Impact of Raw Egg Storage Conditions and *Salmonella* Genomic Traits on Survival of Outbreak Strains

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Abstract

The survival of *Salmonella* spp. in raw eggs is influenced by a complex interplay of environmental conditions and genetic adaptations that enhance pathogen resilience. Storage factors such as temperature, humidity, and duration impact bacterial persistence within different egg compartments, including the shell, albumen, and yolk. The ability of *Salmonella* to withstand hostile conditions is mediated by virulence factors, stress response mechanisms, biofilm formation, and metabolic adaptations. Key serotypes like *S. typhimurium* and *S. enteritidis* exhibit distinct survival strategies, leveraging quorum sensing, efflux pumps, and stress-induced mutagenesis to adapt to fluctuating environments. Notably, ambient storage conditions increase bacterial survival compared to refrigeration, posing a greater risk of contamination. Understanding the molecular mechanisms that enable *Salmonella* to thrive in eggs provides critical insights for developing targeted interventions to enhance food safety and reduce the risk of outbreaks associated with raw egg consumption.

Keywords: Raw eggs, stress response, *S. typhimurium*, *S. enteritidis*, quorum sensing, efflux pumps.

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Introduction

Salmonella is a Gram-negative, rod-shaped, non-spore-forming bacterium with facultative respiratory metabolism that consists of two species: *Salmonella bongori* and *Salmonella enterica* (Tindall et al., 2005; Su and Chui, 2007). 99 % of human and warm-blooded animal cases of salmonellosis are caused by subspecies enterica, one of the six subspecies of *Salmonella enterica* (Issenhuth-Jeanjean et al., 2014; Lamas et al., 2018). All *Salmonella* serotypes other than those that cause typhoid fever, namely *Salmonella typhi* and *Salmonella para typhi*, are classified as non-typhoidal Salmonella (NTS) serovars (Feasey et al., 2012). With an average incidence rate of 1.14% episodes per person, NTS pathogens are major foodborne dangers that impact around 94 million individuals worldwide (Majowicz et al., 2010; Murray et al., 2012).

Overview of *Salmonella* Contamination in the Egg Industry

Globally, the number of salmonellosis cases rose sharply between 1980 and the end of the 1990s. "Salmonella was only found in a very low proportion of table eggs, at levels of 0.3%," according to an EFSA analysis from 2012, which is a 0.5% decrease from 2009. *Salmonella* can penetrate the eggshell and contaminate the contents (De Reu et al., 2006), and the eggs themselves can contaminate other surfaces, so external contamination of the eggshell from exposure to a contaminated environment is crucial. Additionally, fecal deposition on the shell and oviduct contamination might cause external contamination of the egg (Gantois et al., 2009).

Public Health Significance of Egg-related *Salmonella* Outbreaks

Salmonella contamination of eggs is an issue for the poultry and egg industries as well as for public health. In the context of the chicken business, internal Salmonella infection of eggs in the reproductive organs during egg formation results in Salmonella contamination of day-old chicks in the case of viable eggs. In turn, these chicks may operate as a vast bacterial reservoir on farms or in commercial hatcheries (Cox et al 2000). Additionally, *Salmonella* in eggs has been linked to weak day-old chicks, low egg quality and viability, and a high death rate in infected broilers and layers (Davies, 2012).

Importance of Examining both Storage Conditions and Genomic Traits for Outbreak Prevention

Metagenomics, which is the random shotgun sequencing of a community's entire genomic content, and amplicon sequencing, also known as metabarcoding, which entails the amplification and sequencing of marker gene families, are two methods that use NGS technologies to investigate the species and functional diversity of microbial communities without bacterial culture. These two methods are frequently mistakenly grouped together under the phrase metagenomics, thus it's critical to distinguish between them (Forbes et al., 2017).

Overview of international food safety regulations related to egg storage (e.g., FAO, WHO guidelines)

Eggs are consumed as a staple food around the world. They are a complete food that is necessary for health and are regarded by customers as being healthy, adaptable, and balanced in vital nutrients (Jones et al., 2020). Nevertheless, eggs are prone to infection by certain germs and are perishable (Smith & Brown, 2018). Each American consumes 234 eggs annually on average (USDA, 2023). *Salmonella enteritidis* (SE) bacteria can be found in some of these eggs, which can get you sick if you eat them raw or use them in undercooked cuisine (CDC, 2022).

Overview of *Salmonella* in Egg Contamination

When eggs are placed by birds and come into contact with bird droppings (poop), *Salmonella* may infect the eggshells. Because the eggs are cleaned before they are delivered to retailers, touching them at the grocery store is not a major source of sickness. Previous studies on the impact of various poultry management methods on *Salmonella* and other food safety viruses have produced a variety of frequently contradicting findings, making the public health implications of laying hen housing unclear. Nevertheless, prior data has established that the unique inherent characteristics of each housing system present correspondingly unique challenges for pathogen control efforts (Jones et al., 2015). In Pakistan, studies have shown a significant prevalence of *Salmonella* in poultry farms, posing a risk to public health. A study conducted in Punjab found *Salmonella enteritidis* in 18.3% of layer farms, indicating inadequate biosecurity measures (Hussain et al., 2020). Another study reported contamination rates as high as 23.5% in eggshells collected from open markets in Karachi, emphasizing the need for stricter hygiene regulations (Ahmed et al., 2021). The lack of standardized cleaning and disinfection protocols in commercial and backyard poultry farms further exacerbates the risk of *Salmonella* transmission in Pakistan (Khan et al., 2019).

Common *Salmonella* Serotypes linked to Egg Outbreaks

One of the most prevalent serotypes of *Salmonella* that has been recorded globally is *Salmonella enteritidis*, which is frequently associated with eggs. The young chick becomes infected if a viable egg contains *Salmonella enteritidis*. According to estimates, the most common bacterial cause of foodborne illnesses, hospitalizations, and fatalities in the United States is salmonella. An estimated $4.1 billion is spent annually on direct medical expenses, lost productivity, and premature mortality due to foodborne nontyphoidal *Salmonella* strains, which are responsible for 1.03 million infections, 19,300 hospitalizations, and 378 fatalities in the United States (Scallan et al., 2011, United States Department of Agriculture Economic Research Service, 2018).

