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REVIEW ARTICLE

Salmonella infections: immune and non-immune protection with vaccines

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Salmonella enterica in poultry remains a major political issue. *S. enterica* serovar Enteritidis, particularly, remains a world-wide problem. Control in poultry by immunity, whether acquired or innate, is a possible means of containing the problem. Widespread usage of antibiotics has led to the emergence of multiple antibiotic-resistant bacteria. This problem has indicated an increasing requirement for effective vaccines to control this important zoonotic infection. An attempt is made in the present review to explain the relatively poor success in immunizing food animals against these non-host-specific *Salmonella* serotypes that usually produce food-poisoning, compared with the success obtained with the small number of serotypes that more typically produce systemic "typhoid-like" diseases. New examinations of old problems such as the carrier state and vertical transmission, observed with *S*. Pullorum, is generating new information of relevance to immunity. Newer methods of attenuation are being developed. Live vaccines, if administered orally, demonstrate non-specific and rapid protection against infection that is of biological and practical interest. However, from the point of view of consumer safety, there is a school of thought that considers inactivated or sub-unit vaccines to be the safest. The benefits of developing effective killed or sub-unit vaccines over the use of live vaccines are enormous. Recently, there have been significant advances in the development of adjuvants (e.g. microspheres) that are capable of potent immuno-stimulation, targeting different arms of the immune system. The exploitation of such technology in conjunction with the ongoing developments in identifying key Salmonella virulence determinants should form the next generation of Salmonella sub-unit vaccines for the control of this important group of pathogens. There are additional areas of concern associated with the use of live vaccines, particularly if these are generated by genetic manipulation.

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Introduction

Over the past 15 years the animal and public health problems associated with *Salmonella enterica* in poultry have increased to the extent that they have become major political issues of which the general public have become very aware. *S. enterica* serovar Enteritidis, particularly, has become a world-wide problem, arising probably mainly in poultry (Rodrigue *et al*., 1990). In many countries, individual phage types of this serotype have replaced *S*. Typhimurium as the most dominant type in poultry and man. Control in poultry has become a major issue and immunity, whether acquired or, more speculatively, innate, is seen as a possible means of containing the problem. Widespread usage of antibiotics has led to the emergence of multiple antibiotic-resistant bacteria including *S*. Typhimurium. This problem has indicated to the industry and government agencies an increasing requirement for effective vaccines to control this important zoonotic infection.

An attempt is made in the present review to explain the relatively poor success in immunizing food animals against these non-host-specific *Salmonella* serotypes that usually produce food-poisoning, compared with the success obtained with the small number of serotypes that more typically produce systemic "typhoid-like" diseases in a restricted range of host species. Most of our understanding of immunity to salmonellosis arises from experimental work with typhoid-like diseases, usually *S.* Typhimurium infection in mice. Such work may not be entirely relevant to the, largely disease-free, colonization by most *Salmonella* serotypes. Whereas live, attenuated vaccines against host-specific serotypes are highly protective, similarly developed vaccine strains have traditionally been less effective in protecting chickens, calves and pigs against intestinal colonization. Newer methods of attenuation are being developed, which are being exploited. Their success will depend on appropriate attenuation and delivery, and on their use for infection types that have been shown to be amenable to immune control. However, from the point of view of consumer safety, there is a school of thought that considers inactivated or sub-unit vaccines to be the safest. The benefits of developing effective killed or sub-unit vaccines over the use of live vaccines are enormous. Recently, there have been significant advances in the development of adjuvants (e.g. microspheres) that are capable of potent immuno-stimulation, targeting

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different arms of the immune system. The exploitation of such technology in conjunction with the ongoing developments in identifying key Salmonella virulence determinants should form the next generation of Salmonella sub-unit vaccines for the control of this important group of pathogens. The use of microarray technology may also help in this field.

Pathogenesis of Salmonellosis

The development of an effective vaccine is dependent on an understanding of how *Salmonella* organisms infect their hosts and the host response to infection. A major problem with this goal is that *Salmonella* pathogenicity is both *Salmonella* serotype-dependent and host-dependent and the factors influencing serotype-host specificity are not known.

From the point of view of pathogenesis, the *Salmonella* genus can be divided into two major groups. One group typically produces systemic disease and, because in the absence of disease these strains colonize the intestine poorly and do not contaminate the carcass surface, they are rarely involved in human food-poisoning. The other group of serovars typically produces food-poisoning and only produces systemic disease under particular circumstances, such as during parturition, in lay, in very young or old animals, or after some viral infections. A comparison of the biological aspects of the two groups might explain our success in immunological control of the former group and limited success in control of the latter. It might also contribute to our understanding of the immune responses to infection. It is obviously central to any appraisal of experimental work on pathogenesis and immunity that like is compared with like, and that the results for one type of infection are not extrapolated to the other.

