

# **Salmonella in poultry meat and eggs: can we go to a zero level?**

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Abbreviated title: Salmonella in poultry meat and eggs

## **Summary**

Although the increasing trend of human infections caused by consumption of Salmonella contaminated food is stopped, there is still a high number of infections in developed countries. Poultry products constitute an important source for human infections. In the EU, obligatory control plans have been introduced and as a consequence, the poultry industry is applying a range of control measures in laying hen and broiler flocks. Vaccination is definitely an effective tool to combat Salmonella colonization in laying hens and thus to prevent egg contamination. Although the use of vaccines is currently not done in broilers in the EU, novel data show that live attenuated strains can be used to prevent colonization of virulent strains, a process called colonization-inhibition. A wide range of feed additives is on the market, aimed to decrease colonization and shedding of Salmonella in broilers (and layers), but the actual efficacy of many of these products can be questioned. Acids, prebiotics, such as manno-oligosaccharides and fructo-oligosaccharides, competitive exclusion products and probiotic strains are only some examples of currently used products. The numbers of contaminated Salmonella layer and broiler flocks will most likely decrease in the future due to the established action plans, but it is an utopia to eradicate Salmonella, as the pathogen is a commonly found bacterium in the environment. The main issue is however to keep the flock prevalence, the within-flock prevalence and the numbers of bacteria in infected animals at such a low level, that contamination of eggs and meat, and thus

transmission to humans, is only a marginal event. This clearly underlines the need of coordinated action of governments and poultry-related industries (including feed industry and slaughterhouses). Thus the control plans will need to encompass biosecurity and hygienic measures at different stages of the production chain, combined with the use of control measures.

Keywords: Salmonella, poultry meat, eggs, vaccination, control methods

## ***Salmonella* as cause of gastrointestinal disease in humans**

The annual number of *Salmonella* infections in humans is tremendously high worldwide. A worldwide egg-associated salmonellosis pandemic has started in the '70s and is currently fading away, thanks to huge efforts of policy makers and the poultry industry. This pandemic has been caused by the serotype *Salmonella* Enteritidis. Due to its preferential association with hen eggs, combined with the way humans tend to store (room temperature), handle and eat (uncooked) eggs, *Salmonella* Enteritidis had and still has a major impact on human health. While total EU *Salmonella* contamination levels are decreasing in recent years, the antimicrobial resistance of the *Salmonella* isolates is still increasing. Especially serotype Typhimurium is causing concerns, as about 40% of all *Salmonella* Typhimurium strains isolated in 2006 were resistant to 4 or more antimicrobials.

## **Serotype distribution in layers and broilers**

Although, but to a lesser extent, other serotypes can also infect and colonize laying hens, Enteritidis is the predominant serotype found in eggs. Several EU member states have reported data from investigations of table eggs, and the overall EU prevalence in 2006 was 0.8% (EFSA, 2007a). More than 90% of all egg-isolates were strains of the serotype Enteritidis. The other 10% of the isolates were strains from different serotypes but mostly isolated in only one EU member state, indicating that the importance of non-Salmonella Enteritidis isolates in eggs is marginal. The

