Salmonella Control

The role of vaccination in a comprehensive Salmonella control plan

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Introduction

The global poultry industry must constantly evolve to respond to the consumers’ demand for safe and nutritous poultry products. Under this evolving situation the presentation of pathogenic agents causing important challenges for public health, becomes more evident every day. Currently, *Salmonella sp* and *Campylobacter sp*, lead the list of foodborne agents related to poultry products. Additionally, these bacteria can exhibit high resistance to antibiotics; nowadays this condition constitutes a global threat.

Since most Salmonella serotypes do not cause severe disease in chickens, there is a tendency to view its presence as something that does not affect the poultry business. Frequently, this false sense of safety results in the relaxation of control measures and the tendency to accept the presence of these serotypes as something we can live with. Given the versatility of Salmonella, its high ability to adapt, and its persistence for long periods in poultry facilities; failure to consider Salmonella as part of the poultry “landscape” could be a big mistake.

The management of salmonellosis in the poultry industry requires the implementation of comprehensive prevention and control programs. With such a complex disease, a single measure to control Salmonella bacteria does not exist. The long-awaited “silver bullet” is far away from being identified! Therefore, intensive work is required to mitigate, contain and, mainly, prevent the persistence and spread of Salmonella in the chickens and their environment. The management of this disease should focus on the combination of actions that include adequate monitoring and diagnosis with live side interventions: identification and elimination of sources and risk factors, the use of safe vaccines implementing good vaccination plans, high levels of biosecurity, and strategies to achieve the preservation of intestinal integrity and the proper functioning of both the immune system and the gastro-intestinal tract. The concept of a “Salmonella hostile environment” must be implemented both, inside and outside of the bird.

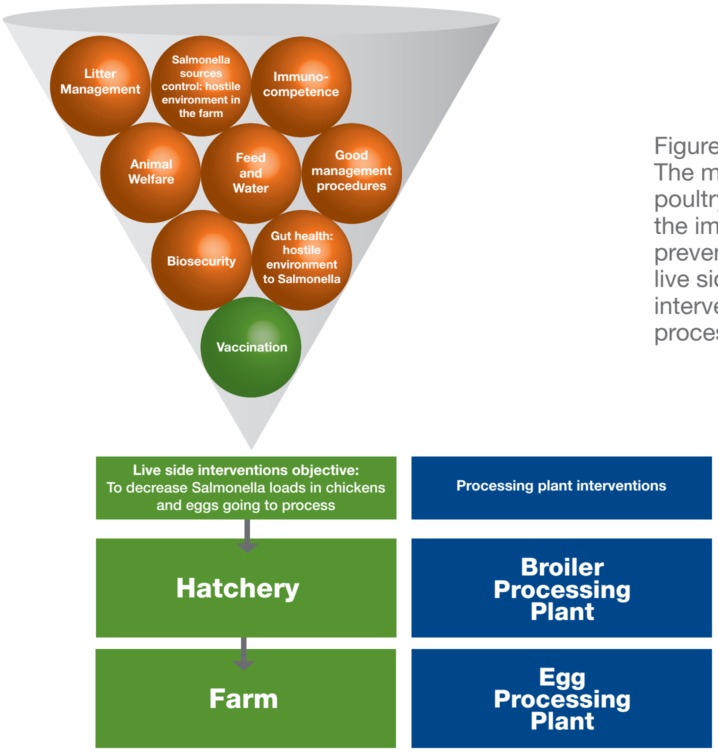


Figure 1.
The management of Salmonella in the poultry industry is complex and requires the implementation of comprehensive prevention and control programs. Multiple live side interventions combined with variable interventions in hatcheries, broiler and egg processing plants are required.

Salmonella diversity and its ability to adapt to adverse environments

Salmonella (S) genus has two species (*Salmonella enterica* and *Salmonella bongori*). The specie *enterica* has 6 subspecies. Among these, the subspecies *enterica* is the most important in terms of public and/or animal health. According with The Institut Pasteur, today 1586 serotypes are included in this subspecies. Among them, 100 may be of real importance to public or animal health, being some serotypes considered as zoonotic and/or related to foodborne illness. Worldwide, *S*. Enteritidis and *S*. Typhimurium are the most important serotypes in terms of public health. In some countries, *S*. Heidelberg is also considered a major threat, and in recent years *S*. Infantis, an emerging Salmonella serotype, has become very important, causing foodborne disease outbreaks in different countries. Despite efforts to control this serotype, its prevalence in poultry environments is getting alarming.