Our current understanding of *Salmonella* pathogenesis and immunity is largely derived from experimental infection studies in inbred laboratory strains of mice, generally with *S.* Typhimurium. This type of infection is characteristic of a small number of serotypes that characteristically produce this severe systemic disease, initially involving the reticulo-endothelial system in a restricted number of host species. Following inoculation of mice with an infective dose, a systemic infection occurs without an extensive enteritis. Thus mice are only a useful model for studying the systemic form of disease. The severity of the infection is dependent on the virulence of the *Salmonella* strain, the route of infection, the innate resistance of the mouse strain in use and its immune status. Similar information on systemic *S.* Dublin infection in cattle, *S*. Choleraesuis in pigs or *S.* Gallinarum in poultry is much less complete. These typhoidal serovars can also show persistent infection in convalescent animals and man, the nature of which is unclear. In birds, *S.* Pullorum particularly persists until sexual maturity when the reproductive tract becomes infected and infected eggs are laid leading to vertical transmission.

The second group comprises the remaining 2000 or so serotypes. They are not restricted to particular host species and their epidemiology can therefore be complex. Most are able to colonize the alimentary tract of animals without production of disease. Extensive carcass contamination at slaughter can result in *Salmonella* organisms entering the human food chain.

Some strains of particular serotypes, most notably *S.* Typhimurium and *S*. Enteritidis, are capable of producing clinical disease under certain circumstances. This is particularly the case for mice, young chickens, calves or piglets or during or after a period of physiological stress, such as after calving, during lay, after liver fluke infection or in cold wet weather. In these cases a systemic disease, again initially involving the reticulo-endothelial system, occurs in addition to faecal excretion.

Thus, host-specific serotypes cause systemic disease with involvement of the monocyte–macrophage series, and generally little initial intestinal colonization, whereas the reverse is true for most of the serovars that are associated with entry into the human food chain, causing food-poisoning (Barrow *et al*., 1994; Uzzau *et al*., 2000).

Immunity to *Salmonella*

Immune responses to *Salmonella* depend on the host species and the *Salmonella* serotype infecting the host. Serotypes that usually induce a self-limiting gastroenteritis in a broad range of unrelated host species, while being capable of inducing systemic disease in a wide range of host animals, are sometimes called un-restricted or broad host-range serotypes. Host-restricted serotypes, such as *S.* Gallinarum in poultry, have a totally different pathogenesis. In the discussion below, data on immunity of mice are given since this is best understood, followed by available data on poultry immunity where this is known.

The presence of two non-motile *Salmonella* serovars causing systemic disease in poultry is suggestive, and there are indications that the main initial signalling event with intestinal cells in chickens is through toll-like receptor 5; the absence of flagella would enable these two serovars to invade without the stimulation of a strong pro-inflammatory response from the host (Iqbal *et al*., 2005), as has been shown previously (Kaiser *et al*., 2000).

It is widely accepted that cell-mediated immunity is more important than humoral responses in protection against *Salmonella* (Collins, 1974; Mastroeni *et al*., 1993), although most of these studies come from *S*. Typhimurium infection in mice where a typical typhoid-like infection is produced and how far this is true for disease-free gut colonization is unclear. In mice, Th1 cytokines, which enhance cell-mediated responses, are crucial for protective immunity against a primary *Salmonella* infection (Eckmann & Kagnoff, 2001; Raupach & Kaufmann, 2001). Evidence for the importance of Th1 responses comes from experiments using interferon (IFN)-γ receptor knockout mice and mice with neutralizing antibodies to interleukin (IL)-12, which are unable to resolve infection by an attenuated *Salmonella* strain, in contrast to mice lacking class-I-restricted T cells, $\gamma\delta$ T cells or Ig-producing B cells that are able to clear the infection (Hess *et al*., 1996; Sinha *et al*., 1997; Mastroeni *et al*., 1998). Moreover, in mouse typhoid, protective roles have been shown for IL-1α, tumour necrosis factor-α, IFN-γ, IL-12, IL-18 and IL-15, whereas IL-4 and IL-10 inhibit host defences against *Salmonella*, again pointing to the importance of the Th1 response in control of *Salmonella* (Eckmann & Kagnoff, 2001). In Ig$\mu^{-/-}$ knockout mice lacking B lymphocytes, it has been shown that control of primary infection with