high prevalence of serotype Enteritidis in table eggs is not completely consistent with the serotype distribution in laying hens. In 2006, 4.8% of the EU laying hen flocks were found *Salmonella* positive, and about 75% of all isolates were serotype Enteritidis strains (EFSA, 2007a). More than 10% were *Salmonella* Typhimurium strains, and a range of other serotypes was found in laying hen flocks. A large-scale baseline study of the European Food Safety Authority (EFSA) in 2005 revealed the presence of *Salmonella* spp. in 30.7% of 4561 large-scale laying hen holdings in the EU (EFSA, 2006a). The actual percentage of positive laying hen flocks in the same year reported by the member states however, revealed a percentage of *Salmonella* positive flocks in the EU of only 3.2%. Thus also the prevalence of 4.8% in 2006 is most likely a serious underestimation, and the sampling method and analytical methods were clearly more sensitive in the baseline study. More than 51% of all *Salmonella* isolates of the EFSA baseline study were *Salmonella* Enteritidis strains. The fact that different non-Enteritidis serotypes can be isolated from 25-50% of the *Salmonella* infected laying hen flocks, while more than 90% of all isolates from eggs are serotype Enteritidis strains (and the other 10% are derived from a minority of member states), implicates that the serotype Enteritidis harbors some intrinsic characteristics that lead to a specific interaction with either the reproductive tract of chickens, or the egg components. Generally, eggs can be contaminated by *Salmonella* on the outer shell and inside the egg. The former could potentially occur due to the presence of *Salmonella* in the hen's environment or passage of the egg through the cloaca. The latter could be a consequence of either shell penetration or colonization of the reproductive tract of laying hens and thus incorporation in the forming egg. There seem to be some virulence traits/factors that are possibly specific for *Salmonella* Enteritidis and that are associated with egg predilection. These are recently reviewed by Gantois et al. (2009). Although the exact reasons for the specific association of the *Salmonella* serotype Enteritidis with the oviduct and/or eggs is unknown, it is clear that one should focus on combating this serotype in layers to prevent egg contamination.

For broilers, a *Salmonella* baseline survey, carried out under supervision of EFSA from October 2005 till September 2006, observed a mean EU *Salmonella* prevalence of 23.7% (EFSA, 2007b). This strong discrepancy between the data from the baseline survey and the data reported by the member states is most likely caused by more sensitive sampling and more sensitive analytical methods used. This thus strongly points to a serious underestimation of the actual *Salmonella* prevalence as reported by the individual member states. The five most frequently isolated *Salmonella* serotypes at the EU level (in the baseline study) were Enteritidis, (37.1%), Infantis (20.4%), Mbandaka (7.9%), Typhimurium (4.6%) and Hadar (4.1%). Many more different serotypes are circulating in the broiler population as compared with laying hen flocks. About 5.6% of all EU broiler meat samples analyzed in 2006 were *Salmonella* positive. When the data from Hungary are excluded (more than 50% of all EU broiler meat samples were from

Hungary), *Salmonella* Enteritidis was the most frequent meat-contaminating serotype, followed by Paratyphi B var. Java, Infantis, Bredeney and Typhimurium. Besides these, many other serotypes can contaminate broiler meat. With respect to prevention of human *Salmonella* infections, in theory all serotypes should be controlled in the primary poultry production, as all of these can potentially be transmitted to humans by meat contamination in the slaughterhouse. There is however not a clear relation between the serotype distribution in broiler flocks and broiler meat, and the proportion of human *Salmonella* infections that is caused by consumption of broiler meat (relative to egg consumption) cannot be easily estimated. Nevertheless, serotypes typically found in broiler flocks and meat, and not in other animal species (such as serotypes Hadar, Infantis and Virchow) cause a certain (low) proportion of human salmonellosis cases. Other serotypes often found in broiler meat, in contrast, are not commonly causing human salmonellosis. This makes it difficult to speculate about the importance of *Salmonella* strains and serotypes present in broiler flocks with regard to human *Salmonella* infection.

## Vaccines are the preferred choice for decreasing prevalence in layer flocks

Vaccination has been compulsory in national control programmes in some EU member states as a consequence of the implementation of the EU legislations. Vaccination can be done using live or inactivated vaccines, and vaccines should a) reduce or prevent the intestinal colonisation resulting in reduced faecal shedding and thus egg shell contamination and b) prevent systemic infection resulting in a decreased colonization of the reproductive tissues, in this way reducing internal egg contamination. Currently used commercial vaccines in the EU mainly claim reduced shedding. It is very well documented that both killed and live vaccines can reduce shedding of *Salmonella* in poultry (reviewed by Van Immerseel et al., 2005).

