Current perspectives in avian salmonellosis: Vaccines and immune mechanisms of protection

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Primary Audience: Veterinarians, Poultry Researchers, Poultry Industry Personnel

SUMMARY

Salmonellosis is one of the most prevalent foodborne diseases worldwide. Food animals have been identified as reservoirs for nontyphoid *Salmonella* infections. In poultry, host-specific *Salmonella* infections cause fowl typhoid and pullorum diseases that produce economic losses in different parts of the world. Several measures have been used to prevent and control *Salmonella* infections in poultry, and vaccination is the most practical measure because it avoids contamination of poultry products and by-products and prevents disease in humans. *Salmonella* vaccines can decrease public health risk by reducing colonization and organ invasion, including invasion of reproductive tissues, and by diminishing fecal shedding and environmental contamination. We review available information on the host-specific and non-host-specific *Salmonella* serotypes found in poultry and the improved understanding of the pathogenesis of and immune responses to infection. We also include some approaches based on updated publications regarding killed and live attenuated vaccines and their immune mechanisms of protection.

Key words: avian salmonellosis, immune response, *Salmonella* vaccine

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INTRODUCTION

Salmonella enterica infects different hosts and is one of the most common causes of food poisoning in humans and a wide variety of animals. Food animals in particular have been identified as reservoirs for nontyphoid *Salmonella* infections. In poultry, host-specific *Salmonella* infections cause systemic disease and are primarily caused by *Salmonella enterica* serovar Gallinarum and serovar Pullorum, which cause fowl typhoid and pullorum disease, respectively [1], and are often avirulent in mammals. These diseases have been eradicated in many developed countries, but they remain responsible for economic losses in the poultry industry in developing countries. By contrast, non-host-specific *Salmonella* are commensal in poultry and can persist in the gastrointestinal tract. They are mainly asymptomatic but are associated with widespread human illness. Food vehicles such poultry, eggs, and poultry by-products are among the most common sources of *Salmonella* infections [2–4]. Contaminated poultry meat has been implicated as a major vehicle for the transmission of the bacteria to humans [5, 6].

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There is continuing interest in finding ways of preventing flock infection and, hence, contamination of poultry products with *Salmonella enterica*. Control measures are difficult to use effectively because numerous potential sources of *Salmonella* infection and product contamination exist in an integrated poultry enterprise [7]. Control of *Salmonella* infections in poultry farms needs to begin with good farming practices and appropriate management associated with strict sanitary measures. Preventive and curative strategies have been widely applied to reduce the incidence of *Salmonella* colonization in chickens at the farm level [8]. Various prophylactic measures have been used to prevent and control *Salmonella* infection in poultry production, and vaccination is the most practical measure to avoid contamination of poultry products and by-products and to prevent the disease in humans. Killed and live attenuated products have been used for controlling *Salmonella* in poultry production, and vaccination with live attenuated products has proved to be effective [9]. This review is a brief overview focusing on *Salmonella* in chickens, vaccines, and immune mechanisms of protection.

SALMONELLA SEROTYPES FOUND IN POULTRY

Host-Specific Salmonella

Salmonella Gallinarum is different from the rest of the known *Salmonella* serovars; it is the only serovar (including the 2 biovars Gallinarum and Pullorum) that is highly specific to fowl [10]. Avian systemic salmonellosis fowl typhoid and pullorum diseases are widely distributed throughout the world and have been eradicated from commercial poultry in many developed countries in Western Europe, the United States, Canada, Japan, and Australia [11], but they remain of high economic importance in the poultry industry in South America and Asia [12]. Although many countries have succeeded in eradicating both diseases by tests and slaughter on infected farms, developing countries often use other strategies for eradication, including the use of vaccines and prophylactic treatment with antibiotics.

Non-Host-Specific Salmonella

Different serotypes of *Salmonella* that infect a wide range of hosts have been reported worldwide in poultry and poultry products, as shown in Table 1. Some of these serotypes appear for a short time and then disappear. Others become established in the "*Salmonella* circle" and are found in a wide variety of poultry products and by-products. Contamination of eggs may occur by direct transmission from an infected ovary or oviduct, or by contamination of the eggshell. Different serotypes of *Salmonella* present in feces can penetrate the interior of eggs and grow during storage [13], and some have been isolated from the ovaries of naturally infected chickens [14].

PATHOGENESIS IN POULTRY

Most of the known *Salmonella* serotypes are pathogenic to humans, animals, or both. Although *Salmonella* pathogenesis has been well characterized in the mammalian model [15], information is limited on specific mechanisms in avian species [2]. Poultry species can be infected by host-specific and non-host-specific *Salmonella* serotypes.

Host-Specific Salmonella

Salmonella Gallinarum and *Salmonella* Pullorum cause severe disease and death of birds compared with other known *Salmonella* serotypes. Chadfield et al. [16] suggested that *Salmonella* Gallinarum invades the bursa, but the process is not time dependent, and they demonstrated no selectivity for a potential port of entry for the host-specific serotype. Avian systemic salmonellosis has 3 phases: invasion, systemic infection, and the resolution of the infection [4]. The third phase can have 3 results: the clearance of the bacteria, death of the birds resulting from infection, and partial clearance of the bacteria, which leads to a subclinical carrier state, as shown in Figure 1. The biology of pullorosis is markedly different when compared with fowl typhoid, which causes high mortality. Pullorosis induces an increase in *Salmonella* in the spleen, resulting in an infection of the reproductive tract [17].

Table 1. Non-host-specific *Salmonella* isolated from poultry or poultry products worldwide

Group	Serotype	Source(s)	Reference(s)
O:4	Agona	Chickens	[58]
		Geese	[59]
		Turkeys	[60]
		Pheasants	[61]
		Frozen chicken, frozen duck	[62]
	Brandenburg	Turkeys	[63]
	Bredeney	Chickens	[64]
	Chester	Turkeys	[65]
	Derby	Chickens	[59, 66]
		Frozen duck	[62]
	Haifa	Chickens	[67, 68]
	Hato	Chickens	[69]
	Heidelberg	Breeders	[70]
		Eggs	[13]
		Chicken meat	[71]
	Indiana	Geese and ducks	[72]
		Turkey and chicken meat	[60, 71]
	Kiambu	Chickens	[73]
		Backyard ducks	[74]
	Kingston	Chicken meat	[71]
	Paratyphi B	Chickens	[75, 76]
	Reading	Turkey	[77]
	Saint-Paul	Chickens	[78]
		Turkeys	[79]
		Turkey and chicken meat	[71]
	Sandiego	Turkeys	[65]
		Chickens	[60]
	Schleissheim	Chickens	[68]
	Schwarzengrund	Chickens	[64, 80]
	Stanley	Chickens	[79]
		Turkeys	[75, 81]
		Frozen duck	[62]
	Typhimurium	Eggs	[70]
		Ostriches	[82]
		Quail	[83]
O:7	Augustenborg	Chickens	[68]
	Bareilly	Chickens	[65]
	Djugu	Chickens	[69]
	Infantis	Chickens	[65]
		Turkey	[79]
	Isangi	Chickens	[60]
	Lille	Chickens	[81, 84]
	Livingstone	Chickens	[64]
		Ostriches	[82]
	Lomita	Chickens	[67]
	Mbandaka	Chickens	[69, 73, 85]
	Mikawasima	Laying hens	[86]
	Montevideo	Chickens and turkeys	[79]
	Ohio	Chickens	[59]
	Oranienburg	Quail	[87]
	Rissen	Poultry	[66]
	Singapore	Chickens	[88]
	Tennessee	Chickens	[60]
		Frozen duck	[62]
	Thompson	Chickens	[65]
		Chickens and ducks	[60]