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avirulent *Salmonella* vaccine strains depends strictly on IFN-γ-producing $CD4^+$ T cells, whereas vaccine-induced protection against infection involves both cell-mediated and humoral responses, the latter in the later stages of infection (McSorley & Jenkins, 2000; Mastroeni *et al*., 2000a; Mittrücker *et al*., 2000; Mastroeni & Menager, 2003). Both cellular and humoral immune responses are stimulated by intraperitoneally administered heat-killed and live *Salmonella* vaccines in mice, the difference being the stimulation of Th1 or Th2 responses, which either direct B cells to switch to IgG2a via live organism stimulation of Th2/IL-4 or switch to IgG_1 following stimulation of IFN-γ, producing Th1 cells by killed *Salmonella* (Thatte *et al*., 1993; Thatte *et al*., 1995). IFN-γ has been found to be essential for reactive oxygen species-mediated killing of virulent *Salmonella*, although not essential for killing of avirulent vaccine strains (Foster *et al*., 2003a).

In contrast to mice, little is known about immune responses to virulent and attenuated *Salmonella* strains in poultry. It is not possible to prove the role of Th1 relative to Th2 immune responses, since there are so far no published studies involving Th2 cytokines. However, the correlation of IFN-γ levels with clearance is indicative (Beal *et al*., 2004a), as is the association with a strong T-cell response (Beal *et al*., 2005). An important role of early $CD8^+$ T cells as a representative population of cell-mediated immunity was shown after primary *Salmonella* infection in young chicks (Berndt & Methner, 2001). The importance of cell-mediated immune mechanisms in systemic clearance of *S.* Enteritidis in chickens was recently investigated by Farnell *et al*. (2001). In this study, intraperitoneal administration of recombinant IFN-γ resulted in a decrease in organ colonization after oral *S.* Enteritidis infection. It is proposed that cell-mediated immunity is more important than humoral responses for tissue clearance of virulent strains in poultry, while IgA responses and polymorphonuclear leukocytes would seem to be the likely key players in intestinal clearance of *Salmonella*, although this has not been proved experimentally and the evidence is confusing (Nagaraja & Rajashekara, 1999). Clearance of *S.* Typhimurium infection in chickens correlates with high cell-mediated responses (delayed type hypersensitivity reaction) and not with high antibody levels (Lee *et al*., 1981; Lee *et al*., 1983). A study of Desmidt *et al*. (1998c) with *S.* Enteritidis-infected, chemically bursectomized chickens showed increased faecal excretion and higher caecal *Salmonella* counts, while having normal counts in internal organs, indicative of a protective effect of IgA against intestinal colonization. Chemical bursectomy has the disadvantage that cell types other than only B cells may be affected. Colonization of the liver and spleen decreased over time in control as well as in bursectomized animals, indicating that other immune mechanisms play a role in systemic clearance of *S.* Enteritidis in chickens (Desmidt *et al*., 1998c). By contrast, surgically bursectomzed chickens, in which only B-cell maturation would be affected, showed clearance of *S*. Typhimurium from the intestine at the same rate as non-bursectomized birds (Beal *et al*., 2006), demonstrating that antibody response is not essential to gut clearance—which poses a number of fundamental and interesting questions about the nature and anatomical site/location of immune clearance. The age of the birds is also important, indicating a requirement for maturation of some component of the host that allows rapid clearance from 5 to 6 weeks of age (Beal *et al*., 2004b) whatever the age of infection.

The basis for disease-free persistent infection in the tissues in convalescent birds is still poorly understood. Using an infection model established by Berchieri *et al*. (2001), Wigley and colleagues showed that *S.* Pullorum persists within macrophages in the spleen (Wigley *et al*., 2001). However, the reason for the absence of clearance has not been fully elucidated. It is possible that *S.* Pullorum induces a response that is more Th2-like than the Th1-type response more normally associated with *S*. Typhimurium or *S*. Enteritidis, and from the IFN-γ responses there is some evidence for this (P. Wigley, unpublished results). Certainly, virulence determinants such as Salmonella Pathogenicity Island-2 are essential for persistent infection (Wigley *et al*., 2002). At the onset of sexual maturity the rapid increase in circulating sex hormones in the female suppresses the capacity to respond specifically to *S.* Pullorum antigens but also non-specifically to other antigens, accounting for the spread of the infection from the spleen to the reproductive tract at this time (Wigley *et al*., 2005).

