

Approved and Implemented in 2023

Understanding the impact of the farm and lairage environments on *Salmonella* contamination in market hogs, University of Wisconsin-Madison, Kansas State University, Texas Tech University, USDA-ARS

Salmonella contamination in market hog tonsils, lymph nodes, feces, and cecal contents likely occurs rapidly, and previous research suggests that the lairage period provides risk for cross contamination to occur. The study objectives are to detect and quantify *Salmonella* from market hogs on-farm, at lairage, carcass swabs, and lymph nodes. The *Salmonella* isolates will be characterized to determine their serotype and the presence of highly pathogenic *Salmonella* at the different stages of sampling. *Salmonella* concentration and serotypes in post-harvest samples will be evaluated to determine if it most closely represents *Salmonella* contamination on-farm or from lairage, and the impact of time spent in lairage.

Funded in part by the National Pork Checkoff.

Survival of African swine fever in pork and processed pork products, Canadian Food Inspection Service, Agriculture and Agri-Food Canada

African Swine Fever (ASF) is a contagious, haemorrhagic viral disease of pigs that is currently spreading westwards throughout Europe and eastwards into China, with significant economic losses along its path. While strict regulatory guidelines are in place to prevent the spread of this virus, little is known about the effectiveness of current meat processing methods in inactivating ASF. In addition, the matrix effect of individual meat products and ingredients on ASF survival is not well documented. This project aims to explore the survival of ASF in different meat products from ASF infected pigs as well as investigate the effect of different ingredients, cooking temperature, storage time and high pressure processing on ASF inactivation in different meat model systems.

Funded in part by the National Pork Checkoff under an ASF Partnership and administered by the Foundation.

Creating Alternative Support for Lethality and Stabilization for Heat Treated and Fully Cooked Meat and Poultry Products, University of Wisconsin, HansonTech

Nearly all meat processors in the United States utilize USDA, FSIS Appendix A and B to ensure adequate thermal lethality and stabilization is achieved for partially and fully cooked products. Through the development and release of updated versions in 2017 and 2021, and the realization that a host of potential food safety vulnerabilities exist, the widespread usefulness and in-plant practical application of these guidance documents has become a significant concern and practical challenge to implement. The primary objective of this study is to develop a scientific-based, regulatory-supported, and industry-useful thermal processing and cooling resource (e.g. cooking and cooling food safety handbook) for validating pathogen destruction and control, and regulatory compliance for partially and fully cooked meat products that can be used in conjunction with or in lieu of USDA, FSIS Appendix A & B.

Funded in part by the Beef Checkoff.

Summarizing the current knowledge and existing knowledge gaps for pre-harvest and post-harvest *Salmonella* contamination in pork, Kansas State University, Triumph Foods

Research on pre-harvest and post-harvest measures to prevent or reduce pathogen contamination have been published. However, knowledge gaps still remain, and a thorough literature review is necessary to fully understand what steps should be taken to address *Salmonella* concerns both preharvest and post-harvest in the swine. Therefore, this project will conduct a thorough search of pre-harvest and post-harvest *Salmonella* research in swine; compile the literature and prepare a written review of the existing knowledge. Knowledge gaps and research recommendations will be identified. NAMI members/volunteers will serve as focus group participants to ensure all current knowledge is considered.

Funded in part by the National Pork Checkoff.

Revealing mechanisms for internal *Salmonella* colonization and persistence in porcine lymphoid and fat tissue, USDA-ARS-NADC - Food Safety and Enteric Pathogens Research Unit

Swine can become persistently infected with *Salmonella*, shedding little to no bacteria in the feces, until subjected to a stressful event, which increases fecal shedding. A clear understanding of the mechanisms of *Salmonella* persistence in porcine immune cells is needed to developing targeted intervention strategies to significantly reduce *Salmonella* carriage in swine and the risk of contamination of products and the environment. The overall hypothesis is that *Salmonella* resides in myeloid-lineage cells in porcine lymphoid tissues and fat, and subsequently modulates the cellular state to limit bacterial clearance. The objectives of this project are to identify the cell types harboring *Salmonella* in pig lymphoid and adipose tissue at various stages of colonization; characterize the cellular response; and identify mechanisms of intracellular colonization. *Funded in part by the National Pork Checkoff.*

Developing a Quantitative *Salmonella* Baseline from Ground Beef in the United States, Texas Tech University, Kansas State University, University of Georgia, USDA-ARS, Meat Animal Research Center, Food Safety Net Services

