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Control of *Salmonella* in poultry: The role of host immunity and vaccines

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ABSTRACT

Salmonella is among the most important foodborne pathogens impacting public health and food safety. In humans, *Salmonella* infections are typically caused by the consumption of raw or undercooked poultry products. Therefore, controlling *Salmonella* at the poultry farm level is crucial. The main focus of this review article is recent advancements in vaccination strategies aimed at reducing *Salmonella* colonization in poultry. To provide essential context, it briefly outlines *Salmonella* transmission, pathogenesis, and host immune responses. The review explores the development and application of various vaccine types, including live-attenuated, killed, subunit, and ghost vaccines, and evaluates their immunological mechanisms, safety, and effectiveness. It also discusses limitations of current vaccines and the need for innovative approaches such as nanoparticle-based oral vaccines. Finally, the article offers recommendations for optimizing vaccination programs to protect both poultry health and food safety.

Introduction

Salmonella infection or salmonellosis is one of the most important foodborne zoonotic diseases that impacts the digestive tracts of both humans and animals (Coburn et al., 2007). Infection begins with the adhesion of bacteria to intestinal epithelial cells, a process facilitated by flagella, fimbriae, and adhesin molecules, leading to a pro-inflammatory response in the host (Ohl and Miller, 2001). In humans, salmonellosis may be caused by consuming raw or undercooked meat, eggs, and other poultry products (Sanchez et al., 2002). Salmonellosis poses a significant risk to food safety and public health, causing an estimated 95.1 million cases of gastroenteritis annually worldwide (Stanaway et al., 2019). Recent estimates indicate that the total economic burden of foodborne illnesses in the United States has reached approximately $75 billion (adjusted to 2023 dollars), with nontyphoidal *Salmonella* identified as the costliest pathogen, responsible for $17.1 billion in losses. A large portion of this burden is attributed to deaths (56%) and chronic health outcomes (31%) (Hoffmann et al., 2024).Therefore, controlling salmonellosis is vital for protecting public health, reducing economic burdens, and ensuring food safety.

Contaminated poultry products (meat and eggs) remain major sources of *Salmonella* transmission to humans (Wigley, 2024) .Poultry can be infected with different *Salmonella enterica* serovar Gallinarum includes two avian-adapted biovars, Gallinarum and Pullorum, both of which are primarily associated with poultry. Human infection with these biovars is extremely rare (Farhat et al., 2023). In contrast, *Salmonella enterica* serovars Enteritidis and Typhimurium are among the most prevalent in poultry and are major contributors to *Salmonella*-related foodborne illnesses in humans (Shivaning Karabasanavar et al., 2020). Poultry can become infected with *Salmonella* through various ways, including contaminated feed, water, or environment. They can also become infected through contact with other infected birds or animals or via vertical transmission from breeding hens to *offspring* (Shaji et al., 2023). Therefore, controlling *Salmonella* and reducing contamination at the farm level is critical to reduce the size of the problem and protect public health and food safety.

A multifaceted approach must be taken to control *Salmonella* at the farm level. This includes implementing hygiene and biosecurity measures, optimizing management practices (such as feed, water, and manure management), incorporating dietary interventions, and adopting vaccination strategies (Galán-Relaño et al., 2023). Vaccination is one of the key strategies to control *Salmonella* in poultry. It provides long-lasting protection and reduces *Salmonella* colonization over time (Barrow, 2007). It also prevents vertical transmission from breeder hens

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to eggs and reduces contamination risks during the early stages of poultry production (Dórea et al., 2010). Fig. 1 presents a schematic representation of a One Health approach required for the effective control of *Salmonella* at the poultry farm level.

This review article primarily focuses on recent advancements in vaccination strategies aimed at reducing *Salmonella* colonization in poultry. It also briefly outlines the key aspects of *Salmonella* transmission, pathogenesis, and the host immune response. Furthermore, the review provides a comparative analysis of different vaccination approaches, evaluating their relative effectiveness and cost-efficiency, and concludes with practical recommendations based on the current evidence.

Transmission

Understanding *Salmonella* transmission pathways is essential for exploring how vaccination can help prevent infection and reduce pathogen spread in poultry flocks (Desin et al., 2013).

The distribution of *Salmonella* serovars varies worldwide. However, *Salmonella* ser. Typhimurium and *Salmonella* ser. Enteritidis remain prevalent globally. While some serovars are host-specific, *Salmonella* can infect a variety of hosts and is widely distributed in both domesticated and wild animals (Shaji et al., 2023). *Salmonella* can cause clinical or subclinical disease, or asymptomatic infection in poultry. The severity of *Salmonella* infection depends on several factors including host age, overall health, immune system, coinfections, gut microbiome, environmental stress, and bacterial load. Carrier birds play a key role in the transmission of *Salmonella* because they can continuously shed the bacteria without displaying clinical signs of infection (Jajere, 2019). Adult birds are more likely to remain asymptomatic while young birds are more susceptible to systemic infection leading to higher mortality rates (Shaji et al., 2023).

Salmonella infection occurs via vertical or horizontal transmission. Vertical or transovarial transmission occurs between a parent bird and its offspring. When a systemic infection occurs, the ovary and oviduct can become infected, passing the bacteria directly to the developing egg. The bacteria infect the yolk, albumen, and vitellin membrane before the formation of the shell (De Reu et al., 2006). Contaminated feces can also infect the egg during or after oviposition. Some pores remain open during the first few minutes following oviposition, leaving the egg more vulnerable to bacterial penetration (Padron, 1990). Horizontal transmission occurs via fecal-oral or aerogenous routes. *Salmonella* is commonly spread via contaminated feces, as it easily spreads throughout the environment, rapidly infecting the entire flock (Shaji et al., 2023). Poultry are often infected by contaminated feed, water, litter, aerosolized bacteria, or other carriers such as rodents, insects, or wild birds as shown in Fig. 1 (Park et al., 2008).

Pathogenesis and immune response

Salmonella pathogenesis consists of several stages: adhesion and invasion of intestinal epithelial cells, intracellular survival, replication, and systemic dissemination. Throughout this process, the pathogen elicits both innate and adaptive immune responses, contributing to localized intestinal and systemic immune activation. Fig. 2 summarizes key host–pathogen interactions during *Salmonella* infection, including epithelial invasion, innate responses, and downstream signaling pathways involved in inflammation and clearance.

When bacteria are orally ingested, it must pass through the digestive system. To survive this acidic environment, *Salmonella* employs a complex acid tolerance response which involves the production of over 50 acid shock proteins allowing *Salmonella* to withstand pH as low as 3.7 (Bearson et al., 2006). Upon entering the small intestine, *Salmonella* must outcompete the present intestinal microbiome and adhere to intestinal epithelial cells. *Salmonella* attaches to intestinal epithelial cells through several mechanisms. Flagella enable the bacteria to move toward the epithelial surface while fimbriae bind to extracellular matrix protein laminin. Adhesins such as SopE, InvA, and OmpX facilitate *Salmonella*'s adherence to intestinal epithelial cells (Gart et al., 2016).

