

GENETIC INTERVENTION IN PIGS TO CONTROL *SALMONELLA* SHEDDING

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## Abstract

Salmonellosis is one of the most important bacterial foodborne infections in the United States resulting in over 1 million illnesses and 375 deaths annually. *Salmonella* serotypes cause several types of disease in humans: gastroenteritis, enteric fever, septicemia, focal infections, and an asymptomatic carrier state. *Salmonella*-shedding pigs are known to constitute a risk factor for contamination of carcasses during the slaughter process. Vaccination has generally not been effective in the prevention of *Salmonella*, partially because of the rapid mutation rate. Previous research has indicated that >70% of farms tested in Iowa were positive for *Salmonella* during 2006-2009. *Salmonella*-colonized pigs are usually asymptomatic carriers of the bacterium and can shed upon exposure to stress causing contamination of pen-mates, trailers used for shipping, and lairage areas at processing facilities. Emergence and dissemination of antimicrobial-resistant pathogens, which antibiotics are commonly used in pig production, have become a public health concern worldwide. For this reason, alternative interventions need to be evaluated for effectiveness. The objective of this report was to determine if there is a genetic basis for host resistance or susceptibility to *Salmonella* through quantitative and/or molecular selection. Genetic improvement of disease resistance and/or tolerance in animals is a potentially effective intervention for addressing pre-harvest food safety issues. Previous research has demonstrated genetic control of the immune response to pathogens. Developing a strong innate response to infection, so the animal does not become ill or become a carrier, is the basis for a genetic intervention for *Salmonella*. Quantitative trait loci for humoral and innate immune response have been detected for *E. coli* through leucocyte counts, cytokine concentration, mitogen-induced proliferation, and levels of pre-infection antibody titers. Single nucleotide polymorphisms have been found and can be exploited for genetic improvement of the innate immune response in pigs when infected with *Salmonella*. Pigs that differentially express polymorphisms and persistently shed the bacteria versus pigs that do not shed or shed little can be used as criteria for selection when developing the intervention.

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## **Chapter 1 - Characteristics of *Salmonella***

*Salmonella* is a bacterium that was responsible for the most frequently reported cause of foodborne illness prior to 2004 (Pedro and Boris, 2005). In more recent years Norovirus has become the number one cause of foodborne illness, with *Salmonella* being the leading cause of hospitalization (Scallan et al., 2011). *Salmonella* is a gram-negative, rod-shaped bacterium that can cause diarrheal illness in humans. This bacteria is the most frequently reported cause of foodborne illness, over 2,500 serotypes have been identified to date. The two most common serotypes are *Salmonella* Enteritidis and *Salmonella* Typhimurium which account for more than half of the reported infections. Dr. Daniel E. Salmon was credited with the discovery of *Salmonella* in 1885 though it was one of his research assistants, Theobald Smith, who was responsible for the discovery (Molbak et al., 2006).

Salmonellosis is the disease caused by any of the numerous serotypes of *Salmonella*. In swine, only a few cause disease, usually as septicemia and/or enterocolitis. Infections in asymptomatic swine serve as a source of *Salmonella* infection to humans via contamination of food products. Salmonellosis occurs when the bacteria survive the pH in the gut and reach the mucosa in the small intestine in an adequate number to cause an infection. Epithelial cells localized in the mucosal layer of the intestine interact with the bacteria, thus stimulating an inflammatory response (Guthrie, 1991).

### **Historical**

*Salmonella* has been documented to be one of the primary causative agents of foodborne illnesses (Cardinale et al., 2005; Garry et al. 2009; Prapas et al., 2008). Scallan et al. (2011) reported that of foodborne illnesses nontyphoidal *Salmonella* represents 11% which is the second most causative agent. These researchers estimated over 1 million cases of non-typhoidal *Salmonella* illnesses per year occur in the United States. The number of outbreaks in underdeveloped countries is much greater than this but is hard to quantify with limited resources for diagnostics. The cost for Salmonellosis was estimated to be \$600 million to \$3.5 billion on an annual basis in the United States (Voetsch et al., 2004; Ter-Hsin et al., 2005).

Historically, water and milk were found to be the first culprits as the agent of enteric fever; however, the agent itself had not been identified (Budd, 1874). *Salmonella enterica* serotype Typhi was discovered in 1880 (Eberth, 1880) and isolated on media in 1884 (Gaffky, 1884) although no credit was given for this discovery. A bacteriologist named Theobald Smith, isolated *S. Choleraesuis* in 1885 from a pig intestine, and the genus name *Salmonella* was given as a credit to D. E. Salmon, the laboratory chief, for the discovery.

Historical data is limited on the prevalence of *Salmonella* in animals, carcasses, or meat, but it is likely to have been lower, due to detection methods and surveillance or just not detected, than what had been found in industrialized countries in the 1980's and 1990's (Molbak et al., 2006). Testing in connection with meat inspection in Copenhagen revealed *S. Typhimurium* on two occasions between 1922 and 1941 (Hansen, 1942). In contrast, a total of 662 cases of *Salmonella* infections were found in Denmark in a 6-year period from 1936 to 1942, with 79% comprising the serotype Typhimurium and 6% comprising the serotype Enteritidis (Harhoff, 1948). Harhoff (1948) concluded that although eggs were often used as a food source, practically speaking they were never seen as a cause of contamination because hens were resistant to *Salmonella* infections (excluding *S. pullorum* and *S. gallinarum*, specific to birds). The linkage of how *Salmonella* could be transmitted was not understood. During this same time period, duck eggs caused most egg-associated outbreaks and meat from sick animals (mainly cattle) were a common cause of foodborne outbreaks. Cheese made from raw milk was a known source of contamination, yet broilers were an uncommon source of foodborne outbreaks (Harhoff, 1948). In 1955, an outbreak in Sweden affected 9,000 individuals and caused 90 deaths, prompting early intervention strategies to control *Salmonella* (Bengtsson et al., 1955).

*Salmonella* Typhi became a large problem in the United States in the early industrial era, while disease pressure associated with non-typhoid *Salmonella* was low prior to World War II (Tauxe, 1999). Improvements made in sanitation nearly eliminated *S. Typhi* as a cause of infection, however, the transition to non-typhoid *Salmonella* infections began a trend that seemed to have peaked around 1990 (Figure 1-1).

The development of serotyping was essential for determining the epidemiology of pathways involved in *Salmonella* infections. During discovery, *Salmonella* serotypes were regarded as different species and were named according to the disease that was caused (i.e. *S. Enteritidis*, *S. Typhi*, *S. Paratyphi*, and *S. Abortus equi*) or the animal from which they were



isolated (Kelterborn, 1967). Recently, the naming has changed to reflect the geographic location where it was isolated for each new antigenically distinguishable type. Most serotypes are considered to belong to a single species, *Salmonella enterica* (Brenner et al., 2000; Popoff, 2001).

The Kaufmann-White scheme, which currently consists of >2,500 serotypes, is the most successful bacterial typing scheme in history (Molbak et al., 2006). This scheme is a system that classifies the genus *Salmonella* into serotypes based on surface antigens. Serotypes are usually correlated with severity (clinically), reservoir, and occurrence of resistance. The information in the database includes: distribution of different serotypes, as well as subtypes, in different species, foods, and humans. This information may be used to quantify the relative importance of sources of *Salmonella* infections (Hald et al., 2004).

### ***Progression***

The past couple of decades have shown an increased incidence of non-typhoid *Salmonella* infections (Figure 1-1). This is difficult to track globally as official numbers of *Salmonella* infections are derived from laboratory-based surveillance and each country has a different protocol for handling samples. Some countries submit isolates to national reference laboratories for serotyping and other characterization, whereas other countries do little beyond reporting outbreaks (Molbak et al., 2006). The figures derived from official reporting do not measure the burden of illness or the degree of surveillance. Usually the reported incidence is a composite of several factors including: true incidence of *Salmonella* infections, healthcare-seeking behavior of patients with gastroenteritis, and the likelihood the physician will request a stool sample. The last item (stool sample) tends to be the biggest hurdle with patients, yet is necessary for serotyping (Molbak et al., 2006).

In countries with data from food production animals, parallel trends can be observed between serotype distribution in human infections and the prevalence and distribution of *Salmonella* in animals and food (Wegener et al., 2003). Industrialization and globalization have been blamed for the emergence of *Salmonella* in poultry production and egg production (Rodrigue et al., 1990; Tauxe, 1999). Baumler et al. (2000) hypothesized that the epidemic of Salmonellosis in humans was triggered by *S. Enteritidis* filling the ecological niche vacated by other avian pathogens such as *S. Gallinarum* and *S. Pullorum*; however, this is contradicted by

other researchers (Riemann et al., 2000; Ward et al., 2000). The emergence of international trade of live animals was thought to further amplify the rise of salmonellosis.

International trade of live animals, which serves as a means to supply the food animal production system with breeder animals to improve genetic stock, is an efficient way to disseminate not just *Salmonella* vertically throughout the system, but viral infections as well. Over the past century, livestock production has evolved from small herd operations to large concentrations of animals (i.e. feedlots and high stocking density buildings). The confinement of large numbers of animals and the growth in productivity due to practices such as nontherapeutic antimicrobial use has given rise to public health concerns regarding foodborne pathogens and the prevalence of antimicrobial-resistant pathogens (Prapas et al. 2008). Chapin et al. (2005) concluded that use of nontherapeutic levels of antibiotics in the production of pigs and other livestock in general has contributed to an outburst of multidrug-resistant pathogens. Though the incidence of multidrug-resistant pathogens has increased slightly, the prevalence coming into plants has remained consistent.

### *Nomenclature*

Enterobacteriaceae is a family of bacteria that cause illness in humans and includes *Salmonella*, *Escherichia*, *Yersinia*, and *Shigella*. Members of the Enterobacteriaceae are referred to as “enteric” bacteria as several of the members live in the intestines of animals. The members of this family are gram-negative, rod-shaped, and are typically 1-5µm in length (Bell and Kyriakides, 2002; Molbak et al., 2006). They are facultative anaerobes and are for the most part a normal part of the gut flora found in the intestines. The optimal temperature for growth ranges from 8°C to 45°C and they tolerate pH levels ranging from 4 to 9. *Salmonella* are heat labile and are inactivated at ordinary cooking temperatures (>70°C); however, inactivation is dependent on cooking time, serotype, and the food matrix (Guthrie, 1991). *Salmonella* do not survive pasteurization but are relatively resistant to freezing (Bell and Kyriakides, 2002). In the farm-to-fork production chain, reduction/kill of *Salmonella* through heat treatment is used in feed, carcasses, processing, and preparation.

Using the Kauffman-White classification scheme, *Salmonella* are classified according to three major antigens: H or flagellar antigen, O or somatic antigen, and Vi antigen (only a few serotypes possess this). The O antigen is located on the cell wall of the bacterium, and bacteria

may possess 2 or more O antigens on the surface. The O antigen type is determined based on polysaccharides associated with lipopolysaccharide. The H antigen is determined based on flagellar proteins. Because *Salmonella* exhibit phase variation (defined as the random switching of phenotype at frequencies that are much higher than classical mutation rates) between motile and non-motile phenotypes, different H antigens may be expressed. The Vi antigen is related to the virulence of the bacterium. Because it is a capsular antigen, its presence enhances virulence.

More than 2,500 serotypes have been found in the gastrointestinal tracts of mammals and birds (Molbak et al., 2006). With regard to food safety the subspecies enterica is of most concern because the strains within these serogroups are known to cause 99% of human infections related to *Salmonella* (Bell and Kyriakides, 2002; Brenner et al., 2000). However, all serotypes of *Salmonella* are considered a potential health hazard to humans.