Finally, in mice it has been shown that polymorphonuclear (PMN) cells play an important role in resistance to *Salmonella* infections (Hargis *et al*., 1999; Fierer, 2001). In chickens, heterophilic granulocytes accumulate in the propria mucosae of the caeca within 18 h following experimental infection with a *S.* Enteritidis field strain (van Immerseel *et al*., 2002a,b). In infection with *S*. Typhimurium this is accompanied by acute enteropathogenic responses characterized by expression of CXC chemokines and a PMN cell influx (Withanage *et al*., 2004, 2005). In response to *S*. Enteritidis, heterophils have been shown to up-regulate mRNA expression for pro-inflammatory chemokines IL-6 and IL-8 as well as the anti-inflammatory cytokine transforming growth factor-β4, whereas expression of IL-18 and IFN-γ was down-regulated (Kogut *et al*., 2003). The bacterial factors that are responsible for this effect have not been fully elucidated but in mice appear to include secreted effector proteins such as SopA, SopB and SopD (Wood *et al*., 2000), SipA (Lee *et al*., 2000) and the flagella protein FliC and probably FljB (Gewirtz *et al*., 2001; Hayashi *et al*., 2001; Reed *et al*., 2002). However, differences occur between different serovars, since the avian typhoid serovar *S.* Gallinarum down-regulates induction of IL-1 and IL-6 in avian epithelial cells (Kaiser *et al*., 2000). There has been considerable discussion about the contribution to enteropathogenicity of PMN cell influx in animals but there is now good evidence that this does not contribute directly *per se* (Foster *et al*., 2003b).

Granulocytopoenic (heterophil-depleted) chickens are much more susceptible to *S.* Enteritidis organ invasion, with the increase in bacterial number in the internal organs being proportionally related to the decrease in number of circulating PMN cells (Kogut *et al*., 1993, 1994). Another result underlining the importance of chicken heterophils in protection against *Salmonella* organ invasion was the finding that intraperitoneal administration of *S.* Enteritidis-immune lymphokines (SE-ILK) to 18-week-old chickens protected the animals from organ invasion by *S.* Enteritidis (Hargis *et al*., 1999; Tellez *et al*., 1993). SE-ILK are soluble products produced by T lymphocytes, derived from

S. Enteritidis-immune hens, cultured in the presence of concanavalin A. Intraperitoneal administration of SE-ILK in chickens resulted in a dramatic increase in the number of heterophilic granulocytes into the peritoneum without changing the numbers of other leukocytes, and administration *in ovo* protected young chicks against organ invasion by *Salmonella* (Kogut *et al*., 1995a,b). These studies indicate not only the importance of this aspect of the innate response to *Salmonella* infection, but also suggest that the course of infections might be modulated by manipulation of these responses.

Vaccines and Adaptive Immunity

Vaccination against host-specific *Salmonella* serotypes, causing severe systemic disease in a particular host species (e.g. *S*. Gallinarum in poultry), induces a strong serotype-specific protective immunity against infection and disease (Smith, 1956; Barrow & Wallis, 2000). In contrast, vaccination against non host-specific *Salmonella* serotypes has yielded variable success rates. The two infection types display very different epidemiological pictures and patterns of pathogenicity that, together with the nature of the immune response to systemic and intestinal infections, may account for these differences. Although the extent of disease and mortality varies according to the strain, with most serotypes there is always some invasion of the intestinal mucosa and reticulo-endothelial system.

The efficacy of vaccine preparations is judged by the level of intestinal and systemic colonization and morbidity and mortality rates after vaccination and experimental infection using the oral or parenteral routes of administration. However, the level of protection depends on the challenge strain, the route of administration, the infection dose, the age of birds and the species/line of birds. Consequently it has been difficult to compare strictly the efficacy of the vaccine preparations currently available.

Vaccination against host non-specific *Salmonella* serotypes has had varying success. As a result of public interest this has been a fruitful area for research over several years. A number of reviews have appeared that summarize our knowledge and understanding up to 5 to 6 years ago (Barrow, 1991; Meyer *et al*., 1992; Zhang-Barber *et al*., 1999; Barrow & Wallis, 2000). This is not the place to present a similar summary, but rather to examine recent literature and to review: (i) current feeling on the use of different types of vaccine in poultry, which will have some relevance to their use in other food animals; and (ii) any consequences for their application in young animals to exploit the early effects covered by this and a previous review (van Immerseel *et al*., 2005).