Pathogen-associated molecular patterns (PAMPs) are molecular

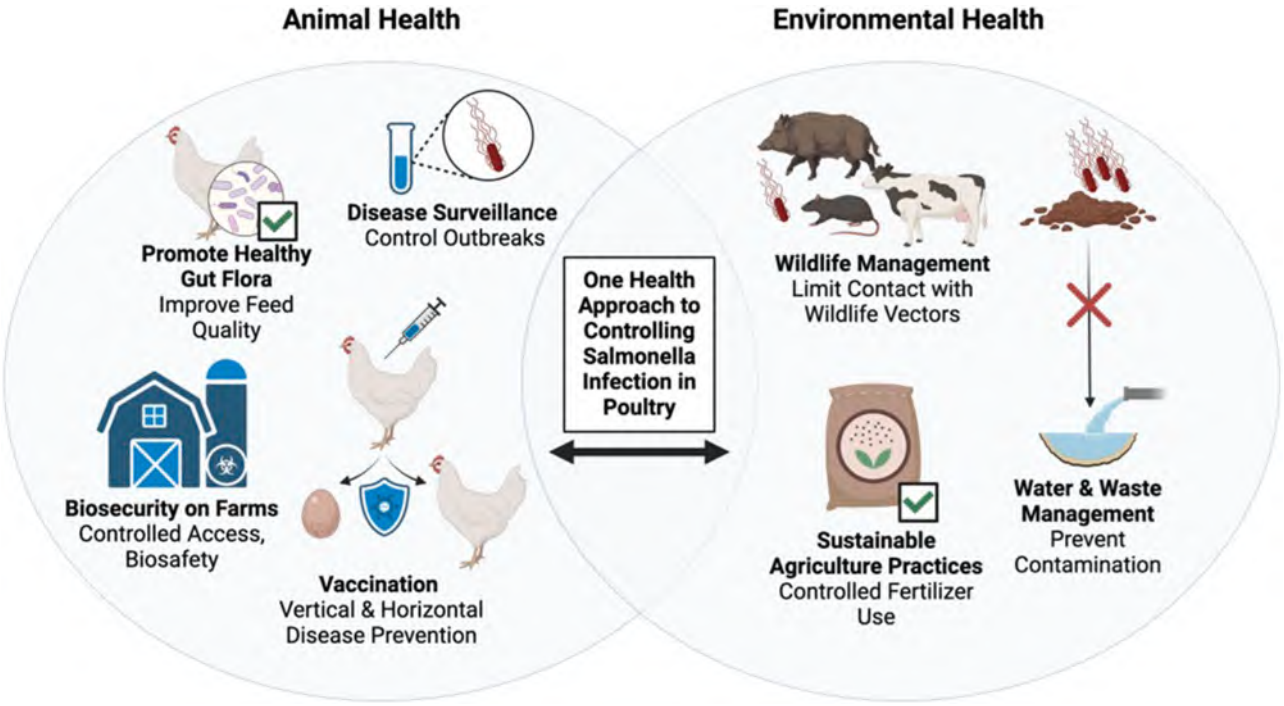

Fig. 1. A One Health approach is required to effectively control *Salmonella* at the farm level. This includes integrated efforts to protect the environment and animal health. Sustainable farming, along with wildlife, water, and waste management, helps prevent contamination and disease spread. Enhancing disease surveillance, feed quality, and biosecurity supports poultry health. Vaccination strategies further reduce *Salmonella* transmission, minimizing early-stage contamination. Figure was created using the BioRender software.

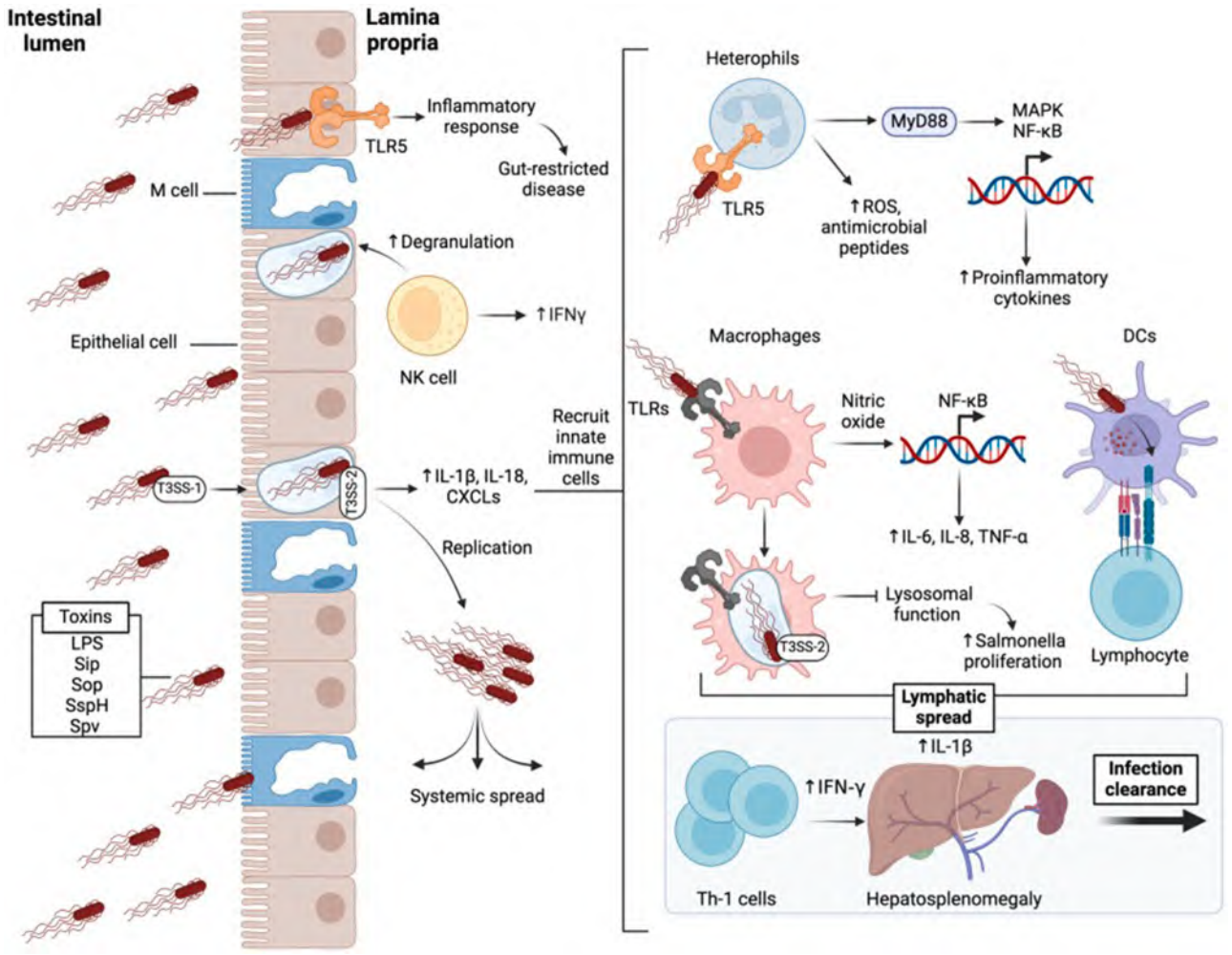

Fig. 2. *Salmonella* infection elicits innate and adaptive immune responses in both gut-restricted and systemic manners. *Salmonella* invades intestinal epithelial cells using outer membrane components like LPS and virulence factors encoded by SPIs, including effector proteins delivered via T3SS-1 and T3SS-2 to form *Salmonella*-containing vacuoles (SCVs). This triggers gut-localized inflammation through TLR5 activation and recruits immune cells such as NK cells, heterophils, and macrophages, which release cytotoxic molecules and proinflammatory cytokines. SCVs enable bacterial survival within macrophages, while dendritic cells facilitate systemic spread and bridge innate and adaptive immunity via Th1 cell activation and IFN-γ–mediated responses. Figure was created using the BioRender software.

structures of microbes that stimulate pattern recognition receptors (PRRs) of hosts (Shaji et al., 2023). PRRs are found on various immune system cells, including phagocytes, dendritic cells, heterophils, as well as on epithelial cells. The interaction between PAMPs and PRRs triggers the release of reactive nitrogen and oxygen species, and cytokines, and initiates immune responses (Werling et al., 2004). *Salmonella* expresses several PAMPs, such as flagellin, a protein component of flagella, and lipopolysaccharide (LPS), a cell wall lipid. LPS is recognized by Toll-like receptor 4 (TLR4), a critical mediator of the inflammatory response to *Salmonella* infection (Shaji et al., 2023). Studies in mice have shown that the absence of functional TLR4 significantly increases susceptibility to infection.

The presence of flagellin appears to have a major impact on disease progression in avian species. Infection of hosts with flagellated serovars is more likely to lead to inflammation in the intestine due to the interaction with TLR5. Non-flagellated serovars such as *Salmonella enterica* ser. Gallinarum biovar Gallinarum or *Salmonella enterica* ser. Gallinarum biovar Pullorum can enter intestinal epithelial cells without activating TLR5 or prompting an inflammatory response. This evasive strategy allows *Salmonella* to invade the host cell without provoking inflammation, enabling disease progression to a systemic infection (Chappell et al., 2009).

