical banding pattern designated as genotype A. Another banding pattern was produced represented by one (7.7%) isolate, and designated as genotype B. Most serotypic isolates were collected from farm EA (7/13 or 53.8%), while other isolates were collected from three farm sources (BT, 3/13 or 23.1%; CF, 2/13 or 15.4%; and EL, 1/13 or 7.7%). Out of 12 genotype A isolates identified, seven (58.3%) were collected from the same farm source (EA, 7/7 or 100.0%) on one collection date during the month of April. Dendrogram analysis indicated an overall composite similarity of 74.7% (Fig. 9).

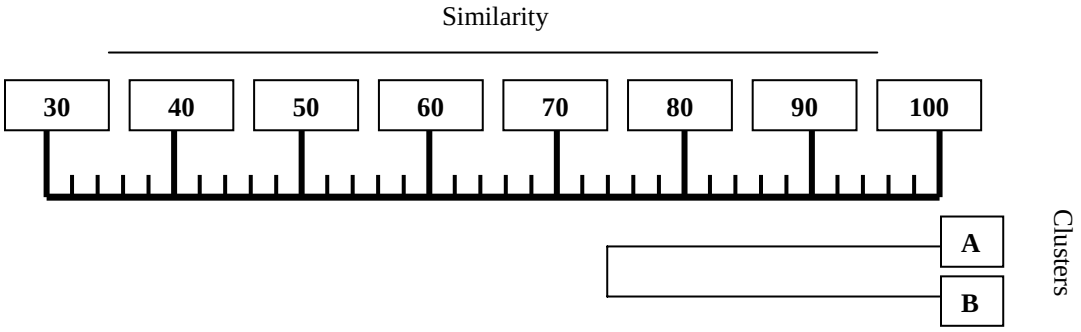


FIGURE 9. *Dendrogram indicating genotypic diversity among* Salmonella muenster *serogroup E1 banding patterns.*

***S. typhimurium* (var. Copenhagen) B.** A total of 11 isolates were collected within this serotype with four banding patterns produced. Out of the 11 isolates collected, seven (63.6%) produced an identical banding pattern designated as genotype D. Three additional banding patterns were produced, each represented by two (18.2%), one (9.1%), and one (9.1%) isolates, designated as genotypes A, B, and C, respectively. Most serotypic isolates were collected from one farm source (EA, 7/11 or 63.6%), while other isolates were collected from two farm sources (CF, 3/11 or 27.3% of serotypic isolates; and EL, 1/11 or 9.1% of serotypic isolates). Out of seven genotype D isolates identified, six (85.7%) were collected from the same farm source (EA, 6/7 or 85.7%) on one collection date during the month of April. Dendrogram analysis indicated an overall composite similarity of 86.5%. The highest percent similarity was 93.8, between genotypic cluster C/D. The percent similarity was 88.3, between genotypic cluster C/D/B (Fig. 10).

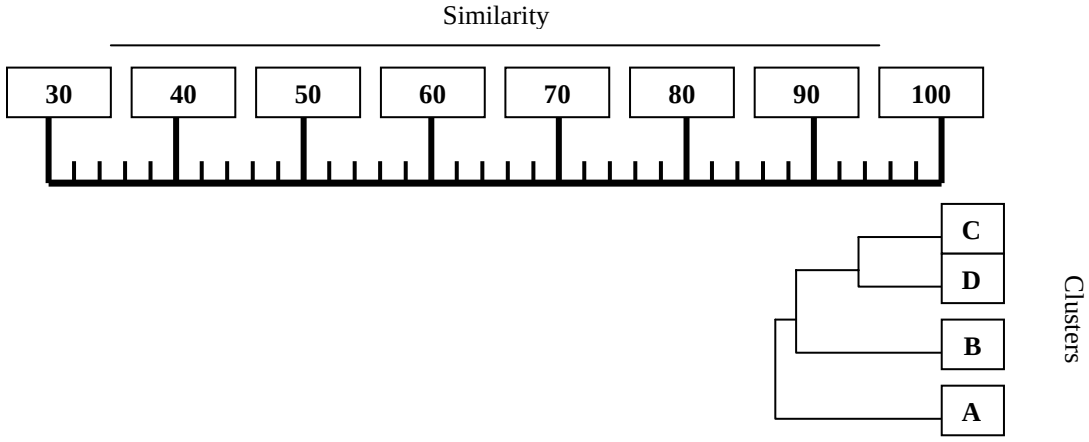


FIGURE 10. *Dendrogram indicating genotypic diversity among* Salmonella typhimurium *(var. Copenhagen) serogroup B banding patterns.*

***S. newport* C2.** A total of seven isolates were collected within this serotype with two banding patterns produced. Out of the seven isolates collected, five (71.4%) produced an identical banding pattern designated as genotype A. Another banding pattern was produced, represented by two (28.6%) isolates and designated as genotype B. Most serotypic isolates were collected from farm BT (5/7 or 71.4%), while other isolates were collected from farm CF (2/7 or 28.6%). Out of five genotype A isolates identified, five (100.0%) were collected from the same farm source (BT, 5/5 or 100.0%) on one collection date during the month of March. Dendrogram analysis indicated an overall composite similarity of 61.3% (Fig. 11).

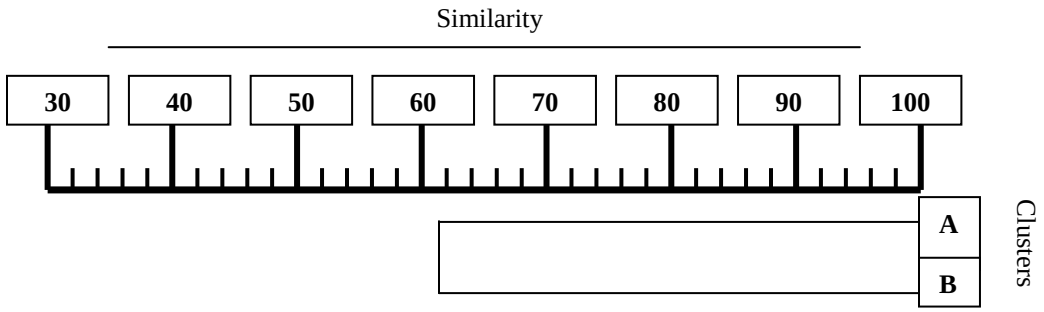
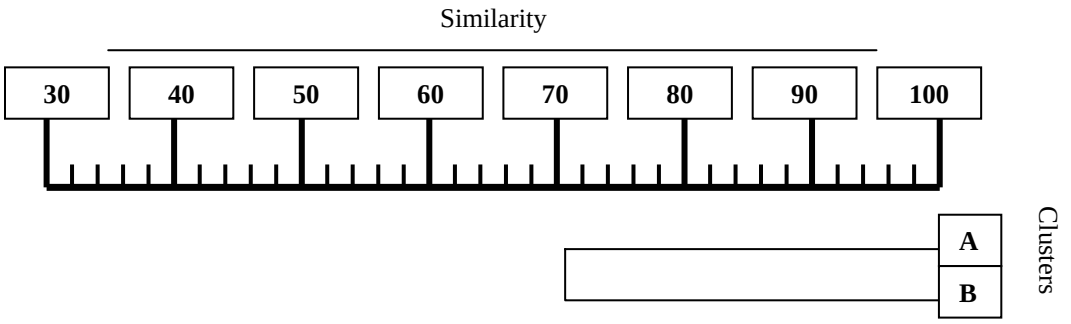


FIGURE 11. *Dendrogram indicating genotypic diversity among* Salmonella newport *serogroup C2 banding patterns*.

***S. braenderup* C1.** A total of three isolates were collected within this serotype with two banding patterns produced. Out of the three isolates collected, two (66.7%) produced an identical banding pattern designated as genotype B. Another banding pattern was produced, represented by one (33.3%) isolate designated as genotype A. All serotypic isolates (regardless of genotypic designation) were collected from one farm source (BT, 3/3 or 100.0%), on three separate collection dates during the months of

March and June. Dendrogram analysis indicates an overall composite similarity of 71.6% (Fig. 12).



FIGRUE 12. *Dendrogram indicating genotypic diversity among* Salmonella braenderup *serogroup C1 banding patterns.*

***S. meleagridis* E1.** A total of three isolates were collected within this serotype with two banding patterns produced. Out of the three isolates collected, two (66.7%) produced an identical banding pattern designated as genotype A. One additional banding pattern was produced, represented by one (33.3%) isolate, designated as genotype B. All serotypic isolates were collected from one farm source (EA, 3/3 or 100.0%), on two separate collection dates during the month of May. Dendrogram analysis indicated an overall composite similarity of 89.1% (Fig. 13).

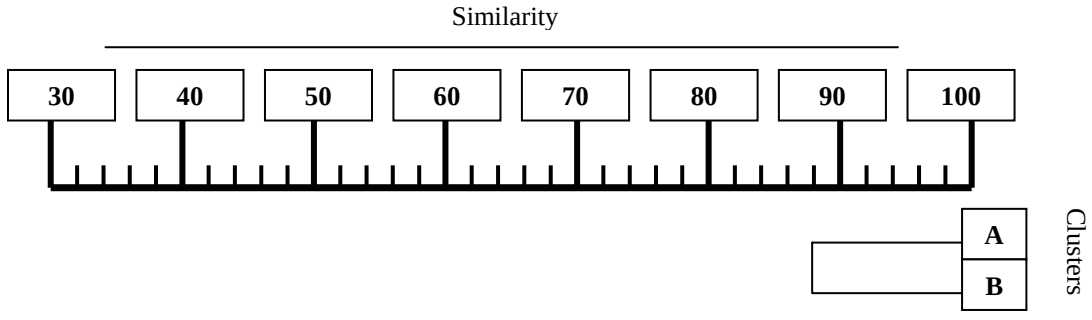


FIGURE 13. *Dendrogram indicating genotypic diversity among* Salmonella meleagridis *serogroup E1 banding patterns.*

***S. uganda* E1.** A total of three isolates were collected within this serotype with two banding patterns produced. Out of the three isolates collected, two (66.7%) produced an identical banding pattern designated as genotype B. One additional banding pattern was produced, represented by one (33.3%) isolate, designated as genotype A. All serotypic isolates were collected from one farm source (EL, 3/3 or 100.0%), on one collection data during the month of October. Dendrogram analysis indicated an overall composite similarity of 88.0% (Fig. 14).