Fig. 1: Medical illustration of non-typhoidal *Salmonella*

Routes of *Salmonella* contamination in egg production and processing

Salmonella, specifically the serovar *S. enteritidis*, can contaminate eggs in two ways: horizontally and vertically. There are two ways that eggs might get contaminated: vertically and horizontally. Horizontal transmission results from external contamination of the eggshell, while vertical transmission is caused by colonization of the reproductive organs (oviduct and ovary) prior to shell formation (De Reu et al., 2006).

Influence of Storage Conditions on *Salmonella* Survival in Eggs

At 22°C, *Salmonella Typhimurium* may survive better on eggshells, but at 4°C, the bacterial burden diminishes with storage time (University of Florida, 2021). *Salmonella Typhimurium* develops best in the yolk at 15 or 22°C; however, it depends on the temperature to grow in albumen (University of Florida, 2021). One strain of each of the *Salmonella enterica* serovars Enteritidis, Typhimurium, and Tennessee has been shown to survive on the surface of table eggs while stored at 4, 8, and 20°C (Figure 1) (University of Florida, 2021).

Effects of Relative Humidity and CO_2 Levels on *Salmonella* stability

Food drying inhibits the development of germs, although low-infectious dose pathogens can persist for long periods of time at low water activity (aW) and result in foodborne disease (Gruzdev et al., 2012). According to estimates, Shiga toxin-producing Escherichia coli (STEC) and Salmonella enterica account for 24% of foodborne disease deaths and 30% of hospitalizations in Canada annually (Health Canada, 2016).

Genomic Traits of *Salmonella* That Enhance Survival in Raw Eggs

MDR strains have become much more prevalent as a result of Salmonella's ongoing genomic evolution, which also increases the bacteria's

pathogenicity and resistance to several antibiotics. The ability of Salmonella Enteritidis strains isolated from eggs to be phylogenomically distinct from those isolated from other sources or to have genomic similarities is yet unclear. The current work was carried out to fill this information vacuum by shedding light on the genetic signatures, virulence, antibiotic resistance, and phylogenetic traits of *Salmonella enteritidis* linked to eggs (Abdelhamid & Yousef, 2023).

Virulence Genes, Stress Response, and Heat Resistance in Salmonella strains

In Shigella flexneri, virulence genes are produced at 37°C and turn off at 30°C or below (Dorman and Porter, 1998). According to Dorman and Porter (1998), the strict regulation of virulence genes by ambient temperature may be a tactic to save energy and stop the virulence genes from being expressed inappropriately outside the host. At 42°C, every virulent gene examined in this investigation significantly increased, except for the sefA gene.

Role of Antibiotic Resistance Genes in Persistence and Survival

Antibiotic persistence is triggered by the stringent response, which is mediated by the alarmone (p) ppGpp, which is present in nearly all bacterial species (Figure 2). In *E. coli*, (p) ppGpp is generated by the bifunctional synthetase/hydrolase SpoT during carbon, nitrogen, iron, phosphate, and fatty acid shortage or by the synthetase RelA, which is triggered by heat shock and a lack of amino acids (Irving & Corrigan, 2018; Ronneau & Hallez, 2019).

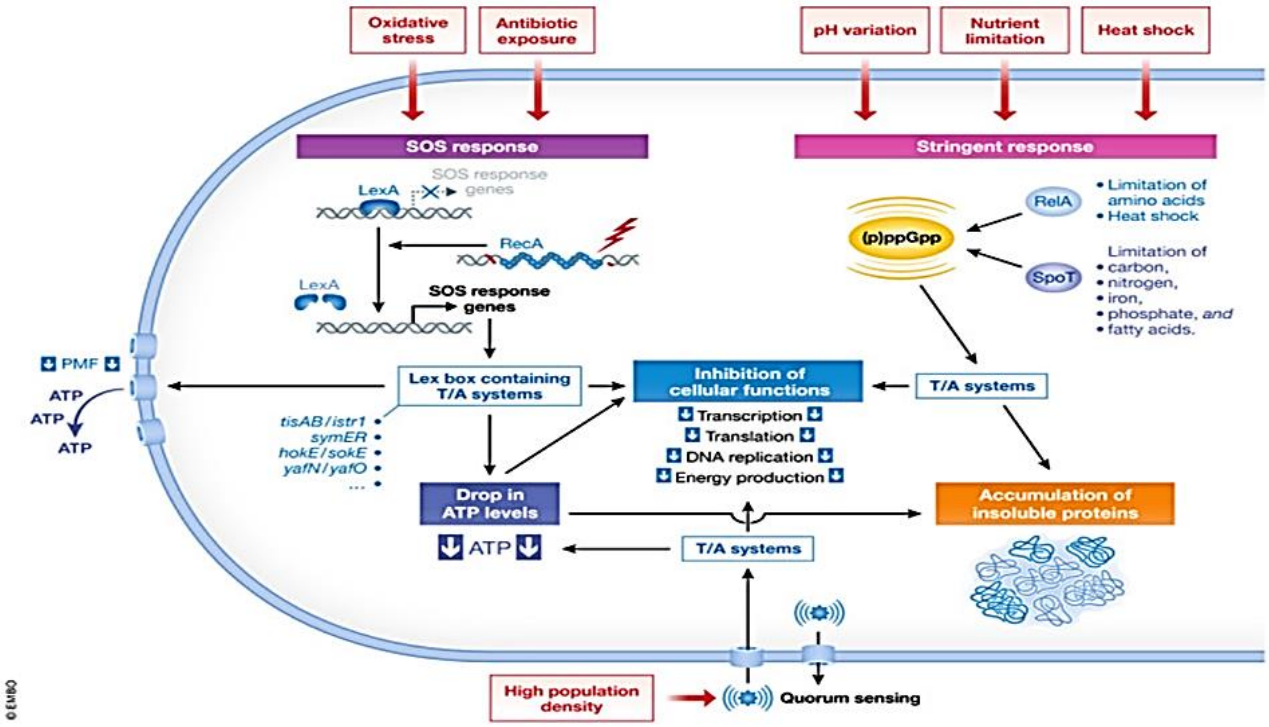

Fig. 2: Environmental triggers of antibiotic persistence and bacterial response mechanisms. (De Oliveira & Sauer, 2021)

Interactions between Egg Storage Conditions and *Salmonella* Genomic Traits

A refrigerator (6°C, 90% RH) used to keep half of the eggs, while the other half were kept at room temperature (25°C, 63%. Beginning at time 0 (the instant following inoculation) and continuing at 2, 4, 6, and 8 weeks after inoculation, two or three eggs per treatment were removed in order to check for Salmonella in the egg contents and on the infected portions of the shell (Okamura et al., 2008).

