



### **Current Research Projects**

#### ***Salmonella* deep serotyping and microbial community profiling through swine production and processing, USDA-ARS-MARC, University of Georgia, Texas Tech University**

This study will investigate the implementation of effective interventions at the most appropriate points in swine production, as well as deepen the understanding of *Salmonella* serotype diversity and complexity at all stages of production. This study will use archived samples of swine finishing barns as well as samples collected under currently funded work, for CRISPR-SeroSeq and microbiome analysis to gain a deeper understanding of the microbiome and *Salmonella* serotype ecology from farm to fork.

*Funded in part by the National Pork Checkoff.*

#### **Understanding *Salmonella* ecology and internal colonization in market hogs, University of Wisconsin-Madison, University of Arkansas**

This study will use barcoded *Salmonella* to inoculated market hogs orally, intranasally, and at various locations intradermally to explore *Salmonella* ecology and internal colonization in market hogs. The findings are intended to address the following research questions: How quickly does *Salmonella* contaminate lymph nodes, tonsils, liver, spleen, cecal contents, and feces of market hogs?; and Does inoculation method (oral vs. intradermal) impact frequency and concentration of *Salmonella* recovered from inoculated market hogs?

*Funded in part by the National Pork Checkoff.*

#### **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.*

### **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.*

### **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.*

### **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.

### **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.

### **Research Funded By the Beef Checkoff and Administered by the Foundation**

#### **From Trim to Table: Tracking *Salmonella* Dynamics, Including Levels and Survival, from Beef Trim to Ground Product, Kansas State University and Cargill**

This research will conduct an inoculation study to evaluate the influence of *Salmonella* levels in beef trim (including lymph nodes) on *Salmonella* levels in ground beef, providing valuable insights for managing *Salmonella* in final product. *Salmonella* survival will be assessed during standard industry storage periods to further understand its persistence and potential risks in products intended for commerce.

#### **Improving and validating the THERM model for predicting growth of *Staphylococcus aureus* in raw meat products during temperature abuse and come-up-time (CUT) deviations, University of Wisconsin – Madison**

The current Temperature History Evaluation for Raw Meats (THERM) model, originally intended for use by small and very small processors who lacked refrigeration in their raw processing areas and now widely used at all levels of the meat processing industry to estimate pathogen growth in raw materials, may overestimate pathogen growth when following USDA temperature recommendations. This study will refine product categories and determine the growth of *Staphylococcus aureus* in uncured model meat products with varying pH levels and temperatures which represent come-up-time ranges where *S. aureus* may grow. A validated predictive model will be developed using a variety of different meat products and formulations.

#### **Reliability and repeatability of digital PCR and VAE assays for the enumeration of *Salmonella* in beef samples, Florida State University and U.S. Meat Animal Research Center USDA-ARS**

This study intends to optimize digital PCR (dPCR) and varying amplification efficiency (VAE) assay workflows for the detection and quantification of *Salmonella* load in beef samples. The reliability and repeatability of dPCR and VAE assays will be validated and their applicability will be compared with an AOAC performance-tested method. The application of a partition-based digital PCR approach will enable highly reliable and repeatable detection and quantification of *Salmonella* load in contaminated beef samples. The VAE assay will enable simple estimation of *Salmonella* levels in beef samples.

#### **Targeting Foodborne Pathogens in the Beef Industry through an Integrated Analysis of the Beef Processing System, Texas Tech University, University of Nebraska, US MARC, and Auburn University**

This research will take a comprehensive approach to targeting *Salmonella* and Shiga Toxin Producing *E. coli* (STEC) conducted through optimization of sampling techniques, microbial detection and microbial quantification methods combined with microbiome and metagenomic analysis to enable mitigation strategies and validation techniques to impact pathogen presence in beef products. *Contract pending*

#### **Effect of organic acid components on the inhibition of *Clostridium perfringens* during extended cooling of uncured meat products and the inhibition of *Listeria monocytogenes* during extended refrigerated storage, University of Wisconsin-Madison Food Research Institute**

This research will compare the relative efficacy and synergistic effect of the active components (lactate, propionate, acetate/diacetate) found in clean label growth inhibitors (such as cultured sugar and vinegar-based products) on the inhibition of *Clostridium perfringens* in a model-uncured beef system, with primary focus on extending Phase 1 cooling (120 to 80°F).