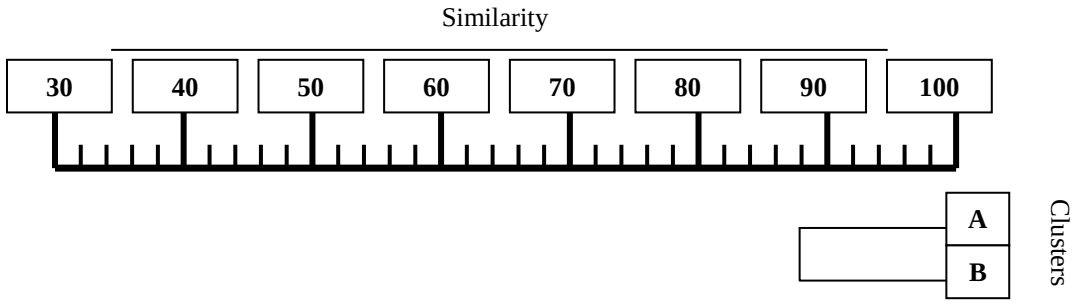


FIGURE 14. *Dendrogram indicating genotypic diversity among* Salmonella uganda *serogroup E1 banding patterns*.

***S. infantis* C1.** A total of two isolates were collected within this serotype with two banding patterns produced designated as genotypes A and B. Genotypic A and B isolates were collected from two different farms (BT and EL) on separate collection dates during the months of March and October, respectively. Dendrogram analysis indicated an overall composite similarity of 67.1% (Fig. 15).

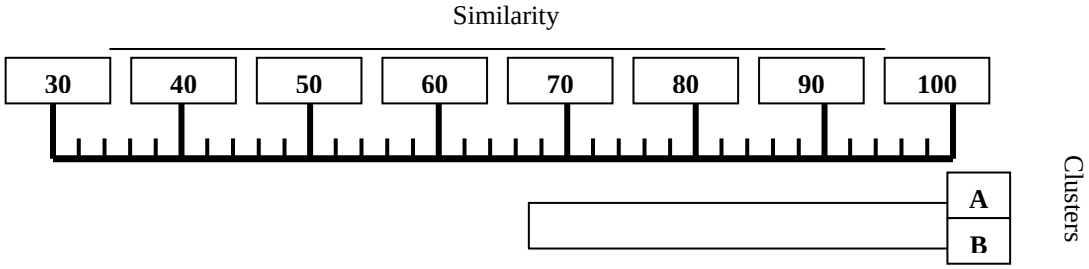


FIGURE 15. *Dendrogram indicating genotypic diversity among* Salmonella infantis *serogroup C1 banding patterns.*

***S. mbandaka* C1.** A total of two isolates were collected within this serotype with two banding patterns produced designated as genotypes A and B. Genotypic A and B isolates were collected from two different farms (BT and EL) on separate collection dates during the months of March and October, respectively. Dendrogram analysis indicated an overall composite similarity of 93.3% (Fig. 16).

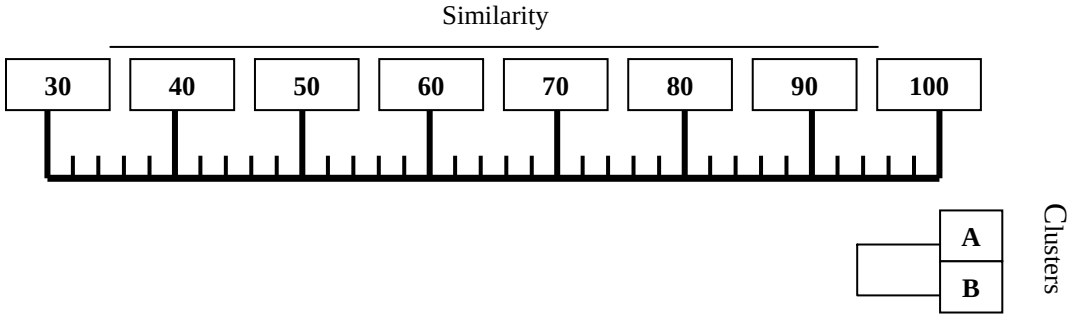


FIGURE 16. *Dendrogram indicating genotypic diversity among* Salmonella mbandaka *serogroup C1 banding patterns.*

DISCUSSION

PFGE was discovered by scientists involved in the typing of eukaryotic organisms. Since then, PFGE has been discovered as a widely applicable typing

technology and is now considered the gold standard of all prokaryotic genotyping methods. When PFGE was first introduced to the scientific community in the 1980's, many aspects of the technology were not yet perfected. Beyond the initial cost of expensive reagents and time-consuming protocols, a switching apparatus capable of alternating electrical current was needed for straight banding lanes. Band resolution was not available, or not applicable to all genotyping situations. Since, scientists have attempted to perfect the technical deficiencies of PFGE and have worked to develop time-saving protocols that standardize the use of PFGE in epidemiological studies, particularly in outbreak situations. The advent of PFGE has helped to revolutionize technologies used in epidemiological studies where the ability to rapidly identify identical or similar strains of prokaryotic organisms (isolates collected from the same geographical region and period belonging to the same clone) from foods or clinical cases are essential. Another application of PFGE that has been stipulated revolves around the idea that bacterial isolates collected from the same geographical region and period can be traced back to their origin. If true, the integral concept of tracing a bacterial isolate to its origin could help scientists instigate intervention strategies (e.g., Hazard Analysis Critical Control Point, (HACCP) program), and thereby reduce bacterial prevalence among various food producing operations. However, successful in-depth epidemiological studies will need to precede the evaluation of potential control strategies (*10*). Other applications of PFGE may combine multiple technologies that would allow enhanced specificity and greater applicability than technologies used in unison, such as the combined use of PFGE and serotyping to characterize salmonellae. According to the CDC, approximately 1.5 million cases of salmonellosis are estimated to occur each year

in the United States, of which 40,000 cases are culture confirmed; and that approximately 600 deaths occur each year due to acute salmonellosis, mainly in children, the elderly, and the immunocompromised (*3*). Salmonellosis has been linked to many origins of contamination such as undercooked foods, cross-contamination, poor sanitation, and contaminated food production facilities. Thus, if intervention strategies could be devised and implemented along the food production chain, food production facilities could enhance the wholesomeness of their products and increase consumer safety.

Previous investigations have suggested that serotyping is insufficient for characterization of salmonellae, in that it lacks discriminatory power and reproducibility (*14*, *41*). In this study, salmonellae of various serotypes (*S. schwarzengrund, S. montevideo, S. agona, S. livingstone, S. derby, S. anatum, S. typhimurium, S. javiana, S. muenster, S. typhimurium* (var. Copenhagen)) subjected to PFGE produced multiple genotypic banding patterns suggesting that multiple strains can exist. Note: only serotypes containing 10 or more isolates were included in this discussion. Interestingly, strain characterization (number of genotypes) using PFGE was serotypically related and not dependent upon the quantity of isolates collected. For example, 71 isolates of *S. schwarzengrund* were subjected to PFGE resulting in a genotypic profile containing four genotypes; whereas, 20 *S. anatum* isolates were subjected to PFGE resulting in a genotypic profile containing seven genotypes. These data support studies conducted by Old *et al*. (*27*) and Zhao *et al*. (*44*), indicating that genotypic characterization is more discriminatory than serotyping, and that genotypic profiles vary serotypically independent of the number of isolates collected. Old *et al.* (*27*), assessed clonal relationships among three *Salmonella* serotypes (*S*. salinatis, *S*. duisburg, and *S*.

sandiego) by use of multiple subtyping methods (biotyping, ribotyping, insertion sequence (IS) 200 fingerprinting, and PFGE), and found PFGE to be superior to other subtyping schemes. Zhao *et al*. (*44*), subjected 87 *S. newport* isolates to PFGE and antimicrobial susceptibility testing finding 35 genotypic patterns, three of which were indistinguishable among isolates collected from humans and animals. Other studies conducted by Bender *et al*. (*2*), and Olive *et al*. (*28*), demonstrated that numerous subtyping methods used to assess *S. typhimurium, Escherichia, Enterococci, Staphylococcus*, *Acinetobacter*, *Neisseria*, and *Psuedomonas* species, were less specific and discerning than PFGE; pointing out the time required to complete procedural analysis was its primary weakness. Collectively, these data suggested that serotyping like other less discriminating technologies, may prove to be more applicable when used as an adjunct to more powerful genomic approaches like PFGE *(14, 41*).

We proposed that salmonellae characterized by PFGE, once identified by serotype, could be analyzed for genotypic similarity, which potentially, may point to a common source or origin of contamination. If isolates of a specific serotype were found to be genetically similar and were collected from the same farm source during multiple collection dates, then it might increase the likelihood of a specific farm as the point of origin. On the other hand, if isolates of a specific serotype were found to be genetically distinct and were collected from different farm sources during multiple collection dates, then the slaughter plant might be suspected as the point of origin.

***S. schwarzengrund* B.** A total of 71 isolates were collected from three farm sources and six sampling dates over a period of nine months (October – June). Fifty-eight isolates were collected from the same farm source over a period of four months

(March – June). Fifty-six of these isolates were identified as being identical (i.e., genetic clones; identified by the author as genotype A). Because 56 genotype A isolates were collected from farm BT on different collection dates these data suggest that farm BT might be the point of origin. Eleven genotype A isolates were also collected from farm CF suggesting that farm CF could be a point of origin, but less significant in terms of overall isolates collected. Note: dendrogram analysis indicated an overall composite similarity of 35.2% among all four genotypes (Fig. 1). These data suggest that a low degree of genetic relatedness existed, indicating a high degree of genetic diversity within the serotype. Genotypic cluster A/B showed moderate similarity, 58.2%. Genotype B was composed of only one isolate collected from farm BT, the same farm source as most genotype A isolates.

***S. montevideo* C1.** A total of 50 isolates were collected from five farm sources and eight sampling dates over a period of eight months (November – June). Thirty-one isolates were collected from farm CF over a period of two months (November – December). Thirty of these isolates were identified as being identical (i.e., genetic clones; identified by the author as genotype B). Thirty genotype B isolates were collected from farm CF on different collection dates, and these data suggest that the farm could be a point of origin. Seven genotype B isolates were collected from a common farm source (BT) that differs from farm CF, thereby suggesting that farm BT could possibly be a point of origin, but less significant in terms of overall isolates collected. Note: dendrogram analysis indicated an overall composite similarity of 44.4% among all five genotypes (Fig. 2). These data suggest that a low degree of genetic relatedness existed, indicating a high degree of genetic diversity. Genotypic cluster D/E/C/B showed

moderate similarity, 57.9%, while clusters D/E and D/E/C showed high similarity, 84.6% and 81.5%, respectively. Also interesting, is that cluster D/E/C shows high similarity (81.5%) and is composed of 12 isolates collected from five farms over a period of seven months (November – May). These data suggest that the slaughter plant could also be a possible point of origin.

