Vaccination of poultry against *Salmonella*: what is the ideal vaccine (strain)?

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Salmonella – data from human and poultry

Salmonelloses belong to the most important food-borne zoonoses throughout the world. According to the reports on zoonoses by the European Commission (EU 2001, 2003), a total of 157,822 cases of human salmonellosis were reported by Member States in 2001 and 135,546 cases in 2003. The decreasing tendency observed over several years has continued. *Salmonella* (S.) Enteritidis and *S*. Typhimurium are now as ever the serovars of *Salmonella* most frequently associated with human food poisoning (78.3 % of all cases in 2003). *S.* Enteritidis accounted for 61.8 % (2002: 67.1 %) of all notified cases, *S.* Typhimurium was on the 2nd place, causing 16.5 % of all cases. The serovars of *Salmonella* having places three to five are *S.* Virchow, *S.* Infantis and *S.* Hadar. However, compared with *S.* Enteritidis and *S.* Typhimurium those serovars are responsible each for only 0.5 % to 0.6 % of the reported human cases of salmonellosis.

The most important source for human infection with non-host adapted *Salmonella* organisms represent contaminated foods. It is concluded that the food categories possibly posing the greatest hazard to public health include raw meat and some meat products intended to be eaten raw, raw or undercooked products of poultry meat, eggs and products containing raw eggs, unpasteurised milk and some products thereof. Sprouted seeds and unpasteurised fruit juices are also of major concern. On the basis of a detailed analysis on the frequency of detection of *Salmonella* serovars in foods and infected humans (Steinbach and Hartung, 1999), it was possible to conclude that ca. 20 % of all human cases of salmonellosis are caused by *Salmonella* originating from swine (mostly *S.* Typhimurium) and that ca. 60 % to 65 % of human infections are caused by *Salmonella* arising from poultry, eggs and egg products (nearly exclusively *S.* Enteritidis).

These conclusion were confirmed by the results of a study on the prevalence of *Salmonella* in laying hen flocks in 25 Member States from 2004 to 2005 (EFSA, 2006). According to these data the EU prevalence of all *Salmonella* serovars in holdings of laying hens amounted to 30.7 %, however, the prevalence of both *S*. Enteritidis and *S*. Typhimurium amounted already to 20.3 %. The analysis of the frequency of isolated *Salmonella* serovars highlights the dominating role of *Salmonella* Enteritidis in the laying hen population. More than 50 % of all isolates belong to *S*. Enteritidis whereas the 2nd most detected serovar *S*. Infantis reached a share of less than 10 % and *S.* Typhimurium was only on the 3rd place with a share ca. 5 %, indicating that this serovar does not play an outstanding role in the laying hen population. Other serovars (Mbandaka, Livingstone, Virchow, Hadar, Ohio, Subsp. I Rough, Braenderup) reached percentages between 1.5 % and 4.4 % of all *Salmonella* isolates.

Furthermore, a study on the prevalence of *Salmonella* in broiler flocks from 2005 to 2006 (EFSA, 2007) could give valuable information for that animal population. The detected *Salmonella* spp. broiler flock prevalence amounted to 23.7 % with a large variety between countries. The observed *Salmonella* spp. flock prevalence in the EU ranged from a minimum of 0 % to a maximum of 68.2 %. The presence of *S.* Enteritidis and/ or *S.* Typhimurium was detected in 13 % of the flocks sampled in the EU, again with a broad range from 0 % to 39.3 %. Also in broilers, the analysis of the frequency of isolated *Salmonella* serovars highlights the dominating role of *Salmonella* Enteritidis (10,9 % of the 23.7 %). *S.* Infantis on the 2nd and *S.* Hadar on the 3rd place reached percentages of 2.2 % or 1.1%, respectively. *S*. Typhimurium responsible for 1.1 % of positive broilers flocks is also in broilers not

predominant and, therefore, *S.* Typhimurium originating from poultry products is responsible only to a less extent for *S.* Typhimurium infections in human beings.

Control of *Salmonella* in poultry

In view of these data there is a strong need to introduce effective measures for controlling *Salmonella* spp. in farm animal population. The primary aim of *Salmonella* control in poultry is to prevent these organisms from entering the food chain via eggs or meat. The successful control of *Salmonella* spp*.* is fundamentally based on the use of Good Farming and Good Hygienic Practices (GFP and GHP) from stable to table (WHO, 1994). Successful control of *Salmonella* infections in poultry starts at the farm and includes qualified management in connection with strictly observed zoosanitary measures. These measures are very important to avoid that hatching eggs, day-old chicks, feed, water, the poultry house, rodents and residual environmental contamination become the source of infection in fresh uninfected stock. Identification of *Salmonella* survival sites, evaluation of disinfectants and development of highly effective cleansing and disinfection regimes are necessary.