Killed vaccines have been used to control host non-specific *Salmonella* infections in poultry with very varying success. These have been used extensively as autologous vaccines and little information is available on their efficacy. Recent work (Timms *et al*., 1994; Liu *et al.*, 2001) supports earlier observations that they may be used to reduce mortality, although this is of little practical significance in the field. The relevance of this decrease in mortality for colonization of organs and shedding is also not clear since *Salmonella* infection in the field is mostly asymptomatic. Earlier experiments with killed vaccines report variable effects on faecal shedding and colonization of the intestine and internal organs. Some work (McCapes *et al*., 1967; Truscott & Friars, 1972) supports earlier contentions that maternal vaccination with bacterins does not reduce significantly excretion of *Salmonella* in the progeny, although mortality can be reduced. However, positive results have been reported. Single oral or intramuscular immunization with formalin-inactivated *S.* Enteritidis bacteria, encapsulated in biodegradable microspheres, at 2 weeks of age decreased faecal shedding and organ colonization of *S.* Enteritidis, after oral infection with 10^9 colony-forming units (CFU) at 6 weeks of age (Liu *et al*., 2001). Intra-vaginal vaccination with an oil-emulsion bacterin of *S.* Enteritidis at 38 weeks, followed by a booster 4 weeks later, reduced colonization of the ovary and spleen and reduced faecal shedding of a *S.* Enteritidis challenge strain (Miyamoto *et al*., 1999). After challenge, 36 of 189 eggs (19.0%) in the vaccinated hens were positive, and this contamination rate was significantly lower than that in the unvaccinated hens (61 of 165 eggs, 37.0%). By contrast, in a field trial in which autogenous bacterins were used for single or double immunization, 10 layer flocks were vaccinated at different time intervals while one flock was left unvaccinated. The percentage of positive environmental samples and samples of internal organs of the vaccinated animals were not decreased relative to the animals of the unvaccinated flock (Davison *et al*., 1999).

A vaccine containing inactivated *S.* Enteritidis that was grown under iron-restricted conditions is available on the market in some European countries (Woodward *et al*., 2002). Also, a vaccine containing *S*. Enteritidis as well as *S*. Typhimurium, both grown under conditions of iron restriction, is also commercially available (Clifton-Hadley *et al*., 2002). Iron-restriction is known to up-regulate bacterial factors that stimulate virulence and thus may stimulate important immunogens. However, given that many other relevant genes are also up-regulated in macrophages (Eriksson *et al*., 2003), it might be more appropriate to produce the vaccines under the conditions experienced in that environment. The inactivated *S.* Enteritidis vaccine was efficient at decreasing egg contamination after intravenous challenge with *S.* Enteritidis (Woodward *et al*., 2002). This work is difficult to evaluate since oral or respiratory challenge would have been more relevant. However, the combined *S*. Enteritidis and Typhimurium vaccine, when given intramuscularly at day 1 and week 4, did decrease shedding after oral challenge with *S.* Typhimurium in a seeder-bird challenge model (Clifton-Hadley *et al*., 2002). Less than 30% of the vaccinated birds shed *Salmonella* bacteria, while at 10 days post-challenge more than 80% of the unvaccinated animals shed *Salmonella*.

Subunit vaccines have also been used in poultry. Outer-membrane protein vaccines with adjuvant have been used to decrease shedding of *S.* Enteritidis in poultry (Meenakshi *et al*., 1999). Khan *et al*. (2003) immunized 9-week-old chickens with two outer membrane proteins subcutaneously, followed by two boost immunizations with time intervals of 2 weeks. These outer membrane proteins were shown to be involved in attachment of *S.* Enteritidis to intestinal epithelial cell lines (Fadl *et al*., 2002). Immunization of either of the outer membrane proteins decreased caecal colonization about 1000-fold when the animals were infected orally

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with 8×10^8 CFU virulent *S.* Enteritidis strain (Khan *et al*., 2003).

Attention has been paid to the development of avirulent vaccine strains of *Salmonella* because of the accumulation of evidence that such strains of *Salmonella* are more immunogenic in mice and in poultry than are killed or subunit vaccines (Collins, 1974; Zhang-Barber *et al*., 1999). Live vaccines have been tested extensively in mice and also in poultry. Although a number of different live *Salmonella* strains have been tested for their efficacy in experimental or semi-field studies, only a few are registered and commercially available for use in poultry in Europe. The commercially available 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 (Vielitz *et al*., 1992; Linde *et al*., 1997; Hahn, 2000). These are negative mutations in essential enzymes and metabolic regulatory centres, as a consequence of which the resulting metabolic processes lead to prolonged generation times and corresponding reductions in virulence (Linde *et al*., 1997). Some of these *Salmonella* live vaccines have been further characterized by molecular methods (Schwarz & Liebisch, 1994).