***S. agona* B.** A total of 39 isolates were collected from four farm sources and seven sampling dates over a period of nine months (October – June). Twenty-six isolates were collected from the same farm source over a period of four months (March – June). Fourteen of these isolates were identified as being identical (i.e., genetic clones; identified by the author as genotype A) and were collected from farm BT on different dates. These data suggest that farm BT could possibly be the point of origin. Four additional genotype A isolates were collected from a common farm source (CF) that differs from farm BT, thereby suggesting that farm CF could possibly be a point of origin, but less significant in terms of overall isolates collected. Note: dendrogram analysis indicated an overall composite similarity of 49.8% among all seven genotypes (Fig. 3). These data suggest that a moderate degree of genetic relatedness existed, indicating some degree of genetic diversity. Genotypic clusters F/G and A/B showed high similarity, while genotypic clusters F/G/E, F/G/E/D, and A/B/C showed moderate similarity, 89.5%, 89.1%, 74.4%, 69.4%, and 74.6%, respectively. Determining a potential point of origin is complicated by the number of genotypes for this serotype. Most serotypic isolates regardless of genotype were collected from farm BT on two collection dates over period of four months (March – June), further supporting the notion that farm BT could possibly be the point of origin.

***S. livingstone* C1.** A total of 35 isolates were collected from four farm sources and seven sampling dates over a period of nine months (October – June). Thirty-two of these isolates were identified as being identical (i.e., genetic clones; identified by the author as genotype A). Note: two isolates did not produce a banding pattern. Seventeen genotype A isolates were collected from farm CF on different collection dates, and these data suggest that farm CF could possibly be the point of origin. Twelve genotype A isolates were also collected from a farm EA, thereby suggesting that farm EA could possibly be another point of origin, but less significant in terms of overall isolates collected. Note: dendrogram analysis indicated an overall composite similarity of 61.3% between both genotypes (Fig. 4). These data suggest that a moderate degree of genetic relatedness existed, indicating less genetic diversity than most of the previously discussed serotypes.

***S. derby* B.** A total of 21 isolates were collected from five farm sources and nine sampling dates over a period of nine months (October – June). Ten isolates were collected from the same farm source over a period of four months (March – June). Nine of these isolates were identified as being identical (i.e., genetic clones; identified by the author as genotype B). Because nine genotype B isolates were collected from farm BT on different collection dates, the initial impression would point to farm BT as a point of origin. However, other genotypes from other farms confuse the issue and make the data inconclusive. For example, seven genotype A isolates were collected from farm sources (EA, EL, and FG), thereby suggesting that the slaughter plant could possibly be a point of origin. Note: dendrogram analysis indicated an overall composite similarity of 81.1% among all three genotypes (Fig. 5). These data suggest that a high degree of genetic

relatedness existed, indicating a low degree of genetic diversity. Thus, the author concludes that because genotypic cluster A/B/C isolates are highly related and were collected from multiple farm sources during multiple collection dates, that the slaughter plant could possibly be the point of origin.

***S. anatum* E1.** A total of 20 isolates were collected from four farm sources and eight sampling dates over a period of nine months (October – June). Note: dendrogram analysis indicated an overall composite similarity of 48.3% among all seven genotypes (Fig. 6). These data suggest that a moderate degree of genetic relatedness existed, indicating some degree of genetic diversity. Collectively, nine isolates were collected from farm BT during multiple collection dates over a period of four months (March – June). These data tentatively suggest that farm BT could be a point of origin. However, genotypic populations were low and the total isolates collected represented four farm sources during multiple collection dates. These data suggest that the results are inconclusive or that the slaughter plant might possibly be a central point of origin.

***S. typhimurium* B.** A total of 16 isolates were collected from two farm sources and four sampling dates over a period of eight months (October – May). Twelve isolates were collected from the same farm source over a period of two months (April – May). Eleven of these isolates were identified as belonging to genotypic cluster C/D, while nine were identical (i.e., genetic clones; identified by the author as genotype D). Because 12 genotype cluster C/D isolates were collected from farm EA on different collection dates, it is possible that the farm was a point of origin. Note: dendrogram analysis indicated an overall composite similarity of 55.1% among all five genotypes (Fig. 7). A moderate degree of genetic relatedness existed, indicating some degree of genetic diversity within

the serotype. However, genotypic cluster A/B showed high similarity, 84.4%. The data are inconclusive for trying to determine a point of origin.

***S. javiana* D1.** A total of 14 isolates were collected from farm EA on one collection date during the month of May. Thirteen of these isolates were identified as being identical (i.e., genetic clones; identified by the author as genotype A). These data suggest that farm EA could possibly be the point of origin. Note: dendrogram analysis indicated an overall composite similarity of 35.2% between both genotypes (Fig. 8). These data suggest that a low degree of genetic relatedness existed, indicating a high degree of genetic diversity.

***S. muenster* E1.** A total of 13 isolates were collected from four farm sources and five sampling dates over a period of seven months (November – June). Twelve isolates were identified as being identical (i.e., genetic clones; identified by the author as genotype A). Because genotype A isolates were collected from three farms and four collection dates, these data suggest the slaughter plant might be the point of origin. Note: dendrogram analysis indicated an overall composite similarity of 74.7% between both genotypes (Fig. 9). A moderate degree of genetic relatedness existed, indicating some degree of genetic diversity.

***S. typhimurium* (var. Copenhagen) B.** A total of 11 isolates were collected from three farm sources and four sampling dates over a period of eight months (October – May). Seven of these isolates were identified as being identical (i.e., genetic clones; identified by the author as genotype D). Six genotype D isolates were collected from farm EA on the same collection date, suggesting that the farm could have been the point of origin. Note: dendrogram analysis indicated an overall composite similarity of 86.5%

among all four genotypes (Fig. 10). These data suggest that a high degree of genetic relatedness existed, indicating a low degree of genetic diversity. Genotypic cluster C/D showed the highest similarity, 93.8%, supporting farm EA as a possible point of origin. Yet, the slaughter plant can't be ruled out as the point of origin due to the existence of isolates collected from multiple farms on multiple collection dates.

Although not conclusive, PFGE profiles of salmonellae in the current study suggested potential origins of contamination, thereby aiding the epidemiological application of this technique. However, more sample data will be needed before points of origin could be determined. Additional variables such as transport, lairage, environment, nutrition, and handling can affect salmonellae carriage rate in swine thereby complicating the interpretation of data (*18, 19, 31*). On the basis of the results of this study, origins of contamination were not clearcut and therefore it would be premature to try to design intervention strategies specifically for the farm or slaughter plant.

CHAPTER IV

CONCLUSION

The purpose of the present study was to test the hypothesis that PFGE, compared to serotyping, shows greater discriminatory power when used to genotypically characterize salmonellae. Also hypothesized, was that origins of contamination could be determined, thereby aiding in the development of intervention strategies designed to reduce bacterial prevalence in the pork food chain. Results from the present study, indicated that genotypic characterization using PFGE was more discriminatory than serotyping, suggesting that serotyping may be insufficient for epidemiologic studies. In this study, salmonellae characterized by PFGE produced multiple genotypic banding patterns indicating that multiple strains exist within a serotype. Dendrogram analysis further reflected the idea that genetic diversity existed among serotypic isolates. Genotypes within some serotypes were closely related (less diverse) whereas genotypes within other serotypes were highly diverse. These findings point out the poor discriminatory power of serotyping. On the basis of PFGE results alone, origins of contamination could not be determined in this study. It is suggested by the author, that origins of contamination could be further defined pending future research, in which in-depth longitudinal studies are included. Overall, this study concludes that PFGE is highly discriminating among many salmonellae. When used as an adjunct to conventional typing methods, PFGE may prove to be a substantial subtyping system in epidemiologic investigations to identify point-of-entry contaminants to the food chain.

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APPENDIX A

RAW - DATA

This appendix contains raw data on 340 *Salmonella* isolates and 32 serotypes. These data will aid as supplements to the tables, figures, and general text of this thesis. Below is a legend in tabular format:

LEGEND	
Isolates……	I
Genotype[a]……...	GT
Cecal Content……..……	CC
Lymph Node……...	LN
Male……	M
Female……	F
Gel[b]……..……	G
Gel Lane[c]……	L
Dendrogram Isolate[d]……...	*
Data Unavailable……	?
No Bands[e]……...	NB

[a] Genotype designations are specific for each serotype and represented by capital letters (i.e., A, B, C, D, E, F, and G).
[b] Gel (pulsed-field gel electrophoresis).
[c] Gel lanes are designated alphabetically (i.e., lane #1,L-A; lane #2, L-B…; and lane #27, L-A^2).
[d] *Salmonella* isolate chosen for dendrogram comparison.
[e] No banding pattern (w/restriction enzyme *Xba*I).

Note: All isolates were collected from swine at slaughter.

Salmonella raw data are presented in column/row format. *Salmonella* serotypes are alphabetically described as genus/serovar/serogroup (i.e., *S. agona* B), and serotypic isolates are separated by genotypic characterization. Column headings are listed and defined as follows:

A. Bacteria – *Salmonella* serovar, serogroup, genotype, and PFGE gel/lane designation.
B. Inventory # – Identity number for each isolate.
C. Date – Month, day, and year isolate was collected.
D. Farm – Farm or origin of isolate followed by sampling sequence (i.e., BT1, BT2, & BT3).
E. Origin of Isolate – Animal collection site for each isolate.
F. Animal # – Pig number of each isolate collected [150 pigs/farm (50 pigs/collection date); FG, 45 pigs/farm (one collection date)].
G. Sex – Sex of pig specific for each isolate.

Note: See appendix B, for specific salmonellae banding patterns (PFGE gel/lane-designations).