## **Pathogenesis**

*Salmonella* infections are zoonotic and can be transferred between humans and animals. Salmonellosis includes several syndromes: gastroenteritis, enteric fevers, septicemia, focal infections, and an asymptomatic carrier state (Guthrie, 1991; Monteville and Matthews, 2008). Certain serotypes have more of a propensity to produce these syndromes compared to others. In general, more serious infections occur in infants and adults over the age of 50. This occurs because of the low expression of interferon gamma (IFN- $\gamma$ ) in individuals at this stage in their life. Juveniles and young adults tend to have higher levels of specific cytokines needed for quick response and eradication of bacterial infections (Coburn et al., 2007).

Once ingested, the bacteria colonize in the ileum and colon, then invade the intestinal epithelium, and proliferate within the epithelium and lymphoid follicles. To colonize a host, the pathogen must contend with the resident intestinal microflora present. After invasion, the organisms multiply intracellularly and then spread throughout the body via systemic circulation. However, depending on the serotype and the effectiveness of the host defenses against that serotype, some of the organisms may infect the liver, spleen, gallbladder, bones, meninges, and other organs.

The period of incubation for *Salmonella* Typhi and Paratyphi ranges from 8 to 28 days and treatment is usually accomplished with ampicillin, cholraphenicol, and trimethoprim-sulfamethoxazole (Monteville and Matthews, 2008). If the infection is from consumption of

food contaminated with non-typhoid *Salmonella*, symptoms appear 8 to 72 hours post ingestion and tend to be less severe than typhoid cases (Monteville and Matthews, 2008).

### ***Transmission***

Most *Salmonella* (non-typhoidal) enter the body when food through ingestion of contaminated food and/or through person-to-person contact. To become pathogenic, certain attributes must be possessed including: ability to invade cells, a complete lipopolysaccharide coat, ability to replicate intracellularly, and in some cases ability to produce toxins. Once ingested, the bacteria colonize in the ileum and colon and then invade the submucosa. Once infected, the pig continues to consume feed and will contaminate the rest of the herd through fecal-oral transmission.

### ***Animals***

The number of potential sources of *Salmonella* infection for populations of animals is seemingly endless. In general, the source of salmonellae for production animals is most likely to be other production animals or environments contaminated by production animals. *Salmonella choleraesuis* is the most frequent porcine isolate from clinically ill pigs, but it is a very infrequent isolate from pig feeds or non-porcine *Salmonella* reservoirs (Schwartz 1991). The conclusion was that infected, shedding pigs and contaminated environments are the major source of new infections of *S. choleraesuis*. When looking at other serotypes the source of infection in pigs was not as clear, since the host and vector range for salmonellae is broad and salmonellae have the capability to survive outside the host (Baggesen et al., 1996; Dahl et al., 1997).

*Salmonella* contamination has been linked to other sources such as feed, especially since it has become more popular to have ingredients of animal origin as a protein source (Schwartz, 1997; Bergstrom et al., 2006; Osterberg et al., 2006). Pelleting of feed, which heats product to >82°C for 15 seconds, is an effective method for reducing the level of contamination (Molbak et al., 2006). In a study by Veldman et al. (1995), it was found that mash feed had a contamination rate of 21% versus pelleted feed at 1.4%, clearly showing the impact that heating has on *Salmonella* survival. However, with all the recent outbreaks in vegetables, the ingredients derived from them could also be a source of contamination (i.e., animal vegetable blends used as an energy source). Detection of new serotypes in pig herds may be partially explained by changes in the feed industry, with a high proportion of imported raw materials having a higher

prevalence of *Salmonella* (Weirup, 2006). In general, water is not a likely source of infection, unless animals consume surface water. In most confined production systems this would not be an issue; however, in beef production where feeder cattle are allowed to roam it is possible they may drink water from the surface and in that case water may be a source of contamination.

Vectors such as birds, insects, rodents, and pets can all act as carriers, as could bedding used in buildings and during transportation. Flies can be vectors of various bacterial and viral pathogens including *Salmonella* (Holt et al., 2007). Flies can transmit bacteria via their mouthparts, body and leg hairs, fecal deposition, or regurgitation (Olsen and Hammack, 2000). Bailey et al. (2001) was able to recover *Salmonella* from 19% of the flies tested at broiler farms. Lysyk and Axtell (1986) determined that flies will travel from 7 to 20 km and because of their affinity for decaying matter, garbage, and feces they pose a significant health hazard. Confinement facilities tend to have large quantities of flies and mice throughout the year; however, most are seen during the warmer months.

### ***Humans***

In humans, transmission of *Salmonella* occurs through ingestion of contaminated foods and/or drinks. Outbreaks of *Salmonella* occur because of improper cooking, preparation, and cross-contamination. Cross-contamination of food can be prevented by washing hands, utensils, and all surfaces that come into contact with food. Most disclaimers about cross-contamination focus on uncooked meat; however, numerous outbreaks have been linked to fruits and vegetables so washing food contact surfaces, hands, and utensils applies to all food products to promote food safety. Contamination can be as simple as an infected person, who does not wash their hands, contacting surfaces that others will use. Though not common, *Salmonella* can be found in the feces of pets, which in turn can infect humans from handling pets and not washing their hands properly. Reptiles and birds commonly have *Salmonella* and the Centers for Disease Control and Prevention (CDC) (2009) reports these pets to be the leading cause of infection of *Salmonella* other than food. The Federation of American Scientists (2013) reported that 40,000 cases of Salmonellosis occur annually with 600 deaths; however, they estimate that this number is actually 30 times higher, or more, since milder cases are not reported or diagnosed. The estimate of 30 times or higher is consistent with Scallan et al. (2011) who reported over 1 million illnesses annually.

## ***Host Response***

There are various host defenses that are important in resisting pathogens and specifically for *Salmonella* intestinal colonization and invasion. The innate immune response is the first line of host defense against infections and includes four types of defensive barriers: anatomic, physiologic, phagocytic, and inflammatory. This response employs a number of different cells, such as natural killer cells, macrophages, dendritic cells, and neutrophils, which have the property of pattern recognition or the ability to recognize molecules as foreign creating a phagocytic response. Once the infection has become an antigenic challenge the adaptive immunity begins take place. The adaptive immune response to a challenge or infection has a high degree of specificity as well as the property of memory. This means any exposure to the same antigen in the future (limited time) will result in a quicker and stronger response for clearing the pathogen. Typically, there is an adaptive immune response within seven days after initial exposure to that antigen.

Since *Salmonella* is a gram-negative bacterium, it has a unique characteristic that is found on the bacteria called lipopolysaccharide (LPS). Cell-associated receptors known as toll-like receptors (TLRs) are proteins involved in the innate response by recognizing LPS and activating an immune cell response. The TLRs play an important role by linking the innate and adaptive immune responses through their presence in dendritic cells and macrophages. The ability to recognize LPS will be important for developing a genetic intervention for *Salmonella*.

## ***Mechanisms Involved in Response***

One of the first defense mechanisms is the gastric acidity (pH < 3.5) in the stomach, which is lethal to salmonellae. In a healthy individual, the number of ingested bacteria is significantly reduced in the stomach, so that few or none will enter the intestinal tract. If some do survive and illicit a response, the innate cells begin to attack the bacteria.

Epithelial cells, dendritic cells, and macrophages are the first cells encountered by *Salmonella*. The interaction between them leads to the synthesis of proinflammatory cytokines and chemokines leading to a massive influx of other neutrophils, macrophages, and dendritic cells. Interferon gamma (IFN- $\gamma$ ) is important for control of bacterial replication in the early stages of infection (Muotiala and Makela, 1990); however, it is not sufficient for the eradication of the bacteria (Muotiala and Makela, 1993). Tumor necrosis factor alpha (TNF- $\alpha$ ) enhances

microbicidal activity synergistically with IFN- $\gamma$  and triggers the production of nitric oxide (Tite et al., 1991). Neutralization of IFN- $\gamma$  results in decreased killing of *Salmonella* whereas neutralization of TNF- $\alpha$  results in increased bacterial replication (Gulig et al., 1997).

Macrophages go through a metabolic process known as the respiratory burst resulting in the activation of a membrane-bound oxidase that catalyzes the reduction of oxygen to superoxide anion, a reactive oxygen intermediate that is toxic to ingested microorganisms (Goldsby et al., 2000). When macrophages are activated with bacterial cell-wall components such as LPS and a T-cell derived cytokine (IFN- $\gamma$ ) they begin to express high levels of nitric oxide. Nitric oxide has potent antimicrobial activity and has demonstrated antimicrobial activity against bacteria, fungi, parasitic worms, and protozoa (Goldsby et al., 2000).

Once the bacteria have caused the host to illicit a response many immune functions take over, specifically, proinflammatory cytokines including: interleukin (IL)-1, IL-6, IL-8, TNF-2, IFN-U, monocyte chemotactic protein-1 (MCP-1), and granulocyte macrophage colony-stimulating factor (GM-CSF). These cytokines invoke an acute inflammatory response which may in turn cause damage to the intestine. Because of this response symptoms of the host will include: fever, chills, abdominal pain, leukocytosis, and diarrhea. The end result of inflammation will be the recruitment of a specific immune response to the area of invasion. The antigenic specificity of the adaptive immune system allows it to distinguish subtle differences among antigens.

A variety of cytokine abnormalities contribute to susceptibility to *Salmonella* infections. Genetic deficiencies in the type I cytokine pathway (IFN- $\gamma$ /IL-12/IL-23) result in increased susceptibility to infection with intracellular pathogens like *Salmonella* and Mycobacteria (Ottenhoff et al., 2002; van de Vosse et al., 2004). Subjects found with abnormalities of these cytokines are more susceptible to the non-typhoidal serovars of *Salmonella* than other subjects. Interleukin-12, which is produced by antigen presenting cells (APC) like macrophages or dendritic cells, induces the production of IFN- $\gamma$  by natural killer cells and T cells which in turn upregulates further production of IL-12 in the APC to fight the infection (Coburn et al., 2007). The IFN- $\gamma$  further enhances the antimicrobial activity in macrophages, natural killer cells, and neutrophils which help clear the infection. Coburn et al. (2007) concluded that IL-12/IL-23 exert protective effects against infection with *Salmonella* independently of induction of IFN- $\gamma$ . This leads to a hypothesis that a possible IFN- $\gamma$  independent mechanism could be the upregulation of

TNF- $\alpha$ , granulocyte-macrophage colony-stimulating factor, and IL-17 by IL-23 leading to enhanced bacterial killing and enhanced nitric oxide production in macrophages.

Infection with serovar Typhimurium rapidly upregulates IFN- $\gamma$  production in gut-associated lymphoid tissue and spleen (Nauciel and Espinasse-Maes, 1992; Ramarathinam et al., 1993). The main producers of IFN- $\gamma$  and TNF- $\alpha$  are macrophages and neutrophils (Kirby et al., 2002); however, natural killer cells can also contribute to early IFN- $\gamma$  production in *Salmonella* that is dependent on IL-12 produced by other APCs (Brigl et al., 2003). While IFN- $\gamma$ , IL-12, TNF- $\alpha$ , IL-18, TGF- $\beta$ , and chemokine ligand 2 (CCL2) have protective functions against *Salmonella*, IL-4 and IL-10 interfere with host defenses which have roles in the adaptive immune response (Eckmann and Kagnoff, 2001).