The ability of *Salmonella* to cause infection largely depends on a set of genes grouped on the bacterial chromosome in regions referred to as *Salmonella* Pathogenicity Islands (SPIs). Twenty-four SPIs have been identified in various *Salmonella* serovars, but SPI-1 and SPI-2 are vital for infection in chickens (Lerminiaux et al., 2020). SPI-1 and SPI-2 encode Type III Secretion Systems (T3SS)-1 and T3SS-2 respectively (Shaji et al., 2023). These protein complexes function as a molecular syringe, injecting effector proteins into host cells to support pathogen survival and proliferation (Coburn et al., 2007). At least 13 proteins are delivered by T3SS-1, including AvrA, SipA/B/C/D, SlrP, SopB/D/E/E2, SptP, and SspH1 (Zhou, 2001). These proteins serve a variety of functions and are essential for initiating host cell invasion, forming *Salmonella*-containing vacuoles (SCVs), and triggering inflammation (Coburn et al., 2007). *Salmonella* invasion proteins, namely SipB/C/D, are essential for the translocation of T3SS-1 effector proteins into the host cell. These proteins form a “translocase” that integrates into the host cell plasma membrane, enabling effector proteins to pass through (Darwin, 1999). Additionally, SipA/C and SopB/E/E2 assist in *Salmonella* invasion by interacting with the actin cytoskeleton. This causes ’ruffling’ of the host cell membranes. Once inside the host cell, SPI-2 is expressed, and T3SS-2 transports proteins such as SpiC, PipB, SseF/G/I/J, SifA/B, SspH1/H2, SlrP, and SopD2 (Foley et al., 2013). These proteins are crucial for SCV maturation, the formation of *Salmonella*-induced filaments, pathogen survival, replication, and systemic dissemination (Coburn et al., 2007).

T3SS-2 also transports *Salmonella* plasmid virulence proteins (Spv), which contribute to bacterial adhesion, colonization, growth, and reproduction. The presence of SpvB and SpvC has been shown to enhance *Salmonella* virulence(Guiney and Fierer, 2011). SpvB plays a crucial role in disrupting the epithelial barrier, facilitating the paracellular translocation of *Salmonella* and leading to systemic infection (Sun et al., 2020). SpvB has also been shown to disrupt the host cell cytoskeleton and contribute to macrophage apoptosis (Guiney and Fierer, 2011). SpvC demonstrates anti-inflammatory effects by inhibiting host cell kinase activity (Guiney and Fierer, 2011), while SpvD promotes intracellular bacterial replication in macrophages (Figueira et al., 2013).

Effector proteins SopE/E2 and SopB activate the NF-κB pathway, triggering the production of pro-inflammatory cytokines such as interleukin (IL)-1β and CXCL8 (C-X-C Motif Chemokine Ligand 8). These cytokines recruit innate immune system cells, including heterophils, macrophages, and dendritic cells, to the site of infection (Ijaz et al., 2021). In contrast, the effector protein SspH1 exhibits an inhibitory effect on the NF-κB pathway, reducing the inflammatory response (Haraga and Miller, 2003). This inhibition is facilitated by SspH1′s interaction with host kinases (PKN1) (Haraga and Miller, 2006). Similarly, the effector SpvD inhibits the NF-κB pathway by disrupting the nuclear translocation of the transcription factor p65 (Rolhion et al., 2016). These anti-inflammatory effects may aid in the survival of *Salmonella* within the intestinal tract (Haraga and Miller, 2003).

Heterophils play a role in limiting the development of *Salmonella* infection by restricting the bacteria to the intestine (Withanage et al., 2004). These cells attack the invading pathogen by releasing oxygen radicals and antimicrobial peptides such as Cathelicidin-2 (Van Dijk et al., 2009; Ijaz et al., 2021). *Salmonella* interacts with heterophils via flagellin receptor, TLR5. This interaction activates MyD88-dependent signaling pathways leading to activation of downstream signal transduction factors (MAPK and NF-κB) and secretion of proinflammatory cytokines (Gewirtz et al., 2001; Ijaz et al., 2021). Upon arrival at the site of infection, dendritic cells take up and process *Salmonella* antigens for presentation to T cells to initiate the adaptive immune response (Kalupahana et al., 2005). Interaction of macrophages and with *Salmonella* at the site of the infection leads to the production of nitric oxide, and activation of the NF-κB pathway which is responsible for the releasing of pro-inflammatory cytokines such as IL-6, IL-8, and TNFα (Setta et al., 2012; Ijaz et al., 2021). When macrophages endocytose *Salmonella*, SCVs form to protect the bacteria inside the macrophages. Inside phagocytic cells, T3SS-2 transports effector proteins to aid the survival of *Salmonella* by inhibiting lysosomal function and promoting bacterial proliferation (Santos et al., 2009). The ability of *Salmonella* serovars to persist inside macrophages and dendritic cells is vital to the spread of infection (Ijaz et al., 2021).

Natural Killer cells (NKs) play a key role in the early response to *Salmonella* infection. NK cells are located between enterocytes and can recognize infected epithelial cells and receive signals from macrophages. It has been shown in mice and humans that activated NK cells release cytoplasmic granules containing perforins and granzymes to kill the infected cells. They also release interferon (IFN)-γ to attract other immune system cells to the site of infection and initiate phagocytosis of *Salmonella*, to prevent further damage (Ijaz et al., 2021). Studies in broiler chickens have also shown an increase in NK cells shortly after infection. It was shown that NK cell degranulation and IFN-γ production were elevated, indicating NK cell activation in response to *Salmonella* infection (Meijerink et al., 2021).

B cells are another important part of the humoral immune response to pathogens. Upon differentiation into antibody-secreting plasma cells, B cells produce various immunoglobulin (Ig) isotypes, including IgY, IgM, and IgA (Shaji et al., 2023). *Salmonella* inoculation in birds has been shown to initially induce the proliferation of T cells and B cells, followed by elevation of IgY, IgM and IgA production approximately 14 days after inoculation, which coincides with clearance of *Salmonella* (Withanage et al., 1998). It has been shown that IgY and IgM levels remain elevated following primary infection but are unaffected by secondary infection, unlike IgA which significantly increased 7 days after secondary infection (Withanage et al., 2005). To evade antibody-mediated immune response, *Salmonella* produces a protein called SiiE which reduces IgG-producing B cells in bone marrow, leading to lower serum IgG levels. It has been reported that inoculation of mice with SiiE-depleted *Salmonella* induced high titers of anti-*Salmonella* IgG (Männe et al., 2019). However, the exact role of B cells in the immune response to *Salmonella* infection remains unclear. Arnold and Holt (1995) performed chemically induced B cell depletion in chickens, demonstrating higher rates of intestinal *Salmonella* shedding (Arnold and Holt, 1995). However, studies conducted with surgically bursectomized birds show *Salmonella* clearance to be independent of antibody-mediated immune response. This discrepancy may be a result of the chemical treatment affecting other cell types, particularly T cell responses to infection (Beal et al., 2006).

Clearance of *S. enterica* ser. Typhimurium infection in chickens is associated with increased IFN-γ expression and T cell proliferation. This suggests that clearance is mediated through T helper (Th)1 responses. Increased IFN-γ expression in the liver also coincides with the formation of follicular lesions in the liver, which is consistent with studies conducted in mice (Withanage et al., 2005).

Vaccination against *Salmonella* in poultry

Vaccination of chickens, along with other control measures, plays a pivotal role in comprehensive *Salmonella* control programs at the farm level. Vaccination is one of the most prominent serovar-specific risk-reduction practices (Huberman et al., 2019; Gast et al., 2024). Immunizing chickens against *Salmonella* is intended to diminish their susceptibility to infection and minimize both vertical and horizontal transmission. This vaccination also reduces environmental contamination in the barn environment and decreases contamination of eggs and meat during egg grading or meat processing, leading to lower food-borne *Salmonella* infections and safer food supply (Huberman et al., 2019; Gast et al., 2024). Additionally, vaccination helps reduce the reliance on antibiotics in poultry farming by lowering the bacterial load at the farm level. While antibiotics may be used for treatment or specific disease control, their overuse has contributed to the emergence of resistant bacterial species that can spread to humans through the food chain, exacerbating the global challenge of antimicrobial resistance (Farhat et al., 2023).