Another live vaccine registered for prophylactic use against *S.* Enteritidis, which was developed initially for immunization against *S.* Gallinarum, is the rough strain *S.* Gallinarum 9R (Smith, 1956). This vaccine strain has been tested more extensively in recent years since is has been shown to give cross-protection against S. Enteritidis (Barrow *et al*., 1991), a member of the same serogroup. The extent of cross-protection against other serotypes, from either the same or other serogroups, remains unclear. In a large field trial in The Netherlands in which 80 commercial flocks were vaccinated with the *S.* Gallinarum 9R vaccine strain, the flock level occurrence of *S.* Enteritidis infections was 2.5% (2/80 flocks). This was significantly less than the flock level occurrence of *S.* Enteritidis infections in unvaccinated flocks (214 out of 1854 flocks, 11.5%) (Feberwee *et al*., 2001a). In 4500 eggs derived from five *S*. Gallinarum 9R vaccinated flocks, no vaccine strain bacteria were detected—while no evidence was found in another study for the faecal spread of the vaccine strain (Feberwee *et al*., 2001b, 2002).

Temperature-sensitive spontaneous *S.* Enteritidis mutants, able to grow well at 28°C but not at 37°C, have been tested as vaccine strains in poultry (Cerquetti & Gherardi, 2000a,b). When the mutant was orally inoculated (10^9 CFU) in chickens at days 1, 2, 3 and 7 post-hatch and these animals were orally challenged at 7 or 14 days after the last vaccination with 10^8 CFU strains of *S.* Enteritidis and Typhimurium, fewer challenge bacteria were recovered from the caecal contents, liver and spleen 14 days post-challenge (Cerquetti & Gherardi, 2000a). An alternative vaccination scheme (10^9 CFU at day 1 and 2 weeks post-hatch, orally) also decreased shedding and colonization of internal organs when the animals were challenged with 10^9 CFU virulent *S.* Enteritidis strain 14 days after the last oral immunization (Cerquetti & Gherardi, 2000b). As with many studies, challenge occurred soon after vaccination and the vaccine strain was still present in the tissues of 54% and 28% of the animals at the time of vaccination. Experiments such as these may be partially explained by the non-specific effects covered later by this review and all work involving short periods between vaccination and challenge must take into account stimulation of innate responses (Maskell *et al*., 1987).

Numerous other live attenuated *Salmonella* vaccine strains have been developed by mutating genes involved in survival in host tissues. Genetic modification of the vaccine strain aims at reducing the risk of spread or persistence in the environment while at the same time inducing an adaptive immune response. It will be apparent that some of the mutations chosen may have consequences for the colonization inhibition effect inducible in the gut of young animals (*vide infra*). The complete genome of *S.* Typhimurium has been sequenced (www.genome.wustl.edu/projects/bacterial/styphimurium) and that for *S*. Enteritidis is now also complete (www.sanger.ac.uk/ projects/*Salmonella*). This will facilitate the construction of completely rational mutations. Genes coding for metabolic functions or virulence factors are the main targets for producing safe vaccine strains. There is a certain rationale for inactivation of housekeeping genes that will reduce bacterial growth and virulence without greatly affecting the expression of key virulence determinants, required for appropriate immunogenicity (Klose & Mekalanos, 1997). Double or even triple mutations can be introduced to increase safety by reducing the risk of reversion by acquisition of genes by horizontal transfer (Tacket *et al*., 1997; Methner *et al*., 2004). Whichever mutations are made, it would seem crucial that the vaccine strains retain the capacity of invasiveness in order to stimulate sufficient immunity to be protective. At the same time the vaccine strain needs to be eliminated before slaughter age in broilers, and before onset of lay in layer and breeder chickens. A number of genes have been mutated for the construction of candidate vaccines, including those involved in the biosynthesis of bacterial lipopolysaccharide (*galE*), regulation of expression of outer membrane proteins (*ompR*), amino acid or purine biosynthesis (e.g. *aro*, *pur*, *guaB*), regulation of carbon source utilization (*cya crp*), virulence factors and many others, such as *htrA*, *phoPQ*, *recA* and *waaN* (Mastroeni *et al*., 2000b). Few mutants have been tested in poultry (Zhang-Barber *et al*., 1999), but the relevance of murine studies to intestinal colonization of poultry is questionable. For example, *phoP*^{c} mutants of *S*. Typhimurium, although poor presenters of antigens *in vitro* (Wick *et al*., 1995), are highly immunogenic in mice (Miller & Mekalanos, 1990; Hopkins *et al*., 1995), largely ascribed to their persistent infection of and efficient presentation by dendritic cells, as opposed to their poor survival in macrophages (Niedergang *et al*., 2000). How well such strains survive in chicken cells is totally unknown but they are certainly immunogenic (Methner *et al*., 2004). *AroA* mutants have been tested extensively in poultry and found to be effective, albeit less protective than the "gold standard" produced in chickens infected with a wild-type strain (Barrow *et al*., 1990; Cooper *et al*., 1990). Given the general consensus that there is little cross-protection between serovars, it is not surprising that Parker *et al*. (2001) found no significant differences in egg or reproductive tract infection when laying hens were vaccinated at the day of hatch, 4 and 22 weeks with an *aroA* mutant of