Molecular and Genomic Techniques for Studying *Salmonella* in Raw Eggs

The development of thorough functional genomics-based methods to identify the genetic prerequisites for Salmonella colonization, infection, and survival in the environmental conditions to which the microorganism is exposed has been made possible by molecular technologies such as sequencing and targeted mutagenesis. The creation and use of such innovative functional genomics methods may uncover hitherto unidentified weaknesses that may be investigated to provide fresh treatments and disrupt the Salmonella transmission chain (Hensel, 2013).

Use of whole genome sequencing (WGS) for characterizing *Salmonella* strains

In public health labs, WGS may generate high-quality sequencing data that facilitates the identification of clinical strains, their association with outbreak strains, and the identification of genes responsible for antibiotic resistance and virulence. WGS, which has already profoundly changed the field of genomics, may allow researchers to examine host-pathogen interactions and test gene

expression by sequencing RNA (Oakeson et al., 2017). WGS may also be used to identify a disease's probable causes and the way it spreads throughout a population.

CRISPR-based methods for gene function analysis in Salmonella

One effective method for modifying the genes in bacteria's genomes is CRISPR/Cas9. Nevertheless, the effective rate of genome editing in bacterial plasmids using CRISPR/Cas9 is quite low since a single untreated plasmid (if any) multiply and restore its original sequences (Figure 3) (Tagliaferri et al., 2020). Removing harmful genes from genomic DNA (Jiang et al., 2013) or curing pathogenic plasmids from clinical infections (Wang et al., 2019; Tagliaferri et al., 2020) are two current applications of CRISPR/Cas9 technology.

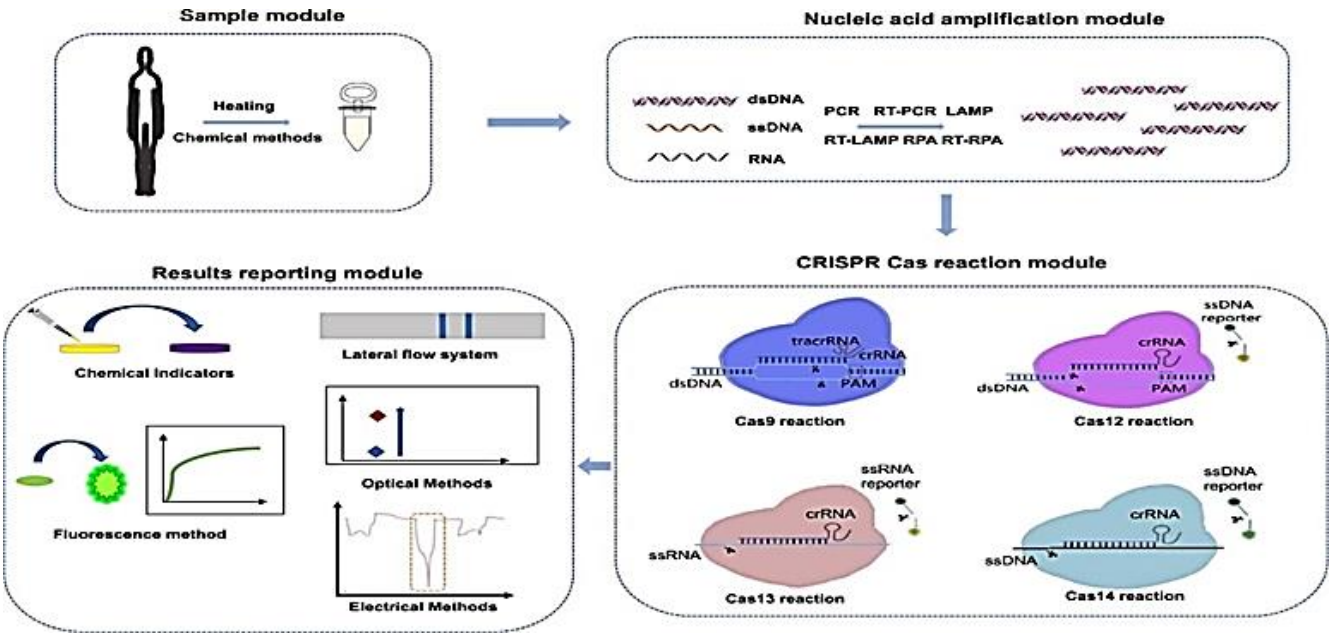

Fig. 3: Real-time PCR and other rapid detection techniques for *Salmonella* in food products (Zhang et al., 2021)

A TaqMan probe and specifically designed primers are used in a real-time PCR approach to detect the presence of a 262-bp fragment of the Salmonella-specific invA gene. The DNA template for qPCR was extracted using the lysis–guanidine isothiocyanate procedure after a pre-enriched sample was kept in buffered peptone water for the whole night. The target pathogen's invA gene was co-amplified during qPCR testing with the addition of foreign internally amplification control (IAC) (U.S. Food and Drug Administration [FDA], 2024)

Impact of *Salmonella* Genomic Variability on Food Safety Risk

One crucial step in incorporating molecular data into risk assessment is filtering genome sequences to find genes pertinent to its virulence. A significant obstacle, though, is the physiological effect of these genes' presence, expression, and interaction. Even though there are a number of techniques (both established and new) for analyzing genome sequences, data mining for virulence genes and forecasting their visible symptoms is still in its early stages (Van Hoorde & Butler, 2018).

Strain-specific Factors that Influence Salmonella Survival and Virulence

Animal sources of food frequently include resistant strains of Salmonella. Antimicrobial resistance in Salmonellae has been facilitated by the misuse of growth promoters in cattle and the improper handling of antimicrobial drugs for human and animal therapy. In addition to influencing the bacteria's pathogenicity, the surface features are also important targets for the human immune system, which puts selection pressure on the development of genetic polymorphisms that code for antigenic variety (Van Boeckel et al., 2015).