Vaccination against *Salmonella* in poultry was first demonstrated to be effective in decreasing poultry mortality due to the poultry-specific *Salmonella* serovar Gallinarum in the 1920s (Smith, 1956; Ruvalcaba-Gómez et al., 2022). This success, along with the outbreak of *Salmonella enterica* ser. Enteritidis in layer hens in Europe and the United Kingdom during the late 1980s, led to the development of a *Salmonella enterica* ser. Enteritidis vaccine. This vaccine has effectively reduced the prevalence of the serovar in poultry and continues to do so to this day (Pavic et al., 2012). Although these poultry-specific *Salmonella* serovars have been eradicated in commercial production in many developed countries, they continue to persist in numerous developing nations (Revolledo, 2018; Farhat et al., 2023; Joaquim et al., 2024).

The production of *Salmonella* vaccines for poultry is generally conducted using Typhimurium and/or Enteritidis serovars (Renu et al., 2020b).

Salmonella vaccines are classified into four categories: live-attenuated, killed (inactivated), subunit, and ghost vaccines. Each type will be discussed in detail in the following sections.

Criteria for an ideal vaccine

The desired characteristics for ideal poultry *Salmonella* vaccines are as follows (Van Immerseel et al., 2005; Desin et al., 2013; Hofacre et al.,

2021; Garcia-Llorens et al., 2024):

Safety. The vaccine should be safe and attenuated for both poultry and humans causing no side effects or disease. Additionally, it should not easily survive in the environment.

Immunogenicity. It should effectively stimulate both cell- and antibody-mediated immune responses to provide strong protection against *Salmonella*. In addition, it should promote immunity transferred from parent to chickens and rapidly stimulate active immunity, even in the presence of maternal antibody.

Efficacy.The vaccine should protect the host against systemic and intestinal infections, reduce intestinal colonization, and consequently lower fecal shedding and eggshell contamination. Furthermore, it should minimize the colonization of reproductive tissues, which helps decrease environmental contamination. It should also inhibit the colonization and invasion of different *Salmonella* strains.

Stability. An ideal vaccine should maintain stability under various storage conditions to ensure its effectiveness until administration. Additionally, it must not possess the capability to revert to a wild type.

Broad Protection. The vaccine should provide protection against prevalent serotypes of *Salmonella* and have the capability to induce cross-protection.

Long-lasting Immunity. It should provide long-term immunity, reducing the need for frequent boosters.

DIVA Capability. The vaccine should support the differentiating infected from vaccinated animals (DIVA), enabling effective surveillance and disease control.

Ease of Administration. A suitable vaccine should be easy to administer, ideally through methods such as drinking water or spray, to facilitate mass vaccination.

Cost-effectiveness. Vaccines must be cost-effective to promote widespread use in the poultry industry.

These characteristics help ensure that the vaccine is both practical and effective in controlling *Salmonella* infections in poultry, ultimately improving food safety and public health.

Live-attenuated vaccines

Live-attenuated vaccines contain bacterial pathogens that have been made non-virulent through methods such as chemical treatment and genetic engineering (Pollard and Bijker, 2021).

The strains used in these vaccines have mutations or deletions in genes that are crucial for metabolism, virulence, or survival within the host (Desin et al., 2013). These changes can impair their ability to produce flagella or fimbria, the expression of virulence-related proteins, or lipopolysaccharides, or the ability to multiply at poultry body temperature (Gast and Porter, 2020). These modifications must ensure that vaccines induce a specific immune response while preventing reversion to wild-type strains and minimizing the risk of environmental spread and persistence (Barrow, 2007). Live attenuated vaccines must persist in tissues long enough to induce protective immune responses; however, they should remain avirulent and be cleared from vaccinated birds within a few weeks of administration (Gast and Porter, 2020).

Various genes have been targeted in studies to develop genetically modified live *Salmonella* vaccines. These include genes involved in lipopolysaccharide biosynthesis (*galE*), outer membrane protein expression (*ompR*), amino acid or purine synthesis (*aro, aroA, pur, guaB*), carbon source utilization (*cya, crp*), virulence factors, and O-antigen expression (*rfaL*). Other genes, such as *nuoG, secC, phoP, rpoS, lon, cpxR, dam*, and *phoA*, have also been explored for this purpose (Figueira et al., 2013; Senevirathne et al., 2020a; Hofacre et al., 2021; Shaji et al., 2023). Live attenuated vaccines can remain in poultry for extended durations, posing a significant risk of reverting to virulent strains (Kwon et al., 2007). Therefore, developing a safe and immunogenic strain is the biggest challenge in creating live *Salmonella* vaccines.

Salmonella is known as an intracellular pathogen, which invades the epithelium of the gastrointestinal tract, penetrates the gut-associated lymphoid tissue and replicates within macrophages and dendritic cells. Virulent *Salmonella* serovars can survive and replicate within intracellular environments, particularly inside macrophages. Therefore, inducing a strong cell-mediated immune response is crucial and is most effectively achieved using live vaccines (Chappell et al., 2009; Huberman et al., 2019). Although antibody-mediated immune responses also play a key role in protection against *Salmonella* infection, it has been found that *Salmonella* can suppress B cells, and immunoglobulin Y (IgY) production as a mechanism to evade and attack the host's immune system. This highlights the critical importance of cellular immunity against *Salmonella* infection (Acevedo-Villanueva et al., 2021a).

It has been shown that a live *Salmonella* vaccine significantly reduces bacteremia during subsequent exposure and limits the distribution of the bacteria among systemic organs. Furthermore, live vaccine immunization enhances bacterial killing during the early stages of infection and provides bacteriostatic control during the first day post-challenge. Although T cell-mediated immune responses elicited by this type of vaccine are not essential for improving bacteriostasis, they are crucial for eliminating *Salmonella* from the bloodstream and tissues (Coward et al., 2014).

Early vaccination and live vaccines

Newly hatched chickens are most susceptible to *Salmonella* infection due to the lack of a protective intestinal microbiota and an immature immune system. This vulnerability can lead to widespread systemic infection and facilitate horizontal disease transmission. Therefore, early vaccination is crucial for protecting chicks against natural infection upon their arrival at farms (Van Immerseel et al., 2005; Kilroy et al., 2015; Huberman et al., 2019). Numerous studies have shown that oral administration of live attenuated vaccines to chicks after hatching can induce rapid protection against environmental challenges, potentially through colonization inhibition or competitive exclusion (Van Immerseel et al., 2005; Gast and Porter, 2020). It should be noted that not all vaccine strains have the same ability to induce an inhibitory effect. Therefore, it is essential to select the appropriate strains that can promote intestinal colonization and invasion-inhibition effects against wild *Salmonella* strains (Hofacre et al., 2021).Studies have shown that early vaccination on the first day post-hatch helps reduce *Salmonella* invasion into organs and its colonization in the ceca within the first 8 weeks (Barrow et al., 2000; Huberman et al., 2019).

Interaction between live vaccines and antibiotics

There is some evidence that antibiotics can significantly impact the colonization of live attenuated *Salmonella* vaccine in chickens. In some parts of the world, poultry flocks are frequently raised under intensive conditions that require substantial antimicrobial use for disease prevention, treatment, and growth promotion. In a study, chickens were treated with various antibiotics, including ceftiofur, amoxicillin, enrofloxacin, and lincomycin-spectinomycin, and then immunized at different intervals after antibiotic withdrawal. The results showed that the highest colonization of the vaccine strain in the cecum occurred when chickens were vaccinated 2 days after ceftiofur withdrawal and 4 days after amoxicillin, enrofloxacin, and lincomycin–spectinomycin withdrawal. It was suggested that the timing of vaccination relative to antibiotic withdrawal is crucial for effective colonization of the live vaccine strains (Hu et al., 2021).

