SALMONELLA ENUMERATION

American Meat Science Association

Salmonella is a genus of bacteria widespread throughout the environment and found across a large variety of food products including meat, poultry, produce, and other products. Salmonellosis is one of the most prominent foodborne diseases worldwide and causes significant medical concerns and financial impacts annually.[1] Likelihood of human illness may be influenced by the serotype of the *Salmonella*, the pathogenicity (virulence of the serotype and presence of pathogenic genes), the number of bacterial cells present (dose), the health status of the host, and the handling and preparation methods of the food.[2,3] To continually improve food safety and public health, data collection and data sharing methods, testing techniques, and quantification methods are important. Constant learning through risk assessments and evolving methodologies will lead to more impactful public health decisions.

Pathogenicity and Virulence

Salmonella is an organism that belongs to the Enterobacteriaceae family of bacteria and can be further subdivided into serotypes based on other cellular characteristics.[4] Its pathogenicity is determined by the presence of specific genes that allow the bacteria to invade and colonize the gut of their hosts.[3] Virulence is described as an ability of an organism to infect the host and cause a disease. Research studies have shown that the severity of salmonellosis and the percentage of infected humans after consumption of food is associated with the level of contamination, displaying a dose-response relationship. Additionally, the infectious dose of *Salmonella* and the severity of the acquired illness depends on the food matrix itself and the immune status of the host. Figure one shows a comparison of dose-response relationships from various food safety and public health organizations. This figure models how the probability of response, or contracting an illness, is dependent on the dosage of viable cells consumed and emphasizes the importance of quantification of bacterial cells to make informed decisions.[5]

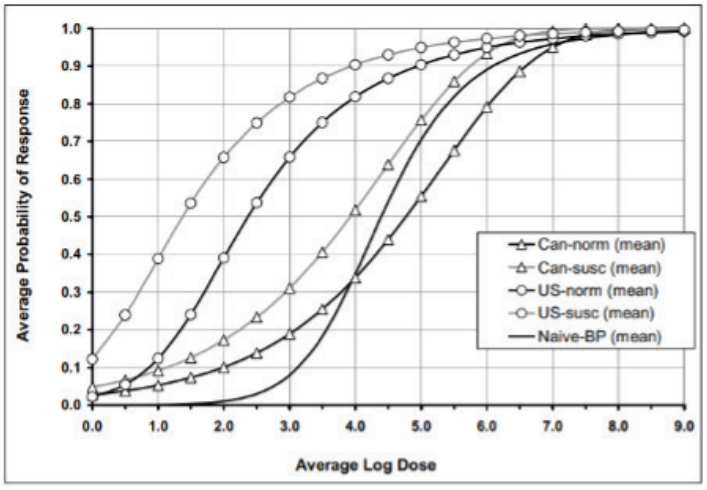

Figure 1. Comparison of dose-response relationship models from various food safety and public health organizations (adapted from FAO, 2002).[5]

Detection and Enumeration of *Salmonella*

Very low numbers of *Salmonella* cells are typically found in food, feed, and environmental samples.[6] Meat and poultry carcasses may become contaminated during the slaughter process, which may result in low levels and uneven distribution of the pathogen. Yet, these low levels of contamination may cause serious illness for various reasons. Conversely, many factors contribute to the likelihood of no illness occurring from low cell counts. To identify critical contamination points and to gather quantitative data during processing steps, cost-effective methods that can quantify low levels of *Salmonella* are needed to fully assess food safety risk.[2] Current quantification methods are not accurate for consistently quantifying numbers of cells under 10 cfu/g.

Detecting the presence and quantity of *Salmonella* is just one piece of information for assessing food safety risk. Highly pathogenic strains are more likely to cause illness even if the number of cells are relatively low. Since pathogenicity varies by serotype, methods to detect the serotype quickly and accurately in production settings are needed.

Methodologies

Detection methods can determine presence or absence while quantification methods measure the total population of *Salmonella* in a sample. Methods with specificity, precision and accuracy are needed to fully understand situations involving *Salmonella*. For instance, detection is critical for obtaining bacteria isolates which can then be explored for whole genome sequencing (WGS), presence of pathogenicity genes, and antimicrobial resistance (AMR genes).