Salmonella is able to survive in different environments. It grows easily in a variety of materials present in a chicken house. Because of the production of biofilms, these bacteria can persist for long periods. Originally in the feces, Salmonella can contaminate water and drinkers, feed and feeders, feathers, and eggs; and its presence in litter, dusty environments, insects, and rodents are common sources of these bacteria for poultry facilities. The range of Salmonella survival on these materials could be hours to months, depending on the serotype and the presence of organic material. The ability of Salmonella to survive at diverse temperatures and different pH, and its presence in hatcheries, farms, feed mills, and processing plants makes it challenging.

Introducing the concept “Salmonella resident serotypes” and the importance of their early identification:

Some Salmonella serotypes can be considered as transient organisms that are occasionally isolated and identified. Most of these serotypes are generally not pathogenic and it is possible to eliminate them with the routine cleaning and disinfection processes. However, some serotypes can get completely established in poultry facilities. These can grow and form resident populations in the chickens and their environment. These “resident serotypes” persist a long time, even after strict sanitation processes.

Some examples of “resident serotypes” include *S*. Kentucky, which often goes overlooked because it does not cause disease in the chickens and there are very few reports of this serotype causing foodborne diseases. However, *S.* Kentucky can be highly prevalent in the poultry industry in various countries, and its importance related with antimicrobial resistance has been identified recently. In some poultry farms, Salmonella serotypes with great importance in terms of public health can become residents; among these *S.* Enteritidis, *S.* Typhimurium, and *S.* Heidelberg are the most important.

Currently, *S.* Infantis is considered one of the most important serotypes in Europe and Asia, with a growing distribution in the American continent, especially in South America. In the US, this serotype was linked to severe outbreaks of foodborne illness in 2018 and its multi-resistance to antibiotics has been clearly documented. This serotype has been isolated from a variety of samples collected in vertical broiler integrations, starting at the grandparent level all the way to the chicken served on a dinner table. Its emergence in commercial layers is also becoming evident. Its ability to persist in poultry environments is amazing, and several poultry companies have observed that once *S.* Infantis occurs for the first time, it can persist for a long time in chickens and different materials and elements commonly used in poultry facilities, mainly when appropriate control measures are not are not promptly implemented after its detection.

There are many reasons why the early and specific identification of Salmonella serotypes present in a poultry facility is essential. However, it is particularly important in terms of the use of vaccines to control Salmonella. This live side intervention could work better when the vaccine contains the serotype present in the chickens or one serotype closely related to it. The more specific the serotype included in the vaccine is, the better protection that can be obtained. Of course, it is not plausible to include all the serotypes detected in one farm. It is necessary to identify the most prevalent ones. Many times the battle will be fought in terms of preventing the presence of serotypes that affect human or animal health. These cannot become residents, because once Salmonella bacteria acquires this condition, their prevalence and spread will increase, and it would be very difficult to eradicate them.

Chicken’s immune response against Salmonella

The chicken’s immune response against Salmonella infection is not fully understood. Cell-mediated immunity and mucosal immunity are the most important responses in terms of protection against Salmonella infections. When this agent gets in contact with the intestinal mucosa, innate immunity is activated, a cell-mediated immunity is induced, accompanied by the synthesis of specific secretory immunoglobilins (IgA), providing a first defense barrier, seeking to prevent its dissemination to other tissues.. The response and defense mechanisms will be activated according to the type of bird, the age, the condition of the immune system and the serotype infecting the chickens.

Bearing in mind that Salmonella invasion occurs mainly by the oral route, another important component to consider is the intestinal microenvironment. The gut microbiota and its balance, and also the action of the lymphoid tissue associated with the gastrointestinal tract (GALT), are essential for an adequate local immunity. At the start of infection, paratyphoid salmonellae (*S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, among others) produce a local inflammatory process in the intestine. Later, some of these bacteria can colonize organs such as liver, spleen, ovary etc., and occasionally can lead to the presentation of a systemic disease.