Live vaccines and Salmonella monitoring programs

A crucial factor to consider when using live vaccines is that the vaccine strains could potentially interfere with *Salmonella* monitoring programs. Diagnosis, surveillance, and control of *Salmonella* in poultry require methods that allow for rapid discrimination between vaccine and non-vaccine isolates (DIVA capability) (Acevedo-Villanueva et al.,

2021a). These tests are crucial for distinguishing between vaccine strains and wild-type *Salmonella* strains. They can be conducted using various methods, including serological, molecular, and bacteriological tests, as well as whole-genome sequencing (Garcia-Llorens et al., 2024; Maurischat et al., 2015; Tang et al., 2019).

In a study, researchers developed and validated real-time PCR (qPCR) and High-Resolution Melting (HRM) assays to differentiate *Salmonella* vaccine strains from wild-type field strains. These methods demonstrated 100% inclusivity, accurately identifying all tested vaccine strains, and 100% exclusivity, with no cross-reactivity to non-vaccine strains (Maurischat et al., 2015). Additionally, the researchers achieved 100% selectivity in distinguishing *Salmonella* vaccine strains from wild-type field strains using TaqMan-qPCR and HRM-qPCR (Maurischat et al., 2015). In another study, the ASAP medium (Selective chromogenic medium for isolating *Salmonella* spp. from food and environmental samples) effectively differentiated *Salmonella enterica* ser. Enteritidis vaccine strains from field strains, achieving 100% concordance with the three previously authorized differentiation assays. This method proved to be faster, easier to implement, and more cost-effective compared to alternative approaches (Garcia-Llorens et al., 2024).

Interaction between live vaccines and gut microbiota

Currently, few studies have investigated the effect of live attenuated *Salmonella* vaccines on the intestinal microbiota of poultry, highlighting the need for further investigation in future research (Park et al., 2017; Jia et al., 2020). In a recent study, the effects of different doses and diluents of a commercial live attenuated *Salmonella enterica* ser. Typhimurium vaccine, as well as the age of the birds, on the gut microbiota of commercial layer chickens were evaluated (Khan et al., 2024). The results showed distinct gut microbial communities between vaccinated and unvaccinated chickens. Additionally, the highest vaccine dose did not significantly alter the abundance of microbial genera. The age of the chickens had a more significant effect on microbiota composition than the vaccine dose and diluent. Overall, the vaccine minimally affected the gut microbiota structure, with more significant changes attributed to the age of the chickens (Khan et al., 2024). In another study, it was shown that the oral administration of a *Salmonella enterica* ser. Typhimurium vaccine to 4-day-old White Leghorn chickens significantly influenced the gut microbiota composition and exerted a strong selective pressure on microbial diversity (Redweik et al., 2020).

Killed or inactivated vaccines

Inactivated (or killed) vaccines are produced by exposing bacterial or viral pathogens to chemical agents (such as formaldehyde, β-propiolactone, acetone, or ethanol), physical treatments (such as heat at 60°C for 1 h), or irradiation (ultraviolet or gamma rays). These processes eliminate the ability of pathogens to replicate while maintaining their antigenic properties, ensuring the immune system can recognize and respond to the pathogens without the risk of active infection (Helmy et al., 2023; Ji et al., 2021; Mak et al., 2013; Rabie and Amin Girh, 2020).

The primary immunological response elicited by inactivated *Salmonella* vaccines is antibody-mediated immunity, characterized by the production of circulating antibodies. Since these vaccines contain non-replicating bacterial components, they are less effective at stimulating a strong cell-mediated immune response compared to live attenuated vaccines (Singh, 2009; Gast and Porter, 2020; Acevedo-Villanueva et al., 2021c; Shaji et al., 2023).

This limitation arises because inactivated pathogens do not actively invade host cells, leading to reduced intracellular antigen presentation and subsequent T-cell activation. Given that an effective defense against *Salmonella*, as an intracellular pathogen, requires robust T-cell responses and cytokine signaling, inactivated vaccines may not provide long-term protection against persistent or systemic infections (Berndt and Methner, 2004).

When an inactivated *Salmonella* vaccine is administered, antigen-presenting cells (APCs), such as dendritic cells and macrophages, engulf the bacterial components and process them for presentation via major histocompatibility complex (MHC) class II molecules. This activates CD4+ helper T cells, which, in turn, stimulate B cells to produce antigen-specific antibodies, primarily IgY. These antibodies neutralize *Salmonella* and prevent its systemic spread (Rabie and Amin Girh, 2020). However, since inactivated vaccines do not engage the MHC class I pathway, they fail to efficiently activate CD8+ cytotoxic T cells, which are crucial for eliminating intracellular pathogens (Ji et al., 2021). Furthermore, the lack of a strong mucosal immune response makes inactivated vaccines less effective at reducing *Salmonella* colonization in the intestines, a key site for bacterial persistence and transmission (Marouf et al., 2022).

To enhance the immune response, adjuvants such as mineral oils or aluminum hydroxide are often incorporated into inactivated vaccine formulations. These adjuvants improve antigen presentation and prolong immune system stimulation (Rabie and Amin Girh, 2020). Despite these enhancements, inactivated vaccines generally do not match the dual immune stimulation provided by live vaccines, which effectively induce both antibody- and cell-mediated responses (Gast and Porter, 2020; Acevedo-Villanueva et al., 2021c; Shaji et al., 2023).

In commercial poultry production, inactivated vaccines are commonly used to control *Salmonella* infections, particularly against prevalent serotypes such as *Salmonella enterica* ser. Enteritidis and *Salmonella enterica* ser. Typhimurium (Gast and Porter, 2020). However, their overall effectiveness depends on multiple factors, including the vaccine administration method, the type and efficacy of adjuvants, and the frequency of booster doses (Rabie and Amin Girh, 2020; Marouf et al., 2022). Therefore, while inactivated vaccines remain a critical component of *Salmonella* control programs, their immunological limitations must be considered when designing comprehensive vaccination strategies.

Inactivated *Salmonella* vaccines are serovar-specific and exhibit optimal efficacy when the antigens of the vaccine strain are homologous to those of the infecting pathogens (Ruvalcaba-Gómez et al., 2022; Shaji et al., 2023). Therefore, to effectively control the spread of various *Salmonella* serovars in poultry, multivalent inactivated vaccines are essential (Crouch et al., 2020; Huberman et al., 2022). In a study, the safety and efficacy of a novel inactivated trivalent *Salmonella enterica* vaccine was evaluated under field conditions. This vaccine consisted of the *Salmonella* serovars Enteritidis (O:9, serogroup D), Typhimurium (O:4, serogroup B), and Infantis (O:7, serogroup C1). For this study, broiler breeder pullets reared under commercial conditions were vaccinated intramuscularly in the breast muscle at 10 and 17 weeks of age. The vaccine demonstrated safety, with no observed local or systemic reactions, and did not negatively impact bird performance. Vaccination led to significant increases in serovar-specific antibodies, which were sustained for at least 56 weeks. Vaccinated birds exposed to homologous challenges during the initial laying period exhibited significantly reduced fecal shedding and organ invasion. Additionally, following a heterologous challenge with S. Hadar (O:8, serogroup C2), fecal shedding was significantly decreased (Crouch et al., 2020).

One of the disadvantages of killed *Salmonella* vaccines is that they must be administered via injection. In the poultry industry, mass vaccination is crucial for maximizing the protection of large poultry flocks. The intramuscular route is time-consuming and impractical for vaccinating commercial poultry flocks. Furthermore, improper administration can lead to focal inflammatory myositis, decreasing tissue quality and potentially causing the removal of the carcass at the slaughterhouse (Acevedo-Villanueva et al., 2021c; Ruvalcaba-Gómez et al., 2022).

Combination of live and killed vaccines

Control of *Salmonella* in poultry is vital, particularly in upstream flocks, where vertical transmission from grandparent or breeder flocks

to progeny via eggs can occur (Gast and Porter, 2020; Hofacre et al., 2021). Preventing *Salmonella* infection in these upstream flocks is essential for reducing its spread throughout the production chain. One of the most effective strategies for controlling *Salmonella* infection in these flocks involves using a combination of live and inactivated vaccines during the rearing period. This approach helps maintain immunity that can be transferred to eggs and progeny during the production period (Bailey et al., 2007; Berghaus et al., 2011; Gast and Porter, 2020; Hofacre et al., 2021).