Continued

Table 1 (Continued). Non-host-specific *Salmonella* isolated from poultry or poultry products worldwide

Group	Serotype	Source(s)	Reference(s)
	Virchow	Chickens	[79]
		Turkeys	[81]
		Quail	[89]
O:8	Albany	Poultry	[66]
	Bardo	Chickens	[59]
	Blockley	Chickens	[64, 66]
		Turkey and chicken meat	[71]
	Bovismorbificans	Chickens	[67]
	Chincol	Chickens	[67]
	Emek	Poultry	[66]
	Hadar	Chickens	[64, 72]
		Quail	[83]
		Frozen chicken, frozen duck, turkey and chicken meat	[62, 71]
	Istanbul	Chickens	[68]
	Kentucky	Layers	[90]
	Kottbus	Ducks	[60]
	Litchfield	Processing plant	[91]
	Muenchen	Chickens	[67]
		Ostriches	[82]
	Newport	Turkeys	[65]
		Chickens	[58, 75]
		Emus and rheas	[82]
		Frozen duck	[62]
	Tallahassee	Poultry	[66]
O:9	Berta	Poultry products	[92]
	Enteritidis	Eggs	[70]
		Quail eggs	[93]
	Javiana	Turkeys	[65]
	Moscow	Ducklings	[94]
	Panama	Turkeys	[65]
		Ostriches	[82]
		Frozen duck	[62]
O:3,10	Amsterdam	Frozen duck	[62]
	Anatum	Chickens	[65]
		Turkeys	[64]
		Ostriches	[82]
	London	Poultry	[66]
	Newlands	Chickens	[60]
	Orion	Backyard ducks	[74]
	Uganda	Backyard ducks	[74]
	Weltervreden	Chickens	[67]
		Frozen duck	[62]
	Zanzibar	Chickens	[60]
O:1,3,19	Niloese	Poultry	[95]
	Parkroyal	Chickens	[60]
	Senftenberg	Frozen duck	[62, 90]
	Taksony	Broiler parent stock	[96]
O:6,14	Fischerkietz	Turkeys	[60]
O:11	Rubislaw	Rheas	[82]
O:13	Kedougou	Chickens	[97]
	Havana	Chickens	[88]
	Poona	Retail chicken carcasses or products	[98]
O:18	Cerro	Chickens	[85]
O:30	Godesberg	Ostriches	[82]
O:35	Alachua	Chickens	[99]
O:40	Johannesburg	Chickens	[99]
O:41	Waycross	Chickens	[75]

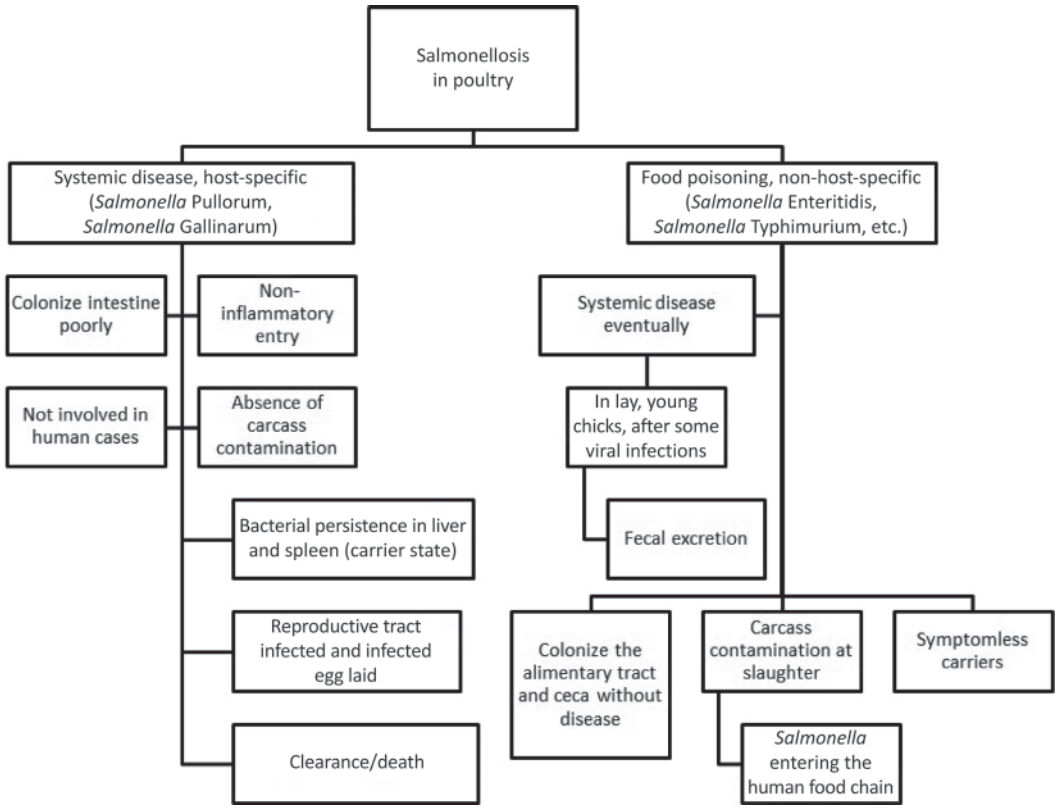

Figure 1. Salmonellosis in poultry, showing differences between host-specific and non-host-specific *Salmonella*.

Non-Host-Specific Salmonella

Salmonella infects poultry and other animals by the oral route. Non-host-specific *Salmonella* in poultry is frequently involved in food poisoning in humans. In chickens, it produces systemic disease in some special cases, such as during the laying period, in chicks in the first 2 wk of life, or after viral diseases. The pathogenesis of non-host *Salmonella* serotypes in poultry is summarized in Figure 1. Salmonellae are not native members of the gut microbiota, but young chicks are readily colonized, and the organisms may persist in the host for some weeks or during the entire rearing period [7]. They become localized in the cecal tonsils and can occur in the upper part of the small intestine and in the gizzard and proventriculus [18]. Because most birds infected with salmonellae become symptomless carriers, they constitute a reservoir of the organisms, which is a potential human health hazard. Additionally, by contaminating the environment, these birds are responsible for increasing the number of infected individuals [7].

IMMUNE RESPONSES AGAINST *SALMONELLA* INFECTION

The immune response to *Salmonella* infections is very complicated and involves the interaction of many components of the immune system, including the innate and the adaptive immune systems [19]. Although progress has been made in understanding immune responses against *Salmonella* infections, further research is needed to understand the complete roles of humoral and cell-mediated immunity because until now, no consistent pattern has been observed. Pathogenic bacteria have evolved mechanisms to invade the epithelial cell barrier and survive within host tissues. *Salmonella* maintains genes organized within pathogenicity islands that encode virulence factors that allow adherence, invasion, and dissemination in the host [20].