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. agona B **18 GT A**						
G 1, L-C	336	31-Mar-98	BT2	CC	16	M
G 1, L-F	366	31-Mar-98	BT2	CC	14	M
G 1, L-J	436	31-Mar-98	BT2	CC	28	M
G 1, L-K	471	31-Mar-98	BT2	CC	33	M
G 1, L-L	476	31-Mar-98	BT2	CC	34	M
G 1, L-N	491	31-Mar-98	BT2	CC	36	M
G 1, L-Q	521	31-Mar-98	BT2	CC	42	M
G 1, L-G	416	31-Mar-98	BT2	CC	22	F
G 1, L-H	426	31-Mar-98	BT2	CC	24	F
G 1, L-P	516	31-Mar-98	BT2	CC	41	F
G 1, L-B	311*	31-Mar-98	BT2	LN	2	M
G 1, L-E	361	31-Mar-98	BT2	LN	11	M
G 1, L-I	431	31-Mar-98	BT2	LN	25	M
G 2, L-K	1396	9-Jun-98	BT3	LN	21	M
G 2, L-B	626	28-Oct-97	CF1	CC	12	M
G 2, L-E	681	28-Oct-97	CF1	CC	37	F
G 2, L-C	671	28-Oct-97	CF1	LN	37	F
G 2, L-D	676	28-Oct-97	CF1	LN	37	F
S. agona B **11 GT B**						
G 1, L-D	346*	31-Mar-98	BT2	CC	9	M
G 1, L-R	551	31-Mar-98	BT2	CC	49	F
G 1, L-O	506	31-Mar-98	BT2	LN	40	M
G 2, L-P	1431	9-Jun-98	BT3	LN	26	M
G 2, L-I	1311	9-Jun-98	BT3	LN	4	F
G 2, L-M	1401	9-Jun-98	BT3	LN	22	F
G 2, L-N	1406	9-Jun-98	BT3	LN	23	F
G 2, L-Q	1471	9-Jun-98	BT3	LN	36	F
G 2, L-G	846	17-Nov-97	CF2	CC	6	F
G 2, L-H	971	17-Nov-97	CF2	LN	36	M
G 2, L-U	1771	19-May-98	EA3	CC	14	M
S. agona B **4 GT C**						
G 1, L-T	596	14-Oct-97	EL1	LN	33	M
G 1, L-U	601	14-Oct-97	EL1	LN	33	M
G 1, L-S	581*	14-Oct-97	EL1	LN	24	F
G 1, L-V	606	14-Oct-97	EL1	LN	35	F

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. agona B, cont. **1 GT D**						
G 2, L-F	756	14-Nov-97	EL2	LN	8	M
S. agona B **3 GT E**						
G 2, L-S	1546	9-Jun-98	BT3	LN	45	M
G 2, L-J	1376*	9-Jun-98	BT3	LN	17	F
G 2, L-T	1766	19-May-98	EA3	LN	14	M
S. agona B **1 GT F**						
G 2, L-O	1411*	9-Jun-98	BT3	LN	24	F
S. agona B **1 GT G**						
G 2, L-R	1501*	9-Jun-98	BT3	LN	41	F
S. anatum E1 **7 GT A**						
G 3, L-B	18*	4-Mar-98	BT1	CC	2	F
G 4, L-B	14	4-Mar-98	BT1	LN	2	F
G 3, L-C	33	4-Mar-98	BT1	LN	4	F
G 3, L-E	821	17-Nov-97	CF2	CC	1	M
G 3, L-D	651	28-Oct-97	CF1	LN	26	M
G 3, L-F	936	17-Nov-97	CF2	LN	25	M
G 3, L-I	1136	2-Dec-97	CF3	LN	11	M
S. anatum E1 **5 GT B**						
G 3, L-G	1051*	25-Feb-98	EL3	LN	23	M
G 3, L-H	1106	5-May-98	EA2	LN	11	M
G 3, L-R	1856	19-May-98	EA3	LN	39	M
G 4, L-C	1798	19-May-98	EA3	LN	23	F
G 3, L-S	1866	19-May-98	EA3	LN	40	F
S. anatum E1 **1 GT C**						
G 3, L-J	1791*	19-May-98	EA3	LN	23	F
S. anatum E1 **1 GT D**						
G 3, L-L	116*	4-Mar-98	BT1	CC	15	M

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. anatum E1, cont. **1 GT D**						
G 3, L-L	116*	4-Mar-98	BT1	CC	15	M
S. anatum E1 **4 GT E**						
G 3, L-P	1426	9-Jun-98	BT3	CC	25	M
G 3, L-N	1321	9-Jun-98	BT3	LN	7	M
G 3, L-M	1291*	9-Jun-98	BT3	LN	1	F
G 3, L-O	1361	9-Jun-98	BT3	LN	15	F
S. anatum E1 **1 GT F**						
G 3, L-Q	1526*	9-Jun-98	BT3	LN	45	F
S. anatum E1 **1 GT G**						
G 3, L-T	1911*	19-May-98	EA3	LN	50	M
S. braenderup C1 **1 GT A**						
G 3, L-V	1491*	9-Jun-98	BT3	LN	40	M
S. braenderup C1 **2 GT B**						
G 3, L-X	496	31-Mar-98	BT2	LN	37	M
G 3, L-W	73*	4-Mar-98	BT1	LN	10	F
S. derby B **2 GT A**						
G 5, L-B	218*	4-Mar-98	BT1	LN	33	F
G 5, L-V	1611	22-Apr-98	EA1	CC	19	M
S. derby B **16 GT B**						
G 5, L-N	1331	9-Jun-98	BT3	CC	8	M
G 5, L-P	1441	9-Jun-98	BT3	CC	31	M
G 5, L-Q	1451	9-Jun-98	BT3	CC	32	M
G 5, L-R	1521	9-Jun-98	BT3	CC	44	M
G 5, L-U	1541	9-Jun-98	BT3	CC	47	M
G 5, L-M	1326	9-Jun-98	BT3	LN	8	M
G 5, L-O	1416	9-Jun-98	BT3	CC	24	F

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. derby B, cont. **16 GT B**						
G 5, L-S	1531	9-Jun-98	BT3	CC	45	F
G 5, L-T	1536	9-Jun-98	BT3	CC	46	F
G 5, L-C	556*	14-Oct-97	EL1	CC	11	F
G 5, L-D	561	14-Oct-97	EL1	CC	11	F
G 5, L-E	616	14-Oct-97	EL1	CC	45	F
G 5, L-J	1066	25-Feb-98	EL3	CC	6	F
G 5, L-K	1116	5-May-98	EA2	CC	30	F
G 5, L-L	1121	5-May-98	EA2	LN	36	F
G 5, L-G	731	27-Jan-98	FG1	CC	44	F
S. derby B **3 GT C**						
G 5, L-F	666*	28-Oct-97	CF1	CC	32	M
G 5, L-H	746	4-Nov-97	EL2	CC	4	F
G 5, L-I	816	4-Nov-97	EL2	CC	28	F
S. havana G2 **5 NB**						
G 5, L-X	741	4-Nov-97	EL2	CC	1	F
G 5, L-Y	806	4-Nov-97	EL2	CC	23	F
G 5, L-Z	811	4-Nov-97	EL2	CC	26	F
G 5, L-A[2]	751	4-Nov-97	EL2	CC	7	F
G 5, L-B[2]	766	4-Nov-97	EL2	CC	12	M
S. heidelberg B **4 GT A**						
G 6, L-B	1806*	19-May-98	EA3	LN	27	M
G 6, L-E	1831	19-May-98	EA3	LN	32	M
G 6, L-C	1816	19-May-98	EA3	LN	28	F
G 6, L-D	1821	19-May-98	EA3	LN	29	F
S. infantis C1 **1 GT A**						
G 6, L-F	386*	31-Mar-98	BT2	LN	16	M
S. infantis C1 **1 GT B**						
G 6, L-G	571*	14-Oct-97	EL1	CC	17	M

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. javiana D1 **13 GT A**						
G 6, L-H	1736*	19-May-98	EA3	CC	2	M
G 6, L-I	1741	19-May-98	EA3	CC	6	M
G 6, L-J	1781	19-May-98	EA3	CC	18	M
G 6, L-K	1786	19-May-98	EA3	CC	22	M
G 6, L-N	1811	19-May-98	EA3	CC	27	M
G 6, L-O	1826	19-May-98	EA3	CC	31	M
G 6, L-P	1836	19-May-98	EA3	CC	33	M
G 6, L-Q	1841	19-May-98	EA3	CC	34	M
G 6, L-T	1886	19-May-98	EA3	CC	44	M
G 6, L-U	1891	19-May-98	EA3	CC	46	M
G 6, L-R	1876	19-May-98	EA3	LN	42	M
G 6, L-M	1801	19-May-98	EA3	CC	23	F
G 6, L-V	1906	19-May-98	EA3	CC	49	F
S. javiana D1 **1 GT B**						
G 6, L-S	1881*	19-May-98	EA3	CC	42	M
S. johannesburg B **1 GT A**						
G 6, L-W	711*	27-Jan-98	FG1	CC	34	F
S. livingstone C1 **32 GT A**						
G 7, L-C	391*	31-Mar-98	BT2	CC	18	M
G 7, L-N	1296	9-Jun-98	BT3	CC	1	F
G 7, L-L	1131	2-Dec-97	CF3	CC	4	M
G 8, L-K	1031	17-Nov-97	CF2	CC	48	M
G 7, L-J	876	17-Nov-97	CF2	LN	14	M
G 8, L-D	956	17-Nov-97	CF2	LN	31	M
G 8, L-E	961	17-Nov-97	CF2	LN	32	M
G 8, L-F	966	17-Nov-97	CF2	LN	35	M
G 8, L-G	991	17-Nov-97	CF2	LN	49	M
G 8, L-H	1006	17-Nov-97	CF2	LN	43	M
G 8, L-I	1016	17-Nov-97	CF2	LN	46	M
G 7, L-F	826	17-Nov-97	CF2	CC	2	F
G 7, L-G	831	17-Nov-97	CF2	LN	30	F
G 7, L-H	851	17-Nov-97	CF2	LN	7	F
G 7, L-I	861	17-Nov-97	CF2	LN	12	F

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. livingstone C1, cont. **32 GT A**						
G 7, L-K	926	17-Nov-97	CF2	LN	22	F
G 8, L-B	941	17-Nov-97	CF2	LN	26	F
G 8, L-C	951	17-Nov-97	CF2	LN	28	F
G 8, L-J	1021	17-Nov-97	CF2	LN	45	F
G 7, L-E	786	4-Nov-97	EL2	CC	30	M
G 7, L-P	1576	22-Apr-98	EA1	CC	10	M
G 7, L-T	1616	22-Apr-98	EA1	CC	21	M
G 7, L-U	1636	22-Apr-98	EA1	CC	24	M
G 8, L-N	1676	22-Apr-98	EA1	CC	31	M
G 7, L-O	1571	22-Apr-98	EA1	CC	9	F
G 7, L-Q	1581	22-Apr-98	EA1	CC	11	F
G 7, L-R	1586	22-Apr-98	EA1	CC	13	F
G 7, L-S	1596	22-Apr-98	EA1	CC	16	F
G 8, L-L	1651	22-Apr-98	EA1	CC	27	F
G 8, L-M	1666	22-Apr-98	EA1	CC	30	F
G 8, L-O	1706	22-Apr-98	EA1	CC	39	F
G 8, L-P	1726	22-Apr-98	EA1	CC	48	F
S. livingstone C1 **1 GT B**						
G 7, L-M	1166*	2-Dec-97	CF3	LN	17	M
S. livingstone C1 **2 NB**						
G 7, L-B	371	31-Mar-98	BT2	LN	13	F
G 7, L-D	621	28-Oct-97	CF1	LN	7	F
S. mbandaka C1 **1 GT A**						
G 7, L-W	151*	4-Mar-98	BT1	LN	22	F
S. mbandaka C1 **1 GT B**						
G 7, L-X	611*	14-Oct-97	EL1	LN	43	M
S. mbandaka ? **1 NB**						
G 17, L-K	511	14-Sep-98	BT2	LN	40	M
S. meleagridis E1 **2 GT A**						
G 7, L-A[2]	1111	5-May-98	EA2	CC	11	M
G 7, L-Z	1091*	5-May-98	EA2	LN	9	F