S. Typhimurium and challenged with *S.* Enteritidis 8 weeks after the final immunization.

Many of the characteristics and claims attributed to the *cya crp* mutant of *S*. Typhimurium, including the high-level cross-protection, require confirmation, and this mutant retains considerable virulence producing enteritis in gnotobiotic pigs (Barrow *et al*., 2001). Dueger *et al*. (2003) also made claims for cross-protection using *dam* mutants, although the degree of cross-protection was fairly small. These studies also highlight the shortcomings of mutations (*cya crp* and *dam*), which demonstrate attenuation in systemic infection but are not tested for their ability to induce gastroenteritis. The exploration of the *sop* and other genes associated with Sip-dependent effector proteins (Wallis & Galyov, 2000) are a logical next stage in the creation of a truly rational live vaccine.

The use of live attenuated *Salmonella* strains to deliver recombinant antigens to the immune system is an attractive additional strategy for the creation of multivalent vaccines for poultry. Multivalent vaccines would decrease the number of vaccinations required in the field. Sustained expression of the heterologous antigen in the tissues in an immunogenic form at levels sufficient for priming a protective immune response is the main target when developing *Salmonella* recombinant vaccines (Mastroeni *et al*., 2000b). Vaccination of chickens with a Δ*cya crp* mutant of *S*. Typhimurium expressing the *Escherichia coli* O78 lipopolysaccharide O-antigens induced antibodies against the O78 lipopolysaccharide O-antigen and against *Salmonella,* and engendered a degree of protection against challenge with a pathogenic *E. coli* O78 strain (Roland *et al*., 1999). *S*. Typhimurium vaccine strains were used as antigen delivery system for oral immunization of chickens against two antigens of the coccidian parasite *Eimeria tenella* (Pogonka *et al*., 2003). However, the delivery of antigens to the immune system is not sufficient *per se* to engender a protective response. A successful vaccination also requires the elicitation of an appropriate type of immune response. Thus, different groups are working on the development of carrier-based vaccination strategy in order to promote this. For example, strains carrying mutations affecting the specific course of infection can be exploited to modify the immune response elicited (Drabner & Guzmann, 2001; Dietrich *et al*., 2003), or the sub-cellular location of recombinant antigen in the vaccine *Salmonella* strain may influence the type of the immune response (Kang & Curtiss, 2003). In addition, the co-delivery of immune stimulatory molecules facilitates triggering a predictable response according to specific needs (Dunstan *et al*., 1996). This type of work has, up to now, been performed only in mice. For example, Igwe *et al*. (2002) constructed a chimeric protein based on the *Yersinia* outer protein E (YopE) comprising the listerial antigens eliciting a cell-mediated immune response. In mice orally immunized with attenuated *Salmonella* vaccine strains expressing the chimeric YopE translocated by the type III secretion system, this novel vaccination strategy led to the induction of a pronounced cytotoxic CD8 T-cell response that conferred some protective immunity against *Yersinia* (Russmann, 2004).

A significant development in the past few years involves the use of *Salmonella* vaccines for the delivery of DNA vaccines. Such vaccines may induce immunity against the *Salmonella* carrier, heterologous antigen(s) from a second *Salmonella* serotype or other pathogen (Darji *et al*., 1997). Consideration is being given to future modulation of the immune response by the co-expression of cytokines. A number of cytokines have been expressed in *Salmonella* vaccine strains, some of which have been shown to have an immunomodulatory effect at least in mice (Dunstan *et al*., 1996; Ianaro *et al*., 1995).