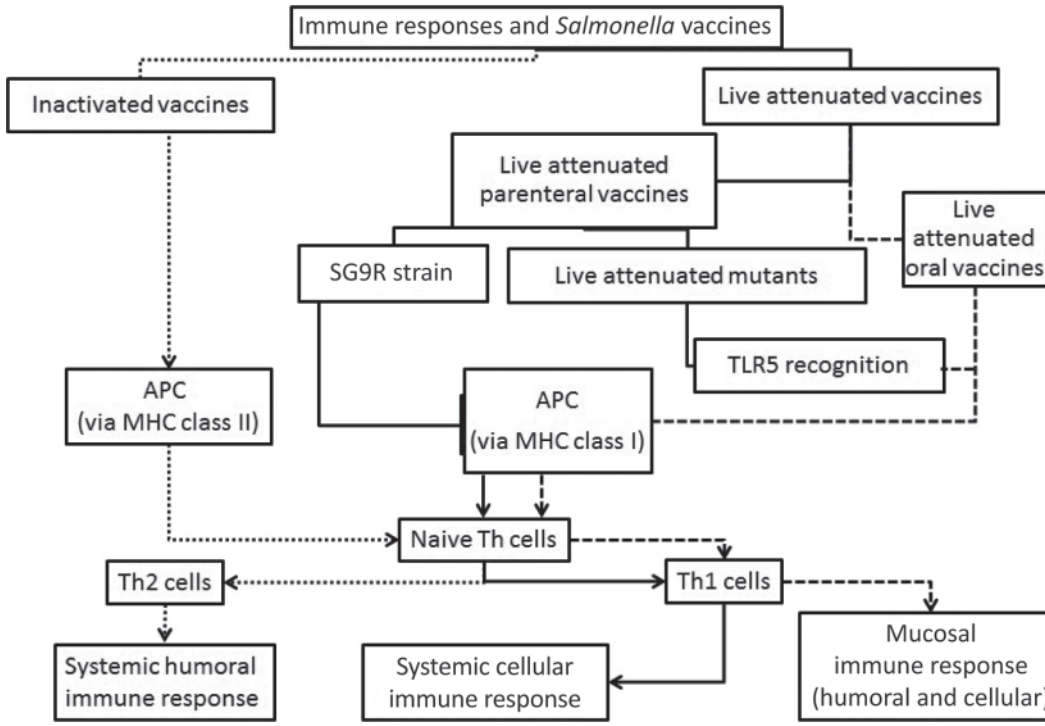

Figure 2. Immune responses when using an inactivated or live *Salmonella* vaccine. Th = T helper cells; SG 9R = *Salmonella* Gallinarum 9 rough strain; APC = antigen-presenting cells; MHC = major histocompatibility complex; TLR5 = Toll-like receptor 5.

Toll-like receptors (**TLR**) are cell receptors that recognize structural motifs on pathogens and initiate signaling cascades controlling the development of innate immune response [21]. These receptors contribute to host resistance to microbial pathogens and can drive the evolution of virulence mechanisms [22] and can promote adaptive immunity through control of dendritic cell maturation [23]. The consequences of *Salmonella* infection on the expression of the different TLR, and particularly TLR4, have been widely studied [24].

Salmonella Gallinarum does not induce an inflammatory response and may not be limited by the immune system, leading to severe systemic disease [25]. Invasion of *Salmonella* Gallinarum results in little or no production of IL-6, suggesting that the pathogenesis and host specificity of *Salmonella* Gallinarum infection in the chicken may be related, to some extent, to the lack of an inflammatory response in the early stages of the infection in the gut [25].

Chickens infected with enteric *Salmonella* serovars show high levels of specific antibodies, a T-cell response, cytokines, and chemokines. Within cell populations, their function can be further discriminated by the presence of cellular determinants, such $CD4^+$ (T helper cells) and $CD8^+$ (T cytotoxic cells), which are associated with helper and cytotoxic functions, respectively [26]. The local immune response in the gut has been shown to be more effectively involved in the clearance of *Salmonella* Enteritidis from the gastrointestinal tract than in the systemic response [27]. An important role of local cell-mediated immunity in the defense of chickens against *Salmonella* exposure has been suggested [28], describing that modifications of T-cell populations, especially $CD8^+TcR1^+(\gamma\delta)$ cells (T-cell receptor-bearing cells) in ceca, occur a few days after the inoculation of 1-d-old chickens with the serovar Typhimurium.

It has been suggested that intestinal secretory IgA (**SIgA**) responses partially contribute to the

later elimination of *Salmonella* Enteritidis from the gut, and the humoral systemic and local immune responses seem to be related to the cecal colonization [29]. Cell-mediated immunity is responsible for tissue clearance, but how this mechanism could be responsible for intestinal clearance remains unclear [30]. The role of T-cell responses in the clearance of enteric salmonellae has not been proven. However, in the absence of an essential role for B cells (bursa-derived cells) and with faster clearance of infection as a secondary challenge, the responses are likely to be important evidence of immune memory [31].

In recent studies of cytokine and chemokine expression in vitro, previous work has been confirmed, showing that paratyphoid species stimulate significant mRNA expression levels of proinflammatory IL-6, inducible nitric oxide synthase, and chemokines [32]. It was suggested that host gene expression and differences between chicken lines in host responses toward the *Salmonella* infection are host dependent [33].

It is interesting that Berndt et al. [34] evaluated the chicken cecum immune response and reported that low quantities of enteric bacteria were present inside the macrophages. Therefore, paratyphoid *Salmonella* serovars can enter and invade the cecal mucosa, affecting the level and character of the immune response. The expression of IL-12, IL-18, tumor necrosis factor α, and inducible nitric oxide synthase in the cecum was correlated with the invasiveness of serovars in the lamina propria. In contrast, IL-2 mRNA expression and changes in the numbers of T-cell receptor 2 and $CD4^+$ cells seem to be more dependent on the infection of intestinal epithelial cells [34]. Crhanova et al. [24] found that chickens respond to natural colonization of cecum by an increased expression of IL-8 and IL-17 in the first week of life. These authors reported that chickens infected with *Salmonella* Enteritidis before, during, and after the IL-8 and Il-17 induction responded through Th1 (T helper cell subset 1) inducing IL-8 and IL-17, whereas birds infected after this point responded more through the Th17 (T helper cell subset 17) branch of the immune response. Therefore, the gut microbiota and expression of some cytokines increase the resistance to *Salmonella* Enteritidis infection.

VACCINES AGAINST *SALMONELLA*: IMMUNE MECHANISMS OF PROTECTION

The regulation and effectiveness of the avian acquired immune response is comparable with that in mammals [35]. For a better understand-

Table 2. *Salmonella* strain vaccines in poultry

Salmonella serotype	Strain vaccine	Reference
Salmonella Gallinarum	Semirough lipopolysaccharide structure	[100]
	aroA mutant	[101]
	nuoG mutant	[102]
	crp mutant	[103]
	metC mutant	[104]
	cobS and *cbiA* mutants	[105]
Salmonella Enteritidis	Auxotrophic double-marker mutant (chemical mutagenesis)	[106, 107]
	Metabolic drift mutation	[45]
	aroA mutant	[108, 109]
	Outer membrane protein (*ompR*)	[110, 111]
	Iron restricted	[112]
	Temperature-sensitive spontaneous mutant	[9]
	cya crp mutants	[113]
Salmonella Typhimurium	Auxotrophic double-marker mutant (chemical mutagenesis)	[106, 107]
	Metabolic drift mutants	[45]
	aroA mutant	[114]
	galE mutant	[115]
	dam mutant	[116]
	phoP rpoS double deletion	[117]
	cya crp mutants	[118]

ing of these mechanisms, an extensive review of vaccines, protection, and the immune dynamics of the avian digestive system is available [15, 36]. In chickens, vaccines should prevent intestinal and cecal colonization, resulting in diminished fecal shedding, and should be effective against systemic infection, preventing vertical transmission and egg contamination. Vaccinations with either inactivated or live products have been applied to reduce the susceptibility of poultry to *Salmonella* infections [37]. Both inactivated and live vaccines are used to protect against *Salmonella* challenge; however, killed products increase humoral immunity and reduce *Salmonella* prevalence but do not significantly decrease *Salmonella* in the farm environment [38].

Vaccines based on dead *Salmonella* bacteria have been used to protect poultry and their progeny against field challenges. However, there are 2 remaining problems with these products when they are administered parenterally. They fail to elicit a cell-mediated immune response, which is considered the most important for clearing the intracellular pathogen [39], and they do not stimulate SIgA responses at mucosal surfaces, which is the key for protection against intestinal colonization [9]. Figure 2 shows the steps in the immune response of an immune system activation using dead *Salmonella* bacteria.