The *Salmonella* level in ground beef across the U.S. is unknown. As a result, risk assessments and understanding the public health impact of potential *Salmonella* control programs across the industry are not always accurate. This study intends to conduct a representative *Salmonella* baseline and develop a blinded quantitative *Salmonella* baseline for the U.S. beef industry representing season and geographical waves. *Funded by the Beef Checkoff and administered by the Foundation.*

Characterizing *Salmonella* Isolates from Ground Beef in the United States, Texas Tech University, Kansas State University, University of Georgia, USDA-ARS, Meat Animal Research Center, Food Safety Net Services

It is hypothesized that *Salmonella* serotypes and presence of highly pathogenic *Salmonella* (HPS) in ground beef will vary by geographic location and season depending on the facility. Samples obtained from a separate study will be analyzed to determine the *Salmonella* serotype(s) present in each positive sample and the presence of HPS associated with U.S. ground beef. *Funded in part by the Beef Industry Food Safety Council.*

Enhanced Characterization of Sequence Differences Among *Salmonella* isolates within SNP Clusters Identified by the NCBI Pathogen Detection System, USDA-ARS, Meat Animal Research Center

This research intends to better understand the full picture of relatedness within critical *Salmonella* serovars of interest by performing a comparative genomic analyses on currently available data within the Pathogen Detection Isolates Browser (PDIB). An analysis pipeline will be developed to catalogue *Salmonella* SNP cluster diversity in the NCBI PDIB with the goal of producing a white paper to enhance industry use and understanding of this tool, and to enhance public health actions and general understanding of *Salmonella* genomics by identifying isolates for closed genome sequencing that are within 50 SNP differences. *This project is funded by the Beef Checkoff and administered by the Foundation.*

A surveillance of *Salmonella* in the lymph nodes of sows and boars, Kansas State University, Texas Tech University

Salmonella contamination remains the leading food safety concern for pork products. The contribution of *Salmonella* in the lymph nodes in sows and boars is unknown. This study will determine *Salmonella* prevalence and concentration in the lymph nodes (subiliac, mesenteric, tracheobronchial, inguinal, axillary, pre-scapular) and tonsils of sows and boars at harvest. The impact of season and region on *Salmonella* prevalence and concentration in the lymph nodes of sows and boars will be evaluated. Positive samples will be serotyped.

Approved for Funding in 2023, Implementation Pending Available Funding

Effect of minimally processed animal protein within the Dietary Guidelines for Americans on biomarkers for cognitive decline, South Dakota State University

Investigators will leverage an ongoing well-designed, randomized, controlled, crossover, feeding study following USDA Dietary Guidelines for Americans to establish the role of lean animal protein in cognitive health promotion. A minimally processed lean meat incorporated diet will be compared with an isocaloric lacto-ovo-vegetarian control. It is expected the addition of animal protein will enhance nutrient adequacy and reduce markers associated with cognitive decline and neurodegenerative diseases.

Determine function of a potential stress-induced genetic regulator associated with DFD beef, Texas A&M University

This research intends to offer new insight into the biological mechanisms and genetic variability that continue to influence the occurrence of the dark, firm, and dry (DFD) phenotype. In vitro experiments will be conducted to determine the function of a genetic regulator (miR-2422) in skeletal muscle in order to understand its role in the stress response of beef cattle. The presence of miR-2422 in blood pre-harvest from cattle will be assessed to determine if it resulted in carcasses containing DFD beef or if its function is restricted to skeletal muscle.

Evaluation of the different dietary patterns on meeting protein and amino acid requirements in young and older adults, University of Arkansas

This project will develop menus reflecting the following dietary patterns: U.S. Western Style; Mediterranean Style; DASH Style; Vegan Style; and Healthy U.S. Style to determine the amino acid composition of each dietary pattern. The amino acid patterns will be compared to current dietary recommendations. The acute meal response to meal compositions of these dietary patterns will be evaluated on postprandial plasma amino acid profiles in young versus older adults.

Current Research Projects

A Cross-Sectional Investigation of *Salmonella* in Market Hog Lymph Nodes & Leveraging a current market hog lymph node study to further understand *Salmonella* transmission and internal colonization, Kansas State University, Texas Tech University, Triumph Foods

A cross-sectional study design will be employed to investigate the prevalence and concentration of *Salmonella* in up to 6 lymph nodes (LN) and tonsils of market hogs. Prevalence and concentration data will be subsequently used to design a risk-assessment mapping of the carcass for prioritization of node-removal for pathogen control. Additionally, research will probe a possible relationship between *Salmonella* antibodies in oral fluids and internal colonization of market hog carcasses by determining if antibody testing of oral fluids can be used as an effective antemortem screening tool to assess a group/lot of pigs for *Salmonella* risk.

Funded in part by the National Pork Checkoff.