Genomic Insights into the Persistence of *Salmonella* in different Egg Storage Environments

Not only do the surface characteristics affect the pathogenicity of the bacteria, but the human immune system also targets them, exerting selection pressure on the development of genetic polymorphisms that code for antigenic variation. Elgroud et al. (2009), Alali et al. (2010), Chinivasagam et al. (2010), Donado-Godoy et al. (2012), Jay-Russell et al. (2018 a,b) and others have reported that a variety of Salmonella strains are present in animal dung, especially chicken manure.

Role of Emerging *Salmonella* Strains and their Potential to Evade Traditional Storage Safety Measures

Salmonella infections are a serious public health issue that also burdens the economy due to the high expenses of both preventing and treating illness. (Crump et al., 2004; Helms et al., 2005; Mkangara, 2023). When food or water is infected with Salmonella, the consequence is salmonellosis, which is brought on by eating contaminated food (Gonçalves-Tenório et al., 2018).

Analysis of Plasmid-mediated Antibiotic Resistance in *Salmonella* strains from Eggs

It is possible that the plasmid is not maintained and may be present in other bacteria. With 50% coverage, discontinuous contigs were added, allowing for the identification of plasmid continuity gaps brought on by insertions, deletions, or other mobile elements (Antipov et al., 2019). Ultimately, the reference plasmid was used to align the shortest contigs (>3,000–<10,000) and BLAST was used to assess them using the generated database. A plasmid draft was created using certain contigs that were taken out of the genome (Roosaare et al., 2023).

Practical Implications for Egg Storage and Handling in Preventing *Salmonella* Outbreaks

The public health burden of salmonellosis and antibiotic resistance linked to poultry products may be lessened by practical interventions such as post-harvest antimicrobial application, biosecurity measures at poultry facilities, and omics tools and surveillance for identifying antibiotic-resistance genes.

Recommendations for Industry Practices in Egg Processing, Packaging, and Distribution

Best practices in the egg processing sector concentrate on maintaining sustainability, safety, and quality at every turn. Maintaining ideal temperatures to stop bacterial development and putting in place stringent quality control and sanitation procedures are essential throughout processing (United States Department of Agriculture [USDA], 2020).

Innovative Technologies for Enhancing Egg Safety

An increasing number of feedings, egg gathering, and packaging systems are automated, which lowers labor expenses and minimizes human mistake. Furthermore, advancements in egg processing techniques like pasteurization and packaging are increasing the eggs' shelf life and guaranteeing their continued safety for human consumption (Market Research Intellect, 2023).

New Approaches to Rapid *Salmonella* Detection and strain differentiation in Eggs

Recently, the International Organization for Standardization (ISO) expanded the scope of the horizontal method for the detection, enumeration, and serotyping of *Salmonella* to include milk and milk products, animal feces, and environmental samples from the primary production stage (ISO 6579-1:2017). *Salmonella*, the bacteria that causes salmonellosis, can cause very serious illness, especially in small children, the elderly, and those with weakened immune systems (WHO, 2016). It is often acquired by eating tainted animal-based food, namely eggs, meat, poultry, and milk; however, other foods have also been linked to its spread (WHO, 2016).

Conclusion

The whole genomic sequencing has become a very promising tool in the investigation of foodborne outbreaks due to the rapid advancement of DNA/RNA sequencing technology. This is because it can be used to determine the temporal, geographical, and evolutionary origins of outbreaks as well as to evaluate the population structure of highly clonal, outbreak-related pathogens at a single-base resolution. The primary source of human *Salmonella* infections is thought to be the chicken production chain. Studies have shown that *Salmonella* contamination is prevalent in poultry production and retail chains. The reported findings emphasize the urgent need for robust surveillance systems and improved hygiene practices to minimize *Salmonella* contamination, ensuring food safety and public health.

References

Abasht, B., et al. (2020). Genomic selection for disease resistance in poultry: A review. *Genetics Selection Evolution, 52*(1), 33. https://doi.org/10.1186/s12711-020-00561-1

Abdelhamid, A. G., & Yousef, A. E. (2023). Egg-associated *Salmonella enterica* serovar *Enteritidis*: Comparative genomics unveils phylogenetic links, virulence potential, and antimicrobial resistance traits. *Frontiers in Microbiology, 14*, 1278821.

Ahmed, S., Khan, M. N., & Ali, R. (2021). Prevalence of *Salmonella* in eggshells from retail markets in Karachi, Pakistan. *Journal of Food Safety, 45*(3), 567-579.

Ahrenfeldt, J., Skaarup, C., Hasman, H., Pedersen, A. G., Aarestrup, F. M., & Lund, O. (2017). Bacterial whole genome-based phylogeny: Construction of a new benchmarking dataset and assessment of some existing methods. *BMC Genomics, 18*, 19. https://doi.org/10.1186/s12864-016-3407-6

Alali, W. Q., Thakur, S., Berghaus, R. D., Martin, M. P., & Gebreyes, W. A. (2010). Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. *Foodborne Pathogens and Disease, 7*(12), 1363–1371. https://doi.org/10.1089/fpd.2010.0566

Almeida, C., Cerqueira, L., Azevedo, N. F., & Vieira, M. J. (2013). Detection of *Salmonella enterica* serovar Enteritidis using real-time PCR, immunocapture assay, PNA FISH, and standard culture methods in different types of food samples. *International Journal of Food Microbiology, 161*(1), 16–22. https://doi.org/10.1016/j.ijfoodmicro.2012.11.014

American Egg Board (2021). *Egg Nutrition Center*. Retrieved from https://www.aeb.org

American Egg Board (2022). *Egg Packaging Guidelines*. Retrieved from AEB Website.