Humoral immunity should be considered as complementary. The primary humoral immune response occurs 7 to 14 days post infection/vaccination through initial production of IgM followed by production of IgY (IgG) that can last for weeks. IgM are the antibodies initially produced. However, IgY are the most abundant antibodies at the intravascular and extravascular level. These are stored in the yolk sac and provide certain levels of humoral immunity to the chick in the first weeks of life. Once the plateau phase is reached, the level of antibodies decreases. If continuous antigenic stimulation is not present, the antibody levels will drop and may even disappear. Immune memory cells produce high, homogeneous, and long-lasting serological IgY titers that are mainly transmitted to the progeny (maternal antibodies) and to the egg, in breeders and commercial layers, respectively.

Types of vaccines

As stated before, to establish a successful Salmonella vaccination program, it is necessary to make a specific diagnosis of the serotypes present in the farm. The severity of the challenge and the type and age of the birds affected, and also the characteristics of the chicken houses, and the vaccines available on the market must be considered. Inclusion of live and inactivated vaccines should also be considered. Using vaccines as part of a comprehensive Salmonella control program will contribute to reduction of the excretion and prevalence of these bacteria. But the strategic choice of the vaccine or vaccines to use, timing, and method of vaccination may be the difference between a good or a bad vaccination program.

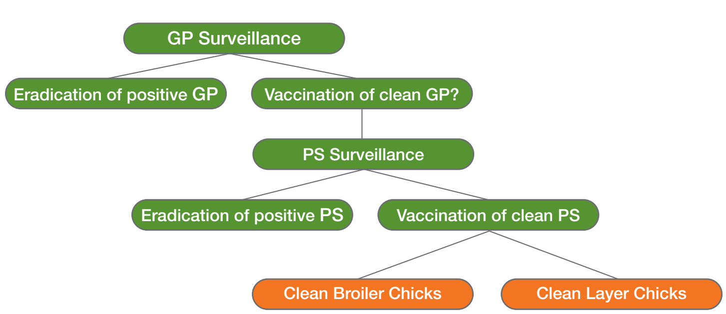
The use of vaccines for the control of Salmonella should be considered as part of a comprehensive control program seeking to prevent intestinal colonization and the subsequent invasion of the reproductive system. By preventing invasion of the ovary and oviduct, vertical or transovarian transmission would be avoided. On the other hand, if the bacterium cannot adhere to the intestinal epithelial cell, it will not be able to multiply, and in this way fecal excretion could be reduced. Consequently, the risk of fecal contamination of the egg is decreased too, reducing the risk of Salmonella transmission through the egg. In broiler chickens, reduction of Salmonella fecal excretion means less Salmonella loads contaminating the chicken house environment and litter, reducing the risk of contamination of feathers and legs. In this way vaccination, as live side intervention, contributes to a better outcome at the processing level by being more effective at eliminating (or reducing) loads of Salmonella from the chicken carcasses.

Live attenuated vaccines

Considering that cell-mediated immunity is the most important immune defense against Salmonella, live vaccines confer local protection by stimulating cell-mediated immunity and mucosal immunity; in addition to a mild stimulus to a long-term humoral response. An additional positive aspect is that live vaccines can cause a colonization inhibition effect, through competitive exclusion.

The use of live vaccines have become a common practice worldwide. However, despite the demonstrated safety of these products, it is necessary to plan a careful vaccination scheme that takes into account the withdrawal periods of the vaccine, to ensure that the final products (meat/eggs) are not contaminated with vaccine bacteria. This comment is specifically based on studies detecting the vaccine strain in 99% of vaccinated birds three days after vaccination. This percentage is gradually reduced at 21 days, when it is difficult (not impossible) to isolate the vaccine bacteria serotype from vaccinated chickens.

Top Down Control System

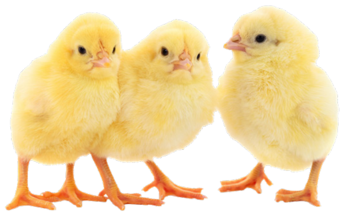


*GP – Grand Parents
**PS – Parent Stocks

Generally speaking, live Salmonella vaccines confer good protection. However, these vaccines have some disadvantages. Among these, the acceptance by the consumer of poultry products, which associates vaccines made with genetic manipulation as a risk to their health. Other criteria to take into account are effective protection against both mucosal infection and systemic infection, low risk with attenuated effects for both humans and animals, efficacy in reducing intestinal colonization, as well as in reducing environmental and egg contamination, easy application, cost-effective and especially compatible with other control measures.