Vaccination of poultry against *Salmonella*

There has been an increasing interest in using *Salmonella* vaccination in poultry especially against the serovars of major public health relevance, *S.* Enteritidis and *S.* Typhimurium.
Vaccination of birds results in a further increase of resistance against *Salmonella* infection beyond the level of birds with developed intestinal flora. Inactivated and/or live *Salmonella* vaccines are in use for poultry in a number of countries.
The aim of vaccination as part of a complex control programmes for *Salmonella* infections in poultry is

- to reduce or prevent the intestinal colonisation resulting in reduced faecal shedding and egg shell contamination and,
- to prevent systemic infection resulting in a diminished localisation in the reproductive tissues.

The basis for protection of poultry against *Salmonella* infection is largely empirical although knowledge relating to the course of the innate and adaptive response to various *Salmonella* infections types is beginning to increase.

***Salmonella* serovars in vaccination and cross protection between serovars**

As *S*. Typhimurium and *S.* Enteritidis are the serovars of *Salmonella* most important for public health in Europe all existing commercially available *Salmonella* live and inactivated vaccines are intended for use against these serovars. Also the indication of a commercial live *S.* Gallinarum vaccine strain is the active immunisation of layers against *S.* Enteritidis. However, for other serovars relevant to human infections no vaccines are available for poultry production.
The salmonellae primarily responsible for enteritis in humans belong to a number of serogroups, including groups B, C and D. There is little evidence for any significant cross-protection between serogroups in mice (Hormaeche et al., 1996), cattle (Meyer et al., 1993) or chickens (Curtiss and Hassan, 1996) although the reason for this remains unclear. Some experimental evidence exists indicating little mutual protection between groups B and D in chickens. No published information exists for group C.
It seems likely that lipopolysaccharide (O-antigen) is a major component of the key immunogenic component and that protection between strains within a serovar is likely to be much greater. This assumption is supported by investigations in poultry under both experimental (Springer et al., 2000) and field conditions. After introduction of large scale vaccination using live *S.* Typhimurium strain in poultry breeding farms (layers and broilers) the detection rate of both *S.* Typhimurium and *S.* Enteritidis dropped considerably. Twelve months after starting vaccination *S.* Typhimurium was no longer detected, indicating the strong homologous immunisation effect. The detection of *S.* Enteritidis was reduced but this heterologous protection was much less effective than the homologous effects against *S.* Typhimurium, suggesting only a partial cross immunity effect between serogroups B and

D (Vielitz, 1993). As consequence of the observation that homologous immunity between strains of the same serovar is considerably stronger than between strains of different serovars, live *S.* Enteritidis vaccines were developed.

Live and inactivated *Salmonella* vaccines

Both live and inactivated *Salmonella* vaccines are available for poultry and a variety of vaccine preparations has been developed and tested for their protective efficacy in poultry (Barrow et al., 1991; Cooper et al., 1992; Vielitz et al., 1992; Methner, et al., 1994; Curtiss and Hassan, 1996; Hahn, 2000; Springer et al., 2000; Feberwee et al*.,* 2001).

Although a number of different **live** *Salmonella* strains have been tested for their efficacy in experimental or semi-field studies only a few are authorised and commercially available for use in poultry in Europe. The accessible live *S.* Typhimurium and *S.* Enteritidis vaccine strains are either auxotrophic double-marker mutants derived through chemical mutagenesis (Meyer et al., 1993; Springer et al., 2000) or developed on the basis of the principle of metabolic drift mutations (Linde et al., 1997; Hahn, 2000). Some of these *S.* live vaccines were further characterised by molecular methods (Schwarz and Liebisch, 1994). Another live vaccine authorised for prophylactic use against *S.* Enteritidis is based on a rough strain of *S.* Gallinarum without further molecular characterisation (Feberwee, et al. 2001).

Also a number of different **inactivated** preparations of *Salmonella* organisms have been tested for their efficacy against *Salmonella* challenge in poultry. However only one commercial inactivated *S.* Enteritidis based vaccine against *S.* Enteritidis infection in breeders and laying type chickens (Feberwee et al., 2001) is used in different countries and one commercial inactivated bivalent *S.* Enteritidis and Typhimurium dual vaccine against both *S.* Enteritidis and Typhimurium has been authorised (Clifton-Hadley et al., 2002). These killed vaccine types are based on bacterial cells cultured under conditions of iron depletion.

Vaccination schemes using a **combination of live and inactivated** *Salmonella* **vaccines** have been shown to be effective. Usually live vaccines are administered orally via drinking water in very young chicks during the rearing period followed by parenteral injection of inactivated vaccines before the beginning and during the laying period. However, immunisation schemes that do not use combination of live and inactivated vaccines are also used (Vielitz, et al., 1993; Feberwee et al., 2001).

Although most of the *Salmonella* vaccines used in Europe are of commercial origin, also **autologous** vaccines can be applied in some countries. An autologous vaccine is made by isolating a local strain of *Salmonella* spp. from a poultry house or animals, and producing a specific inactivated vaccine for this poultry farm. These vaccines comprise extracts of a culture killed by various processes (heat, formalin, etc.) and various adjuvants. Their efficacy is very controversial and their therapeutic efficiency has never been proven. These types of vaccines can only be produced for the farm of concern.