The complement system aids the ability of antibodies and phagocytic cells to clear pathogens. After activation, in a highly regulated cascade, the components interact to carry out several functions including: lysis of bacteria, opsonization which promotes phagocytosis, inflammation, and immune clearance. The complement system is generally effective in lysing gram-negative bacteria. However, some of these bacteria have mechanisms for evading complement. In particular, *E. coli* and *Salmonella* have some resistance to complement because of the structural phenotype of each that have long polysaccharide side chains in the cell-wall LPS. It has been proposed that the increase of LPS in the wall of resistant strains may prevent insertion of the membrane-attack complex into the bacterial membrane, so that the complex is released from the cell rather than forming a pore. The membrane-attack complex forms a large channel through the membrane of the target cell, enabling ions and small molecules to diffuse freely across the membrane.

### **Incidence/Prevalence**

The incidence rate of a disease is defined as the number of new cases of a disease that occur during a specified period of time in a population at risk for developing the disease (Gordis, 2009). In contrast, prevalence is defined as the number of affected subjects present in the population at a specific time divided by the number of subjects in the population at that time, that is, what proportion of the population is affected by the disease at that time (Gordis, 2009). Clarifying the difference between the two is important for assessing impacts in the food industry. A simple differentiation is prevalence is a snapshot or a slice through the population at a point in



time to determine who has the disease and who does not. Conversely, incidence provides a measure of the risk of getting the disease.

### *Species*

The distributions of *Salmonella* in a variety of farm types (i.e., swine, poultry, beef, and dairy) have been characterized (Rodriguez et al., 2006). It was determined that feed, soil, bedding, litter, and feces were notable sources of *Salmonella* contamination for all farm types. The prevalence in poultry was 3% and in pigs was 10.7%. Beetles found outside the barns in some farms tested positive for *Salmonella* representing 40% of the positive isolates. This illustrates the difficulty at the farm to eliminate *Salmonella* once it has been established.

### *Pork*

Of the top 20 most common serotypes associated with human illnesses from *Salmonella*, 4 are commonly isolated from pigs. These common serotypes are S. Typhimurium, S. Heidelberg, S. Agona, and S. Infantis (CDC, 2009). In the United States there are approximately 185 million hogs raised annually stemming from approximately 82,000 farms (USDA, 2004). The prevalence of *Salmonella*-positive animals tends to be variable. Barber et al. (2002) found that 1.4 to 3.2% of pigs on farms that were sampled were positive. Other results found a greater prevalence ranging from 3.4 and 33% of animals sampled were positive for *Salmonella* (Davies et al., 1998; Rodriguez et al., 2006). Davies et al. (1998) found type of farm was significantly different, with gilt development farms being 3.4% positive and breeder farms between 18 – 22% positive.

Gebreyes et al. (2004) examined pigs from five farms and followed them through the processing plant. The percentage of on-farm positive fecal samples ranged from 0 to 42% as a percent of positive animals, whereas the percentage of positive samples at slaughter (mesenteric lymph node and cecal samples) ranged from 0 to 77%. The herds with the highest levels at the farm also had the highest levels at slaughter. A study across the United States at randomly selected abattoirs by the United States Department of Agriculture (USDA) was conducted from 2003 to 2006 (USDA, 2007). The results of swabs ranged from 2.5 to 4% positive annually with each year getting lower. From these results a conclusion can be determined that the pathogen reduction mandated by the government by plant interventions has had an impact on reducing *Salmonella* contamination at slaughter plants.

## ***Poultry***

*Salmonella* can frequently be isolated from most species of live poultry, such as broilers, turkeys, ducks, and geese (Molbak et al., 2006). A large number of *Salmonella* serotypes have been associated with poultry meat and egg products and are capable of infecting live birds. Contaminated poultry meat and eggs are important vehicles of *Salmonella* transmission. Several factors can affect the susceptibility of poultry to *Salmonella* colonization (Bailey, 1988). These include both animal and bacterial factors: country, age, serotype and dose level, stress, feed additives (antimicrobial agents), survival at low pH, competition within gut flora, compatible colonization site, and genetic makeup. Numerous studies have been done on each factor and interactions of these factors (Lahellec and Colin, 1985; Opitz et al., 1993; Hoover et al., 1997; Amick-Morris, 1998).

Poultry do have some host-specific serotypes, *S. Pullorum* and *S. Gallinarum*, but have several that are human related, *S. Typhimurium*, *S. Enteritidis*, and *S. Heidelberg*, *S. Infantis* (Molbak et al., 2006). Like pigs, poultry have a lot of variation for positive farms. The percentage of *Salmonella*-positive birds and fecal samples at the farm was 5 to 100% (Bailey et al., 2001). Bailey et al. (2001) also found that samples taken from hatcheries had the greatest percentage of positive samples and breeder farms had the lowest percentage. Samples (carcass and rinse water) taken at the slaughter plant ranged from 8 to 34% positive. A USDA study conducted similar to the pork testing found that between 1998 – 2006 the percentages increased annually for positive samples, from 10.9 to 16.3% (USDA, 2007). After the pathogen reduction mandate passed similar testing was done a year later and results were 11.4% which was down 16.3% from the previous year (USDA, 2009).

## **Food Safety Risk Assessment**

Risk assessment is the scientific evaluation of the probability of occurrence and severity of known or potential adverse health effects resulting from exposure to a hazard. This means risk assessments take into account both the magnitude of the harm and the likelihood of exposure to a hazardous agent. An important part of looking at risks is looking at both the frequency or likelihood and the significance or impact. Both aspects have to be brought together to adequately assess risk. In the food chain there tends to be uncertainty in the probability of

occurrence, where areas trying to be assessed and managed lack data for the assessment making proper assessment difficult.

Hazard Analysis and Critical Control Point (HACCP) is a systematic approach to food safety that identifies physical, chemical, and biological hazards in production processes that would cause finished product to be unsafe (Scott and Stevenson, 2006). HACCP was started by the National Aeronautics and Space Administration (NASA) who chartered Pillsbury to design and manufacture foods to be safe during space travel (Pillsbury, 1973). Since this inception the food industry has adopted this program as a science-based food safety system. There are seven principals in the HACCP program: conduct a hazard analysis, identify critical control points, establish critical limits for each critical control point, establish critical control point monitoring, establish corrective actions, establish procedures for verification, and establish record keeping. The most important concept of the HACCP program is prevention rather than inspection.

Risk assessment is similar to HACCP, but focuses on the impact of the risk on the consumer. They are typically broken into four parts: hazard identification, hazard characterization (dose response), exposure assessment, and risk characterization (NRC, 1983).

### ***Risk***

Risk, as defined by the World Health Organization (WHO) (1995) is the scientific evaluation of known or potential adverse health effects from human exposure to foodborne hazards. The definition includes quantitative (assigns fixed numerical values to the probability and utility of an outcome), qualitative (represents the probability and utility of an outcome on an interval scale), and uncertainties.

### ***Hazard Identification***

The first step in risk assessment is to describe the association between the microbial pathogen in a food and human illness. Pork is a major cause of foodborne salmonellosis throughout the world. *Salmonella* in processing facilities, specifically pork and poultry, are diverse, however the serotypes tend to be more common in these environments. In poultry the main culprit is *S. Enteritidis* (CDC, 2009; FAO/WHO, 2002), whereas in pork the main culprits are *S. Typhimurium*, *S. Derby*, *S. Brandenburg*, and *S. London* (Delhalle, 2009; Dorr et al., 2009). A study of fresh pork in retail stores found 9.6% of samples were contaminated (Duffy et al., 2000). In the United States fewer cases of *Salmonella* are linked to pork than other protein

sources (beef, chicken, dairy) because of the fear of *Trichinella* resulting in people overcooking pork (Morrow and Funk, 2001).

### ***Hazard Characterization (Dose Response)***

*Salmonella* infections are acquired through the fecal-oral route, although swallowing of contaminated aerosols may cause infections in rare situations (Fannin et al., 1985). The number of organisms ingested, the vehicle of infection, and several host factors are important in determining the outcome of exposure. Some serotypes of *Salmonella* are more successful in infecting animals than others.

In a review of epidemiological data in humans, Glynn and Bradley (1992) concluded that there was evidence of a correlation between dose and severity for several common serotypes including: Typhimurium, Enteritidis, Infantis, Newport, and Thompson. As dose increased, the median incubation period decreased and greater proportions reported symptoms. Increased dose was also associated with increases in weight loss, subjective rating of illness, and the number of days confined to bed. Mintz et al. (1994) concluded that ingested dose was an important determinant of the incubation period in an outbreak.

In one outbreak with chocolate, it was shown that under certain circumstances very small inocula have been sufficient to cause disease (Kapperud et al., 1990). In this outbreak of *S. Typhimurium* it took  $\leq 10$  organisms per 100g of chocolate to be infective. Lehmacher et al. (1995) found in an outbreak spread by paprika that an infective dose was estimated at 4-45 organisms with an attack rate of 1 in 10,000 exposed persons. An outbreak caused from a liquid premix transported in tanker trucks used for making ice cream, which had previously hauled liquid raw eggs, had an infective dose of 6 organisms in a serving (Hennessy et al., 1996). These three outbreaks confirm that a low-level dosage of *Salmonella* in food can cause disease in humans. Modeling a threshold is not practical because of the low-level dose needed to cause infection and single-hit models provide better estimates (Haas, 2002).

### ***Exposure Assessment***

The exposure assessment is an estimate of the likelihood of ingesting a pathogen and an adequate dose of the organism at the time of consumption. The most important element of an exposure assessment is the data on the prevalence of a pathogen in the final product and the

relevant consumption data for that product (Lammerding and Fazil, 2000). Any factor affecting the presence and level of the agent up to the point of consumption should be included. Typically, data available at the exact time of consumption are limited if they even exist. Estimations are derived from what is known about the contamination at the time of ingestion. Abusive conditions, like temperature, will impact microbial levels and make it difficult to model correctly.

### ***Risk Characterization***

The final step in risk assessment combines the information generated in hazard identification, exposure assessment, and hazard characterization. This estimate should reflect the range of contamination of a food product, factors affecting growth, and the variability of the human response to the pathogen. Modeling will require some simplification and the use of assumptions. These steps should be clearly stated and should be understood during the process. If observed data are available comparisons should be made for relevance.

Risk assessments should be based on scientific knowledge and evidence as much as possible. In some instances, conflicting data or the lack of data for a particular measurement occurs. In developing the final plan uncertainty and the variability of the data must be considered. The degree of confidence in the final estimation of risk depends on the uncertainty factors identified in previous steps. In a farm-to-fork process rarely is there access to high quality quantitative data available.

### ***Case Fatality***

Mortality from *Salmonella* gastroenteritis is low. Cohen and Tauxe (1986) estimated the acute phase mortality to be 1.3% in the United States. The average case-fatality rate reported to FoodNet was lower at 0.5% (Scallan et al., 2011). In a Danish study conducted by Helms et al. (2003), the mortality rate was 3.1%, which suggests that the mortality rate after *Salmonella* infections may be underestimated. The CDC reported in the Morbidity and Mortality Weekly Report (2012) that the case-fatality rate was 1.3% in 2008 and 1.2% in 2010, which is consistent with the findings by Cohen and Tauxe (1986). Though *Salmonella* has the highest incidence rate of foodborne pathogens for bacteria, it has a low case-fatality rate. The Federation of American

Scientists (2013) reported that untreated cases can have a case-fatality rate between 12 to 30% for typhoid illnesses. If the illness is treated there is less concern for fatality and the treatments available are effective for eliminating the pathogen.