Antao, T., Lopes, A., Lopes, R. J., Beja-Pereira, A., & Luikart, G. (2008). LOSITAN: A workbench to detect molecular adaptation based on an *F*st-outlier method. *BMC Bioinformatics, 9*, 323. https://doi.org/10.1186/1471-2105-9-323

Antipov, D., Hartwick, N., Shen, M., Raiko, M., Lapidus, A., & Pevzner, P. A. (2019). Plasmid detection and assembly in genomic and metagenomic datasets. *Genome Research, 29*(6), 961–968. https://doi.org/10.1101/gr.241299.118

Arndt, D., Grant, J. R., Marcu, A., Sajed, T., Pon, A., Liang, Y., et al. (2016). PHASTER: A better, faster version of the PHAST phage search tool. *Nucleic Acids Research, 44*(W1), W16–W21. https://doi.org/10.1093/nar/gkw387

Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., et al. (2008). The RAST server: Rapid annotations using subsystems technology. *BMC Genomics, 9*, 75. https://doi.org/10.1186/1471-2164-9-75

Baker, M., Lee, D., & Bell, C. (2020). Genomic epidemiology of *Salmonella*: Implications for public health. *International Journal of Food Microbiology, 320*, 108536. https://doi.org/10.1016/j.ijfoodmicro.2019.108536

Bakker, H. C., Switt, A. I., Cummings, C. A., Hoelzer, K., Degoricija, L., Rodriguez-Rivera, L. D., et al. (2011). A whole-genome single nucleotide polymorphism-based approach to trace and identify outbreaks linked to a common *Salmonella enterica* subsp. *enterica* serovar Montevideo pulsed-field gel electrophoresis type. *Applied and Environmental Microbiology, 77*(24), 8648–8655. https://doi.org/10.1128/AEM.06538-11

Batz, M. B., Richardson, L. C., Bazaco, M. C., Parker, C. C., Chirtel, S. J., Cole, D., Golden, N. J., Griffin, P. M., Gu, W., & Schmitt, S. K. (2021). Recency-weighted statistical modeling approach to attribute illnesses caused by four pathogens to food sources using outbreak data, United States. *Emerging Infectious Diseases, 27*(1), 214. https://doi.org/10.3201/eid2701.191716

Benjamini, Y., & Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. *Annals of Statistics, 29*(4), 1165–1188. https://doi.org/10.1214/aos/1013699998

Beyer, M., & Verreet, J.-A. (2005). Germination of *Gibberella zeae* ascospores as affected by age of spores after discharge and environmental factors. *European Journal of Plant Pathology, 111*(4), 381–389. https://doi.org/10.1007/s10658-004-5737-1

Bibek, R. (2001). *Fundamental food microbiology* (2nd ed.). The CRC Press Ltd.

Boyen, F., Haesebrouck, F., Maes, D., Van Immerseel, F., Ducatelle, R., & Pasmans, F. (2008). Non-typhoidal *Salmonella* infections in pigs: A closer look at epidemiology, pathogenesis, and control. *Veterinary Microbiology, 130*(1–2), 1–19. https://doi.org/10.1016/j.vetmic.2007.12.017

Canada. (n.d.). Yearly foodborne illness estimates for Canada. Retrieved from https://www.canada.ca/en/public-health/services/food-borne-illness-canada/yearly-food-borne-illness-estimates-canada.html

Cao, Y., Fanning, S., Proos, S., Jordan, K., & Srikumar, S. (2017). A review on the applications of next-generation sequencing technologies as applied to food-related microbiome studies. *Frontiers in Microbiology, 8*, 1829. https://doi.org/10.3389/fmicb.2017.01829

Chousalkar, K. K., Sexton, M., McWhorter, A., Hewson, K., Martin, G., & Shadbolt, C., et al. (n.d.). [Complete citation details needed]

Cox, N. A., Berrang, M. E., & Cason, J. A. (2000). *Salmonella* penetration of eggshells and proliferation in broiler hatching eggs: A review. *Poultry Science, 79*, 1571–1574.

Cox, N. A., Berrang, M. E., & Cason, J. A. (2000). Salmonella penetration of eggshells and proliferation in broiler hatching eggs—a review. *Poultry Science, 79*, 1571–1574.

Crump, J. A., Luby, S. P., & Mintz, E. D. (2004). The global burden of typhoid fever. *Bulletin of the World Health Organization, 82*(5), 346–353. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2622843/

Currie, A., MacDougall, L., Aramini, J., Gaulin, C., Ahmed, R., & Isaacs, S. (2005). Frozen chicken nuggets and strips and eggs are leading risk factors for *Salmonella Heidelberg* infections in Canada. *Epidemiology and Infection, 133*(4), 809–816. https://doi.org/10.1017/S0950268805004081

De Oliveira, A. P., & Sauer, S. (2021). The influence of microbiota on immune fitness and health. *EMBO Reports, 22*(3), e51034. https://doi.org/10.15252/embr.202051034

European Commission. (2017). *Fipronil in eggs: Factsheet - December 2017*. Retrieved from https://ec.europa.eu/jrc/en/publication/brochures-leaflets/fipronil-eggs-factsheet-december-2017

Falguera, V., Quintero, J. P., Jiménez, A., Muñoz, J. A., & Ibarz, A. (2011). Edible films and coatings: Structures, active functions, and trends in their use. *Trends in Food Science & Technology, 22*(6), 292–303.

Fierer, J., & Swancutt, M. (2000). Non-typhoid *Salmonella*: A review. *Current Clinical Topics in Infectious Diseases, 20*, 134–157.

Foley, S., Lynne, A., & Nayak, R. (2008). *Salmonella* challenges: Prevalence in swine and poultry and potential pathogenicity of such isolates. *Journal of Animal Science, 86*(Suppl_14), E149–E162.

Foley, S., Lynne, A., & Nayak, R. (2008). Salmonella challenges: Prevalence in swine and poultry and potential pathogenicity of such isolates. *Journal of Animal Science, 86*(suppl_14), E149–E162.

Food and Agricultural Organization of the United Nations/World Health Organization. (2002). *Risk assessments of Salmonella in eggs and broiler chickens: Interpretive summary*. FAO/WHO.

Forbes, J. D., Knox, N. C., Ronholm, J., Pagotto, F., & Reimer, A. (2017). Metagenomics: The next culture-independent game changer. *Frontiers in Microbiology, 8*, 1069. https://doi.org/10.3389/fmicb.2017.01069

Gilchrist, C. A., Turner, S. D., Riley, M. F., Petri, W. A., & Hewlett, E. L. (2015). Whole-genome sequencing in outbreak analysis. *Clinical Microbiology Reviews, 28*(3), 541–563. https://doi.org/10.1128/CMR.00075-13

González-Fernández, C., et al. (2023). Antimicrobial coatings for food packaging: A review of recent advances and future perspectives. *Food Control, 142*, 109218. https://doi.org/10.1016/j.foodcont.2022.109218

Gross, U., Tschäpe, H., Bednarek, I., & Frosch, M. (1998). Antibiotic resistance in *Salmonella enterica* serotype *Typhimurium*. *European Journal of Clinical Microbiology & Infectious Diseases, 17*(6), 385–387.