Live and attenuated vaccines have been used worldwide, and their efficacy has been demonstrated in challenge trials [40, 41]. The objective in using a live attenuated vaccine should be to reduce the bacterial virulence while maintaining its immunogenicity. Attenuation frequently involves the mutation of genes encoding metabolic enzymes or the deletion of essential virulence factors. The inactivation of the metabolic gene has the advantage that the bacteria still express virulence determinants that play a key role in eliciting a protective immune response [42]. The intestinal epithelial surface is a physical barrier and represents the key in controlling the gut immune response to antigens delivered by oral administration.

It is important to evaluate the behavior of the attenuated bacteria strains in the environment, focusing on the attenuated strain obtained by gene deletions, because they can acquire genes from other microorganisms and recover their virulence. The *Salmonella* Gallinarum 9 rough strain (**SG 9R**) vaccine strain still results in systemic disease, with pathology in the liver and spleen, and bacteria persist for several weeks at these sites [43]. A lipopolysaccharide defect may be one of the major mechanisms of SG 9R attenuation [44], but this defect could induce partial recognition by Toll-like receptor (**TLR**) 4. Possession of intact SPI-2 (*Salmonella* pathogenicity island 2) and spv (*Salmonella* plasmid virulence) *C*, *B*, *A*, and *R* virulence genes may be associated with residual SG 9R virulence [44] Mutations should be introduced to increase safety by reducing the risk of reversion [45].

Vaccines should establish a long-lasting immunity by manipulating the cytokine milieu to induce the appropriate effector mechanisms for each particular pathogen and by creating a large pool of long-lived memory cells [46]. Vaccines used in poultry against *Salmonella* infections have been effective, but they were empirically designed and are not based on detailed information about the immune responses of protection because efficacy is frequently evaluated in challenge trials. Table 2 shows some of the most important host-specific and non-host-specific strains that are tested as vaccine candidates in poultry.

An important difference must be established in *Salmonella* attenuated vaccines regarding the immune response, and that is the administration route. Parenteral vaccines stimulate a strong humoral response, whereas oral live attenuated vaccines generate both mucosal and systemic immunity [47, 48], as shown in Figure 2. Mucosal memory T-cell phenotypes differ substantially depending on the regimen of immunization, with a secondary response resulting in preferential accumulation of memory T cells in the lamina propria after mucosal vaccination [14].

The first step in initiating an immune response in the gut surface by oral vaccines is based on the signals sent by receptors for pathogen-associated molecular patterns via pathogen recognition receptors such as TLR. The TLR in chickens are very similar to those in mammals; however, some differences in recognition patterns related to TLR5, which recognize flagellin, are observed in host-specific and non-host-

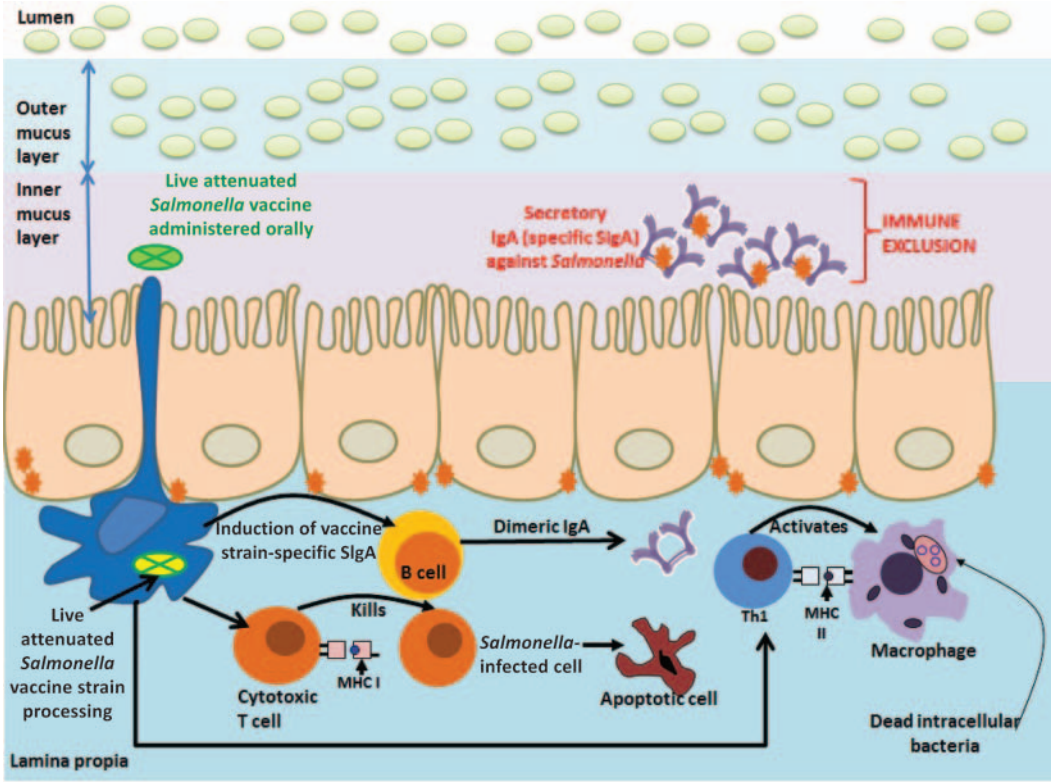

Figure 3. Mucosal immune response after oral attenuated live *Salmonella* vaccine administration. Th1 = T helper cell subset 1; MHC = major histocompatibility complex. Color version available in the online PDF.

specific *Salmonella* strains [49]. In response to the flagellin lamina propria dendritic cell, the differentiation of naïve B cells into IgA plasma cells occurs via a mechanism that is independent of the gut-associated lymphoid tissue [50]. Recognition of *Salmonella* Typhimurium is largely mediated by TLR2, TLR4, and TLR5 [51, 52], and TLR9 is involved in regulating the replication of the bacterium. Lipopolysaccharide of *Salmonella* is recognized by TLR4 expressed at the surface of the immune cells and in the cytoplasm of intestinal epithelial cells [24]. The second step in the immune response is related to the ability of the antigen to cross the epithelial barrier and to be presented to antigen-presenting cells, especially dendritic cells. Mucosal dendritic cells play a central role in the induction of protective immunity against invasive pathogens. Unique dendritic cell subsets are responsible for antigen presentation after mucosal vaccination [53]. In the chicken, multiple lymphoid follicles exist, and they are made up of B cells embedded in a network of follicular dendritic cells [54]. The chicken epithelial barrier can be crossed using 3 mechanisms: endocytosis in the intestinal epithelial cells, transcytosis crossing M cells (microfold cells), and directly through the intraepithelial lymphocytes. The third step in the immune response produced by oral vaccines is the processing by the dendritic cells and the presentation to the T cells. Dendritic cells in the gut can be activated by epithelial cells, which produce cytokines based on the invasiveness of the bacteria, and directly by noninvasive bacteria [55]. *Salmonella* vaccines administered orally must induce Th1 and Th2 (T helper cell subset 2) responses, stimulating cell-mediated immunity and B-cell activation to produce SIgA (Figure 3), which blocks the attachment of the bacteria to mucosal surfaces. To achieve protection, it is important that memory cells are generated in sufficient numbers and persist as a functional long-lived population [46]. According to some authors, it would seem crucial that the

vaccine strains retain the capacity of invasiveness to stimulate sufficient immunity to be protective [15, 56]. However, the immune response in the intestinal mucosa has revealed new possibilities because oral antigens induce effector and memory cells that express certain receptors only on lymphocytes of intestinal mucosa [20]. These cells might be exploited to develop new live attenuated vaccines, inducing a broad repertoire of immune responses against intracellular pathogens [57].