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. meleagridis E1, cont. **1 GT B**						
G 7, L-B[2]	1851*	19-May-98	EA3	CC	38	F
S. menhaden E3 **1 GT A**						
G 7, L-C[2]	1216*	2-Dec-97	CF3	LN	31	M
S. mono 4.5.12:I **5 GT A**						
G 8, L-T	981	17-Nov-97	CF2	LN	40	F
G 8, L-U	1076	25-Feb-98	EL3	LN	19	M
G 8, L-V	1081	25-Feb-98	EL3	LN	8	M
G 8, L-R	761*	4-Nov-97	EL2	LN	11	F
G 8, L-S	791	4-Nov-97	EL2	LN	31	F
S. monophasic B **1 GT A**						
G 8, L-W	566*	14-Oct-97	EL1	LN	16	F
S. montevideo C1 **1 GT A**						
G 9, L-B	131*	4-Mar-98	BT1	LN	18	F
S. montevideo C1 **37 GT B**						
G 9, L-C	136*	4-Mar-98	BT1	LN	18	F
G 9, L-D	146	4-Mar-98	BT1	LN	20	F
G 9, L-E	186	4-Mar-98	BT1	LN	29	F
G 9, L-G	401	31-Mar-98	BT2	LN	20	M
G 9, L-F	321	31-Mar-98	BT2	LN	5	F
G 10, L-V	1306	9-Jun-98	BT3	LN	3	M
G 10, L-W	1366	9-Jun-98	BT3	LN	15	F
G 9, L-Q	866	17-Nov-97	CF2	LN	13	M
G 9, L-R	881	17-Nov-97	CF2	LN	15	F
G 9, L-S	911	17-Nov-97	CF2	LN	19	F
G 9, L-T	921	17-Nov-97	CF2	LN	21	F
G 9, L-U	931	17-Nov-97	CF2	LN	23	F
G 9, L-X	1141	2-Dec-97	CF3	LN	11	M
G 9, L-Y	1146	2-Dec-97	CF3	LN	13	M
G 9, L-Z	1151	2-Dec-97	CF3	LN	14	M
G 9, L-A[2]	1156	2-Dec-97	CF3	LN	15	M
G 9, L-B[2]	1161	2-Dec-97	CF3	LN	16	M
G 10, L-C	1181	2-Dec-97	CF3	LN	23	M
G 10, L-E	1196	2-Dec-97	CF3	LN	26	M

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. montevideo C1, cont. **37 GT B**						
G 10, L-G	1211	2-Dec-97	CF3	LN	29	M
G 10, L-H	1221	2-Dec-97	CF3	LN	32	M
G 10, L-J	1231	2-Dec-97	CF3	LN	35	M
G 10, L-K	1236	2-Dec-97	CF3	LN	38	M
G 10, L-R	1271	2-Dec-97	CF3	LN	47	M
G 10, L-U	1286	2-Dec-97	CF3	LN	50	M
G 9, L-W	1126	2-Dec-97	CF3	LN	2	F
G 10, L-B	1176	2-Dec-97	CF3	LN	20	F
G 10, L-D	1186	2-Dec-97	CF3	LN	24	F
G 10, L-F	1201	2-Dec-97	CF3	LN	26	F
G 10, L-I	1226	2-Dec-97	CF3	LN	33	F
G 10, L-L	1241	2-Dec-97	CF3	LN	39	F
G 10, L-M	1246	2-Dec-97	CF3	LN	41	F
G 10, L-N	1251	2-Dec-97	CF3	LN	42	F
G 10, L-P	1256	2-Dec-97	CF3	LN	43	F
G 10, L-Q	1266	2-Dec-97	CF3	LN	46	F
G 10, L-S	1276	2-Dec-97	CF3	LN	48	F
G 10, L-T	1281	2-Dec-97	CF3	LN	49	F
S. montevideo C1 **4 GT C**						
G 9, L-H	411*	31-Mar-98	BT2	LN	21	M
G 9, L-I	461	31-Mar-98	BT2	LN	32	M
G 9, L-J	501	31-Mar-98	BT2	LN	39	M
G 9, L-K	536	31-Mar-98	BT2	LN	45	M
S. montevideo C1 **6 GT D**						
G 9, L-V	1026	17-Nov-97	CF2	CC	47	M
G 9, L-N	776	4-Nov-97	EL2	LN	15	M
G 10, L-X	1746	19-May-98	EA3	CC	8	F
G 10, L-Y	1751	19-May-98	EA3	CC	10	F
G 9, L-L	721*	27-Jan-98	FG1	CC	37	F
G 9, L-M	726	27-Jan-98	FG1	LN	43	F
S. montevideo C1 **2 GT E**						
G 9, L-O	781*	4-Nov-97	EL2	LN	20	M
G 9, L-P	796	4-Nov-97	EL2	?	20	M

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. muenchen C2						
1 GT A						
G 10, L-Z	976*	17-Nov-97	CF2	LN	37	M
S. muenchen C2						
1 NB						
G 10, L-A[2]	1551	9-Jun-98	BT3	LN	50	M
S. muenster E1						
12 GT A						
G 11, L-B	446*	31-Mar-98	BT2	LN	30	M
G 11, L-G	1381	9-Jun-98	BT3	LN	18	M
G 11, L-F	1356	9-Jun-98	BT3	LN	13	F
G 11, L-D	1171	2-Dec-97	CF3	LN	18	M
G 11, L-E	1191	2-Dec-97	CF3	LN	25	F
G 11, L-K	1661	22-Apr-98	EA1	CC	29	M
G 11, L-L	1671	22-Apr-98	EA1	CC	31	M
G 11, L-M	1711	22-Apr-98	EA1	CC	42	M
G 11, L-H	1561	22-Apr-98	EA1	CC	5	F
G 11, L-I	1566	22-Apr-98	EA1	CC	8	F
G 11, L-J	1656	22-Apr-98	EA1	CC	28	F
G 11, L-N	1716	22-Apr-98	EA1	CC	44	F
S. muenster E1 **1 GT B**						
G 11, L-C	771*	4-Nov-97	EL2	CC	14	M
S. multi serotypes **1 GT A**						
G 11, L-P	946*	17-Nov-97	CF2	LN	27	F
S. newbrunswick E2 **1 GT A**						
G 11, L-Q	886*	17-Nov-97	CF2	CC	15	F
S. newport C2 **5 GT A**						
G 11, L-S	341	31-Mar-98	BT2	LN	8	M
G 11, L-T	351	31-Mar-98	BT2	LN	10	M
G 11, L-U	441	31-Mar-98	BT2	LN	29	M
G 11, L-R	326*	31-Mar-98	BT2	LN	5	F
G 11, L-V	451	31-Mar-98	BT2	LN	31	F
S. newport C2 **2 GT B**						
G 11, L-X	636	28-Oct-97	CF1	CC	14	M
G 11, L-W	631*	28-Oct-97	CF1	LN	14	M

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. orion E1 **2 GT A**						
G 11, L-Z	1316	9-Jun-98	BT3	LN	6	M
G 11, L-Y	176*	4-Mar-98	BT1	CC	27	F
S. schwarzengrund B **68 GT A**						
G 12, L-C	28	4-Mar-98	BT1	CC	3	M
G 12, L-E	43	4-Mar-98	BT1	CC	5	M
G 12, L-H	68	4-Mar-98	BT1	CC	9	M
G 12, L-I	78	4-Mar-98	BT1	CC	11	M
G 12, L-M	98	4-Mar-98	BT1	CC	13	M
G 12, L-O	106	4-Mar-98	BT1	CC	14	M
G 12, L-Y	206	4-Mar-98	BT1	CC	31	M
G 13, L-D	231	4-Mar-98	BT1	CC	35	M
G 13, L-I	271	4-Mar-98	BT1	CC	43	M
G 13, L-J	276	4-Mar-98	BT1	CC	45	M
G 13, L-M	306	4-Mar-98	BT1	CC	50	M
G 12, L-B	23*	4-Mar-98	BT1	LN	3	M
G 12, L-D	38	4-Mar-98	BT1	LN	5	M
G 12, L-G	63	4-Mar-98	BT1	LN	9	M
G 12, L-L	93	4-Mar-98	BT1	LN	13	M
G 12, L-N	103	4-Mar-98	BT1	LN	14	M
G 12, L-P	111	4-Mar-98	BT1	LN	15	M
G 12, L-X	201	4-Mar-98	BT1	LN	31	M
G 13, L-E	236	4-Mar-98	BT1	LN	36	M
G 13, L-F	241	4-Mar-98	BT1	LN	37	M
G 13, L-G	261	4-Mar-98	BT1	LN	41	M
G 13, L-H	266	4-Mar-98	BT1	LN	42	M
G 13, L-N	316	31-Mar-98	BT2	LN	4	M
G 13, L-O	331	31-Mar-98	BT2	LN	7	M
G 13, L-R	486	31-Mar-98	BT2	LN	36	M
G 13, L-S	526	31-Mar-98	BT2	LN	43	M
G 13, L-T	531	31-Mar-98	BT2	LN	45	M
G 13, L-U	541	31-Mar-98	BT2	LN	46	M
G 14, L-L	1346	9-Jun-98	BT3	LN	11	M
G 14, L-M	1351	9-Jun-98	BT3	LN	12	M
G 14, L-N	1391	9-Jun-98	BT3	LN	20	M
G 14, L-O	1421	9-Jun-98	BT3	LN	25	M
G 14, L-Q	1446	9-Jun-98	BT3	LN	32	M
G 14, L-R	1456	9-Jun-98	BT3	LN	33	M