Live attenuated vaccines localize in mucosal tissues, preventing invasion by environmental strains and inducing both antibody- and cell-mediated immune responses. This provides extensive protection against various serovars. On the other hand, killed or inactivated vaccines are generally safer, particularly for immunocompromised birds, and more stable than live vaccines.

In several countries, the implementation of combination vaccination programs in laying hens has contributed to a decline in the number of reported *Salmonella enterica* ser. Enteritidis cases in humans (Cogan and Humphrey, 2003; Gantois et al., 2006). However, a lesser effect has been observed on fecal shedding and environmental contamination within poultry houses (Sharma et al., 2018; Gast and Porter, 2020).

To optimize protection and minimize adverse reactions, vaccination programs should incorporate the sequential use of live attenuated vaccines followed by inactivated vaccines (Gast and Porter, 2020; Renu et al., 2020b; Huberman et al., 2022). The use of inactivated vaccines after live vaccine administration generates strong and uniform antibody levels, enhancing the immune response against the target pathogen (Gast and Porter, 2020; Huberman et al., 2022). This combination approach can lead to extended protection and potentially reduce costs for producers by decreasing the likelihood of *Salmonella* outbreaks over time (Van Immerseel et al., 2005; Hofacre et al., 2021). To achieve a high level of immunity, it is recommended to administer live vaccines at least twice during the rearing period and to use an inactivated vaccine containing multiple *Salmonella* serotypes before the onset of production (Gast and Porter, 2020; Hofacre et al., 2021; Joaquim et al., 2024).

A recent study demonstrated that an inactivated trivalent *Salmonella* vaccine, formulated with strains of *Salmonella enterica* ser. Enteritidis, *Salmonella enterica* ser. Typhimurium, and *Salmonella* Infantis, effectively controls *Salmonella enterica ser. Typhimurium* and *Salmonella* Infantis, while significantly reducing the excretion of *Salmonella enterica* ser. Enteritidis. Combining live vaccines with at least one dose of the inactivated trivalent vaccine also addresses the protection gap against *Salmonella enterica* ser. Enteritidis, leading to a significant reduction in fecal shedding (Huberman et al., 2022).

Salmonella poultry vaccines require a withdrawal period of 21 days prior to the slaughter age in broilers or the onset of egg production in layer and breeder chickens. Administering a live vaccine followed by a killed vaccine booster can eliminate the time constraints associated with live vaccine boosters, allowing producers to adhere to the withdrawal period requirements (Acevedo-Villanueva et al., 2021c; Hofacre et al., 2021).

Subunit vaccines

Subunit vaccines consist of protein or polysaccharide components from a pathogen that can induce a protective immune response. These vaccines contain key antigenic components that elicit immunity without the risk of causing disease (Micoli and MacLennan, 2020; Helmy et al., 2023). These vaccines contain one or more recombinant peptides, proteins, or polysaccharides derived from the structure of the target pathogen. When combined with an appropriate adjuvant, they elicit a robust antibody-mediated immune response (Liljeqvist and Ståhl, 1999). Recent studies have demonstrated that vaccines derived from outer membrane proteins (OMPs), outer membrane vesicles (OMVs), and flagellin proteins (FliC) of *Salmonella enterica* ser. Enteritidis are highly immunogenic in chickens. These vaccines have demonstrated the ability to elicit a significant antigen-specific immune response against *Salmonella* infections and reduce bacterial shedding in poultry (Acevedo-Villanueva et al., 2022; Ruvalcaba-Gómez et al., 2022; Helmy et al., 2023; Shaji et al., 2023). Most subunit *Salmonella* vaccines are administered via intramuscular or subcutaneous injection (Ruvalcaba-Gómez et al., 2022).

Oral vaccines in poultry flocks are generally considered safe, easy to administer, and capable of inducing both systemic and mucosal immune responses (Acevedo-Villanueva et al., 2021a, 2021c). However, there is currently no commercially available oral inactivated vaccine for *Salmonella* in poultry (Gast and Porter, 2020). Developing such vaccines is challenging due to the harsh gastrointestinal environment and mucus barriers, which require a safe and efficient delivery system. In recent years, nanoparticle-based delivery systems have emerged as ideal vectors for oral vaccine development. Successful immunization requires the delivery of intact antigens to the gastrointestinal tract (GIT), efficient transport across the mucosal barrier, and effective activation of antigen-presenting cells (APCs) (Acevedo-Villanueva et al., 2021a). However, the primary challenges associated with oral antigen delivery in killed vaccines include ensuring the successful delivery of antigens to the GIT and their subsequent uptake by APCs (Acevedo-Villanueva et al., 2021a). The acidic environment of the GIT poses a challenge for delivering unprotected vaccine antigens to the target intestinal tissues, as it may lead to degradation or denaturation of the antigens (Homayun et al., 2019). In recent years, researchers have successfully developed innovative subunit nanoparticle vaccines against *Salmonella* for use in poultry. These vaccines are designed to protect vaccine antigens from the extreme pH of the gastrointestinal tract while effectively delivering them to the intestinal Peyer's patches (PPs) (Acevedo-Villanueva et al., 2021a). The nanoparticle-based vaccine delivery system protects the encapsulated antigens from degradation and promotes their uptake through endocytosis by dendritic cells in the mucosal tissues (Dolatyabi et al., 2024; Geevarghese et al., 2023). These vaccines have been shown to be safe and effective in eliciting substantial antigen-specific immune responses as well as reducing the intestinal *Salmonella* load in both broilers and layers (Renu et al., 2020b; Acevedo-Villanueva et al., 2021a; Dolatyabi et al., 2024).

Nano vaccines

Nano vaccines are cutting-edge vaccines that use nanoparticles as delivery vehicles or adjuvants to enhance vaccine stability, targeted delivery, and immune response effectiveness (Gheibi Hayat and Darroudi, 2019). Nanoparticle-based vaccines are being studied as a promising alternative to traditional *Salmonella* vaccines for poultry. These polymeric vaccines are made up of colloidal particles ranging from 10–500 nm in size (Han et al., 2018). They include chitosan-based nanoparticles (a natural biodegradable copolymer) and polyanhydride-based nanoparticles (a synthetic biodegradable copolymer), both of which possess mucoadhesive properties (Tang et al., 2014; Khobragade and Puranik, 2015).

Some of the advantages of using polymeric nanoparticles as delivery vehicles for *Salmonella* vaccines in poultry are as follows: (a) Protection of antigens from the acidic environment of the gastrointestinal tract (GIT), (b) Their small size facilitates uptake by APCs and allows them to cross the GIT mucosa, (c) Nanoparticles provide controlled and sustained release of the antigen, acting as a booster dose, (d) Can function as adjuvants and possess intrinsic immunomodulatory activity, (e) They enhance both antibody- and cell-mediated immune responses, (f) They are suitable for administration via the oral route in poultry flocks, (g) They reduce the need for cold chains to maintain bioactivity (Acevedo-Villanueva et al., 2021a).

Salmonella chitosan-based nanoparticles vaccine

Recent studies have explored the potential for mass delivery of the *Salmonella* chitosan nanoparticle (CNP) vaccine. This vaccine is designed to include *S.* Enteritidis outer membrane proteins (OMPs),

flagellin protein, and a flagellin surface coating. The vaccine has been administered through various methods, including oral gavage, water, feed, in-ovo, gel spray, and in a combined live-followed-by-killed vaccination scheme (Acevedo-Villanueva et al., 2022; Acevedo-Villanueva et al., 2021a, 2021b, 2021c; Han et al., 2020b; Renu et al., 2020a; Shaji et al., 2023). The ability of the CNP vaccine to induce both innate and adaptive immune responses following oral inoculation has been analyzed in several studies. The results demonstrate its capability to block the primary stage of *Salmonella* infection in both broilers and layers while inducing an effective antigen-specific recall response (Akerele et al., 2020; Han et al., 2020c; Renu et al., 2020b; Acevedo--Villanueva et al., 2021a).