Current techniques have opportunities for improvement in time, labor, reliability, and useability in production settings. Isolation of the bacteria from samples enables tracking to

the source of illness and for learning about survival skills of the bacteria.[7]

Detection Methods

- Molecular-based assays (Polymerase Chain Reaction (PCR))
- Immunological techniques (i.e., ELISA)
- Conventional culture methods
- Electrochemical biosensor
- Mass spectrometry
- Spectroscopy
- Optical phenotyping

Quantification Methods

- Most Probable Number (MPN)
- Direct plating
- Conventional plating
- Quantification PCR

Status of Regulations

The United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) conducts routine testing of meat and poultry products for the presence of *Salmonella*. USDA-FSIS currently has performance standards in place for *Salmonella* for chicken and turkey products with proposed standards for ground beef and pork. A performance standard sets a criterion which establishments must meet on select product. Current raw poultry standards establish prevalence limits (presence/absence in a 52 week moving window), without considering pathogenicity as determined by serotype.[8] While USDA-FSIS conducts further testing to determine the serotype, all *Salmonella* is considered "equal" by the agency for regulatory purposes with no consideration to serotype or pathogenicity in terms of performance standards or monitoring.

Zero Tolerance Explained

A zero-tolerance standard indicates that a substance (in this case, *Salmonella*) is present in or on a product, that product will be considered adulterated and unfit for human consumption, which will result in either the diversion of the product to cooking or the destruction of the product. A zero-tolerance policy for pathogens such as Shiga Toxin Producing E. coli (STEC) in place for raw non-intact beef, is scientifically valid due to the manner in which beef products are consumed, the specificity of the pathogen, availability of real-time detection methods for pathogenic strains, and the ability of the pathogen to cause illness with a small number of cells. Currently, data are lacking to implement a zero-tolerance policy for *Salmonella*. There are key differences between *Salmonella* and STEC such as the infectious dose for *Salmonella* is generally higher than that of STEC's.

Also, *Salmonella* presence may include approximately 2600 serotypes and not account for concentration of the pathogen.[7] STEC regulations are based timely specificity testing.

Not all non-Typhoidal *Salmonella* serotypes cause illnesses. Methodologies are commercially available to detect the presence of *Salmonella* as a species, but additional testing time is needed to correctly identify the serotype and to identify pathogenicity, which, makes it impractical for facilities to conduct these tests, currently.

Action and Research to Improve Food Safety

To aid the industry in additional *Salmonella* control strategies that will ultimately impact human health, research and information needs include:

- Establishing national baselines using quantification to understand the risk and patterns of *Salmonella* contamination among FSIS-regulated products.
- Continued development of rapid quantification methods to improve detection of *Salmonella*.
- Developing methods to identify pathogenicity genes which will aid in a better understanding of the virulence of different *Salmonella* strains.
- Creating rapid methods to identify the most pathogenic *Salmonella* strains – those which pose the greatest risk to human health.
- Identifying specific potential contamination points within meat and poultry processing to target *Salmonella* control efforts effectively.
- Understanding the dynamics of *Salmonella* in the meat and poultry processing environment, including factors that affect its growth and survival of *Salmonella*.

Key Points

- The "dose" or quantity of viable cells of *Salmonella* impacts potential to cause human illness.
- *Salmonella* serotype and the presence of pathogenic genes are factors determining the ability to cause illness
- Rapid, precise quantification methods are critical to determine if type (serotype) and amount (quantification) of bacteria create a public health risk.
- Data is currently lacking for establishing a "zero-tolerance" policy and for considering *Salmonella* an adulterant

References

1. The United States Department of Agriculture Food Safety Inspection Services, *Salmonella* Questions and Answers, 2021
2. Food and Agriculture Organization. (2019). Hazard characterization and exposure assessment of *Salmonella* spp. in broilers and eggs. Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods. Retrieved February 27, 2023, from https://www.fao.org/3/x8124e/x8124e06.htm
3. Harhay, D. M. (2020). *Multiplex PCR assay to identify highly pathogenic Salmonella (HPS)*. United States Meat Animal Research Center.
4. Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B. *Salmonella* nomenclature. J Clin Microbiol. 2000 Jul;38(7):2465-7. doi: 10.1128/JCM.38.7.2465-2467.2000. PMID: 10878026; PMCID: PMC86943.
5. Food and Agriculture Organization and World Health Organization. 2002. Risk assessments of *Salmonella* in eggs and broiler chickens. Food and Agriculture Organization and World Health Organization, Rome, Italy.
6. Blodgett, R. February 2006, posting date. Most probable number from serial dilutions. In FDA's bacteriological analytical manual online, appendix 2.
7. Grimont, P.A.D. and Weill, F.X. (2007) Antigenic Formulae of the Salmonella Serovars. 9th Edition, World Health Organization Collaborating Center for Reference and Research on Salmonella, Institut Pasteur, Paris.
8. Food Safety and Inspection Service. Performance Standards *Salmonella* Verification Program for Raw Poultry Products | Food Safety and Inspection Service. (n.d.). Retrieved February 27, 2023, from https://www.fsis.usda.gov/policy/fsis-directives/10250.2

American Meat Science Association
302 S Platte Clay Way, Suite 107
Kearney, MO 64060
meatscience.org