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. schwarzengrund B, cont. **68 GT A**						
G 14, L-S	1466	9-Jun-98	BT3	LN	35	M
G 14, L-V	1516	9-Jun-98	BT3	LN	44	M
G 12, L-F	58	4-Mar-98	BT1	CC	8	F
G 12, L-K	88	4-Mar-98	BT1	CC	12	F
G 12, L-R	126	4-Mar-98	BT1	CC	16	F
G 12, L-S	141	4-Mar-98	BT1	CC	18	F
G 12, L-T	156	4-Mar-98	BT1	CC	22	F
G 12, L-W	171	4-Mar-98	BT1	CC	26	F
G 13, L-B	221	4-Mar-98	BT1	CC	33	F
G 13, L-K	291	4-Mar-98	BT1	CC	48	F
G 12, L-J	83	4-Mar-98	BT1	LN	12	F
G 12, L-U	161	4-Mar-98	BT1	LN	23	F
G 12, L-V	166	4-Mar-98	BT1	LN	24	F
G 13, L-C	226	4-Mar-98	BT1	LN	34	F
G 13, L-L	296	4-Mar-98	BT1	LN	49	F
G 13, L-P	398	31-Mar-98	BT2	LN	19	F
G 13, L-Q	421	31-Mar-98	BT2	LN	23	F
G 13, L-V	546	31-Mar-98	BT2	LN	47	F
G 14, L-J	1336	9-Jun-98	BT3	LN	9	F
G 14, L-K	1341	9-Jun-98	BT3	LN	10	F
G 14, L-P	1436	9-Jun-98	BT3	LN	29	F
G 14, L-T	1481	9-Jun-98	BT3	LN	38	F
G 14, L-B	871	17-Nov-97	CF2	CC	13	M
G 14, L-D	896	17-Nov-97	CF2	CC	17	M
G 13, L-Z	841	17-Nov-97	CF2	LN	50	M
G 13, L-A[2]	856	17-Nov-97	CF2	LN	8	M
G 14, L-G	1011	17-Nov-97	CF2	LN	44	M
G 14, L-I	1206	2-Dec-97	CF3	LN	28	M
G 13, L-Y	836	17-Nov-97	CF2	CC	4	F
G 14, L-H	1036	17-Nov-97	CF2	CC	49	F
G 14, L-C	891	17-Nov-97	CF2	LN	16	F
G 14, L-E	916	17-Nov-97	CF2	LN	19	F
G 14, L-F	986	17-Nov-97	CF2	LN	41	F
G 13, L-X	736	27-Jan-98	FG1	CC	44	F
S. schwarzengrund B **1 GT B**						
G 12, L-Q	121*	4-Mar-98	BT1	LN	16	F

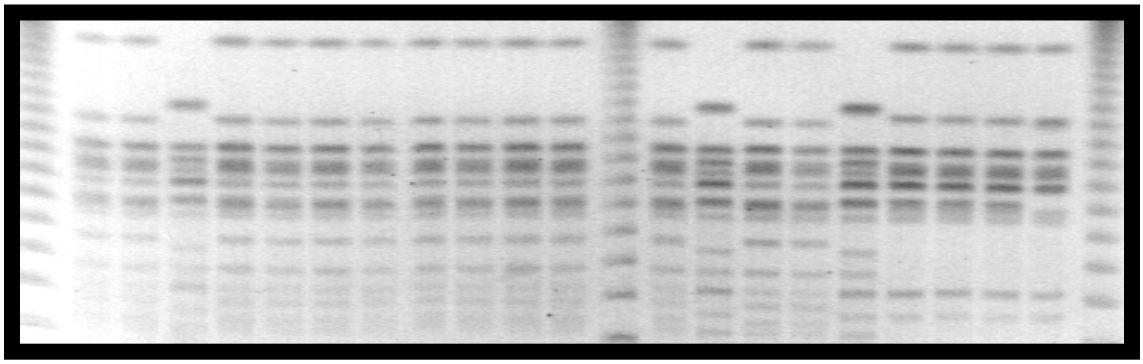
Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. schwarzengrund B, cont. **1 GT C**						
G 13, L-W	716*	27-Jan-98	FG1	CC	36	F
S. schwarzengrund B **1 GT D**						
G 14, L-U	1486*	9-Jun-98	BT3	LN	39	M
*S. typhimurium (*var. Copenhagen) B **2 GT A**						
G 15, L-C	696*	2-Oct-97	CF1	LN	43	M
G 15, L-D	701	28-Oct-97	CF1	LN	43	M
S. typhimurium (var. Copenhagen) B **1 GT B**						
G 15, L-E	1056*	15-Feb-98	EL3	LN	49	M
S. typhimurium (var. Copenhagen) B **1 GT C**						
G 15, L-F	1096*	5-May-98	EA2	CC	10	M
S. typhimurium (var. Copenhagen) B **7 GT D**						
G 15, L-G	1556*	22-Apr-98	EA1	LN	3	M
G 15, L-H	1591	22-Apr-98	EA1	LN	14	M
G 15, L-I	1626	22-Apr-98	EA1	LN	23	M
G 15, L-J	1631	22-Apr-98	EA1	LN	24	M
G 15, L-K	1646	22-Apr-98	EA1	LN	26	M
G 15, L-B	641	28-Oct-97	CF1	LN	15	F
G 15, L-L	1691	22-Apr-98	EA1	LN	36	F
S. tennessee C1 **1 GT A**						
G 15, L-M	1071*	25-Feb-98	EL3	CC	3	F
S. thompson C1 **2 GT A**						
G 15, L-N	191*	4-Mar-98	BT1	CC	29	F
G 15, L-O	211	4-Mar-98	BT1	LN	32	F
S. typhimurium B **2 GT A**						
G 16, L-B	656*	28-Oct-97	CF1	LN	27	M
G 16, L-C	661	28-Oct-97	CF1	LN	27	M

Bacteria	Inventory no.	Date	Farm	Origin of Isolate	Animal no.	Sex
S. typhimurium B, cont. **2 GT B**						
G 16, L-D	691*	28-Oct-98	CF1	LN	39	M
G 16, L-E	706	28-Oct-97	CF1	LN	46	M
S. typhimurium B **2 GT C**						
G 16, L-I	1606	22-Apr-98	EA1	LN	19	M
G 16, L-F	1086*	5-May-98	EA2	CC	3	F
S. typhimurium B **9 GT D**						
G 16, L-G	1101*	5-May-98	EA2	CC	10	M
G 16, L-K	1641	22-Apr-98	EA1	CC	25	M
G 16, L-H	1601	22-Apr-98	EA1	LN	17	M
G 16, L-J	1621	22-Apr-98	EA1	LN	22	M
G 16, L-L	1681	22-Apr-98	EA1	LN	26	M
G 16, L-N	1701	22-Apr-98	EA1	LN	38	M
G 16, L-P	1731	22-Apr-98	EA1	LN	50	M
G 16, L-M	1696	22-Apr-98	EA1	LN	37	F
G 16, L-O	1721	22-Apr-98	EA1	LN	48	F
S. typhimurium B **1 GT E**						
G 16, L-Q	1776*	19-May-98	EA3	CC	17	F
S. uganda E1 **1 GT A**						
G 16, L-R	576*	14-Oct-97	EL1	LN	20	F
S. uganda E1 **2 GT B**						
G 16, L-S	586*	14-Oct-97	EL1	LN	28	F
G 16, L-T	591	14-Oct-97	EL1	LN	28	F
S. untypable E **1 GT A**						
G 16, L-U	686*	28-Oct-97	CF1	LN	38	M
S. urbana N **1 GT A**						
G 16, L-V	1061*	25-Feb-98	EL3	CC	30	F
S. worthington G2 **1 GT A**						
G 16, L-W	1261*	2-Dec-97	CF3	LN	44	F

APPENDIX B

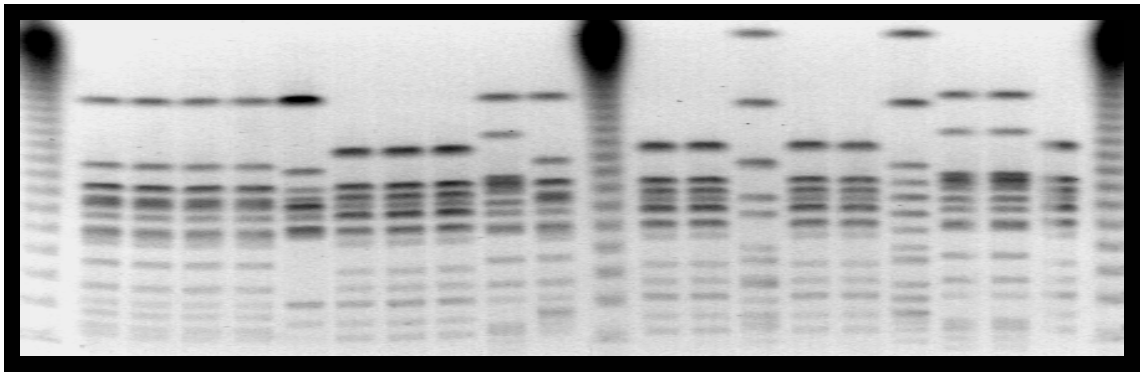
Appendix B consists of pulsed-field gels. Represented, are all salmonellae included in the present study and their genotypic banding patterns. Refer to appendix A for salmonellae and their specific gel number/lane designation. Note: lanes are designated alphabetically (i.e., A, B, C, …Z, A^2, B^2, etc…).

A B C D E F G H I J K L M N O P Q R S T U V W



Gel no. 1.

A B C D E F G H I J K L M N O P Q R S T U V



Gel no. 2.