CONCLUSIONS AND APPLICATIONS

1. Vaccines against *Salmonella* infections in chickens and other food-producing animals require protection at both the local and systemic levels. The ideal product should be administered via drinking water to mimic natural infection and stimulate both the mucosal and systemic immune responses.
2. Vaccination is becoming the best way to prevent these bacteria in food animals. Chicks should be vaccinated in the hatchery or at hatch, obtaining protection against *Salmonella* field challenges in the first weeks of life.
3. The additional advantage of oral administration in poultry production is supported by the fact that oral vaccination also contributes to the quality assurance programs related to animal welfare and decreases the vaccination cost factor.
4. Multivalent vaccines might be constructed based on the knowledge of *Salmonella* interactions with specific hosts because the expression and virulence of each strain depends on the host.
5. Further research is needed to evaluate immunological interactions among the host and *Salmonella*, avoiding empirical methods in developing new vaccines and investigating ways to prevent the infection. New design and delivery strategies for eliciting mucosal and systemic immune responses are needed to develop more efficacious vaccines against *Salmonella* for preventing infection in poultry and contamination in poultry products.

REFERENCES AND NOTES

1. Chappell, L., P. Kaiser, P. Barrow, M. A. Jones, C. Johnston, and P. Wigley. 2009. The immunobiology of avian systemic salmonellosis. Vet. Immunol. Immunopathol. 128:53–59.

2. Dunkley, K. D., T. R. Callaway, V. I. Chalova, J. L. McReynolds, M. E. Hume, C. S. Dunkley, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2009. Foodborne *Salmonella* ecology in the avian gastrointestinal tract. Anaerobe 15:26–35.

3. Gantois, I., R. Ducatelle, F. Pasmans, F. Haesebrouck, R. Gast, T. J. Humphrey, and F. Van Immerseel. 2009. Mechanisms of eggs contamination by *Salmonella* Enteritidis. FEMS Microbiol. Rev. 33:718–738.

4. Sadeyen, J. R., J. Trotterau, P. Velge, J. Marly, C. Beaumont, P. A. Barrow, N. Bumstead, and A. C. Lalmanach. 2004. *Salmonella* carrier state: Comparison of expression of immune response genes between susceptible and resistant animals. Microbes Infect. 6:1278–1286.

5. Galanis, E., D. M. Lo Fo Wong, M. E. Patrick, N. Binsztein, A. Cieslik, T. Chalermchikit, E. Aidara-Kane, A. Ellis, F. J. Angulo, and H. C. Wegener.. 2006. Web-based surveillance and global *Salmonella* distribution, 2000–2002. Emerg. Infect. Dis. 12:381–388.

6. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607–625.

7. Revolledo, L., A. J. P. Ferreira, and G. Mead. 2006. Prospects in *Salmonella* control: Competitive exclusion, probiotics, and enhancement of avian intestinal immunity. J. Appl. Poult. Res. 15:341–351.

8. Vandeplas, S., R. Dubois Duphin, Y. Beckers, P. Thonarty, and A. Théwis. 2010. *Salmonella* in chicken: Current and developing strategies to reduce contamination at farm level. J. Food Prot. 73:774–785.

9. Cerquetti, M. C., and M. M. Gherardi. 2000. Vaccination of chickens with a temperature-sensitive mutant of *Salmonella* enteritidis. Vaccine 18:1140–1145.

10. Eswarappa, S. M., J. Janice, S. V. Balasundaram, N. M. Dixit, and D. Chakravortty. 2009. Host-specificity of *Salmonella enterica* serovar Gallinarum: Insights from comparative genomics. Infect. Genet. Evol. 9:468–473.

11. World Organization for Animal Health (OIE). 2008. Manual of diagnostic tests and vaccines for terrestrial animals. OIE, Paris, France. Accessed Jan. 2010. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.11_FOWL_TYPHOID.pdf.

12. Wigley, P., S. Hulme, C. Powers, R. Beal, A. Smith, and P. Barrow. 2005. Oral infection with the *Salmonella* enteric serovar Gallinarum 9R attenuated live vaccine as a model to characterize immunity to fowl typhoid in the chicken. BMC Vet. Res. 1:2. http://dx.doi.org/10.1186/1746-6148-1-2.

13. Schoeni, J. L., K. A. Glass, J. L. McDermot, and A. C. L. Wong. 1995. Growth and penetration of *Salmonella enteritidis*, *Salmonella heidelberg* and *Salmonella typhimurium* in eggs. Int. J. Food Microbiol. 24:385–396.

14. Barnhart, H. M., D. W. Dreesen, R. Brastien, and O. C. Pancorbo. 1991. Prevalence of *Salmonella enteritidis* and other serovars in ovaries of layer hens at time of slaughter. J. Food Prot. 54:488–491.

15. Barrow, P. A. 2007. *Salmonella* infections: Immune and non-immune protection with vaccines. Avian Pathol. 36:1–13.

16. Chadfield, M. S., D. Brown, S. Aabo, J. P. Christensen, and J. E. Olsen. 2003. Comparison of intestinal invasion and macrophage response of *Salmonella* Gallinarum and other host-adapted *Salmonella enterica* serovars in the avian host. Vet. Microbiol. 92:49–64.

17. Wigley, P., S. D. Hulme, C. Powers, R. K. Beal, A. J. Berchieri, A. Smith, and P. Barrow. 2005. Infection of the reproductive tract and eggs with *Salmonella enterica* serovar Pullorum in the chicken is associated with suppression of cellular immunity at sexual maturity. Infect. Immun. 73:2986–2990.

18. Fanelli, M. J., W. W. Sadler, C. E. Franti, and J. R. Bronwell. 1971. Localization of salmonellae within the intestinal tract of chickens. Avian Dis. 15:366–375.

19. Nagarajan, A. G., S. V. Balasundaram, J. Janice, G. Karnam, S. M. Eswarappa, and D. Chakravortty. 2009. *sopB* of *Salmonella enterica* serovar Typhimurium is a potential DNA vaccine candidate in conjugation with live attenuated vaccine . Vaccine 27:2804–2811.

20. Aziz, M. A., S. Midha, S. M. Waheed, and R. Bhatnagar. 2007. Oral vaccines: New needs, new possibilities. Bioessays 29:591–604.

21. Chaussé, A. M., O. Grepinet, E. Bottreau, Y. Le Vern, P. Menanteau, J. Troterau, V. Robert, Z. Wu, D. Kerboeuf, C. Beaumont, and P. Velge. 2011. Espression of TLR4 and downstream effectors in selected cecal cell subpopulations of chicks resistant or susceptible to *Salmonella* carrier-state. Infect. Immun. 79:3445–3454.

22. Arpaia, N., J. Godec, L. Lau, K. E. Sivick, L. M. McLaughlin, M. B. Jones, T. Dracheva, S. N. Peterson, D. N. Monack, and G. M. Barton. 2011. TLR signaling is required for *Salmonella typhimurium* virulence. Cell 144:675–688.

23. Iwasaki, A., and R. Metzhitov. 2004. Toll-like receptor control of the adaptive immune responses. Nat. Immunol. 5:987–995.

24. Crhanova, M., H. Hradecka, M. Faldynova, M. Matulova, H. Havlickova, F. Sisak, and I. Rychlik. 2011. Immune response of chicken gut to natural colonization by gut microflora and to *Salmonella enterica* serovar Enteritidis infection. Infect. Immun. 79:2755–2763.