A study by Acevedo-Villanueva et al. (2021) evaluated the effects of administering a live *Salmonella* vaccine followed by a killed chitosan nanoparticle (CNP) vaccine booster on broilers' immune responses. The findings demonstrated that both single and combined administration of the *Salmonella* CNP vaccine effectively induced an antigen-specific immune response against *S*. Enteritidis, activated the intestinal mucosal immune system, and significantly reduced *S*. Enteritidis loads in the ceca. Furthermore, production performance parameters remained unaffected at 28 days of age (Acevedo-Villanueva et al., 2021c). The findings of this study indicate that the CNP vaccine is flexible in its application, as it can be used either as an initial dose or as a booster. This adaptability makes it a promising alternative vaccine candidate for protecting broilers against *S*. Enteritidis (Acevedo-Villanueva et al., 2021c).

Multiple studies have shown that CNP vaccines can significantly boost antigen-specific mucosal IgA production in both broilers and layers. These enhanced immune responses have been observed at various time points, both post-vaccination and post-challenge, highlighting the potential of CNP vaccines to stimulate robust mucosal immunity and improve disease resistance (Renu et al., 2020b; Acevedo-Villanueva et al., 2021b; Dolatyabi et al., 2024).

A study assessed the impact of encapsulating the immunogenic OMPs and flagellin (FLA) of *Salmonella enterica* ser. Enteritidis within mannose-chitosan nanoparticles (OMPs-FLA-mCS NPs). Administration of this nanoparticle via oral inoculation using a prime-boost regimen (on day -3 and again 3 weeks later), successfully elicited mucosal immunity and reduced *S*. Enteritidis colonization by over 1 log10 CFU in broiler chickens (Han et al., 2020b).

Another study investigated whether *Salmonella enterica* ser. Enteritidis antigens encapsulated in OMPs-FLA-mCS NPs could induce cross-protection against *Salmonella enterica* ser. Typhimurium in broilers. The results revealed that the encapsulated vaccine elicited stronger cross-protective antibody responses compared to the commercial Poulvac *S. enterica* ser. Typhimurium vaccine, which contains a modified-live *S. enterica* ser. Typhimurium strain. This suggests that the OMPs-FLA-mCS NPs vaccine could offer broader protection against multiple *Salmonella* serovars in poultry (Dolatyabi et al., 2024). When compared to the commercial vaccine group, the OMPs-FLA-mCS NP vaccine led to a notable increase in the production of secretory IgA and IgY antibodies specific to OMPs and FLA in specimens obtained following both vaccination and subsequent challenge. It was suggested that the orally administered CNP vaccine elicited cross-protective mucosal immune responses against *S. enterica* ser. Typhimurium colonization in broilers (Dolatyabi et al., 2024). These results were consistent with findings from other researchers (Renu et al., 2020a, 2020b). Therefore, it was proposed that this CNP vaccine could be a viable alternative to both live and killed *Salmonella* vaccines for birds (Han et al., 2020a; Renu et al., 2020b; Dolatyabi et al., 2024).

Numerous studies have demonstrated that, upon thriving in the acidic pH of the GIT, CNP vaccines adhere to the mucosal surface and are taken up by ileal Peyer's patches (PPs) and lamina propria immune cells. In contrast, CNPs without a flagellin surface coating are poorly taken up by the PPs (Acevedo-Villanueva et al., 2021a).

The results of another study indicated that CNP vaccines can also be mass-administered via the in-ovo route. Findings demonstrated that in-ovo vaccination with the CNP *Salmonella* vaccine induces antigen-specific systemic and mucosal immune responses without negatively affecting hatchability or the performance of broilers. Additionally, this approach reduced *S.* Enteritidis cecal colonization in birds and did not impact production performance, CD4+/CD8+ T-cell frequencies, pro- and anti-inflammatory cytokine levels, or iNOS levels (Acevedo-Villanueva et al., 2021b). While further studies are needed to explore this vaccination method, successful immunization against *Salmonella* at the embryonic stage could serve as a valuable prevention strategy for the poultry industry.

Acevedo-Villanueva et al. (2022) evaluated the efficacy of a CNP vaccine for *Salmonella* control in broilers, delivered via gel spray. The vaccine did not negatively impact production performance and induced strong systemic and mucosal immune responses. However, it successfully triggered a recall immune response against *S*. Enteritidis flagellin and other *Salmonella* antigens such as *S.* Enteritidis heat-killed antigen (HKA), *S. enterica* ser. Typhimurium HKA, and *S.* Litchfield HKA, reduced *Salmonella* loads in the ceca, spleen, and small intestine, and preserved gut permeability. Additionally, it increased the expression of some cytokines like IL-6, IL-17, and tumor necrosis factor-alpha (TNF-α), highlighting its potential as an effective *Salmonella* control strategy in poultry (Acevedo-Villanueva et al., 2022). Nevertheless, further research is needed to explore the vaccine's potential for providing cross-protection against both homologous and heterologous *Salmonella* serovars in future studies.

Salmonella polyanhydride-based nanoparticles vaccine

Compared to chitosan-based nanoparticle vaccines, there have been significantly fewer studies on the use of poly(methyl vinyl ether-co-maleic anhydride) (PVM/MA)-based polymeric nanoparticle *Salmonella* vaccines (Renu et al., 2018; Ochoa-Repáraz et al., 2021).

A *Salmonella* subunit vaccine was developed using poly(methyl vinyl ether-co-maleic anhydride) (PVM/MA) nanoparticles containing *Salmonella enterica* ser. Enteritidis outer membrane proteins (OMPs) and flagellin, with a surface flagellin coating (Renu et al., 2018). The vaccine demonstrated resistance in acidic environments and exhibited ideal physicochemical properties for oral delivery, including optimal particle size, charge, morphology, biocompatibility, and pH stability. This vaccine enhanced OMP-specific immune responses by increasing IgY production and IFN-γ secretion. Furthermore, administration of the vaccine upregulated TLR2, TLR4, TGF-β, and IL-4 gene expression in cecal tonsils. Importantly, it cleared cecal *Salmonella* colonization in 33% of vaccinated birds (Renu et al., 2018).

In a study by Ochoa-Repáraz et al. (2021), a *Salmonella* PVM/MA nanoparticle vaccine containing a heat extract (HE) fraction from the *S*. Enteritidis cell surface was formulated. The researchers assessed vaccine stability in drinking water and its efficacy in layer hens following experimental infection. Administering two doses of the PVM/MA nanoparticle vaccine orally to six-week-old chickens significantly decreased *Salmonella* excretion in vaccinated layer hens (Ochoa-Repáraz et al., 2021). Given the efficacy of this treatment in reducing bacterial excretion, the researchers concluded that HE nanoencapsulation derived from *S*. Enteritidis is a promising new vaccination strategy against salmonellosis in farm settings (Ochoa-Repáraz et al., 2021).

It is important to note that particle size uniformity is a crucial factor that influences the internalization of nanoparticles by immune system cells (Acevedo-Villanueva et al., 2021a). However, the average nanoparticle size can vary from batch to batch in different studies (Akerele et al., 2020; Han et al., 2020b; Renu et al., 2020b). This variation occurs due to the challenges of synthesizing particles that remain homogeneous in both shape and size in a laboratory setting (Acevedo-Villanueva et al., 2021a). Addressing this issue is essential, as it could potentially affect antigen uptake by dendritic cells, leading to varying immune response outcomes (Acevedo-Villanueva et al., 2021a). Additional obstacles in nanoparticle vaccine development include the high costs associated with

particle production, low drug encapsulation efficiency, challenges with initial burst release or incomplete antigen release, and the lack of standardized testing protocols tailored to each material, as well as the absence of reference particles for validation (Su and Kang, 2020; Acevedo-Villanueva et al., 2021a).

The development of polymeric nanoparticle vaccines for poultry is still in its early stages and more studies are needed before they can be used as commercial vaccines in the poultry industry. However, these vaccines have shown promising results for mass administration of vaccines against *Salmonella* in both broiler and layer chickens (Renu et al., 2018; Renu et al., 2020a, 2020b; Han et al., 2020a; Acevedo-Villanueva et al., 2021a, 2021b; Ochoa-Repáraz et al., 2021; Dolatyabi et al., 2024).

Ghost vaccines

Ghost vaccines are a type of vaccine that uses empty bacterial cell envelopes, known as bacterial ghosts (BGs), to stimulate an immune response. These vaccines are produced by eliminating the cytoplasmic content of bacterial cells while maintaining the structural integrity of the cell envelope. This characteristic makes them safe and non-replicative, establishing them as a promising platform for vaccine development (Batah and Ahmad, 2020; Park, 2023).