Gruzdev, N., Pinto, R., & Sela Saldinger, S. (2012). Persistence of *Salmonella enterica* during dehydration and subsequent cold storage. *Food Microbiology, 32*, 415–422. https://doi.org/10.1016/j.fm.2012.08.003

Guard-Petter, J. (2001). The chicken, the egg, and *Salmonella enteritidis*. *Environmental Research, 3*, 421–430.

Hassan, R., Buuck, S., Noveroske, D., Medus, C., Sorenson, A., Laurent, J., Rotstein, D., Schlater, L., Freiman, J., & Douris, A. (2019). Multistate outbreak of *Salmonella* infections linked to raw turkey products—United States, 2017–2019. *Morbidity and Mortality Weekly Report, 68*(46), 1045.

Havelaar, A. H., Ivarsson, S., Lofdahl, M., & Nauta, M. J. (2013). Estimating the true incidence of campylobacteriosis and salmonellosis in the European Union. *Epidemiology and Infection, 141*(2), 293–302. https://doi.org/10.1017/S0950268812000568

Hensel, M. (2013). The FUN of identifying gene function in bacterial pathogens; insights from *Salmonella* functional genomics. *Current Opinion in Microbiology, 16*(1), 1–9.

Hodges, R. J., & Stathers, T. E. (2013). Facing the food crisis: How African smallholders can reduce postharvest cereal losses by supplying better quality grain. *Outlooks on Pest Management, 24*(5), 217–221.

Hofer, U. (2021). *Salmonella enteritidis*: Chicken or egg? *Nature Reviews Microbiology, 19*, 682. https://doi.org/10.1038/s41579-021-00632-6

Hossain, M. I., et al. (2021). The role of probiotics in controlling *Salmonella* infections in poultry. *Poultry Science, 100*(3), 100–115. https://doi.org/10.1016/j.psj.2020.11.019

Huang, H.-W., Hsu, C.-P., & Wang, C.-Y. (2020). Healthy expectations of high hydrostatic pressure treatment in the food processing industry. *Journal of Food and Drug Analysis, 28*, 1–13. https://doi.org/10.1016/j.jfda.2019.10.002

Hull, D. M., Harrell, E., Harden, L., & Thakur, S. (2022). Multidrug resistance and virulence genes carried by mobile genomic elements in *Salmonella enterica* isolated from live food animals, processed, and retail meat in North Carolina, 2018–2019. *International Journal of Food Microbiology, 378*, 109821. https://doi.org/10.1016/j.ijfoodmicro.2022.109821

Hussain, R., Mahmood, M. S., & Akhtar, M. (2020). Detection and characterization of *Salmonella enteritidis* in commercial layer farms of Punjab, Pakistan. *Poultry Science Journal, 38*(4), 112-120.

International Egg Commission. (2021). *Egg industry best practices*. IEC Website.

International Organization for Standardization (ISO). (2020). *Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella* (ISO 6579-1:2020). https://www.iso.org/standard/56712.html

International Organization for Standardization. (2017). *ISO 6579-1:2017. Microbiology of the food chain — Horizontal method for the detection, enumeration, and serotyping of Salmonella — Part 1: Detection of Salmonella spp.* Retrieved March 29, 2025, from https://www.iso.org/standard/56712.html

Irving, S. E., & Corrigan, R. M. (2018). Triggering the stringent response: Signals responsible for activating (p)ppGpp synthesis in bacteria. *Microbiology, 164*(3), 268–276. https://doi.org/10.1099/mic.0.000622

Issenhuth-Jeanjean, S., Roggentin, P., Mikoleit, M., Guibourdenche, M., De Pinna, E., & Nair, S., et al. (2014). Supplement 2008–2010 (No. 48) to the White-Kauffmann-Le Minor Scheme. *Research in Microbiology, 165*(7), 526–530. https://doi.org/10.1016/j.resmic.2014.07.004

Issenhuth-Jeanjean, S., Roggentin, P., Mikoleit, M., Guibourdenche, M., De Pinna, E., Nair, S., Fields, P. I., & Weill, F. X. (2014). Supplement 2008–2010 (No. 48) to the White–Kauffmann–Le Minor scheme. *Research in Microbiology, 165*(7), 526–530.

Jansen, A., Frank, C., & Stark, K. (2007). Pork and pork products as a source for human salmonellosis in Germany. *Berliner und Münchener Tierärztliche Wochenschrift, 120*(9–10), 340–346.

Jay-Russell, M., Chen, Y., Rivadeneira, P., Pouillot, R., Aminabadi, P., Pandey, P., et al. (2018). Prevalence and levels of shiga toxin-producing *Escherichia coli* and *Salmonella* in untreated cattle and poultry manure in the West Coast of the United States. *International Association for Food Protection (IAFP)*. https://iafp.confex.com/iafp/2018/onlineprogram.cgi/Paper/18452

Jiang, W., Bikard, D., Cox, D., Zhang, F., & Marraffini, L. A. (2013). RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nature Biotechnology, 31*(3), 233–239. https://doi.org/10.1038/nbt.2508

Jirtle, R. L., & Skinner, M. K. (2007). Environmental epigenomics and disease susceptibility. *Nature Reviews Genetics, 8*, 253–262.

Jones, D. R., Cox, N. A., Guard, J., Fedorka-Cray, P. J., Buhr, R. J., Gast, R. K., Abdo, Z., Rigsby, L. L., Plumblee, J. R., Karcher, D. M., Robison, C. I., Blatchford, R. A., & Makagon, M. M. (2015). Microbiological impact of three commercial laying hen housing systems. *Poultry Science, 94*(3), 544–551. https://doi.org/10.3382/ps/peu010

Kaminski, J., Christiaensen, L., & Gilbert, C. L. (2016). Seasonality in local food markets and consumption: Evidence from Tanzania. *Oxford Economic Papers, 68*(3), 736–757. https://doi.org/10.1093/oep/gpw005

Keerthirathne, T. P., Ross, K., Fallowfield, H., & Whiley, H. (2016). A review of temperature, pH, and other factors that influence the survival of *Salmonella* in mayonnaise and other raw egg products. *Pathogens, 5*(4), 63. https://doi.org/10.3390/pathogens5040063

Khan, S. H., et al. (2019). Epidemiological study on *Salmonella* prevalence in poultry supply chain in Lahore, Pakistan. *Poultry Science, 98*(3), 1234-1241.