### ***Outbreaks***

Outbreak investigations have proven to be an important means for identifying new serotypes of *Salmonella* and new vehicles of transmission. Investigations have to be prompt and thorough when an outbreak occurs to determine identification of etiological agents, sources and vehicles. In some cases a pathogen is not identified because of incomplete or delayed laboratory examination. Once the analysis is completed, it can be evaluated for trends, vehicles used, and any handling concerns.

The CDC (2013) has reported over 40 major outbreaks of *Salmonella* from 2006 to 2012 in the United States. The implicated vehicles or sources of these incidents range from: fruits, vegetables, live animals, dog food, restaurants, peanut butter, hedgehogs, ground meat, nuts, and eggs. The serotype most linked is Enteritidis; however, several others are reported frequently. Though conventional methods of intervention for *Salmonella* are effective if monitored, the continuous outbreaks of *Salmonella* push for intervention strategies that would not require processing steps that must be monitored to be effective.

### ***Prevention***

Preventing *Salmonella* from contaminating food during the farm-to-fork process remains a challenge. Processors, both pork and poultry, are regulated by government monitoring programs administered by the USDA. The Food Safety and Inspection Service (FSIS) is an agency within the USDA that conducts monitoring to ensure food is safe and wholesome. Reduction of *Salmonella* or prevention of its growth throughout the farm-to-fork chain are important factors in modeling the infection risk to humans because these factors have potential as control measures against salmonellosis.

HACCP plans are an effective tool for implementing strategies to ensure safety. Interventions, which demonstrate effective reductions in the occurrence and levels of pathogenic bacteria at different steps in the processing flow, have been implemented in production flows to keep critical points in the process under control. Examples of interventions are: temperature at which product can be stored, washes in the process flow that may or may not have bactericide,

irradiation, ultraviolet light, and steam (Bautista et al., 1997; Bell and Kyriakides., 2002). *Salmonella* interventions can be implemented at three stages in the production chain: pre-harvest (farm), harvest (slaughter), and post-harvest (fabrication). Reducing pH can be used to control the growth of *Salmonella*. Organic acids can be used as feed additives to lower the pH and reduce counts.

Routes of transmission (feed, equipment, facilities, personnel) have been studied to determine the epidemiology of *Salmonella* in pigs (Magistrali, 2008). Positive results have been found on equipment used in processing facilities (dehairing, hide puller, shackles) and finished products indicating cross contamination during the process. One study found that 33% of the samples collected from heads, feet, ears, and meat were positive for *Salmonella* (Meneses, 2010). Interventions are routinely put in place during the harvest and post-harvest steps to reduce or kill *Salmonella*; however, some organisms may still pass through. Little research has been done at the pre-harvest step where a genetic intervention, selecting animals that are tolerant or do not shed, would potentially help reduce the risk of contaminated food to the consumer. Vegetables have recently been identified as being responsible for the most outbreaks of foodborne illnesses (CDC, 2013) and the same theory could be applied to select for tolerance as proposed for animals to help prevent further outbreaks.

### ***Pre-harvest Interventions***

Preventing the introduction of *Salmonella* into herds is a prerequisite to keeping livestock *Salmonella*-free. It is clearly documented how farms get contaminated and how cross-contamination can easily occur. However, developing interventions pre-harvest that are as effective as post-harvest are not well established. Skov et al. (1999) demonstrated that applying the all-in-all-out production principle and ensuring that barns are totally emptied, cleaned, and disinfected prior to new animals arriving can prevent cross-contamination. In commercial settings (i.e., feedlots) this is usually not practical. Vaccination to boost immunity to *Salmonella* has been tried in poultry, where live attenuated vaccines have been applied on a large scale (Hassan and Curtiss, 1997). The researchers found that the vaccination does not offer complete protection for the birds against infection, but it may increase the animals' resistance to infection/colonization. Similarly, Dodd et al. (2011) found no difference in the amount of shedding in cattle for *Salmonella* when vaccinated versus the control. This is important as it will

reduce the number of animals harboring *Salmonella* and/or the levels of *Salmonella* shed by individuals within the group.

Control of *Salmonella* in pigs consists of monitoring *Salmonella* at the herd level, and implementation of *Salmonella* reduction measures in infected herds through hygiene, separation of animals, feeding strategy, and strict control over *Salmonella* in the supply chain (Molbak et al., 2006). Transportation, holding, and slaughter are all areas of cross-contamination as rarely are these areas properly cleaned and disinfected between every load/pig. A Danish program has shown some progress in reducing *Salmonella* in herds during the period 1996 to 2001; however, it has not eliminated *Salmonella* (Wegener et al., 2003).

### ***Post-harvest Interventions***

Post-harvest controls include food safety programs (HACCP), quality control measures, sanitation, and proper storage conditions. The pathogen level on carcasses and final products can be reduced/eliminated by numerous methods: hot water, steam, irradiation, acid washes, and temperature (Molbak et al., 2006).

In a study by Delhalle et al. (2008), it was determined that several of the steps in processing had a meaningful impact on reducing *Salmonella* (>1 log CFU/cm<sup>2</sup> reduction). The researchers found that variation between slaughterhouses existed and *Salmonella* reductions were not consistent. Disinfection of knives using water >82°C was not monitored properly at all slaughterhouses and caused cross-contamination. The time period between killing and scalding increased the number of bacteria. Although scalding was found to have a significant reduction in bacterial counts, monitoring of the water temperature is imperative to the success in reduction of bacteria. The singeing process ranged from 5 to 16 seconds. When singed appropriately (carcass surface temperature reaching 100°C) a reduction of 2.2 to 2.5 log CFU/cm<sup>2</sup> can be achieved (Pearce et al., 2004).

Evisceration is the most critical step for cross-contamination prevention. Pearce et al. (2004) reported this step as demonstrating the greatest increase in cross-contamination. Practices by operations during this step are responsible for 55 to 90% of all *Salmonella*-positive carcasses (Berends et al., 1997). Washing of the carcasses after evisceration was effective (>2 log CFU/cm<sup>2</sup> reduction) as an intervention in *Salmonella* reduction (Bolton et al., 2002; Spescha et al., 2006). This clearly illustrates the effectiveness of the HACCP program; however, this still leaves the question of interventions prior to harvest.



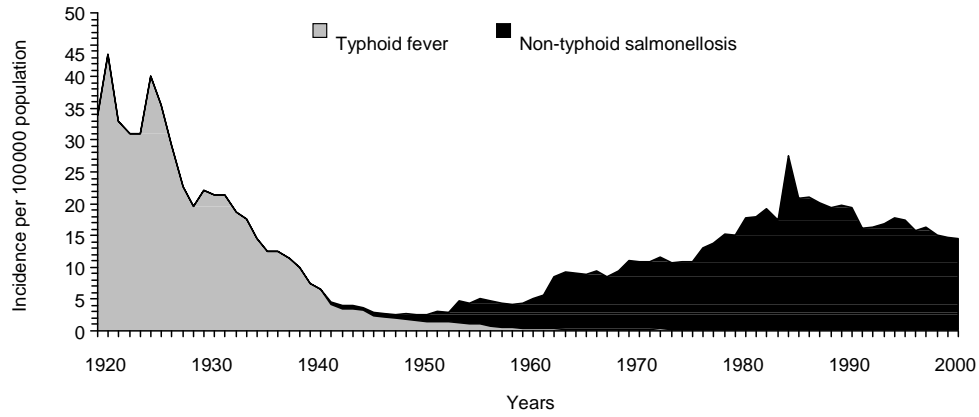
## ***Monitoring***

Resistance to antimicrobials and multidrug resistance is an emerging problem in Enterobacteriaceae (Schwartz and White, 2005). It is believed that resistant microorganisms have emerged as a result of improper use of antibiotics in agriculture practices (Khachatourians, 1998). In the United States, it has been reported that some of the antibiotics produced are fed to production animals as growth promoters and to obtain a more favorable feed conversion (Goldman, 2004). In the pork industry, low levels of chlorotetracycline, oxytetracycline, lincomycin, neomycin, or tylosin are added to feed (Khachatourians, 1998). Over time if this practice is conducted antimicrobial resistance of microorganisms will evolve defense mechanisms, therefore making these drugs less effective (Goldman, 2004).

*Salmonella* has been documented to have resistance to some antibiotics used for medical treatment as an estimate of 4,760 deaths in the United States (1998) were attributed to it (Khachatourians, 1998). One study (Meneses, 2010) detected resistance to tetracycline in *S. Anatum*, *S. Heidelberg*, *S. Typhimurium*, *S. Newport*, *S. Mbandaka*, and *S. Urbana*. In this same study, multidrug resistance was detected on *S. Typhimurium*, which was resistant to sulfoxazole-ampicillin-chloramphenicol. *Salmonella* are adapting to survive within macrophages (Fields et al., 1986). Mouse studies as well as studies from human patients with rare immunodeficiencies have demonstrated the need for cell-mediated immunity as being important for protection against invasive *Salmonella* (Blanden et al., 1966; Mackaness et al., 1966; MacLennan et al., 2004; Bustamante et al., 2008).

The National *Salmonella* Surveillance System is based on data collected by territory/state since the 1970's. This is a passive surveillance of laboratory confirmed isolates. Other systems at the CDC conduct surveillance for *Salmonella* infections as well. The National Notifiable Diseases System (NNDS, 2013) collects and compiles reports of infectious diseases, including salmonellosis. The National Antimicrobial Resistance Monitoring System (NARMS, 2013) monitors antimicrobial resistance among enteric bacteria from humans, retail meats, and food animals. Since 2002 there has been an increase (1.6% - 2002, 2.1% - 2011) in percentage positive of the samples tested for antimicrobial resistance, which is a 31.25% increase in the amount present from when this was first started being tracked. The importance of monitoring systems to detect emerging antibiotic resistance is critical, thus highlighting the need for new interventions such as genetic intervention.

**Figure 1-1. The fall and rise of reported *Salmonella* infections in the United States, 1920 to 2001. (Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance Data, 2013.)**



## Chapter 2 - Genetics of Disease Resistance

Genetic selection for resistance to pathogens is possible, but can be difficult and costly. Selection for resistance has been seen only as a means of combating infectious diseases; however, for many diseases there is evidence that resistance has a genetic component. Experiments have shown that it is possible to exploit genetic differences for resistance (Mallard et al., 1998; Moris, 1998, Petry et al., 2007). Currently, few pre-harvest interventions are commercially available and only novel technologies are being explored (Baer et al., 2013). A need for a pre-harvest intervention is needed. Though there is no genetic intervention used to stop *Salmonella* shedding this chapter focuses on the evidence of genetic variation for pathogen response.

Considerable evidence for genetic variation in pigs in response to pathogens or challenges exist (Duchet-Suchaux et al., 1991; Wilkie and Mallard, 1999; Henryon et al., 2002; Petry et al., 2005). Visscher et al. (2002) concluded that there is opportunity for genetic improvement of the immune capacity based on substantial genetic variation between pigs. Bertschinger et al. (1993) conducted an experiment with *E. coli* and concluded that colonization is controlled by a dominant allele and resistance is controlled by a recessive allele. Meijerink et al. (1997) developed a restriction fragment length polymorphism (RFLP) test for the mutation in the encoding gene; however, most diseases do not have tests for markers of genes involved in immune response. A preliminary examination of allelic variation in porcine reproductive and respiratory syndrome (PRRSv) virus response genes revealed the presence of single nucleotide polymorphism (SNP) in 50% of 52 genes, which is consistent with the possibility of extensive genetic variability in the immune response of pigs to PRRSv (Hawken et al., 2001).