Efficacy of vaccination under experimental and field conditions

The aim of vaccination in poultry is both the prevention and reduction of intestinal colonisation resulting in reduced faecal shedding and egg shell contamination and also in the diminished colonisation of reproductive tissues produced by the induction of an **adaptive immune response**. Therefore, these criteria are generally included in potency testing of vaccines using quantitative and qualitative microbiological examinations of caeca, caecal content, cloacal swabs and different internal organs. Usually, the basis for testing the efficacy of any vaccine preparation are experimental studies. Results from these experiments may be supplemented by field- or semi-field studies.

Efficacy of vaccine preparations as judged by the level of intestinal and systemic colonisation as well as morbidity and mortality rates after vaccination and experimental infection using the oral or parenteral route of administration are examined. In general it is accepted that live *Salmonella* vaccines are more effective against both intestinal and systemic infection than are inactivated vaccine preparations (Lillehoj et al., 2000) largely, because they stimulate both the cellular and humoral arms of the immune system. However the level of protection measured depends on a number of factors (challenge strain, route of administration of the challenge strain, infection dose, parameters used to evaluate the course of infection, age of

birds, species of birds), therefore, it is not possible to compare the efficacy of the vaccine preparations available.
One important reason for the difficulties to compare the protection level is also the fact that an infection of chickens by the natural route even with an non-attenuated *Salmonella* wild-type strain will not result in absolute protection, e.g. another exposure of these birds with *Salmonella* organisms might result in a short-term intestinal colonisation. However, it was clearly demonstrated that vaccination of chickens results in a considerable quantitative reduced level and duration of intestinal colonisation and a diminished systemic invasion by *Salmonella* challenge organisms (Barrow et al., 1991; Cooper et al., 1992, 1993; Curtiss and Hassan, 1996; Hahn, 2000; Springer et al., 2000; Feberwee et al., 2001, Clifton-Hadley et al., 2002). Reduction of egg contamination following vaccination has been shown with some vaccines under experimental conditions (Gantois et al., 2006). Compared to the use of *Salmonella* wild-type strains as vaccines as the "gold standard" to verify the efficacy of live vaccines (Methner et al., 1995b) a number of registered *Salmonella* live vaccines revealed a high protective level (Hahn, 2000; Springer et al., 2000; Methner et al., 2001).
In addition to the development of an adaptive immune response, oral administration of live *Salmonella* bacteria to day-old chicks provides protection against re-infection with closely related *Salmonella* organisms within a matter of hours by **intestinal colonisation-inhibition** (Barrow et al., 1987a,b; Methner et al., 1997, 1999). This can prevent colonisation by wild-type *Salmonella* strains similar to the competitive exclusion effect, however, current vaccine strains contain mutations which abolish this effect, suggesting that currently available vaccines may be effective as immunogens but will not be effective as competitive exclusion preparations.
Parenteral administration of inactivated *Salmonella* vaccines to breeder birds will induce a strong production of antibodies. These antibodies will be transferred to the progeny. The **maternally transferred antibodies** persist a few weeks and, although there seems to be some protective effect against disease in the early post-hatch period, there is little effect on intestinal colonisation by challenge strains (Methner et al., 1994; Methner and Steinbach, 1997) and it is therefore possible to immunise effectively day-old chicks from vaccinated breeder birds with live *Salmonella* vaccines (Methner et al., 2002).
Studies on immunisation against *Salmonella* under field conditions also demonstrated the reduction of egg contamination and faecal shedding by both live and inactivated *Salmonella* vaccines (Linde et al., 1997; Feberwee et al., 2001; Davies and Breslin, 2004). However, it is very difficult to prove reduction of egg contamination following vaccination under field conditions owing to the low and variable percentage of contaminated eggs laid and the missing of appropriated non-vaccinated control flocks. Nevertheless, it could be shown experimentally that the number of shell contaminated eggs declines with the reduction of intestinal *Salmonella* colonisation and faecal excretion in layers (Gast and Beard, 1990; Methner et al., 1995a), therefore, it can be concluded that this effect will also occur after successful vaccination. The highest degree of protection was found in case of low *Salmonella* exposure to the flocks, in a highly *Salmonella* contaminated environment (poor cleaning and disinfection, insufficient control of rodents etc.) the level of protection in vaccinated flocks was diminished (Davies and Breslin, 2004).

What is the ideal *Salmonella* vaccine (strain) in future?
The *Salmonella* vaccines currently registered for use in the Member States have been authorised on the basis of national regulations, the mutual recognition procedure and several international guidelines, and therefore, fulfil the according requirements.
However, in order to improve the efficacy of *Salmonella* vaccination in poultry, an ideal *Salmonella* vaccine (strain) should possess following characteristics:

- a high degree of protection against systemic and intestinal infection
- a high protective potential against a variety of important serovars (serogroups)
- 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 administer and need to have markers enablling 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
- 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 as prerequisite to induce the invasion inhibition effect between *Salmonella* organisms

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

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