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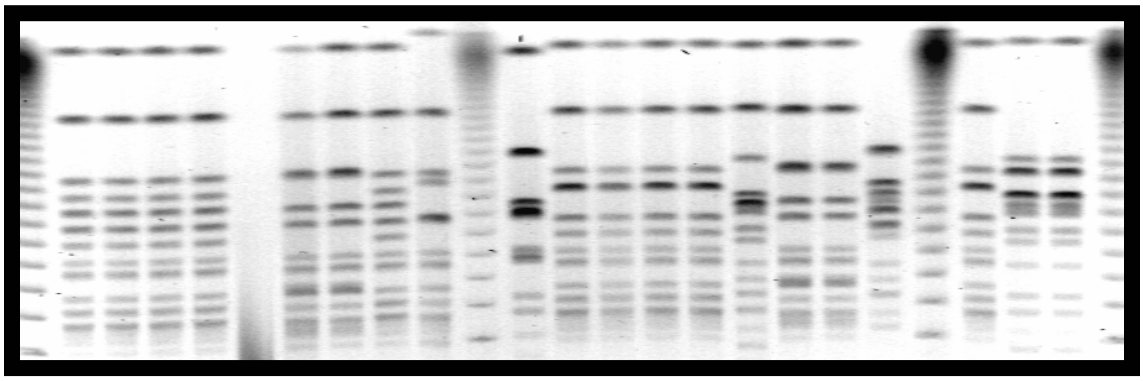
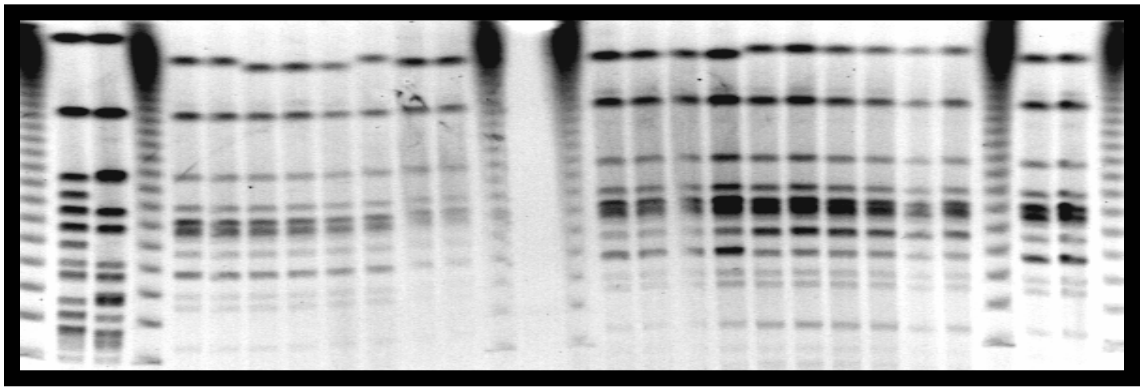


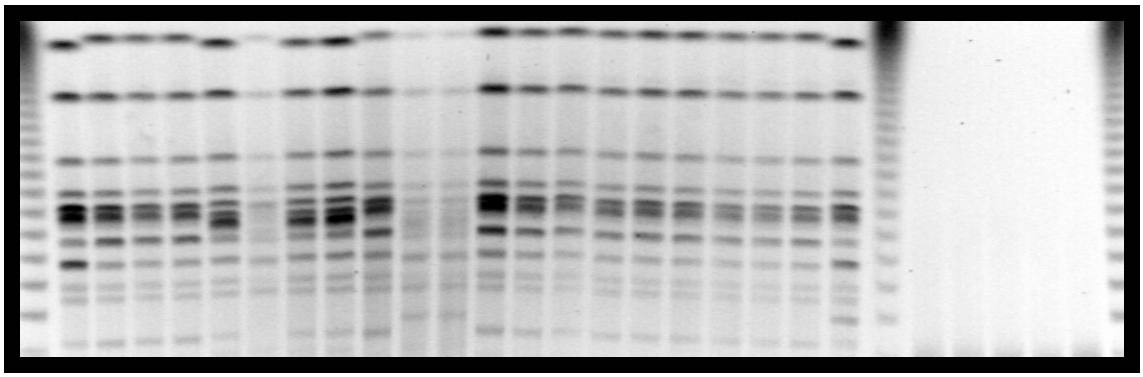
Gel no. 3.

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z A^2 B^2



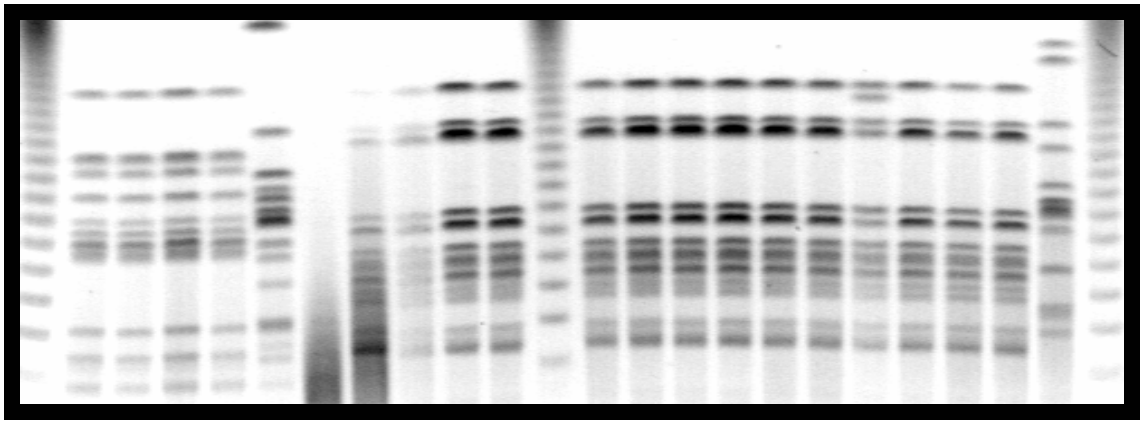
Gel no. 4.

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z A^2 B^2 C^2



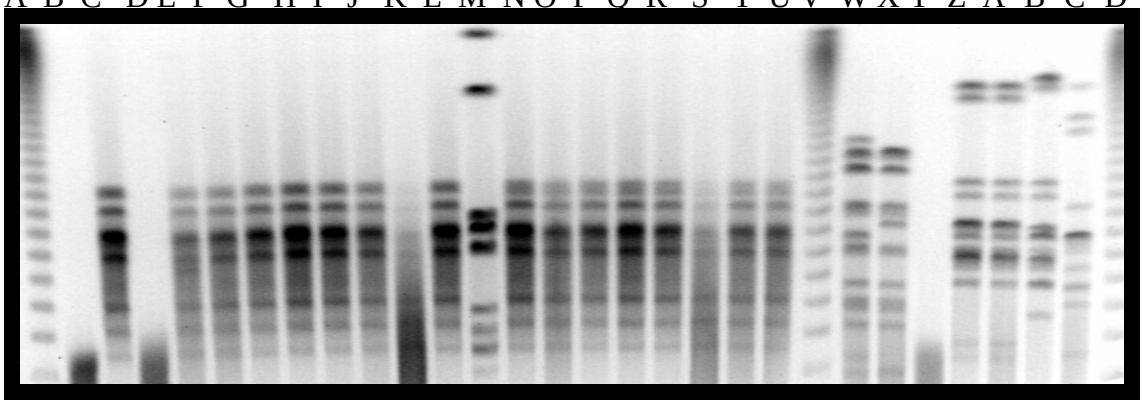
Gel no. 5.

A B C D E F G H I J K L M N O P Q R S T U V W X



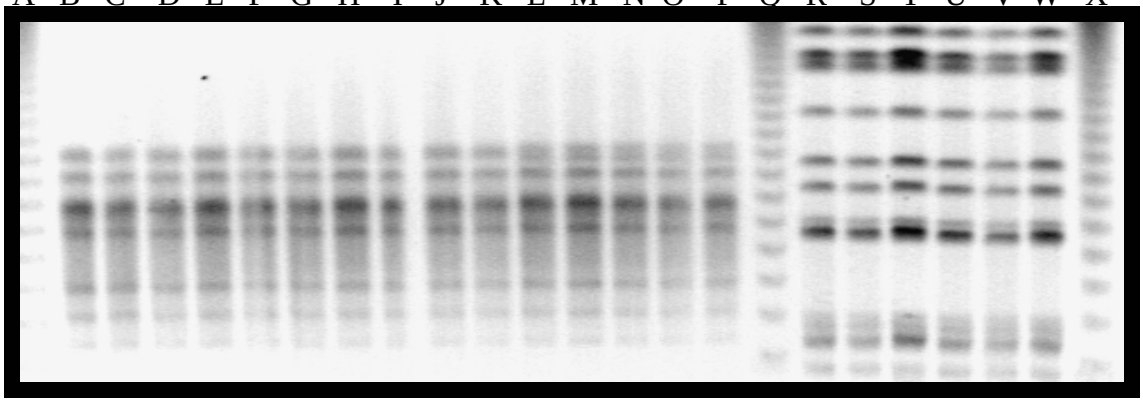
Gel no. 6.

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z A^2 B^2 C^2 D^2



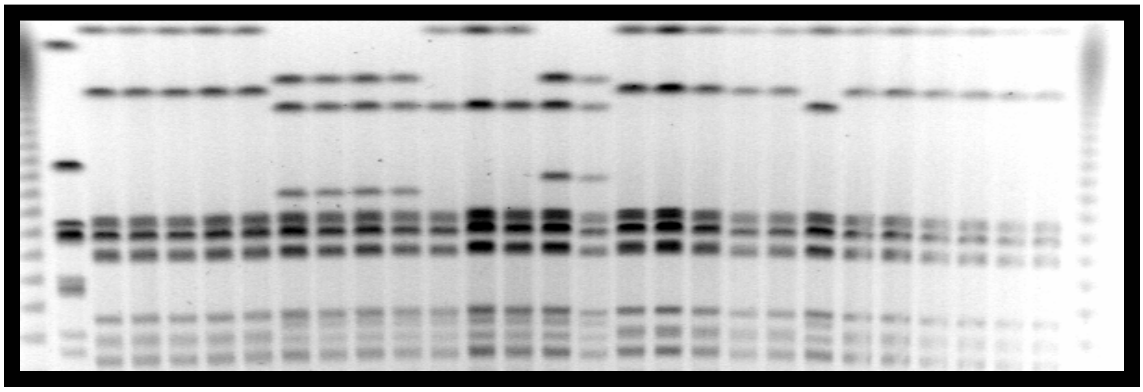
Gel no. 7.

A B C D E F G H I J K L M N O P Q R S T U V W X



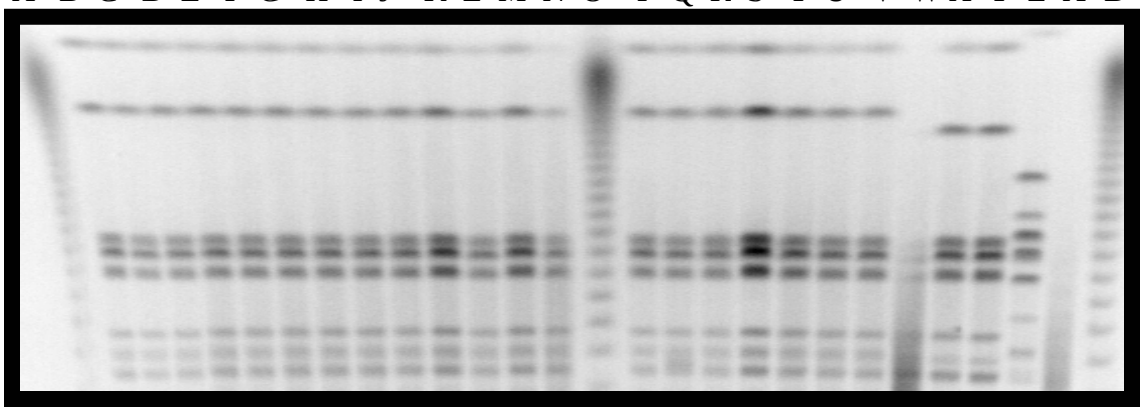
Gel no. 8.