Khan, Z., Tariq, J., & Raza, S. (2019). Hygienic practices and microbial contamination in commercial poultry farms of Pakistan. *International Journal of Veterinary Research, 42*(2), 98-107.

Kruglyak, L. (2008). The road to genome-wide association studies. *Nature Reviews Genetics, 9*, 314–318. https://doi.org/10.1038/nrg2316

Kültz, D. (2005). Molecular and evolutionary basis of the cellular stress response. *Annual Review of Physiology, 67*, 225–257. https://doi.org/10.1146/annurev.physiol.67.040403.103635

Lambert, C. G., & Black, L. J. (2012). Learning from our GWAS mistakes: From experimental design to scientific method. *Biostatistics, 13*(2), 195–203. https://doi.org/10.1093/biostatistics/kxr055

Leekitcharoenphon, P., et al. (2016). Whole genome sequencing for the surveillance of foodborne pathogens: The case of *Salmonella*. *Trends in Food Science & Technology, 57*, 43–56. https://doi.org/10.1016/j.tifs.2016.09.011

Leekitcharoenphon, P., Nielsen, E. M., Kaas, R. S., Lund, O., & Aarestrup, F. M. (2014). Evaluation of whole genome sequencing for outbreak detection of *Salmonella enterica*. *PLoS ONE, 9*(2), e87991. https://doi.org/10.1371/journal.pone.0087991

Lienau, E. K., Strain, E., Wang, C., Zheng, J., Ottesen, A. R., Keys, C. E., et al. (2011). Identification of a salmonellosis outbreak by means of molecular sequencing. *New England Journal of Medicine, 364*, 981–982. https://doi.org/10.1056/NEJMc1100443

Long, P. L. (1970). Some factors affecting the severity of infection with *Eimeria tenella* in chicken embryos. *Parasitology, 60*, 435–447.

Magneschi, L., & Perata, P. (2009). Rice germination and seedling growth in the absence of oxygen. *Annals of Botany, 103*(2), 181–196. https://doi.org/10.1093/aob/mcn118

Maher, B. (2008). Missing heritability. *Nature, 456*, 18. https://doi.org/10.1038/456018a

Market Research Intellect. (2023). *The Future of Egg Quality Control: The Rise of Automatic Egg Grading Equipment*. Retrieved March 29, 2025, from https://www.marketresearchintellect.com/blog/the-future-of-egg-quality-control-the-rise-of-automatic-egg-grading-equipment/

Merhej, V., Royer-Carenzi, M., Pontarotti, P., & Raoult, D. (2009). Massive comparative genomic analysis reveals convergent evolution of specialized bacteria. *Biology Direct, 4*, 13. https://doi.org/10.1186/1745-6150-4-13

Messens, W., Grijspeerdt, K., & Herman, L. (2005). Eggshell penetration by *Salmonella*: A review. *World's Poultry Science Journal, 61*(1), 71–85. https://doi.org/10.1079/WPS200444

Mongan, N. P., Emes, R. D., & Archer, N. (2019). Detection and analysis of RNA methylation. *F1000Research, 8*.

Oakeson, K. F., Wagner, J. M., Mendenhall, M., Rohrwasser, A., & Atkinson-Dunn, R. (2017). Bioinformatic analyses of whole-genome sequence data in a public health laboratory. *Emerging Infectious Diseases, 23*, 1441–1445. https://doi.org/10.3201/eid2309.170416

Okamura, M., Kikuchi, S., Suzuki, A., Tachizaki, H., Takehara, K., & Nakamura, M. (2008). Effect of fixed or changing temperatures during prolonged storage on the growth of *Salmonella enterica* serovar *Enteritidis* inoculated artificially into shell eggs. *Epidemiology and Infection, 136*(9), 1210–1216. https://doi.org/10.1017/S0950268807009612

Pan, Z., Bingol, G., Brandl, M. T., & McHugh, T. H. (2012). Review of current technologies for reduction of *Salmonella* populations on almonds. *Food and Bioprocess Technology, 5*, 2046–2057. https://doi.org/10.1007/s11947-012-0789-6

Petrin, S., Mancin, M., Losasso, C., Deotto, S., Olsen, J. E., & Barco, L. (2022). Effect of pH and salinity on the ability of *Salmonella* serotypes to form biofilm. *Frontiers in Microbiology, 13*, 1186.

Pontel, L. B., Audero, M. E. P., Espariz, M., Checa, S. K., & Soncini, F. C. (2007). GolS controls the response to gold by the hierarchical induction of *Salmonella*-specific genes that include a CBA efflux-coding operon. *Molecular Microbiology, 66*, 814–825. https://doi.org/10.1111/j.1365-2958.2007.05963.x

Pontel, L. B., Audero, M. E. P., Espariz, M., Checa, S. K., & Soncini, F. C. (2007). GolS controls the response to gold by the hierarchical induction of *Salmonella*-specific genes that include a CBA efflux-coding operon. *Molecular Microbiology, 66*, 814–825. https://doi.org/10.1111/j.1365-2958.2007.05963.x

Rabsch, W., Tschäpe, H., & Bäumler, A. J. (2001). Non-typhoidal salmonellosis: Emerging problems. *Microbes and Infection, 3*, 237–247.

Rahman, H., et al. (2020). *Salmonella* contamination in poultry farms: A biosecurity concern. *Veterinaria Italiana, 56*(1), 17-26. Retrieved from https://www.veterinariaitaliana.izs.it/index.php/VetIt/article/view/2392

Raveendran, R., Datta, S., & Chand, W. (2010). Drug resistance in *Salmonella enterica* serotype *Typhi* and *Paratyphi A*. *International Journal of Medical Sciences & Advances, 23*, 21–24.

Ronneau, S., & Hallez, R. (2019). Make and break the alarmone: Regulation of (p)ppGpp synthetase/hydrolase enzymes in bacteria. *FEMS Microbiology Reviews, 43*, 389–400.