Little is known about the magnitude of variation, genetic or environmental, for most diseases because quantification of health in breeding herds is difficult (Mallard et al., 1992; Wilkie and Mallard, 1998). Experiments have been conducted to determine whether genetic variation for resistance to disease caused by other pathogens (Mallard et al., 1998; Wilkie and Mallard, 1999; Dawson et al., 2004; Petry et al., 2005). Halbur et al. (1998) and Petry et al. (2005) found that breeds selected for lean growth that were infected with PRRSv had higher

enzyme linked immunosorbent assay (ELISA) ratios, lower average daily gain (ADG), and an increased severity of PRRSV-induced lesions in the lung than pigs selected for reproduction.

Studies not related to *Salmonella* conducted by Mallard et al. (1998) and Wilkie and Mallard (1999) reported results of eight generations of selection for antibody and cell-mediated immune responses in pigs. High, low and control lines diverged for growth rate, antibody response to various antigens, and response to *Mycoplasma hyorhinis*. They concluded that genetic variation in response to certain antigens and to *Mycoplasma hyorhinis* exists. Though pathways and mechanisms involved in resistance were not characterized, it was concluded that the genetic variation was polygenic, regulating both innate and acquired immunity. The importance of this finding is that genetic variation is associated with another type of bacteria that utilizes pathways similar to those of *Salmonella*. When looking for models that can have a food safety impact it is important to find similarities among different bacteria.

Dawson et al. (2004) infected pigs with *Toxoplasma gondii*, a single-celled parasite that causes multi-organ infection, and found upregulation of some cytokine protein levels, mainly interferon gamma (IFNG), in various tissues. The earliest detection of IFNG was in the liver and lymph node; however, expression of upstream regulatory factors controlling IFNG expression were assessed and found to be upregulated at early stages of infection, but less upregulated at later stages of the infection. This further illustrates the critical role of timing of the pathways play in immune responses.

Another issue in selecting for disease resistance is the fact that herd immunity against one serotype of *Salmonella* may not protect pigs against a challenge from a different strain. Therefore, an initial detection of the *Salmonella* strain in a production system should provide the needed information for management to develop methods for controlling what exists.

## **Biology of Disease**

The innate immune system is the first line of defense for the host during infection and therefore, plays a critical role in the recognition and triggering of a proinflammatory response to invading pathogens (Medzhitov and Janeway, 2000). The adaptive response eliminates the pathogen in late phase of the infection and participates in the creation of immunological memory. This allows the adaptive response to have specificity, developed by clonal gene rearrangement from a broad repertoire of antigen-specific receptors on lymphocytes. Because of

these properties it is important to distinguish which pathways are used during an invasion. Bacterial and viral infections do differ in the pathways used by the immune system during the innate response (Kaczorowski et al., 2010).

### ***Bacterial versus Viral***

Bacteria are single-celled microorganisms that thrive in many different types of environments. In general, bacteria are ubiquitous and can be found in extreme cold and heat. Commonly seen bacterial infections in everyday life include strep throat, urinary tract infections, or foodborne illness. These types of illnesses are usually treated with antibiotics; however, inappropriate use of antibiotics can lead to strains of bacteria that are resistant.

Viruses require a living host such as humans, animals, or plants to replicate. Common viral infections include upper respiratory infections, influenza, croup, ear infections, and cold sores. These types of illnesses can't be treated with antibiotics, which have been misused in the past before knowledge it was known that overuse of the antibiotics could cause mutations in bacteria allowing them to become more resistant.

Toll like receptors (TLR) are a family of transmembrane receptors that play a role in signal transduction in the innate immunity (Akira et al., 2006). They have the ability to recognize microbial motifs, signal to the host, and initiate an inflammatory response (Kapetanovic and Cavillon, 2007). As previously discussed toll like receptor 4 (TLR4) starts the signal transduction for bacteria and TLR3 initiate the signal in viruses (Coburn et al., 2007). Interventions for food safety are focused on bacteria rather than viruses. This means an understanding of the pathways involved in bacterial infection is important so these mechanisms can be genetically selected for to develop an immune response that will stop the replication.

### ***Diversity of Strains***

Popoff (2001) estimated that *Salmonella enterica* has  $\geq 70$  different O antigens, which are surface antigens found on the outer surface of the bacteria. Extensive genetic diversity at the O antigen biosynthetic gene (*rfb*) locus (Brown et al., 1991; Milkman et al., 2003), which encodes enzymes directing O antigen synthesis, has been attributed to frequency-dependent selection imposed by the host immune system (Reeves, 1995; Kingsley and Baumler, 2000). Different

expressions of lipopolysaccharide (LPS) molecules on the surface of the cell helps explain O antigen variation for some bacteria.

A study by Rajabi et al. (2011) estimated the genetic relatedness of *Salmonella* from water samples in the Suwannee River. They conducted a replicate analysis of the same strains and demonstrated a 95% similarity. Therefore, isolates with >95% similarity were considered to be clonal and a total of 499 strains were examined using polymerase chain reaction (PCR), and all strains were >60% similar. The strains segregated into 16 geno-groups using the criteria of >85% deoxyribonucleic acid (DNA) similarity for more than two strains, while 14 strains were not found to be related.

### ***Host Defense***

Expression of different LPS molecules through gene regulation allows invading bacteria to escape host immunity, survive, and proceed through the life cycle. *Haemophilus influenza* and *Neisseria meningitides* are commensal bacteria of the upper respiratory tract in pigs that can cause life-threatening diseases once they invade their host (van Deuren et al., 2000; Yung et al., 2003; Pathan et al., 2003). Upon entering the host, *H. influenza* and *N. meningitids* replicate in the blood stream, resulting in a steadily increasing bacteremia within hours after infection (van Deuren et al., 2000; Snyder et al., 2001).

Bacterial survival within the host's blood stream depends on the ability to escape the innate and adaptive immune systems, and *H. influenza* and *N. meningitids* have multiple genes under control of phase variation that result in antigenic variants arising every generation, allowing for immune evasion (Snyder et al., 2001; Bayliss et al., 2001; Emonts et al., 2003). A concern for genetic intervention against *Salmonella* is LPS phase variation, which is by means of contingency loci that allows the bacteria to express different LPS after each generation, giving the bacteria an ability to survive and escape host immune cell recognition (Jennings et al., 1999; Hosking et al., 1999; Bayliss et al., 2001). A strong selective pressure from the immune system during a bacterial invasion is believed to be the driving force of LPS variation among these bacteria (Wildschutte et al., 2004).

### ***Environmental Impacts***

Polygenic effects in pigs can be influenced by the environment in which phenotypes are measured (Mulder and Bijma, 2005). This effect is called genetic x environment (G x E)

interaction. The environment can be many things including: type of housing, temperature, humidity levels, altitude, sanitary status, feed type, and management practices. It is not practical to genetically select in all of these types of environments but should be considered in the overall scheme of the genetic program.

Long et al. (2008) found that mortality rate in broilers for a 5,000 whole-genome SNP chip differed in the SNP subsets found to be significant in the initial experiment. This was due to across-environment predictive ability or extent of linkage disequilibrium between the subsets. The study purposefully set up environments that were different, one being of high health and the other being challenged. The results were a 30% reduction in early mortality and 20% reduction in late mortality. This clearly illustrates that when modeled properly the G x E can be differentiated for making genetic improvement though environmental effects exists.

### ***Immune Function***

The innate immune system provides recognition of pathogens for host defense. In this innate pathway, TLRs play a key role in host defense, providing a mechanism to respond to highly conserved pathogen-associated molecular patterns (Akira et al., 2001). Lipopolysacchride is one of these conserved patterns and is specific to *Salmonella*. Lipopolysacchride activates TLR 4, then cascades numerous innate immune cells that activate other mediators such as interleukins. Cytokines play a role in inflammatory processes, which is determined by the pathogen structure and the binding receptor through which signaling and activation of gene transcription occurs.

Wu et al. (2001) reported variation in interleukin 4, which plays a critical role in T cell mediated immune response in humans. In this study, 24 SNPs were identified that had genetic variation for interleukin 4. Mikacenic et al. (2013) reported variation in TLR 1, 2, 6, and 10. A whole genome study was used to identify whole blood cytokine responses to the TLR 1/2 lipopeptide agonist. A strong association ( $P < 1 \times 10^{-27}$ ) was found between genetic variation within the TLR 1/6/10 locus on chromosome 4. This association accounted for 35% of the variation in the population variance for this phenotype. These studies show that genetic variation within the innate immune system exists and can be exploited for genetic improvement when using whole genome selection.

## **Genetic Variability**

Genetic variability is a measure of the tendency of individual genotypes in a population to vary. The variability of a phenotype describes how much variation is in response to genetic and environmental influences. The problem with selection programs is that each generation that is selected reduces the total variation available. This means as evolution of a genetic program continues, it will one day run out of potential change and animals will be alike. This concept is the fixation of genes in a population. The only way then to get more variability is to introduce an unrelated line of germplasm into the mix to give gene rearrangement and diversity. Because this variability exists, it allows geneticists to select candidates with a favorable phenotype (Figure 2-1).

Over 2,500 *Salmonella* serotypes have been classified (Molbak et al. 2006). Though molecularly these serotypes are similar, the structure of each can impact how the host will respond. This variability means that when selecting candidates to be more resistant to the pathogen the focus needs to be on the innate response and not the adaptive response. As more serotypes are discovered, it is important to classify them and use several different serotypes in a selection program. Genetic progress should be measured using multiple serotypes to ensure animals being developed are resistant to any and all serotypes.

## ***Mutation Rates***

Mutation is the ultimate source of genetic variation for evolutionary change and for the most part occurs at a very low rate ( $1 \times 10^{-9}$  per replication) (Hartl and Clark, 1997). Pathogenic bacteria rely on genetic variability to combat host defenses, including mutation. Several researchers have found the rate for mutation in *Salmonella* to be  $3 \times 10^{-3}$  per replication (Stoker, 1949), which is significantly more frequent than usual genomic mutations. Because mutations are more common in *Salmonella*, numerous serotypes are expected and new ones will arise.

One of the concerns related to this frequent mutation rate is antibiotic resistance. Mutation rates can increase for a given antibiotic depending on its concentration during selection (Kohler et al., 1997). Physiological conditions, such as the availability of a carbon source or bacterial stress, may regulate the mutation rate in bacteria (Foster, 1993; Hughes and Andersson, 1997). The capability of some antibiotics to increase the mutation rate with an antibiotic resistance phenotype greatly complicates research of the effects of population dynamics on the



emergence of antibiotic resistant mutants in bacteria (Ren et al., 1999). Because some pig producers continue to feed antibiotics during the course of production when not necessarily needed, the mutation rate for antibiotic resistant *Salmonella* will continue to increase giving rise to the need for a genetic intervention to combat this issue (Martinez and Baquero, 2000).

### ***Heritability/Variance Components***

There are two parts of inheritance: heritability and environmentability. Heritability is defined as the ratio of additive genetic variance to phenotypic variance (Falconer and Mackay, 1996). The additive genetic value of an individual, also known as the breeding value, is the sum of the average effects of all the alleles the individual carries (Falconer and Mackay, 1996). According to the principals of Mendelian segregation, one allele from each locus is present in each gamete which is passed on from parent to progeny making up the additive genetic variance. Because each progeny receives a different set of alleles from its parents, half of the additive genetic variance in the population occurs within families. The second part of inheritance is contributed by the environment. Together the sum of the additive genetic variance and the environmental variance should equal the total variance.