It will also validate inhibition of *Listeria monocytogenes* during extended refrigerated storage of a model deli meat system formulated with organic acid salts used to control *C. perfringens*. *Contract pending*

**Assessing *Salmonella* status (prevalence, load, pathogenicity, and serotype complexity) through beef processing, U.S. Meat Animal Research Center USDA-ARS, University of Georgia, and University of Wisconsin-Madison**

This study will integrate four advanced laboratory techniques, e.g. PiLOT, CRISPR-SeroSeq, to improve understanding of *Salmonella* dynamics in beef processing. Specifically, these data and techniques will enable (1) identifying steps in the processing chain which change the *Salmonella* communities either by load or loss or gain of subtypes (serotype, pathogenicity), or both and (2) describing which *Salmonella* subtypes (serotype, pathogenicity) are selected for, or which are lost, during processing steps. Findings will help build the research foundation needed for food safety protocols to produce integrated data sets. This data can then be applied to ensure that proposed regulatory frameworks meaningfully improve beef safety. *Contract pending*

**Rapid identification of ground beef and beef trim samples exceeding a *Salmonella* threshold level, U.S. Meat Animal Research Center USDA-ARS, Kansas State University and Cargill**

The study will determine the impact of the *Salmonella* inoculation method on various *Salmonella* quantification and limit of detection (LOD) methods. Additionally, the study will determine the reliability and repeatability of various *Salmonella* quantification and LOD methods for the identification of non-intact beef samples harboring *Salmonella* equal to or above a threshold. Findings will address beef industry concerns regarding specific methods. *Contract pending*

**Reliability and repeatability of digital PCR and VAE assays for the enumeration of *Salmonella* in beef samples, Florida State University and US MARC**

This study intends to optimize digital PCR (dPCR) and varying amplification efficiency (VAE) assay workflows for the detection and quantification of *Salmonella* load in beef samples. The reliability and repeatability of dPCR and VAE assays will be validated and their applicability will be compared with an AOAC performance-tested method. The application of a partition-based digital PCR approach will enable highly reliable and repeatable detection and quantification of *Salmonella* load in contaminated beef samples. The VAE assay will enable simple estimation of *Salmonella* levels in beef samples. *Contract pending*

**Historical analysis of *Salmonella* using pre-existing data: trends in outbreaks, genomics, and geographic factors, USDA-ARS Meat Safety and Quality Research Unit, and University of Georgia**

Emerging pathogen monitoring programs, such as wastewater monitoring for COVID-19, have gained critical focus as a possible way to predict the next outbreak and assist in development of management tools before the first wave of illnesses hits. These approaches can be extended to beef-related *Salmonella* outbreaks by leveraging the wealth of surveillance data that is collected into the NCBI Pathogen Detection Isolates Browser. This study will develop a framework for understanding where, why, and how certain *Salmonella* strains emerge to cause outbreaks. A short-list of warning signs and monitoring recommendations will be developed to predict the next outbreak before it happens. *Contract pending*

**Analysis of beef *Salmonella* outbreaks using the USMARC SNP analysis pipeline, USDA-ARS, Meat Animal Research Center**

Using the pipeline developed in a previously funded project (see Enhanced Characterization of Sequence Differences Among *Salmonella* isolates within SNP Clusters Identified by the NCBI Pathogen Detection System), genomes from previous beef *Salmonella* outbreaks will be evaluated to characterize the genomic variation between sequenced isolates related to the outbreak. The findings will detail the reliability of Beef *Salmonella* outbreak traceback.

### **Recently Completed Research**

#### **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. This project conducted a thorough review of pre-harvest and post-harvest *Salmonella* research in swine and identified knowledge gaps and research recommendations.

*Funded in part by the National Pork Checkoff.*

#### **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 was employed to evaluate which lymph node (LN) type presented the highest risk for containing *Salmonella* and at what level of contamination, while also investigating the seasonal and regional variability of *Salmonella* in market hog LNs. Findings show *Salmonella* prevalence varies by LN type in different seasons and regions. Tonsils and mesenteric LNs were associated with the greatest overall *Salmonella* prevalence and concentration; however they are not commonly associated with ground pork contamination. The highest prevalence was observed in tonsils during the spring in the eastern region. The results from this study reinforced data previously reported in the literature by indicating that market hog LNs harbor *Salmonella* in various lymphoid tissue. The risk of *Salmonella* contaminated LNs is greatest when the interior of a LN is exposed.

*Funded in part by the National Pork Checkoff.*

### **Recently Completed Research Funded by the Beef Checkoff and Administered by the Foundation**

#### **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 overall prevalence of *Salmonella enterica* from ground beef samples was 3.67% over the course of 14 months. There was quarterly variation with the peak prevalence occurring in the Spring and Fall seasons. Over the study period, the median count of *Salmonella* per unit of ground beef was 2.73 log CFU/g while the median count of *Salmonella* per sample of ground beef was 5.31 log CFU/sample among the samples that yielded quantitative results which were few. Demographic information indicated that *Salmonella* testing was not commonly done on beef trimmings prior to grinding or on final product.