25. Kaiser, P., L. Rothwell, E. E. Galyov, P. A. Barrow, J. Burnside, and P. Wigley. 2000. Differential cytokine expression in avian cells in response to invasion by *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella gallinarum*. Microbiology. 146:3217–3226.

26. Jeurissen, S. H. M., F. Lewis, J. D. Van der Klis, Z. Mroz, J. M. J. Rebel, and A. A. H. M. Huurne. 2002. Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality. Curr. Issues Intest. Microbiol. 3:1–14.

27. Desmidt, M., R. Ducatelle, J. Mast, B. M. Goddeeris, B. Kaspers, and F. Haesebrouck. 1998. Role of the humoral immune system in *Salmonella enteritidis* phage type four infection in chickens. Vet. Immunol. Immunopathol. 63:355–367.

28. Berndt, A., and U. Methner. 2001. Gamma/delta T cell response of chickens after oral administration of attenuated and non-attenuated *Salmonella typhimurium* strains. Vet. Immunol. Immunopathol. 78:143–161.

29. Berthelot-Hérault, F., F. Mompart, M. S. Zygmunt, G. Dubray, and M. Duchet-Suchaux. 2003. Antibody responses in the serum and gut of chickens lines differing in cecal carriage of *Salmonella* enteritidis. Vet. Immunol. Immunopathol. 96:43–52.

30. Zhang-Barber, L., A. K. Turner, and P. A. Barrow. 1999. Vaccination for control of *Salmonella* in poultry. Vaccine 17:2538–2545.

31. Smith, A. L., and R. Beal. 2008. The avian enteric immune system in health and disease. Pages 243–271 in Avian Immunology. F. Davison, B. Kaspers, and K. A. Schat, ed. Academic Press, London, UK.

32. Setta, A. M., M. A. Jones, and P. A. Barrow. 2009. Cytokines and chemokines expression in avian cells infected with *Salmonella enterica*. Pages 65–66 in Abstracts and Reviews Tri. Soc. Annu. Conf. Soc. Leukocyte Biol., Int. Cytokine Soc., and Int. Soc. Inteferon and Cytokine Res., Cell and Cytokine Interact. Healt. Dis., Lisbon, Portugal.

33. van Hemert, S., A. J. W. Hoekman, M. A. Smits, and J. M. J. Rebel. 2006. Gene expression responses to a *Salmonella* infection in the chicken intestine differ between lines. Vet. Immunol. Immunopathol. 114:247–258.

34. Berndt, A., A. Wilhelm, C. Jugert, J. Pieper, K. Sachse, and U. Methner. 2007. Chicken cecum immune response to *Salmonella enterica* serovars of different levels of invasiveness. Infect. Immun. 75:5993–6007.

35. Erf, G. F. 2004. Cell-mediated immunity in poultry. Poult. Sci. 83:580–590.

36. Korver, D. R. 2006. Overview of the immune dynamics of the digestive system. J. Appl. Poult. Res. 15:123–135.

37. Gast, R. K. 2007. Serotype-specific and serotype-independent strategies for preharvest control of food-borne *Salmonella* in poultry. Avian Dis. 51:817–828.

38. Berghaus, R. D., S. G. Thayer, J. J. Maurer, and C. L. Hofacre. 2011. Effect of vaccinating breeder chickens with a killed *Salmonella* vaccine on *Salmonella* prevalence and loads in breeder and broiler chicken flocks. J. Food Prot. 74:727–734.

39. Lax, A. J., P. A. Barrow, P. W. Jones, and T. S. Wallis. 1995. Current perspective in salmonellosis. Br. Vet. J. 151:351–377.

40. Atterbury, R. J., R. H. Davies, J. J. Carrique-Mas, V. Morris, D. Harrison, V. Tucker, and V. M. Allen. 2010. Effect of delivery method on the efficacy of *Salmonella* vaccination in chickens. Vet. Rec. 167:161–164.

41. Papezova, K., H. Havlickova, F. Sisak, V. Kummer, M. Faldyna, and I. Rychlik. 2008. Comparison of live and inactivated *Salmonella* Typhimurium vaccines containing combinations of SPI-1 and SPI-2 antigens in poultry. Vet. Med. Prague 53:315–323.

42. Detmer, A., and J. Glenting. 2006. Live bacterial vaccines—A review and identification of potential hazards. Microb. Cell Fact. 5:23.

43. Silva, E. N., G. H. Snoeyenbos, O. M. Weinack, and C. F. Smyser. 1981. Studies on the use of 9R strain of *Salmonella* Gallinarum as a vaccine in chickens. Avian Dis. 25:38–52.

44. Kwon, H. J., and S. H. Cho. 2011. Pathogenicity of SG 9R, a rough vaccine strain against fowl typhoid. Vaccine 29:1311–1318.

45. Linde, K., G. C. Fthenakis, and A. Fitchner. 1998. Bacterial live vaccines with graded level of attenuation achieved by antibiotic resistance mutations: Transduction experiments on the functional unit of resistance, attenu-

ation and further accompanying markers. Vet. Microbiol. 62:121–134.

46. Chabalgoity, J. A., A. Baz, A. Rial, and S. Grille. 2007. The relevance of cytokines for development of protective immunity and rational design of vaccines. Cytokine Growth Factor Rev. 18:195–207.

47. Bouvet, J. P., N. Decroix, and P. Pamosinlapatham. 2002. Stimulation of local antibody production: Parenteral or mucosal vaccination? Trends Immunol. 23:209–213.

48. Mastroeni, P., F. Bowe, R. Cahill, C. Simmons, and G. Dougan. 1999. Vaccines against gut pathogens. Gut 45:633–635.

49. Salazar-Gonzalez, R. M., and S. J. McSorley. 2005. *Salmonella* flagellin, a microbial target of the innate and adaptive immune system. Immunol. Lett. 101:117–122.

50. Uematsu, S., and S. Akira. 2009. Immune responses of $TLR5^{+}$ lamina propria dendritic cells in enterobacterial infections. J. Gastroenterol. 44:803–811.

51. Uematsu, S., M. H. Jang, N. Chevrier, Z. Guo, Y. Kumagai, M. Yamamoto, H. Kato, N. Sougawa, H. Matsui, H. Kuwata, H. Hemmi, C. Coban, T. Kawai, K. J. Ishii, O. Takeuchi, M. Miyasaka, K. Takeda, and S. Akira. 2006. Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal $CD11c^{+}$ lamina propria cells. Nat. Immunol. 7:868–874.

52. Vazquez-Torres, A., B. A. Vallance, M. A. Bergman, B. B. Finlay, B. T. Cookson, J. Jones-Carson, and F. C. Fang. 2004. Toll-like receptor 4 dependence of innate and adaptive immunity to *Salmonella*: Importance of Kupffer cell network. J. Immunol. 172:6202–6208.

53. Belyakov, I. M., and J. D. Ahlers. 2009. What role does the route of immunization play in the generation of protective immunity against mucosal pathogens? J. Immunol. 183:6883–6892.

54. Brisbin, J. T., J. Gong, and S. Sharif. 2008. Interactions between commensal bacteria and the gut-associated immune system of the chicken. Anim. Health Res. Rev. 9:101–110.

55. Rimoldi, M., M. Chieppa, M. Vulcano, P. Allavena, and M. Rescigno. 2004. Intestinal epithelial cells control dendritic cell function. Ann. N. Y. Acad. Sci. 1029:66–74.

56. Van Immerseel, F., U. Methner, I. Rychlik, B. Nagy, P. Velge, G. Martin, N. Foster, R. Ducatelle, and P. A. Barrow. 2005. Vaccination and early protection against non-host-specific *Salmonella* serotypes in poultry: Exploitation of innate immunity and microbial activity. Epidemiol. Infect. 133:959–978.