An ideal *Salmonella* vaccine (strain) should possess the following characteristics:

- a high degree of protection against systemic and intestinal infection
- adequate attenuation for poultry, other animal species, humans and the environment as well as animal welfare issues
- the inactivated and live vaccines should not affect growth of the animal
- vaccine strains should not be resistant to antibiotics
- vaccines should be easy to be administer and need to have markers facilitating the differentiation from *Salmonella* wild-type strains
- application of vaccines should not interfere with *Salmonella* detection methods

- humoral antibody response after vaccination should be distinguishably from a *Salmonella* wild-type response to allow the use of serological detection methods

Based on multiple scientific and field reports, it is clear that *Salmonella* vaccines are very useful in laying hen flocks and can contribute to a decrease in colonization, shedding, and egg contamination, when the above mentioned vaccine characteristics are fulfilled. Recently multiple scientific groups have reported a phenomenon, in which oral administration of *Salmonella* wild type and attenuated strains can confer resistance to infection by a virulent *Salmonella* challenge strain within 24 h of administration. This 'competitive exclusion'-like phenomenon is called colonization-inhibition (Van Immerseel et al., 2005; Bohez et al., 2008). These data suggest that it might be possible to administer to newly hatched chicks live *Salmonella* vaccine strains such that they would colonize the gut extensively and very rapidly, inducing a profound resistance to colonization by other *Salmonella* strains of epidemiological significance, which may be present in the poultry house or may also have arisen from the hatchery (Van Immerseel et al., 2005). Colonization of the gut by the colonization-inhibition strains (live vaccines strains) would prevent gut colonization by virulent strains, while invasion in the gut tissue would evoke an inflammatory response that would prevent invasion to the internal organs by virulent strains. Thus also these two characteristics can be included in the list of vaccine criteria:

- attenuated live *Salmonella* vaccine strains should be able to induce the colonisation inhibition effect between *Salmonella* organisms
- attenuated *Salmonella* vaccine strains should have preserved the ability to invade the gut

In view of the complete list of both already realised and novel characteristics the key question in developing the ideal live *Salmonella* vaccine is to find the balance between an accepted level of attenuation and an unaffected ability to induce protection. This will best be accomplished by molecular genetics to produce defined deletion mutants.

## A brief overview of other control methods for broilers and layers

Control of *Salmonella* to the point that all EU targets are met, will not be possible by implementing vaccination as the sole control measure, especially in broilers. Indeed, a combination of different preventive and curative measures is necessary for the control of *Salmonella* infections in broilers. First of all, good farming and hygienic practices need to

be implemented, in order to avoid introduction of *Salmonella* on the farm or reduce the infection pressure when *Salmonella* is present. Hygienic measures at all levels of the production chain (pre-harvest (during life), harvest (catching and transport) and post-harvest) are essential for successful *Salmonella* control. Hygienic measures should take into account feed, birds, drinking water, environment, management, cleaning and disinfection. This can imply physical and chemical decontamination treatments of feed, drinking water, the environment of the birds, etc..

Eradication of contaminated flocks has not been shown to be an effective measure. The high density of broiler farms in certain regions within the EU is a major handicap. In addition, in the EU decontaminating treatments of chicken meat (or eggs) are hitherto prohibited. Therefore, prevention and monitoring during the life phase is very important in Europe and additional control measures to increase the resistance of the birds against *Salmonella* and to decrease the shedding and colonization by *Salmonella* field strains are of utmost importance. Vaccination of the parent broiler flocks can be used to decrease the susceptibility of the offspring by stimulating an immune response through maternal antibodies (Hassan *et al.*, 1996; Methner *et al.*, 1997). Also the use of a genetically more resistant chicken line might help to control *Salmonella*, although it remains difficult to reconcile selection for disease resistance and selection for performance. (Sadeyen *et al.*, 2004). More important however will be the application of control products in the laying hen or broiler flocks. Mainly feed additives are of importance in this regard, since they can have effects on intestinal colonization by *Salmonella*. These are mainly of importance in broiler flocks, as these are the only measures that can be applied in these animals during the live phase, as vaccination is not relevant seen the low slaughter age and thus the lack of time to build up a protective immune response (although the colonization-inhibition principle can in theory be used). There are an impressive number of commercially available compounds that can be used as feed or drinking water additives to control *Salmonella*. Some have well documented effects, some less well documented. Most have been selected by trial and error, and the observed effects are on an empirical basis. Since *Salmonella* is a bacterial infection, the most obvious tool for the control of these infections is to use antibiotics. The prophylactic or curative use of antibiotics for the control *Salmonella* infections in poultry however is prohibited under the E.U. regulation N° 2160/2003, amended by E.U. regulation N° 1177/2006. Therefore, most known products applied to control *Salmonella* in broilers flocks are acidic compounds (short- and medium-chain fatty acids), prebiotics and probiotics (less used in the field).

Prebiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health (Gibson and Roberfroid, 1995). In this definition it is understood that certain bacterial species multiplying in the colon can have a beneficial effect on host health. These bacterial species can also be administered

through the feed. These are then called probiotics (see below). Most prebiotics are carbohydrates. Most well known prebiotic products added to poultry feed are manno-oligosaccharides (MOS), glucans, fructo-oligosaccharides and guar gum. Many preparations are on the market, and for most products experimental proof and validation of effects on *Salmonella* colonization of the broiler gut is controversial.

Probiotics by definition are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). The original idea was launched over 30 years ago, when Nurmi and Rantala (1973) administered a suspension of gut contents derived from healthy adult chickens to newly hatched chicks, thereby protecting the chicks against *Salmonella* Enteritidis gut colonization. This concept is called competitive exclusion. Commercial products based on this concept have been on the market for a number of years and are very effective. One major handicap of this concept however is that the microbiota, which are included in these products, are undefined. Moreover these competitive exclusion products hitherto are not commonly included in feed. Therefore over the years considerable efforts have been made to identify specific micro-organisms that confer a similar protection. The many reports on the successful application of lactobacilli as probiotics, beneficial not only to the growth and performance, but also to the resistance of broilers against *Salmonella* infections, suggest a profound effect of these microorganisms on the intestinal ecosystem (Pascual et al., 1999; Van Coillie et al., 2006). A limited number of probiotic products is on the market.

Acidic compounds are more and more used to combat *Salmonella* infections. Not only drinking water acidification, but also acid release in the proximal gastro-intestinal tract (powder as feed additive) or the distal parts of the gastro-intestinal tract (coated or encapsulated acids in feed) is widely used. Medium chain fatty acids (MCFA) are strongly bactericidal towards many gram-positive and gram-negative bacteria, including *Salmonella* (Nakai and Siebert, 2003). Even at concentrations as low as 10mM MCFA still show a bacteriostatic effect on *Salmonella* (Van Immerseel et al., 2004). Short chain fatty acids (SCFA) are the major bacterial fermentation products in the large intestine (Tuohy et al., 2005). SCFA are also commonly added to feed and drinking water. At high concentrations (1%) these products have an antimicrobial effect in moist environment. This microbial growth inhibition is traditionally explained by the ability of these acids to pass across the bacterial cell membrane in undissociated form, dissociate in the neutral bacterial cell interior and thereby acidify the bacterial cell cytoplasm (Kashket, 1987). When the acid-treated feed is eaten by the chickens, it is both warmed and moistened, and thus the activity of the SCFA should increase. It has long been known however that up to 95% of the SCFA produced during carbohydrate fermentation may be taken up and utilized by the host (Cummings et al., 1987). Thus SCFA added to feed in powder form or added to drinking water may exert their activity in the lumen of the crop and gizzard but not further down the gastrointestinal tract (Thompson and Hinton,