In summary, the use of live vaccines for the control of Salmonella should be decided based on a judicious diagnosis of the situation, the possibility of introducing live agents to a region should be carefully studied, but the most important thing is to use "safe vaccines safely".

Inactivated vaccines



Salmonella inactivated vaccines are also called bacterins or killed vaccines. Autogenous inactivated vaccines are also included in this group. In these vaccines, Salmonella has been inactivated and usually suspended in a water-oil emulsion or with an aluminum hydroxide adjuvant. Most of these vaccines are administered intramuscularly or subcutaneously. These products stimulate high levels of circulating antibodies. Inactivated vaccines induce a very low cell-mediated immune response. One of the most important advantages of these vaccines is the significant reduction of fecal excretion of Salmonella, as well as a decrease in the persistence of this agent in some organs of birds (especially liver, spleen and reproductive tract) and in eggs. These vaccines are generally used in long-lived birds, like breeders and commercial layers.

The administration of inactivated products can reduce systemic infection by inducing a good humoral response.

Although bacterins confer partial protection against intestinal colonization and fecal excretion, some negative aspects of the use of these vaccines are the individual application (cost and stress) and the risk of eventual injuries due to the inflammatory reaction at the application site (by adjuvants or by inappropriate application). The interference with serological monitoring in prevention and control programs has been also discussed as a disadvantage.

Understanding the classification according to somatic antigens, the organization by groups and its relationship with Salmonella cross protection.

The way Salmonella serotypes are classified has changed over time. Historically Salmonella groups were classified using the letters of the alphabet. However, due to the large number of serotypes present in this genus, the letters became insufficient and The Institut Pasteur established the current classification of Salmonella using numbers to designate somatic groups O. A total of 67 somatic groups are currently recognized. Although letter designation still prevails in practice, groups should be designated by numbers. Table 1 shows some examples of important somatic groups for poultry O:4; O:7,O:8 and O:9; groups B, C1, C2-C3, and D1, respectively

Somatic Group (O)	O:4 (group B)	O:7 (group C1)	O:8 (group C2-C3)	O:9 (group D1)
Antigenic formula	**1, 4,{5},12**	**6, 7, 14**	**6,8 (C2); 8 (C3),20**	**1, 9,12**
# of serotypes in the group*	**~118**	**~169**	**~155**	**~77**
Examples	**Typhimurium**	**Infantis**	**Kentucky**	**Enteritidis**
	Heidelberg	**Braenderup**	**Newport**	**Gallinarum - Pullorum**
	Reading	Cholera suis	Hadar	Dublin
	Schwarzengrund	Mbandaka	Virginia	Berta
	Saintpaul	Virchow	Manhattan	Sendai
	Bredeney	Montevideo	Muenchen	Javiana
	Brandenburg	Tennessee	Gatuni	Alabama

Table 1.
Examples of classification of *Salmonella sp*. by somatic groups O: some serotypes included in somatic groups O: 4, O: 7 and O: 9 are shown (in bold type: important serotypes for chickens).

The importance of classifying Salmonella by group remains, among other things, in basic concepts related to protection using homologous and heterologous vaccines. According to the structure of the O antigen, Salmonella serotypes are different. Additionally, the flagellar antigen (H) expressed in two phases, also contributes to this differentiation.

- Homologous vaccines are those that have a degree of similarity which can indicate a common origin or ancestor. These vaccines use serotypes included in the same antigenic group as antigens, attenuated by laboratory techniques.

  The strains chosen to develop the vaccines must be strains with high immunogenic power. As an example, *S.* Enteritidis vaccine can confer partial or complete cross immunity between serotypes included in group O:9 (see table 2). However, cross protection will be reduced if there are differences in the somatic, flagellar, or phage antigens included in the same group (Chacana and Terzolo, 2010).

- Heterologous vaccines have a different origin. These vaccines contain a serotype of Salmonella that is in a different somatic O group, but can present common antigenic factors, which stimulate specific defenses. (see table 2). Ex. *S.* Typhimurium in group O:4, can provide heterologous protection for *S.* Enteritidis (group O:9). The immunity conferred by heterologous vaccines is not as strong as the one provided by homologous vaccines.