57. Titball, R. W. 2008. Vaccines against intracellular bacterial pathogens. Drug Discov. Today 13:596–600.

58. Synnott, M. B., M. Brindley, J. Gray, and J. K. Dawson. 1998. An outbreak of *Salmonella agona* infection associated with precooked turkey meat. Commun. Dis. Public Health 1:176–179.

59. Machado, J., and F. Bernardo. 1990. Prevalence of *Salmonella* in chicken carcasses in Portugal. J. Appl. Microbiol. 69:477–480.

60. Hoszowki, A., and D. Wasyl. 2002. *Salmonella* serovars found in animals and feeding stuffs in 2001 and their microbial resistance. Bull. Vet. Inst. Pulawy 46:165–178.

61. Myoujin, Y., R. Yona, S. Umiji, T. Tanimoto, K. Otsuki, and T. Murase. 2003. *Salmonella enterica* subsp. *enterica* serovar Agona infections in commercial pheasant flocks. Avian Pathol. 32:355–359.

62. Bangtrakulnonth, A., S. Pornreongwong, C. Pulsrikarn, P. Sawanpanyalert, R. Hendriksen, and F. M. Aarestrup. 2004. *Salmonella* serovars from humans and other sources in Thailand, 1993–2002. Emerg. Infect. Dis. 10:131–136.

63. Public Health Agency of Canada. 2010. Canada integrated surveillance report online 2010. Accessed Jan. 2010. http://www.phac-aspc.gc.ca/cipars-picra/2010/q3-eng.php.

64. Limawongpranee, S., H. Hayashidani, A. T. Okatani, C. Hirota, K. Kaneko, and M. Ogawa. 1999. Prevalence and persistence of *Salmonella* in broiler chicken flocks. J. Vet. Med. Sci. 61:255–259.

65. Sadler, W. W., and R. E. Corstvet. 1965. Second survey of market poultry for *Salmonella* infection. Appl. Microbiol. 13:348–351.

66. Vo, A. T. T., E. van Duijkeren, A. C. Fluit, M. E. O. C. Heck, A. Verbruggen, H. M. E. Maas, and W. Gaastra. 2006. Distribution of *Salmonella enterica* serovars from humans, livestock and meat in Vietnam and the dominance of *Salmonella* Typhimurium phage type 90. Vet. Microbiol. 113:153–158.

67. Rusul, G., J. Khair, S. Radu, C. T. Cheah, and R. M. Yassin. 1996. Prevalence of *Salmonella* in broilers at retail outlets, processing plants and farms in Malaysia. Int. J. Food Microbiol. 33:183–194.

68. Ishihara, K., T. Takahashi, A. Morioka, A. Kojima, M. Mijima, T. Asai, and I. Tamura. 2009. National surveillance of *Salmonella enterica* in food-producing animals in Japan. Acta Vet. Scand. 51:35 http://dx.doi.org/10.1186/1751-0147-51-35.

69. Pieskus, J., J. Milius, I. Michalskiene, and G. Zagrebneviene. 2006. The distribution of *Salmonella* serovars in chicken and humans in Lithuania. J. Vet. Med. A Physiol. Pathol. Clin. Med. 53:12–16.

70. Smyser, C. F., N. Adinarayanan, H. Van Roekel, and G. H. Snoeyenbos. 1966. Field and laboratory observations on *Salmonella* Heidelberg infection in three chicken breeding flocks. Avian Dis. 10:314–329.

71. Antunes, P., C. Réu, J. C. Sousa, L. Peixe, and N. Pestana. 2003. Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. Int. J. Food Microbiol. 82:97–103.

72. Jørgensen, F., R. Bailey, S. Williams, P. Henderson, D. R. A. Wareing, F. J. Bolton, J. A. Frost, L. Ward, and T. J. Humphrey. 2002. Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. Int. J. Food Microbiol. 76:151–164.

73. Rodrigo, S., A. Adesiyun, Z. Asgarali, and W. Swanston. 2006. Occurrence of selected foodborne pathogens on poultry and poultry giblets from small retail processing operations in Trinidad. J. Food Prot. 69:1096–1105.

74. Rampersad, J., J. Johnson, G. Brown, M. Samlal, and D. Ammons. 2008. Comparison of polymerase chain reaction and bacterial culture for *Salmonella* detection in the Muscovy duck in Trinidad and Tobago. Pan. Am. J. Public Health 23:264–267.

75. Veladaupillai, T. 1964. Salmonellae in bones of slaughtered cattle and poultry. Med. Microbiol. Immunol. (Berl.) 150:10–12.

76. Van Immerseel, F., L. Meulemans, J. De Buck, F. Pasmans, P. Velge, E. Bottreau, F. Haesebrouck, and R. Ducatelle. 2004. Bacteria-host interactions of *Salmonella* Paratyphi B dT+ in poultry. Epidemiol. Infect. 132:239–243.

77. Esteban, E., K. Snipes, D. Hird, R. Kasten, and H. Kinde. 1993. Use of ribotyping for characterization of *Salmonella* serotypes. J. Clin. Microbiol. 31:233–237.

78. Galbraith, N. S., J. F. Archer, and G. H. Tee. 1961. *Salmonella saint-paul* infection in England and Wales in 1959. J. Hyg. (Lond.) 59:133–141.

79. Reilly, W. J., G. I. Forbes, J. C. M. Sharp, S. I. Oboegbulem, O. W. Collier, and G. M. Paterson. 1988. Poultry-borne salmonellosis in Scotland. Epidemiol. Infect. 101:115–122.

80. Chen, M. H., S. W. Wang, W. Z. Hwang, S. J. Tsai, Y. C. Hsih, C. S. Chiou, and H. Y. Tsen. 2010. Contamination of *Salmonella* Schwarzengrund cells in chicken meat from traditional marketplaces in Taiwan and comparison of their antibiograms with those of the human isolates. Poult. Sci. 89:359–365.

81. Gupta, B. R., and J. C. Verma. 1990. Prevalence of *Salmonella* in farms animals and birds in India. Ind. J. Comp. Microbiol. Immunol. Infect. Dis. 11:169–174.

82. Vanhooser, S. L., and R. D. Welsh. 1995. Isolation of *Salmonella* species in ratites. J. Vet. Diagn. Invest. 7:268–269.

83. Sander, J., C. R. Hudson, L. Dufour-Zavala, W. D. Waltman, C. Lobsinger, S. G. Thayer, R. Otalora, and J. J. Maurer. 2001. Dynamics of *Salmonella* contamination in a commercial quail operation. Avian Dis. 45:1044–1049.

84. Kauffmann, F., R. Buttiaux, and R. Gaumont. 1954. A new *Salmonella* type (S. Lille) from chickens. Acta Pathol. Microbiol. Scand. 34:99–100.

85. Zancan, F. T., A. J. Berchieri, S. A. Fernandes, and N. M. S. Queiroz Gama. 2000. *Salmonella* spp. investigation in transport boxes of day-old birds. Braz. J. Microbiol. 31:229–232.

86. Marin, C., A. Hernandez, and M. Lainez. 2009. Biofilm development capacity of *Salmonella* strains isolated in poultry risk factors and their resistance against disinfectants. Poult. Sci. 88:424–431.

87. Edwards, P. R. 1936. The occurrence of *Salmonella*, Oranienburg type, in an infection of quail. J. Bacteriol. 32:259–263.

88. Soerjadi-Liem, A. S., and R. B. Cumming. 1984. Studies of the incidence of *Salmonella* carriers in broiler flocks entering a poultry processing plant in Australia. Poult. Sci. 63:892–895.