1997). Coated and encapsulated products, in different formulations, are on the market. These formulations aim to bring the SCFA further down in the gastro-intestinal tract, and to release the acids at the site of colonization of *Salmonella*. For drinking water acidification and powder form feed additives, formic and acetic acid are most widely used. In coated and encapsulated products, butyrate is of particular importance, as it provides part of the daily energy requirements of the gastrointestinal mucosa, playing an important role in proliferation and differentiation of the epithelial cells (Macfarlane et al., 1997). Butyrate upregulates the expression of tight junction proteins, thereby enhancing the barrier function of the intestinal epithelium (Bordin et al., 2004). It also inhibits inflammation through the NF- $\kappa$ B pathway (Hodin, 2000). Sodium butyrate administered in feed in concentrations up to 0.2% increases feed conversion ratio, daily weight gain and intestinal villus length in broilers (Hu and Guo, 2007). Butyrate and propionate (as opposed to acetate and formate), at concentrations similar to those naturally produced in the lower gastrointestinal tract of the chicken, reduce invasiveness of *Salmonella* in intestinal epithelial cells in vitro (Van Immerseel et al., 2004). This phenomenon of reduced invasion by butyrate is mediated by a specific down-regulation of the genes encoded on the *Salmonella* pathogenicity island 1 (Gantois et al., 2006). Coating butyric acid on a carrier protects the acid from absorption in the upper digestive tract, transporting the acid down to the caeca, where *Salmonella* bacteria are known to colonize and invade the mucosa. As expected, coated butyric acid in feed protects chickens from caecal colonization of *Salmonella* (Van Immerseel et al., 2005). Considering the above mentioned characteristics, it may be advantageous to enhance butyric acid production by the endogenous microbiota using prebiotics. In this regard, it can be advantageous to add the prebiotics as well as the probiotic strains that utilize the prebiotics and thus produce the desired end products. This is called synbiotics, thus, in its simplest definition, a combination of probiotics and prebiotics. This combination could improve the survival of the probiotic organism, because its specific substrate is available for fermentation. This could result in advantages to the host offered by the live microorganism and the prebiotic.

## Can we reduce *Salmonella* prevalence in chicken flocks to zero?

Seen the recent EU Regulations and Directives and the implementation of the regulations by the member states, the awareness of the governments and the poultry industry to control *Salmonella* in poultry has increased. Seen the strict Community Targets and the deadlines at which the targets need to be reached (see above), member states have

established or are establishing control plans in the primary production. It is clear that the number of contaminated *Salmonella* layer and broiler flocks will most likely decrease in the future due to the established action plans. As an example, after introduction of the obligatory vaccination programme for layers in Belgium, the prevalence in layer flocks decreased and the number of human *Salmonella* Enteritidis cases decreased with more than 70% in 3 years. Although not all member states of the EU report this kind of spectacular decreases, the overall trend is a decrease in numbers of contaminated layer flocks. In broilers, some countries have huge contamination levels and the decreasing trend is not observed yet. It is a utopia to eradicate *Salmonella*, and to bring the prevalence of *Salmonella* in broiler and layer flocks to zero, as the pathogen is a commonly found bacterium in the environment. *Salmonella* will thus most likely never be totally eradicated in the poultry industry, but the main issue is to keep the flock prevalence, the within-flock prevalence and the numbers of bacteria in infected animals at such a low level, that contamination of eggs and meat, and thus transmission to humans, is only a marginal event. This will need a coordinated action of governments and poultry-related industries (including feed industry and slaughterhouses). Thus the control plans will need to encompass biosecurity and hygienic measures at different stages of the production chain, combined with the use of control measures. Concerning layer flocks, vaccination is a very good tool to control *Salmonella* to a certain level. For broilers, vaccination is not an option and the use of effective feed additives is an important tool. It is clear that all this will demand an enormous effort that will cost a huge amount of money. Furthermore, one will need to be constantly aware of the probability that new serotypes emerge. In addition, a possibly low prevalence in the future and consequent drop in the effort to control *Salmonella* will increase the risk of a re-emergence. Indeed, *Salmonella* bacteria can be carried by a host at low levels. The current and future control measures aiming at a reduction of *Salmonella* replication in the gut and reduction of shedding may lead to more cases of low level carriage. A large-scale study carried out by the authors of this overview already showed that despite the fact that overshoes and faecal samples were *Salmonella* negative in most flocks, thorough analysis of caecal samples of the same flocks in about 50% of the flocks results in *Salmonella* positive findings (at enrichment level) in a small percentage (1 to 5%) of the animals. Thus even zero prevalence in the EU monitoring schemes will not guarantee that *Salmonella* is not present within a poultry flock. Under conditions of stress and especially in case the control measures would be relieved, re-excretion may occur.

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