These vaccines are particularly promising because they can efficiently present antigens to the immune system and trigger immune responses (Hajam et al., 2017). Since the natural structure of OMPs in bacteria, such as fimbriae, lipopolysaccharides, and peptidoglycan, remains unchanged during the production of BGs, these empty bacterial envelopes retain high immunogenicity (Jalava et al., 2002).

BGs can be produced through genetic engineering or chemical induction. Recent advancements in genetic engineering and chemical biotechnology have facilitated the development of various types of BGs (Chen et al., 2021).

Genetic engineering method

This method which is limited to Gram-negative bacteria and uses bacteriophage-encoded proteins to generate BGs. Specific lysis genes, such as the *E* gene from bacteriophages, are inserted into the bacterial genome. Protein E-mediated lysis induces the formation of transmembrane tunnel structures by fusing the inner and outer membranes of Gram-negative bacteria, resulting in the release of cytoplasmic contents. Importantly, the structural integrity of the bacterial cell envelope remains intact, a feature critical for the vaccine's efficacy (Jawale and Lee, 2014; Hajam et al., 2017; Batah and Ahmad, 2020; Park, 2023).

Chemical method

This method is applicable to both Gram-positive and Gram-negative bacteria. The "sponge-like" method is a widely used chemical approach for generating BGs. In this process, chemical reagents such as sodium hydroxide (NaOH), hydrochloric acid (HCl), sulfuric acid (H2SO4), sodium dodecyl sulfate (SDS), nitric acid (HNO3), hydrogen peroxide (H2O2), and Tween-80 are employed to create pores in the bacterial cell walls. These reagents are applied at their minimum inhibitory concentrations (MIC) or minimum growth concentrations to disrupt the bacterial cell membrane and facilitate the release of cytoplasmic contents. The released contents are subsequently separated through centrifugation, effectively removing them while preserving the structural integrity of the bacterial cell envelope (Hajam et al., 2017; Batah and Ahmad, 2020; Park, 2023).

Several studies have demonstrated that bacterial ghosts generated from pathogenic bacteria can protect against fatal Gram-negative infections. *E. coli, Salmonella spp.*, and *Vibrio cholerae* are among the most used bacterial species for ghost production (Jalava et al., 2002; Batah and Ahmad, 2020).

To date, all ghost vaccines developed against *Salmonella enterica* ser. Enteritidis and *Salmonella enterica* ser. Typhimurium in poultry have been created using genetic engineering methods (Peng et al., 2011; Jawale and Lee, 2014; Senevirathne et al., 2020b; Senevirathne et al., 2021).

Recent studies have demonstrated that *Salmonella* ghost vaccines can elicit robust immune responses in poultry. For example, ghosts of *Salmonella enterica* serovar Enteritidis induced higher levels of specific antibodies (IgY), IFN-γ and IL-4 secretion compared to inactivated vaccines (Peng et al., 2011). Ghosts carrying adjuvants such as *Escherichia coli* heat-labile enterotoxin B subunit (LTB) protein showed comparable efficacy to commercial vaccines, reducing internal organ colonization and egg contamination without adverse effects (Jawale and Lee, 2014). Moreover, *S. enterica* serovars Enteritidis and Typhimurium ghosts engineered with FliC antigen induced antibody- and cell-mediated immune responses, leading to reduced systemic bacterial loads (Senevirathne et al., 2020b). Similar enhancement of immunogenicity was observed in *S. enterica* serovar Gallinarum ghosts expressing FliC, which protected chicks against lethal challenge (Hajam et al., 2018). These findings suggest that FliC decoration can enhance the potency of ghost vaccines, making them a promising and safe strategy for preventing salmonellosis in poultry.

Despite the limited studies conducted to date, the data presented above underscore the critical need for further research into ghost vaccines as potential candidates for *Salmonella* vaccination in poultry. It is crucial to point out that, while ghost *Salmonella* vaccines are considered safe and capable of enhancing both cell- and antibody-mediated immune responses, they consist of non-living cells and lack the ability to penetrate the gut epithelium, which is necessary to trigger a potent mucosal immune response. This limitation may pose a significant challenge to the effective use of ghost vaccines for controlling *Salmonella* in chickens (Shaji et al., 2023).

Conclusions and recommendations

Salmonella infection remains one of the major challenges for the poultry industry, not only due to the potential mortality and reduced production efficiency caused by clinical or subclinical infections in birds, but also because it is a leading cause of foodborne illness in humans worldwide. The prevalence of *Salmonella* in commercial poultry flocks has been linked to the incidence of human salmonellosis, and as a result, international poultry markets are increasingly enforcing food safety-driven restrictions. Therefore, controlling salmonellosis is vital for protecting public health, reducing economic burdens, and ensuring food safety. Vaccination plays a pivotal role in comprehensive *Salmonella* control programs at the farm level, helping to prevent human transmission through poultry products.

Over the past few decades, significant progress has been made in developing novel strategies to create potential *Salmonella* vaccines for poultry. Modified live attenuated, killed, subunit, and bacterial ghost vaccines have demonstrated high efficacy in various studies. Currently, most commercially licensed *Salmonella* vaccines for poultry are live attenuated or killed formulations. However, a few oral subunit vaccines such as polymeric nanoparticle-based formulations or those using outer membrane proteins have also been developed and, in some cases, commercialized in certain countries or approved for clinical use in other contexts (Siddique et al., 2024; Figueira et al., 2013; Najahi-Missaoui et al., 2020; Acevedo-Villanueva et al., 2021a; Curtiss, 2024).

To achieve optimal protection against *Salmonella* infection in poultry, vaccination programs should incorporate the sequential use of live attenuated vaccines followed by multivalent inactivated vaccines. However, current commercial vaccines have certain disadvantages. Live attenuated vaccines carry the risk of strain reversion to virulence, environmental shedding, and potential transmission to humans. They may also interfere with salmonellosis monitoring programs and require a strict cold chain to maintain their efficacy. On the other hand, killed vaccines are generally preferred for their safety profile but induce weaker cell-mediated immune responses compared to live vaccines. Additionally, their intramuscular administration is time-consuming,

causes tissue damage, and incurs additional costs.

Polymeric nanoparticle vaccines have shown promising results for the mass administration of vaccines against *Salmonella* in poultry. Administering these vaccines orally as killed vaccines eliminates the risk of bacterial reversion to a virulent form, reduces labor costs, enhances birds' welfare, and improves mucosal, cell- and antibody-mediated immune responses. Furthermore, they do not require a cold chain for preservation. Therefore, polymeric nanoparticle vaccines have the potential to be a viable alternative in *Salmonella* control strategies for poultry.

Ghost *Salmonella* vaccines are considered safe and can enhance both cell- and antibody-mediated immune responses. However, they cannot trigger a potent mucosal immune response. Furthermore, their administration via injection presents a drawback for these vaccines. Therefore, these limitations may pose a significant challenge to the effective use of ghost vaccines in controlling *Salmonella* in chickens.

Future studies can investigate the influence of live attenuated *Salmonella* vaccines on the host intestinal microbiota, as well as the effect of gut microbiota on vaccine efficacy. Furthermore, the research could explore immunization against *Salmonella* at the embryonic stage using in-ovo vaccination, the development of ghost vaccines produced through chemical methods, and the feasibility of mass vaccination with ghost vaccines in poultry farms. Since the development of polymeric nanoparticle vaccines for poultry is still in its early stages, further studies are essential before they can be commercialized in the poultry industry. These studies should focus on ensuring uniformity of size and shape of nanoparticles between batch-to-batch formulations, evaluating vaccine efficacy and performance, exploring cross-protection capabilities conferred by vaccines, and reducing production costs.

CRediT authorship contribution statement

Ali Tolooe: Writing – original draft, Writing – review & editing. **Mohammadali Alizadeh:** Writing – original draft, Writing – review & editing. **Katherine Blake:** Writing – original draft. **Janan Shoja Doost:** Writing – original draft. **Shayan Sharif:** Writing – review & editing.

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