89. Aarestrup, F. M., H. Hasman, and L. B. Jensen. 2005. Resistant *Salmonella* Virchow in quail products. Emerg. Infect. Dis. 11:1984–1985.

90. Li, X., J. B. Payne, F. B. Santos, J. F. Levine, K. E. Anderson, and B. W. Sheldon. 2007. *Salmonella* populations and prevalence in layer feces from commercial high-rise houses and characterization of the *Salmonella* isolates by serotyping, antibiotic resistance analysis, and pulsed field gel electrophoresis. Poult. Sci. 86:591–597.

91. Morris, G. K., and J. G. Wells. 1970. *Salmonella* contamination in a poultry-processing plant. Appl. Microbiol. 19:795–799.

92. Roy, P., A. S. Dhillon, L. H. Lauerman, D. M. Schaberg, D. Bandli, and S. Johnson. 2002. Results of *Salmonella* isolation from poultry products, poultry, poultry environment, and other characteristics. Avian Dis. 46:17–24.

93. Erdroğrul, Ö. T., N. Özkan, and E. Çakiroğlu. 2002. *Salmonella enteritidis* in quail eggs. Turk. J. Vet. Anim. Sci. 26:321–323.

94. Truscott, R. B. 1956. *Salmonella* Moscow isolated from ducks in Ontario. Can. J. Comp. Med. 20:345–346.

95. World Health Organization (WHO). 1954. Joint WHO/FAO Expert Committee on Zoonoses. Tech. Rep. Ser. 169. WHO, Geneva, Switzerland.

96. Brown, D. J., J. E. Olsen, and M. Bisgaard. 1992. *Salmonella enterica*: Infection, cross infection and persistence within the environment of a broiler parent stock unit in Denmark. Zentralbl. Bakteriol. 277:129–138.

97. Snow, L. C., R. H. Davies, K. H. Christiansen, J. J. Carrique-Mas, A. J. C. Cook, C. J. Teale, and S. J. Evans. 2008. Survey of the prevalence of *Salmonella* on commercial broiler farms in the United Kingdom, 2005/06. Vet. Rec. 163:649–654.

98. Capita, R., M. Alvarez-Astorga, C. Alonso-Calleja, B. Moreno, and M. del Camino García-Fernandez. 2003. Occurrence of salmonellae in retail chicken carcasses and their products in Spain. Int. J. Food Microbiol. 81:169–173.

99. Rigby, C. E., J. R. Pettit, M. F. Baker, A. H. Bentley, M. O. Salomons, and H. Lior. 1980. Flock infection and transport as sources of salmonellae in broiler chickens and carcasses. Can. J. Comp. Med. 44:328–337.

100. Smith, H. W. 1956. The use of live vaccines in experimental *Salmonella gallinarum* infection in chickens with observations on their interference effect. J. Hyg. (Lond.) 54:419–432.

101. Griffin, H. G., and P. A. Barrow. 1993. Construction of an *aroA* mutant of *Salmonella* serotype Gallinarum: Its effectiveness in immunization against experimental fowl typhoid. Vaccine 11:457–462.

102. Zhang-Barber, L., A. K. Turner, G. Dougan, and P. A. Barrow. 1998. Protection of chickens against experimental fowl typhoid using a *nuoG* mutant of *Salmonella* serotype Gallinarum. Vaccine 16:899–903.

103. Rosu, V., M. S. Chadfield, A. Santona, J. P. Chrinstensen, L. E. Thomsen, S. Rubino, and J. E. Olsen. 2007. Effects of *crp* deletion in *Salmonella enterica* serovar Gallinarum. Act. Vet. Scan. 49:14.

104. Shah, D. H., S. Shringi, A. R. Desai, E. J. Heo, J. H. Park, and J. S. Chae. 2007. Effect of *metC* mutation on *Salmonella* Gallinarum virulence and invasiveness in 1-day-old White Leghorn chickens. Vet. Microbiol. 119:352–357.

105. Penha Filho, R. A., J. B. de Paiva, M. D. da Silva, A. M. de Almeida, and A. J. Berchieri. 2010. Control of *Salmonella* Enteritidis and *Salmonella* Gallinarum in birds using live vaccine containing attenuated *Salmonella* Gallinarum mutant strain. Vaccine 28:2853–2859.

106. Meyer, H., H. Koch, U. Methner, and G. Steinbach. 1993. Vaccines in salmonellosis control in animals. Zentralbl. Bakteriol. 278:407–415.

107. Springer, S., J. Lehmann, T. Lindner, J. Thielebein, G. Alber, and H. J. Selbitz. 2000. A new live *Salmonella* Enteritidis vaccine for chickens—Experimental evidence of its safety and efficacy. Berl. Munch. Tierarztl. Wochenschr. 113:246–252.

108. Cooper, G. L., R. A. J. Nicholas, G. A. Cullen, and C. E. Hormaeche. 1990. Vaccination of chickens with *Salmonella enterica aroA* live oral *Salmonella* vaccine. Microb. Pathog. 9:255–265.

109. Cooper, G. L., L. M. Venables, R. A. J. Nicholas, G. A. Cullen, and C. E. Hormaeche. 1992. Vaccination of chickens with chicken derived *Salmonella enteritidis* phage type 4 *aroA* live *Salmonella* vaccines. Vaccine 10:247–254.

110. Meenakshi, M., C. S. Bakshi, G. Butchaiah, M. P. Bansal, M. Z. Siddiqui, and V. P. Singh. 1999. Adjuvant outer membrane protein vaccine protects against infection with *Salmonella* Enteritidis. Vet. Res. Commun. 23:81–90.

111. Khan, M. I., A. A. Fadl, and K. S. Venkitanarayanan. 2003. Reducing colonization of *Salmonella* Enteritidis in chicken by targeting outer membrane proteins. J. Appl. Microbiol. 95:142–145.

112. Woodward, M. J., G. Gettinby, M. F. Breslin, J. D. Corkish, and S. Houghton. 2002. The efficacy of Salenvac, a *Salmonella enterica* susbsp. *enterica* serotype Enteritidis iron-restricted bacterin vaccine, in laying chickens. Avian Pathol. 31:383–392.

113. Hassan, J. O., and R. Curtiss III.. 1990. Control of colonization by virulent *Salmonella typhimurium* by oral immunization of chickens with avirulent Δ*cya*Δ*crp S. typhimurium*. Res. Microbiol. 141:839–850.

114. Tan, S., C. L. Gyles, and B. N. Wilkie. 1997. Evaluation of an *aroA* mutant *Salmonella typhimurium* vaccine in chickens using modified semisolid Rappaport Vassiliadis medium to monitor fecal shedding. Vet. Microbiol. 54:247–254.

115. Pritchard, D. G., S. C. Nivas, M. D. York, and B. S. Pomeroy. 1978. Effect of Gal-E mutants of *Salmonella typhimurium* on experimental salmonellosis in chickens. Avian Dis. 22:562–575.

116. Dueger, E. L., J. K. House, D. M. Heithoff, and M. J. Mahan. 2003. *Salmonella* DNA adenine methylase mutants prevent colonization of newly hatched chickens by homologous and heterologous serovars. Int. J. Food Microbiol. 80:153–159.

117. Methner, U., P. A. Barrow, D. Gregorova, and I. Rychlik. 2004. Intestinal colonization-inhibition and virulence of *Salmonella phoP*, *rpoS* and *ompC* deletion mutants in chickens. Vet. Microbiol. 98:37–43.

118. Hassan, J. O., S. B. Porter, and R. Curtiss III.. 1993. Effect of infective dose on humoral immune responses and colonization in chickens experimentally infected with *Salmonella typhimurium*. Avian Dis. 37:19–26.

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