The heritability of a trait is important in selection programs for making improvements in performance. The most meaningful change or improvement produced by selection is the change of the population mean. This is known as the response to selection (Figure 2.1). It is defined as the difference of mean phenotypic value between the offspring of the selected parents and the whole of the parental generation before selection (Falconer and Mackay, 1996). The measure of the selection applied is the average superiority of the parents selected, known as the selection differential.

Heritabilities can range from 0 to 1, however most will be between 0.05 to 0.50 (NSIF-FS3, 2002). Reproductive traits tend to have lower heritabilities ~0.10, whereas growth and carcass composition will be higher ~0.30 to 0.50 (NSIF-FS3, 2002). Disease and pathogen response heritabilities have not been well estimated to date due to the difficulty in measuring phenotypes and defining what phenotype is relative.

Edfors-Lilja et al. (1994) estimated heritabilities of immune function in Yorkshire pigs. In this study, white blood cell counts were measured and percentages of lymphocytes and polymorphonuclear leukocytes determined. In addition, the innate immunity was determined by

interferon- $\alpha$  level. Estimates of heritabilities were moderate (0.20 to 0.40) for these traits meaning selection can be done to improve pathogen resistance/tolerance. de Craen et al. (2005) estimated heritabilities for interleukin production to be high (0.53 to 0.86), however no pathogen challenge was linked to these estimates. Association studies are needed to validate that high production of interleukins reduces shedding of *Salmonella*. Because cytokines are necessary for signal transduction when the host is pathogenically challenged, a hypothesis can be made that linkage between levels of *Salmonella* shedding are associated with areas in the genome relevant to cytokine production.

For the most efficient genetic improvement animal breeders use selection index methodology for ranking animals. The index is comprised of measuring several traits and utilizing the relationship between the traits and relatives. The index is the best linear prediction of the breeding value of an individual based on all the sources of information. Genetic correlations, which tell how much of the influence on different traits is genetic, are used by geneticists to maximize improvement in multiple traits. When the genetic correlations are different from 0, then the two traits are influenced by common genes. However, it is possible to have unfavorable correlations, e.g., growth rate and backfat in pigs 0.18 (Bryner et al., 1992). This means as pigs grow faster they also get fatter. A producer who gets paid on lean would lose value when pigs are sold for being fat, but would gain money for the quicker growth. Using selection index, geneticists can use these relationships to maximize improvement for producers.

Utilizing an index has an impact on selecting for *Salmonella* shedding because it may be antagonistic to other production traits. The economic impact of *Salmonella* shedding does not directly affect what a producer gets paid when those respective animals are marketed. However, the impact of having pigs that are contaminated with *Salmonella* costs the consumer. Because this contamination exists, processing plants have food safety interventions in place to eliminate bacteria. These interventions are expensive (internal plant data) and must be monitored on a frequent basis, as defined by the hazard analysis and critical control point (HACCP) plan. Thus, adding costs to the final product that consumers purchase.

## **Genomics**

All living things are made up of cells that are made up of genetic material called DNA. This molecule is made up of a long chain of nitrogen containing bases of: adenine, cytosine, guanine, and thymine. On the genome, only a small region is made up of genes that code for proteins involved in pathways, while the remainder and bulk of the DNA represents non-coding sequences, a role that is not clearly understood (Ruane and Sonnino, 2007). By being able to study the genetic makeup of animals at the cellular level, geneticists have a tool to understand mechanisms involved in economically important traits.

Most of the phenotypes considered in genetic improvement programs are quantitative, which are controlled by many genes together along with environmental factors. In classical genetic improvement, selection is carried out based on observable phenotypes of the candidates for selection and their pedigree but without knowing which genes are being selected. Genomics is used by scientists to understand the structure of the genome, which includes mapping of genes and sequences of the DNA. Over the past decade full sequences of animals have become available for public use. Because variation exists in the DNA structure, known as polymorphisms, the use of genomic breeding values can be used to exploit more efficient genetic progress on lowly heritability traits.

### ***Genomic Development***

Substantial advances have been made over the past decades through the application of molecular genetics in the identification of loci and chromosomal regions that contain loci that affect traits of importance in livestock production (Andersson, 2001). This has enabled opportunities to enhance genetic improvement programs in livestock by direct selection on genes or genomic regions that affect economic traits through marker-assisted selection (MAS) (Figure 2.2) (Dekkers and Hospital, 2002). Genomic selection is a form of marker-assisted selection in which markers across the whole genome that have linkage disequilibrium are used for improvement of accuracy on breeding values. The development of whole genome selection became feasible because of new methods to efficiently genotype large numbers of SNP. Previous to whole genome selection individual markers were used in panels that had the greatest effect on accuracy for breeding values.

### *Single Nucleotide Polymorphisms and Accuracy Improvement*

The concept of adding information through markers is to improve the accuracy or explain the variation of a phenotype by its genotype. However, if adding this information does not re-rank the animals retained as breeders for the next generation then little is gained by using this information. Dekkers (2007) estimated accuracy improvements of >50% using markers on rates of inbreeding. Villanueva et al. (2005) reported an increase in accuracy as the number of markers increased in the analysis. As technology has improved the use of small marker panels is being transitioned to whole genome selection. Currently, in pig breeding the 64k chip from Illumina (San Diego, CA) is available for commercial use. Meuwissen and Goddard (2010) reported accuracies of between 0.80 and 0.90 using 33,000 markers versus 0.40 to 0.50 using 1,000 markers. This is evidence that using the whole genome has a greater impact on predicting an accurate breeding value versus traditional marker panels. In lowly heritable traits such as pathogen response, using the whole genome approach can greatly improve the efficiency of selection.

Measuring 64k genotypes is still costly to do in breeding herds, though has been shown to be effective. Huang et al. (2012) developed a method called alphaImpute to calculate probabilities of each parental allele, which are combined to estimate the missing genotype. Among the selection candidates not fully genotyped, between 93 and 95% of the genotype variation was recovered using a unique strategy of 384 SNP. This means progeny from parents can be fully genotyped with only 384 markers and imputed to 64k for a fraction of the cost. One of the key components of this unique methodology is that the 384 markers used need to be equally spaced across the genome to give high reliability. This gives scientists the ability to use the whole genome selection approach at a fraction of the cost with ~95% reliability. Because this type of approach is commercially available, implementing a selection for reduced shedding is possible. Breeders tend to focus on traits that directly impact economics rather than traits like *Salmonella* shedding. The ability to use actual rather than assumed relationships will improve the efficiency with which progress can be made. Normally an assumption would be made that the full sibling relationship is  $\frac{1}{2}$  since they both inherited their respective genes from the same parents. However, this is not always this case. The whole genome selection approach allows researchers to understand the true relationship of inheritance and exploit the full value of the relationship. As pigs are infected with *Salmonella* and begin shedding, they can be tracked until

shedding ceases to determine associations with all regions in the genome. This is a powerful tool for implementing a program for reducing *Salmonella* shedding in pigs.

Figure 2-1 Response (R) of Selection (S) in a selection experiment.

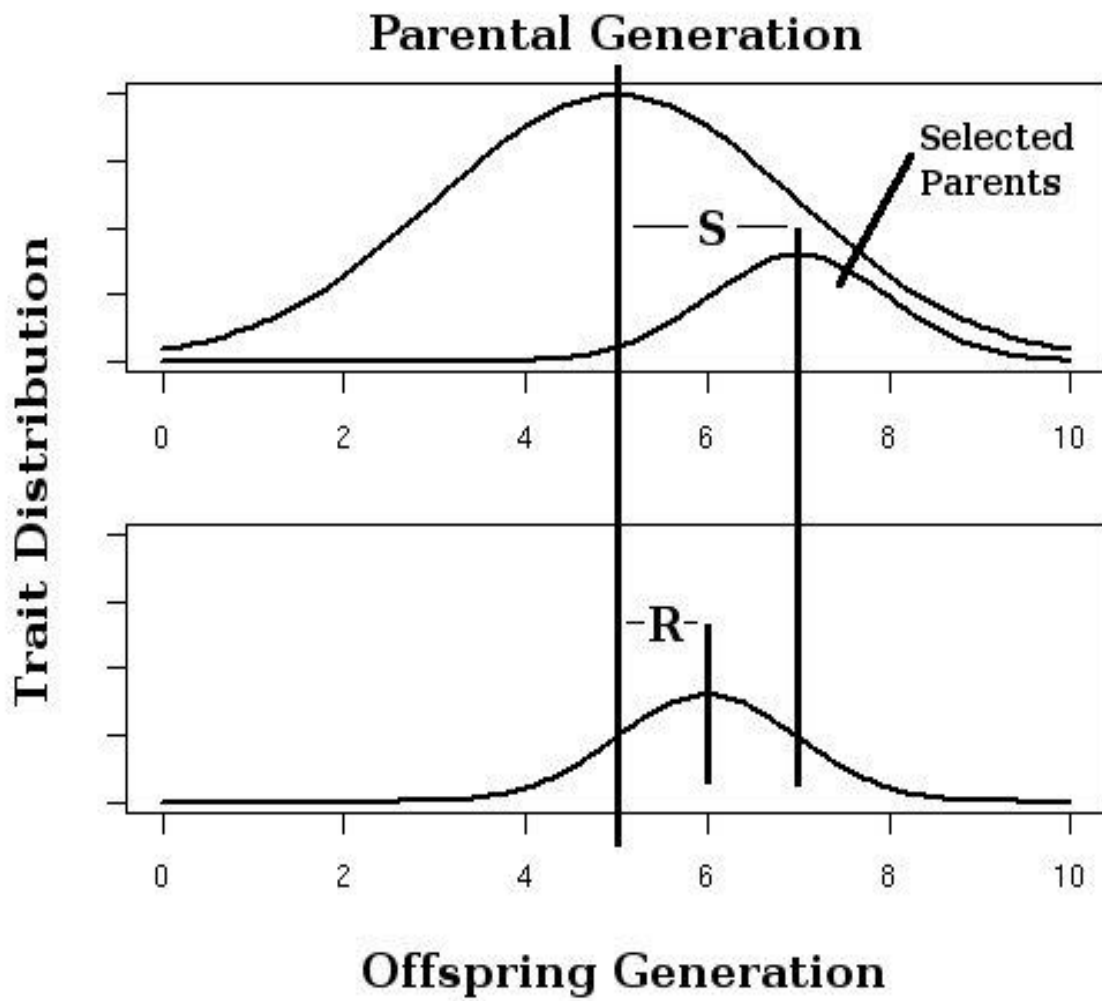
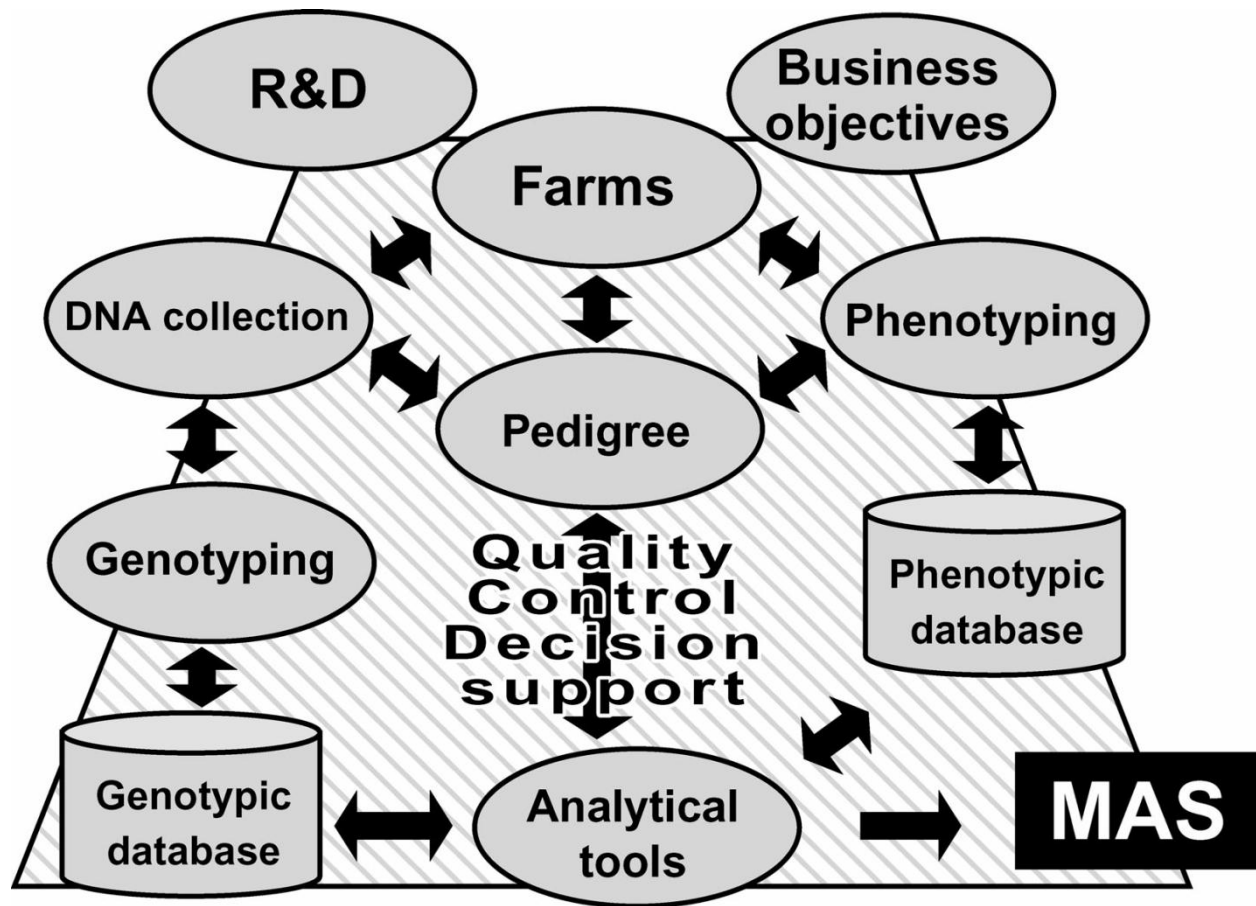