Antigenic Group (O)	O:4 (group B)	O:7 (group C1)	O:9 (group D1)
Antigenic formula	1,4,{5},12	6,7,14	1,9,12
# of serotypes in the group*	~118	~169	~77
Examples	Homologous protection → **Typhimurium**	Homologous protection → **Infantis**	Homologous protection → **Enteritidis**
	Heidelberg	**Braenderup**	**Gallinarum - Pullorum**
	Reading	Cholera suis	Dublin
	Schwarzengrund	Mbandaka	Berta
	Saintpaul	Virchow	Sendai
	Bredeney	Montevideo	Javiana
	Brandenburg	Tennessee	Alabama

Homologous in the same group. Partial cross protection

Cross protection by groups with similarities in antigenic formula: Heterologous protection

Table 2.
Examples of homologous protection using the same serotype, homologous protection using one serotype in the same group, and heterologous protection in among groups with similar antigenic formula.

Some inactivated bivalent or trivalent vaccines including *S.* Enteritidis, *S.* Typhimurium, and *S.* Infantis are commercially available. These vaccines can provide homologous (most important) and/or heterologous protection for serotypes included in groups O:9, O:4, and O:7; respectively. But its possible protection for other somatic groups is uncertain. Sometimes it is suggested that live vaccines may have effect against Salmonella serotypes from other groups (Ex. *S.* Typhimurium (O:4) conferring defenses for *S.* Enteritidis (O:9) and /or *S.* Infantis (O:7)). This concept may apply to those serotypes having antigenic similarities (Ex. *S.*Typhimurium and *S.* Enteritidis). However, for serotypes in different groups (Ex. *S.* Infantis, group O:7), this effect could be low and not specific; possibly related to competitive exclusion, cellular response, or mucosal mediate immunity.

Do we vaccinate or immunize?

When talking about effective vaccination programs against Salmonella, it is necessary to include concepts on the correct application of vaccines. It is not enough to just have a very good quality vaccine. It is also essential to have good application systems where the training of the vaccination personnel, the knowledge of the equipment used, and how the vaccine should be handled being the most critical components of a successful vaccination program.

Many of the criteria taken into account for the application of viral vaccines are valid when making recommendations on the application of live vaccines for Salmonella. Considering that live vaccines available for the control of Salmonella do not spread from bird to bird, the coverage when applying a Salmonella live vaccine must be 100%.

Inactivated vaccines (commercial and autogenous vaccines) are applied parenterally. As stated before, these vaccines require very well-trained vaccination personnel. Application under optimal hygienic conditions is necessary to avoid adverse local reactions; the use of dirty needles would produce a severe inflammatory reaction that can hinder the good diffusion of the vaccine. During the vaccination process, constant supervision will be key for a proper application of the vaccine. In the same way, the form of vaccination must be constantly verified: is the needle removed too quickly? Do the two layers of skin pass through, meaning is the vaccine being placed outside of the body? Does the vaccine leak from the injection site? A good practice is to check the injection site one hour after application to verify if the application of the vaccine was correct.

Final remarks:

1. Vaccination is an essential live side intervention contributing to a high percentage of Salmonella comprehensive prevention and control programs. These programs must also include monitoring and diagnosis, identification and elimination of sources and risk factors, high biosecurity levels, preservation of intestinal integrity, good quality of feed and water, and the proper functioning of both the immune system and the gastro-intestinal tract.

2. For long lived birds, the most effective vaccine programs for the control of Salmonella combine the application of several live vaccines, with at least one inactivated vaccine. To protect against those strains for which no live vaccine is available, i.e. Salmonella in somatic groups O:7 and O:8 or under severe challenge conditions, the administration of two inactivated vaccines is recommended.

3. Salmonella homologous vaccines provide protection against specific serotypes. Heterologous vaccines may provide partial cross protection for serotypes from different antigenic groups sharing some similarities in its antigenic formula. These vaccines can be combined effectively to ensure more adequate protection.

4. Vaccination against Salmonella reduces the number of carrier birds and the prevalence of these bacteria in the flock. Correct handling and application of vaccines are needed to avoid Salmonella persistence.

5. Salmonella resident serotypes are those that get completely established in poultry facilities, persisting for a long time. Early identification and comprehensive Salmonella control programs must be established to avoid Salmonella resident populations in the chickens and their environment.

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