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z A^2 B^2 C^2



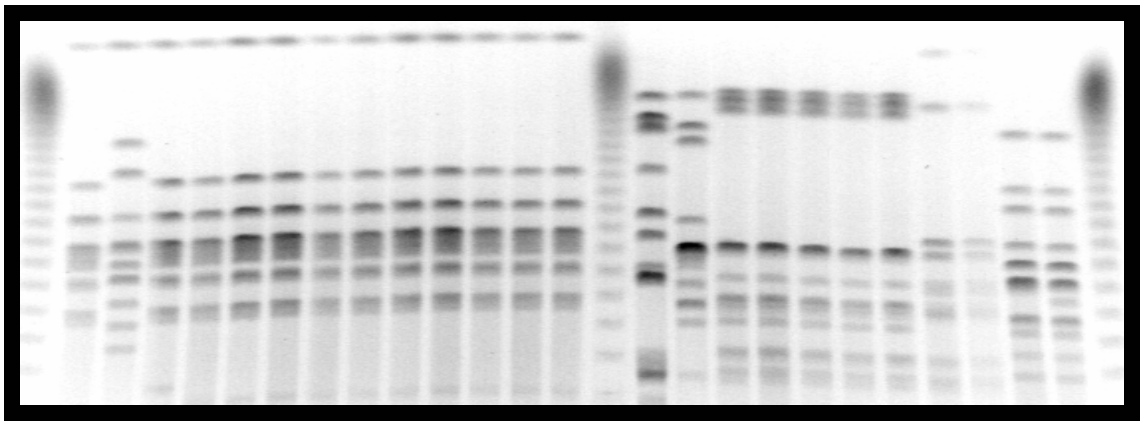
Gel no. 9.

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z A^2 B^2



Gel no. 10.

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z A^2



Gel no. 11.

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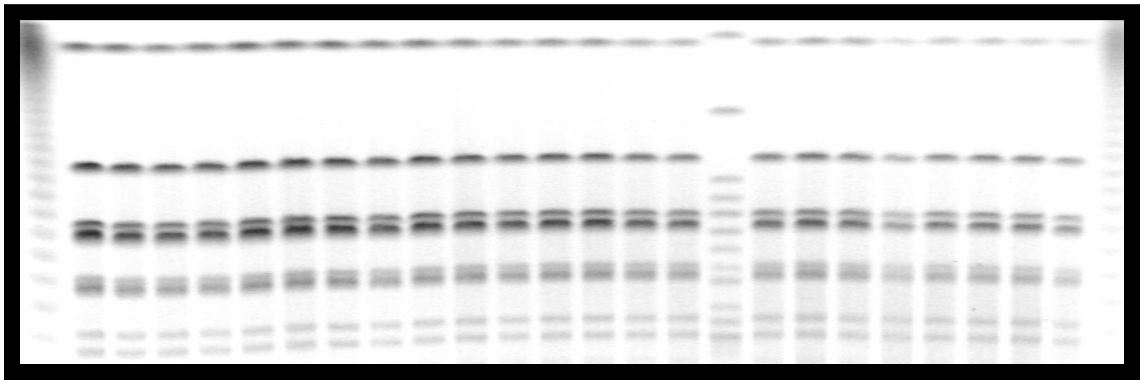
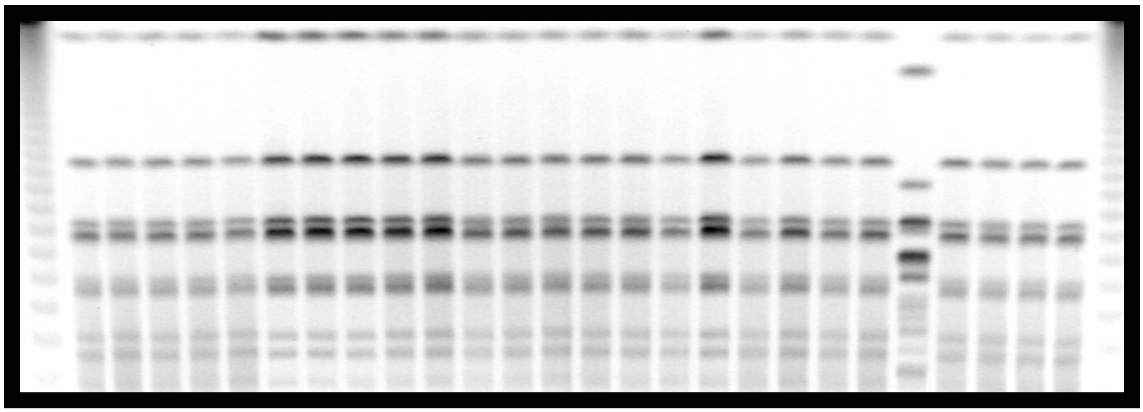


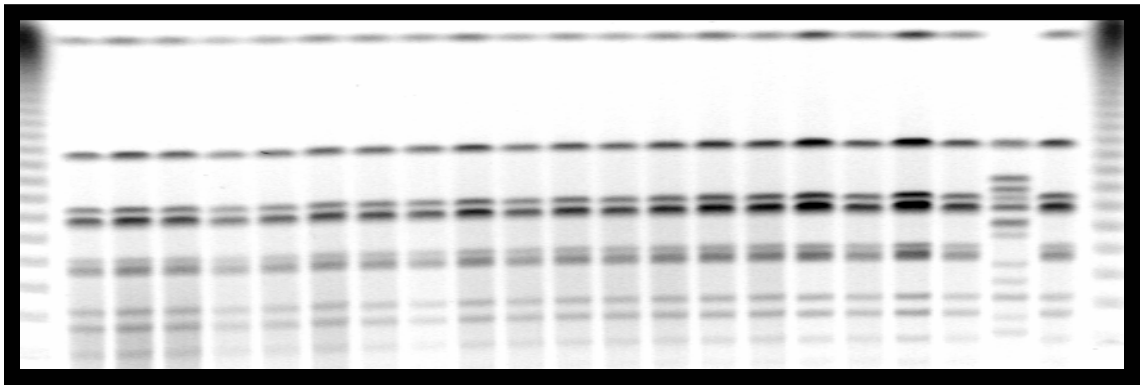
Gel no. 12.

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z A^2 B^2



Gel no. 13.

A B C D E F G H I J K L M N O P Q R S T U V W



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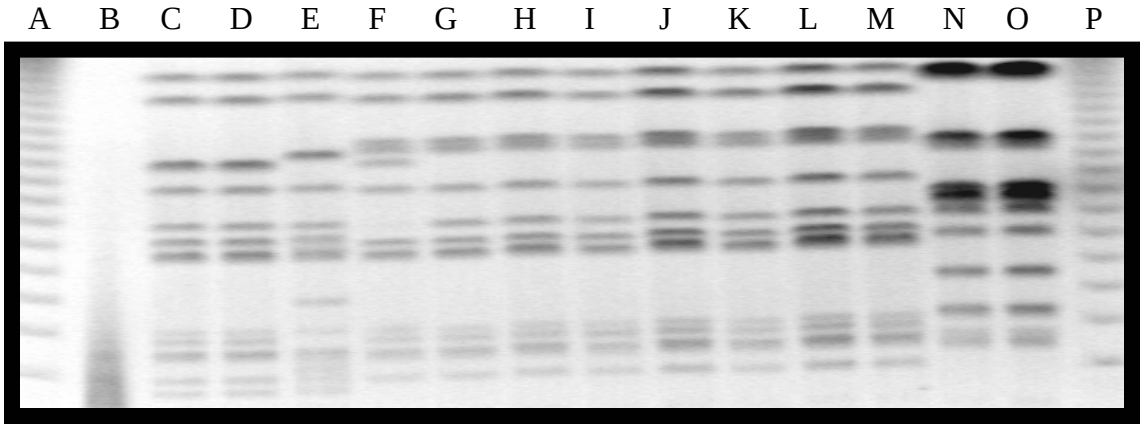
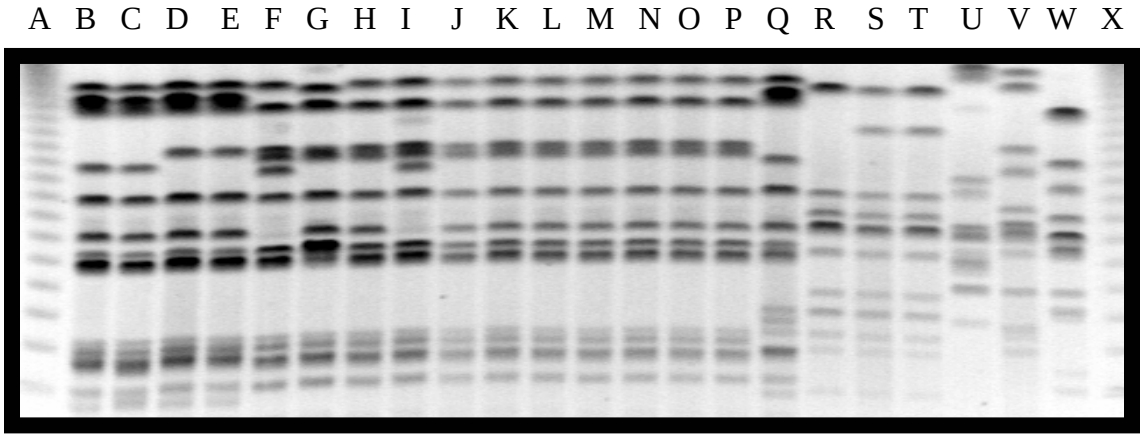


Gel no. 15.



Gel no. 16.

VITA

Damon L. J. Drinnon

Born:

July 27, 1976
Abilene, TX - Taylor County

Home Town:

Anson, TX – Jones County

Eagle Scout:

December 29, 1993
Hamlin, TX - Jones County

Married:

June 15, 1996
Rotan, TX - Fisher County

Education:

Bachelor of Science (Cum Laude)
Biomedical Science & Animal Science (Sci.-Opt.), Texas A&M University, 2001

Master of Science
Veterinary Microbiology, Texas A&M University, 2004

Permanent Address:

800 San Pedro Apt. D
College Station, TX
77845