Figure 2-2 Components of an integrated system for the use of molecular genetic information in breeding programs for marker-assisted selection (MAS). (Dekkers, 2004)



## **Chapter 3 - Genetic Selection for *Salmonella* Shedding**

*Salmonella* contamination in the food chain goes beyond contaminated meat. Fresh produce can be contaminated if it is fertilized with manure from animals infected with *Salmonella* as fertilizer (Guan and Holley, 2003). Baer et al. (2013) reported that novel technologies such as bacteriophages, feed additives, and high-pressure processing are being explored as interventions against pathogens in fresh pork, but none are in practice. Thus, an intervention to reduce on-farm *Salmonella* prevalence is necessary to improve pre-harvest food safety for fresh meat products and fruits/vegetables grown using animal fertilizers.

### **Quantitative Selection**

When selection is applied to improve the economic value of animals, it is applied to several traits simultaneously, not just one trait. Economic value depends on more than one trait and the relationships that exist between the traits are called genetic correlations. It is important to understand how the traits interact in order to maximize economic return. It is difficult to place accurate economic weightings in an index for traits like *Salmonella* shedding which do not impact producers directly. Bacterial and viral infections do differ in the pathways used by the immune system during the innate response (Kaczorowski et al., 2010) and typically farmers focus on viral infections because of the difficulty to eradicate them. Also, viral infections impact animal health, whereas *Salmonella* shedding less-so and is more of a food safety concern. Selecting for less *Salmonella* shedding is a polygenic effect and can be incorporated into a selection index.

### ***Best Linear Unbiased Predictor (BLUP)***

Prediction of breeding values is a fundamental component of breeding programs, as selection candidates with the highest value are retained as breeders for the next generation. The concept of BLUP was introduced by Henderson (1950) and incorporates both fixed and random effects to form a mixed model. Because computing power has increased in the past couple of decades, an animal model (equation 1) is used enabling simultaneous prediction of breeding



values for all traits of individuals regardless of age, location, number of records, and number of relatives.

Equation 1 
$$y = X\beta + Za + e$$

Where X and Z are design matrices,  $\beta$  is a vector of fixed effects (gender, year, contemporary group), a is a vector of random effects (breeding values), and e is a vector of random errors. Because selection candidates can be compared at frequent intervals (contemporary group design), with overlapping generations it is possible to select continuously over time. These breeding values are used in an economically weighted index to rank animals so the geneticist can select animals that will be saved to be parents for the next generation (Figure 3-1).

It is difficult to determine how much improvement can be made by selecting for *Salmonella* shedding. This is true because the amount of variation available in a population has not been determined. As each population will have differing amounts of variation available to select from some populations will achieve quicker progress than others. In a hypothetical scenario if it is assumed that the heritability is 0.20 and the phenotypic standard deviation is 0.50 log then using the breeders equation (equation 2) an amount of improvement can be calculated.

Equation 2 
$$\Delta G = \frac{h^2 * \sigma_p * \left(\frac{i(m+f)}{2}\right)}{t\left(\frac{m+f}{2}\right)}$$

Where i is the intensity of selection for males (m) and females (f), and t is the generation interval for m and f. For this scenario it will be assumed i and t are 1.00. The amount of improvement in one generation of selection would be 0.10 log less shedding of *Salmonella*. However, geneticists use selection index theory when making genetic improvement. This means multiple traits are put into the equation and selected simultaneously so only a percentage of the amount calculated would be observed. If the weighting in the index was 20% for shedding, then the amount of improvement would be 0.02 log less shedding per generation. This is only true if the genetic correlation with all of the other traits in the index were 0, which is never the case. If some of the other traits are negatively correlated, then less of a response would be observed and if some of the traits were positively correlated, then more progress can be made.

In food safety a 90% reduction in live numbers of bacteria or 1 log reduction is considered significant (FDA, 1999). This means that 100 bacteria would be reduced to 10. In the above scenario 100 bacteria would be reduced to 91 in one generation. In order to achieve a

1 log reduction it will require 10 generations of selection. However, the average log count on pigs coming into plants today is ~3 logs, which is 1,000 colonies. This type of reduction is not as great as interventions used in processing plants which usually will reduce 90 to 99% (sometimes greater) of the live bacteria (internal data from processing plant).

### ***Inheritance***

Selection for certain traits has led to unfavorable changes in others, typically those that are associated with fitness (i.e., fertility, leg strength, and liveability). Hill and Zhang (2009) reported that adding liveability and leg strength into the index and ensuring they do not go in the the wrong direction will slightly slow down response in other traits but will prevent fitness issues. This demonstrates the necessity of the index to include all important traits, though they may not seem to have a direct economic value.

Modern breeding programs involve populations of limited size so that effective multi-trait selection can be utilized and practiced. The genetic nucleus is on the top of the multiplication pyramid from which point new improved genetics are dispersed. Though we have millions of market pigs processed in the United States the ancestry of these animals is limited to smaller nucleus populations. The relationships throughout the pedigree are used for calculating the estimated breeding value (Figure 3-2). This distribution of the population becomes part of the effective number of the population, which is what truly drives genetic progress. In a given pig population usually 30 to 50 boars will be retained from the candidates as breeders for the next generation. This means short term gains will be high but over time will decrease the effective number of the population and likely long-term progress (Villanueva et al., 2006). Muir et al. (2008) reported that in Red Jungle Fowl (a population considered to be native) about one-half of the alleles present have been lost after domestication.

### **Molecular Selection**

High density mapping of single nucleotide polymorphisms (SNP) using chips provide a method to estimate genetic variances. On average, full siblings will share 50% of alleles. However, because linked genomic regions are transmitted the actual proportion shared will vary. Visscher et al. (2006) estimated a standard deviation of 4% for humans. Because actual transmittance can be measured using genomics, the genetic variance can be estimated within families from the regression of phenotypic resemblance of family members for a trait on the

actual proportion of the genome shared without confounding of environmental differences or maternal genetic effects (Visscher et al., 2006, 2007).

### ***Discovery***

The first step in using molecular technologies is the discovery work. Association studies are used for finding linkage between a phenotype and the DNA (Figure 3-3). This is where scientific trials are designed to collect specific phenotypes of interest. If the goal is to reduce/eliminate *Salmonella* shedding in pigs, the design of a genetic program could be to infect pigs with *Salmonella* and measure response. The most difficult part of the program is defining the phenotype that will give the desired result. Other studies in the literature measure viremia (the host's ability to replicate the virus), weight gain, cytokine levels, white blood cell counts, and macrophage efficiency. Since the defined objective is to reduce shedding the optimal phenotype would be to measure shedding daily post infection to determine which candidates stop shedding the quickest. One caveat to this is that a follow up stress test should be given to ensure the pigs are not carriers and only express shedding during this time.

Petry et al. (2007) demonstrated in a porcine reproductive and respiratory syndrome challenge that viremia is variable and has genetic control. However, this is a virus and not a bacterium though the author showed variation starting at day four post-infection. This means the innate system had an impact on stopping the replication of the virus prior to an adaptive response. As discussed previously, different mechanisms are involved in the pathway of infection for viruses and bacteria. Since this type of selection has not been done it is hypothesized that mechanisms of the innate immune system are genetically controlled for both viruses and bacteria and can be used to select candidates that shed less than others.

### ***Validation***

Once markers have been found to be linked to a phenotype in a controlled study, a more global study could be performed to validate the effect. The difficulty in doing molecular work is that each population is unique and must go through its own discovery and validation process. Some of the difficulty in earlier studies was the attempt to link markers with highly heritable traits that are easily measured giving little benefit in using this technology. However, as a proof of concept model, it was successful in demonstrating that accuracies are improved by adding a genomic estimated breeding value (EBV) versus a traditional polygenic EBV (Dekkers, 2004).

Validation studies should focus on a more commercial type setting. For *Salmonella* shedding, a validation study should include the initial dose and how the pig responds to the pathogen. Because a pig can have in a carrier state of *Salmonella*, measuring these at the plant would be important to ensure the mechanisms selected are linked to markers having the effect as proposed in the discovery stage. Once this stage is complete and validation is proven then the selection program can begin making improvement that could be realized at a commercial level.

### ***Markers***

The availability of marker panels of thousands of SNPs dose pose a paradigm shift in the prediction of breeding values (Meuwissen et al., 2001). Rather than looking for a single gene that controls a phenotype this approach looks at several areas in the genome. This is important for complex traits that are not controlled by a single gene like *Salmonella* shedding. As described previously many mechanisms are involved in how *Salmonella* interacts with the host.

Markers are used to try and incorporate most variants using historical linkage disequilibrium in the population. This information is used to assess the relatedness of the genome by relatives and to weigh the genotypes according to a favorable phenotypic response associated with a region and the imprecision of estimating these effects. In close linkage of the marker and true gene driving the response it is unlikely to change rapidly (unless doing single trait selection) over generations, so it may be possible to use less dense marker panels (Habier et al., 2009).