Development and validation of an antimicrobial database to predict microbial load reduction on raw pork components against *Salmonella*, University of Illinois at Urbana-Champaign

This study will implement a high-throughput miniature assay to evaluate *Salmonella* reduction after pork carcass wash with antimicrobial treatments. Response surface methodology will be used to determine synergistic or antagonistic interactions between antimicrobials and optimize combinations to reach desired *Salmonella* reductions. The results are intended to validate the predicted interactions between antimicrobials in laboratory experiments, as well as build an antimicrobial database in which additional antimicrobial treatments data can be added as new compounds become relevant against *Salmonella* in pork.

Funded in part by the National Pork Checkoff.

Effects of proportioning meat and plant-based protein-rich foods within the U.S. Healthy Eating Pattern on cardiovascular disease risk factors, Purdue University

This project will assess the effects of consuming different proportions of red meat and plant-based, protein-rich foods incorporated into a U.S. Healthy Eating Pattern on cardiovascular disease risk factors in adults at high risk of developing a heart-related disease.

Funded in part by the Beef Checkoff.

Exploring the use of ProbiCon as a direct-fed microbial to reduce the *Salmonella* burden in market hogs, Kansas State University, USDA-ARS-U.S. Meat Animal Research Center, Triumph Foods

This study will evaluate the influence of direct fed microbials (DFM) on pig performance, morbidity, and mortality throughout the feeding period. The feces and mesenteric lymph nodes of market hogs fed a control or DFM augmented diet will be collected to establish the impact of each diet on *Salmonella* internalized in the lymphatic system. By determining *Salmonella* serotype and presence of highly pathogenic *Salmonella* (HPS), it evaluates whether *Salmonella* diversity and/or presence of HPS is impacted by probiotic administration. *Funded in part by the National Pork Checkoff.*

Novel TaqMan assays for the specific detection and simultaneous differentiation of virulent and avirulent non-O157 Shiga toxin-producing *Escherichia coli* strains, Florida State University, USDA-ARS, U.S. Meat Animal Research Center

This study intends to standardize six multiplex TaqMan assays for the identification of virulent strains of *E. coli* O26, O111, O45, O103, O121, and O145 serogroups. Further, it will demonstrate the applicability of the standardized assays in inoculated food samples and red meat enrichments from national red meat surveillance programs through a direct comparison with the FSIS MLG 5C.01 reference method. *This project is funded by the Beef Checkoff and administered by the Foundation.*

Recent Research Findings

Dietary modeling the nutritional impact of removing/adding/substituting meat and poultry servings to the healthy dietary patterns, Nutrition Impact LLC, NutriScience LLC

This project modeled the effect of removing or adding a serving of minimally processed and further processed meat and poultry or substituting a serving of various foods with a serving of minimally processed and further processed meat and poultry on nutrient profiles in the healthy dietary patterns identified in the Dietary Guidelines for Americans, 2020-2025. *Funded in part by the Beef Checkoff.*

Tests of *Salmonella* Sub-unit Proteins as Vaccines for Broiler Chickens, USDA-ARS U.S. National Poultry Research Center

The overall project goal was to identify the *Salmonella* protein antigens that could induce humoral immune response in broilers, and consequently use these antibodies to prevent *Salmonella* colonization in the broiler gastrointestinal tracts. This project generated and purified recombinant *Salmonella* proteins that involve colonization and invasion in the gastrointestinal tracts and found that these proteins could provoke the immunized chickens to generate IgG, IgM and IgA antibodies. However, these antibodies seemed to enhance the colonization of challenged *Salmonella* in the gastrointestinal tracts.

Impact of sanitization and natural biofilm communities on *Salmonella* prevalence at processing plants, USDA-ARS, U.S. Meat Animal Research Center

This project evaluated the efficacy of commercial sanitizers against *Salmonella* harbored within environmental mixed biofilms by measuring biofilm forming ability and community structure of environmental biofilms before and after sanitization. It then compared environmental microbial communities and *Salmonella* survival in mixed biofilms before and after sanitization to determine the impact of different sanitizers on controlling *Salmonella*. *This project is funded by the Beef Checkoff and administered by the Foundation.*

Risk assessment model to assess the impact on public health of pork based on the contamination level and presence of highly virulent or multidrug resistant strains, University of Minnesota

This study used quantitative microbial pathogen enumeration to estimate the annual cases of salmonellosis due to consumption of contaminated ground pork in the US. It also investigated the use of contamination thresholds as a risk mitigation method that could inform ground pork performance standards moving forward to meet national salmonellosis reduction goals. *This project is funded by the National Pork Checkoff and administered by the Foundation.*