Roosaare, M., Puustusmaa, M., Möls, M., Vaher, M., Remm, M., & Lepamets, M. (2023). PLASMe: A tool to identify plasmid contigs from short-read assemblies using a hybrid approach. *Nucleic Acids Research, 51*(15), e83. https://doi.org/10.1093/nar/gkad467

Salmonella Typhimurium in the Australian egg industry: Multidisciplinary approach to addressing the public health challenge and future directions. (2017). *Critical Reviews in Food Science and Nutrition, 57*(12), 2706–2711.

Sapkota, A. R., Kinney, E. L., George, A., Hulet, R. M., Cruz-Cano, R., Schwab, K. J., et al. (2014). Lower prevalence of antibiotic-resistant *Salmonella* on large-scale U.S. conventional poultry farms that transitioned to organic practices. *Science of the Total Environment, 476–477*, 387–392. https://doi.org/10.1016/j.scitotenv.2013.12.005

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States – Major pathogens. *Emerging Infectious Diseases, 17*(1), 7–15. https://doi.org/10.3201/eid1701.091101p1

Schmid-Hempel, P. (2017). Evolutionary parasitology: The integrated study of infections. *Current Opinion in Microbiology, 38*, 44–48.

Schrader, K. N., Fernandez-Castro, A., Cheung, W. K., Crandall, C. M., & Abbott, S. L. (2008). Evaluation of commercial antisera for *Salmonella* serotyping. *Journal of Clinical Microbiology, 46*, 685–688. https://doi.org/10.1128/JCM.01808-07

Schroeder, S., Harries, M., Prager, R., Höfig, A., Ahrens, B., Hoffmann, L., Rabsch, W., Mertens, E., & Rimek, D. (2016). A prolonged outbreak of *Salmonella Infantis* associated with pork products in central Germany, April–October 2013. *Epidemiology and Infection, 144*, 1429–1439.

Shi, C., Singh, P., Ranieri, M. L., Wiedmann, M., & Moreno Switt, A. I. (2015). Molecular methods for serovar determination of *Salmonella*. *Critical Reviews in Microbiology, 41*, 309–325. https://doi.org/10.3109/1040841X.2013.837862

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). *Campylobacter* spp. as a foodborne pathogen: A review. *Frontiers in Microbiology, 2*, 200. https://doi.org/10.3389/fmicb.2011.00200

Singh, R., & Kumar, S. (2023). The role of IoT in cold chain management for perishable food products: A review. *Journal of Food Engineering, 329*, 111107. https://doi.org/10.1016/j.jfoodeng.2023.111107

Skinner, M. K., Manikkam, M., & Guerrero-Bosagna, C. (2010). Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends in Endocrinology & Metabolism, 21*(4), 214–222. https://doi.org/10.1016/j.tem.2009.12.007

Skyberg, J. A., Logue, C. M., & Nolan, L. K. (2006). Virulence genotyping of *Salmonella* spp. with multiplex PCR. *Avian Diseases, 50*(1), 77–81.

Smyth, G. K. (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Statistical Applications in Genetics and Molecular Biology, 3*, 1–25. https://doi.org/10.2202/1544-6115.1027

Swayne, D. E., & Beck, J. R. (2018). Vaccination of chickens against *Salmonella*: A review. *Poultry Science, 97*(8), 2664–2677. https://doi.org/10.3382/ps/pey081

Tan, C. K., et al. (2021). Prevalence and antimicrobial resistance of *Salmonella* in poultry processing environments in Malaysia. *Journal of Food Safety, 41*(2), e12896. Retrieved from https://pubmed.ncbi.nlm.nih.gov/8930704

Tesfaye, W., & Tirivayi, N. (2018). The impacts of postharvest storage innovations on food security and welfare in Ethiopia. *Food Policy, 75*,

52–67.
Tewari, S., Sehrawat, R., Nema, P. K., & Kaur, B. P. (2016). Preservation effect of high-pressure processing on ascorbic acid of fruits and vegetables: A review. *Journal of Food Biochemistry, 41*(1), e12319.
U.S. Department of Health and Human Services. (2022). *Healthy people 2030: Reduce infections caused by Salmonella—FS 04*. Retrieved October 24, 2022, from https://health.gov/healthypeople/objectives-and-data/browse-objectives/foodborne-illness/reduce-infections-caused-salmonella-fs-04
U.S. Food and Drug Administration. (2017). *Food Code 2017*. https://www.fda.gov/food/fda-food-code/food-code-2017
U.S. Food and Drug Administration. (2024). *Protocol for Screening of Salmonella in Foods and on Environmental Surfaces.* Retrieved from https://www.fda.gov/media/168834
United States Department of Agriculture Economic Research Service. (2018). *Cost estimates of foodborne illness (2020): Salmonella.* Retrieved October 24, 2022, from https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses
University of Florida. (2021). *Understanding Salmonella infections in Florida.* Retrieved from https://epi.ufl.edu/2021/04/22/understanding-salmonella-infections-in-florida/
Van Hoorde, K., & Butler, F. (2018). Use of next-generation sequencing in microbial risk assessment. *EFSA Journal, 16*(S1), e16086.
Whiley, H., & Ross, K. (2015). *Salmonella* and eggs: From production to plate. *International Journal of Environmental Research and Public Health, 12*, 2543–2556. https://doi.org/10.3390/ijerph120302543
World Health Organization. (2016). *Salmonella (non-typhoidal) Fact Sheet*. Retrieved March 29, 2025, from https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal)
World Wildlife Fund. (2021). *Sustainable practices in agriculture: Egg production.* Retrieved from WWF website.
Yadav, M. P., & Shukla, V. (2023). Biodegradable packaging materials for sustainable food preservation: A review. *Sustainable Materials and Technologies, 31*, e00300. https://doi.org/10.1016/j.susmat.2022.e00300
Zhang, M., Zhang, R., Zhang, F., Wang, X., & Li, C. (2021). CRISPR-based biosensors for detecting foodborne pathogens: Principles, applications, and challenges. *Foods, 10*(10), 2402. https://doi.org/10.3390/foods10102402
Zhao, Y., & Zhang, H. (2023). Effects of modified atmosphere packaging on the quality and shelf life of eggs. *Journal of Food Science and Technology, 60*(4), 1082–1091. https://doi.org/10.1007/s11483-022-02757-2