The problem with using markers is: understanding the relationship they have with a mechanism involved for a favorable phenotypic response. Epistasis is a phenomenon in which the phenotype expressed is due to the presence of one or more genes. These genes can play many roles in making the desired phenotype including signal transduction, transcription, and/or transvection.

Genetic variation for pathogen response has been described in the literature (Petry et al., 2007; Reiner et al., 2008; Clapperton et al., 2009, Galina-Pantoja et al., 2009). In these studies, variability of genetic control of the porcine immune response to pathogens was shown to exist, meaning genetic improvement of disease resistance in pigs can be exploited for addressing preharvest food safety issues. Uthe et al. (2011) found 2,527 SNPs associated with *Salmonella* colonization in pigs. Wang et al. (2008) was able to characterize the porcine transcriptional

response to *Salmonella* and also demonstrated that differences between the host (pig) and their respective phenotypic response on a genomic level.

In the hypothesized genetic program, markers can be used to increase accuracy of candidates for *Salmonella* shedding. Once the markers are validated in the population to be selected, they can be applied as assistance to the polygenic breeding value. As marker-assisted selection has evolved, it is clear that selection can't be done on markers alone but needs on-going phenotypes to maximize improvement and estimate gene frequencies in the population. As markers are being used over time genes will become fixed and will bring little value for improvement.

### ***Whole Genome Selection***

The newest iteration of molecular technologies is using whole genome selection (WGS). Until recently this was not an option as the cost was too high. In pigs, a 64k chip is available that spans the entire genome. This allows the investigator to use all regions of the genome for improvement not just the ones with the most influence. The approach of WGS helps take into account epistatic effects because markers are represented in areas that previously may not have been accounted for that influence other regions.

The basic assumption is that many loci contribute to the phenotype and without knowing the whole genome will not give the response desired (Purcell et al., 2009). van Raden et al. (2009) described how not knowing the genome can lower the accuracy in milk yield. The researchers concluded that through typical polygenic BLUP the accuracy was 0.35, with markers it was 0.56, and then incorporating whole genome it was 0.69. This was a 100% increase in accuracy of selection, thus causing animals to be re-ranked as candidates.

One advantage to using the whole genome approach is the ability to understand inheritance between relatives. Conducting pathogen response trials is expensive and experimental units tend to be limited. However, using this approach allows the investigator to use specific inheritance of family relationships through the genome to calculate breeding values for this type of trait.

In the conceptual model of selecting for shedding of *Salmonella*, the WGS methodology takes advantage of both the relationships of relatives and the direct selection of shedding. As previously discussed, heritabilities for pathogen response phenotypes are low to moderate.

Accuracy of breeding values for these traits are also usually low since collecting phenotypes is expensive and takes resources. With the combination of WGS and alpha-impute calculating, accuracies and relationships for pathogen response are more likely to be impactful than previous estimates.

As an intervention for food safety it is important to utilize this tool to maximize the improvement made per generation. In pigs, one generation is approximately one year, so improvement needs to be as rapid as possible. Given the scenario described in the quantitative selection part of this paper utilizing true inheritance relationships and higher accuracies through knowledge of the genome will improve the rate of genetic progress. Since pathogen responses tend to have low accuracies, using genomic information may increase the amount of accuracy by 100% or greater. van Raden et al. (2009) demonstrated near 100% improvement in accuracy in milk yield which normally has low accuracy.

**Figure 3-1 Sample calculation of estimated breeding values and ranking on an economically weighted index.**

BreedTag	AnimalId	INDEX	EBV_BF	EBV_LMD	EBV_WT	EBV_NBA	EBV_SS
431934	2640000047759	2.37	-0.07	0.25	18.04	1.14	-2.10
431811	2640000035333	1.94	-0.03	0.33	17.09	1.03	-1.91
432013	2640000050608	1.93	-0.07	0.20	30.09	0.76	-1.04
428923	2030000116286	1.77	-0.08	0.34	13.29	0.87	-1.51
428997	2030000117674	1.03	-0.06	0.33	11.13	0.83	-0.21
433204	2030000125790	0.99	-0.08	0.10	17.59	0.97	0.51
429526	2640000049922	0.96	0.02	0.30	23.13	0.82	-0.94

EBV\_BF = Estimated breeding value for backfat

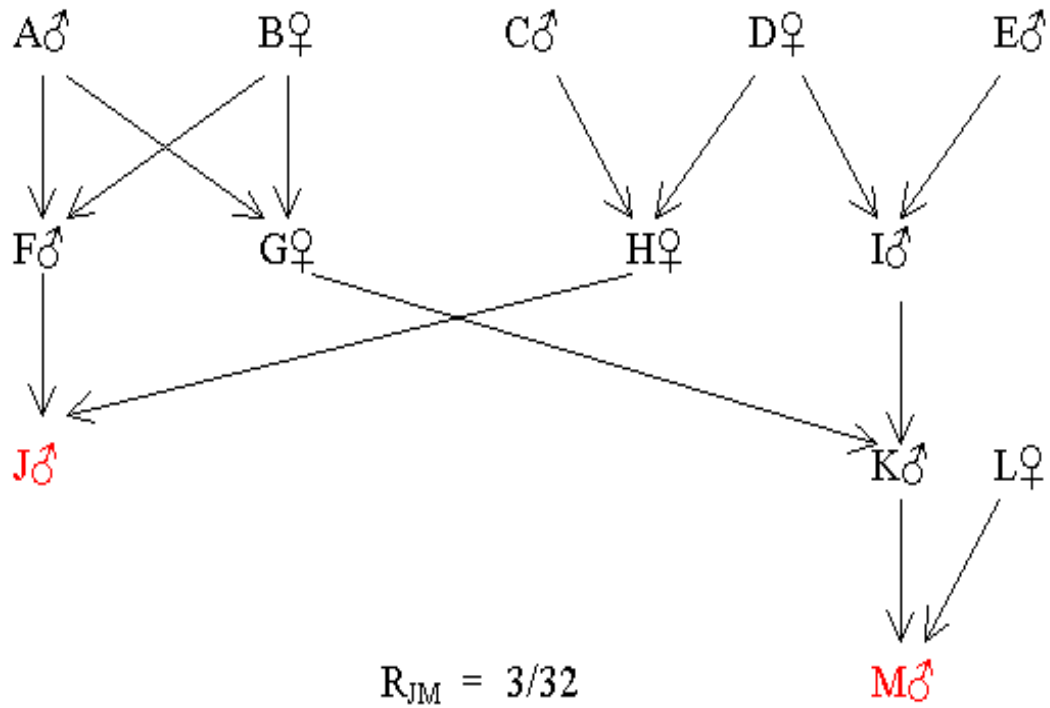
EBV\_LMD = Estimated breeding value for loin muscle depth

EBV\_WT = Estimated breeding value for growth rate

EBV\_NBA = Estimated breeding value for number born alive

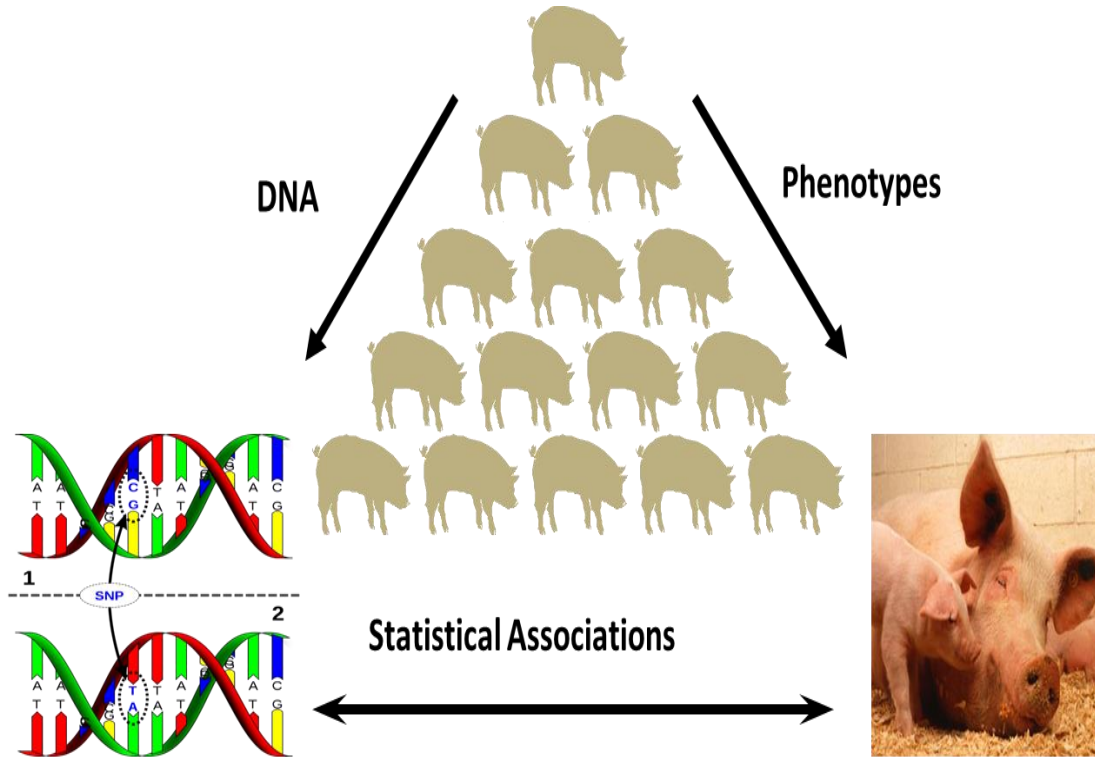
EBV\_SS = Estimated breeding value for less *Salmonella* shedding

Figure 3-2 Sample calculation of relationships between individuals.





**Figure 3-3 Association study design.**



## Chapter 4 - Conclusion

Genetic variation for pathogen response exists and can be selected for reduced *Salmonella* shedding. It is possible that genetic selection can be an intervention for shedding of *Salmonella* though reductions will be slower than what is seen in post-harvest interventions. Since an intervention is defined as a “reduction in intended organisms” (FSIS, 6410.1, 11/3/11) a protocol of continually challenging pigs with a common load to naïve candidates (a pig that has not been challenged or pathogenically compromised) could demonstrate a reduction in shedding, however pigs will need to be stressed at a later point to ensure they are not carriers. The amount of reduction per generation is significantly lower than what can be done during harvesting steps but reducing the amount of *Salmonella* coming into the processing plants can only benefit food safety for the consumer. The difficulty in genetic selection is that over a period of time variation will decrease, meaning the amount of reduction in shedding each generation will decline. Since little to no pre-harvest interventions are in place for pig production this offers a way to reduce (not eliminate) the amount of *Salmonella* shedding coming into processing plants and also the amount of contamination in the manure that is applied to fields as fertilizer.

The amount of improvement that can be made will be determined by what traits are put into the index and the respective weightings and genetic correlations for each trait. In a single trait selection model the amount of improvement would be the highest possible; however, typically geneticists will have multiple traits in an index to make improvement on several traits simultaneously. This means the amount of improvement would not be the highest possible, but would move in the right direction as long as a desired gain of reducing shedding was emphasized. There are practical hurdles that prevent genetic improvement for *Salmonella* shedding from becoming a reality. One of these hurdles is the amount of progress that will be given up in other traits when adding shedding into the index. Another hurdle is the lack of an economic incentive for breeding companies to make improvement for shedding. The cost of collecting data on *Salmonella* shedding is expensive and will have to have dedicated resources to conduct such research not attached to the nucleus farm. For these reasons little work has been done for genetically reducing shedding.

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