Live versus Killed Vaccines

As stated above, most data on vaccine-induced protection are derived from mice studies and care should be taken in extrapolating these data to poultry. Although killed vaccines can be efficacious in reducing *Salmonella* in poultry, live vaccines are thought to have some advantages over killed vaccines, including stimulation of both cell-mediated and humoral immune arms and expression of all appropriate antigens *in vivo*, while the latter stimulate mainly antibody production and express only the antigens present at the time of *in vitro* harvesting (Collins, 1974; Barrow & Wallis, 2000). Killed vaccines may also be destroyed rapidly and eliminated from the host; they may be poorly, immunogenic in unprimed hosts and unable to induce cytotoxic T cells (Nagaraja & Rajashekara, 1999). Live vaccines have been shown to be more effective in increasing lymphocyte proliferation in response to *S.* Enteritidis antigens in laying hens (Babu *et al*., 2003). They also have additional protective effects, particularly when administered orally, which can be exploited during their development and application. These include genus-specific colonization inhibition (competitive exclusion) demonstrated to be primarily an effect of microbial metabolism, and the stimulation of primed PMN cells in the gut (see below for both; see also van Immerseel *et al*., 2005). Killed vaccines are unable to induce these effects. It seems unlikely at the moment that more effective killed or sub-unit vaccines will be produced in the next few years because many basic questions relating to identification of the major protective immunogens and the nature of the immune response in the chicken remain unanswered. Live vaccines have some disadvantages, including, perhaps most significantly, those associated with public acceptability, particularly where genetic manipulation has been used to produce the vaccine. This is a major issue that should be addressed since the safety requirements are different for live vaccines to those for inactivated vaccines.

The criteria for an ideal vaccine have been discussed previously (Pritchard *et al*., 1978; Barrow, 1999) and they include: (1) effective protection against both mucosal and systemic infection; (2) attenuation for animals and man; (3) efficacy in reducing intestinal colonization, and thus reduced environmental contamination, and egg infection; (4) compatibility with other control measures; and (5) cost-effective application. As indicated above, it is already possible to attenuate strains in a number of ways but the inability to induce gastroenteritis is not always evaluated. It should be possible in the next few years to produce live, attenuated strains that are immunogenic for poultry and other food animals but that maintain attenuation in man and other non-target species. This will, by necessity, require molecular genetics as a tool. The alternative is that live, attenuated vaccines are produced, as currently, by undefined chemical mutagenesis with strains possessing

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a combination of uncharacterized lesions, including antibiotic resistance, whose cumulative effects may also not be completely known. The vaccines currently in use in Europe and elsewhere are highly safe, but it is anomalous that it is acceptable to allow their widespread dissemination while being extremely cautious over the use of defined deletion mutants produced by genetic manipulation, but where each deletion is nevertheless known and characterized and where antibiotic resistance genes are not present. The environmental issues associated with the genetic modification of plants and also some food animals that may escape to the wild, are very different issues to the use of bacterial deletion mutants, with no additional DNA added. One advantage of the current widespread application of the vaccines that are already in use is that, because they are widely distributed, data will now accumulate on any reversion and other potential risks to man, target animals and the environment.

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Colonization Inhibition

Vaccination is regarded as an essentially prophylactic measure whose protective effect begins after a period of maturation of the B-cell and T-cell response. Thus, after vaccination of 1-day-old chicks, production of significant amounts of specific antibody responses against *Salmonella* takes more than 10 days (Desmidt *et al*., 1998b). For infections that may occur before this time, such as those arising from hatchery infection, this window of susceptibility is too long. However, orally administered live *Salmonella* organisms can induce a very rapid form of protection early in the life of the bird as a result of their colonization-inhibiting activity.

Colonization inhibition, or competitive exclusion, as it is more commonly known, can also be induced by the administration of normal gut flora preparations to newly hatched chicks. Young birds are highly susceptible to infection with *Salmonella*, as a consequence of the absence of a protective gut flora and immaturity of the immune system (Friedman *et al*., 2003; van der Wielen *et al*., 2000). The first can be overcome by the application of competitive exclusion products based on cultures of normal flora obtained from pathogen-free adult birds (Nurmi & Rantala, 1973), which, according to the recommendation of the World Health Organization, should be applied as early as possible to 1-day-old chicks in the hatchery or by spraying eggs and in preference to administration via the first drinking water. However, treatment with undefined flora is not permitted in many countries due to the potential risk of transmission of pathogens, although this can be avoided by appropriate testing of the product. The use of undefined flora and probiotics to control Salmonella in poultry will not be covered in detail in this review.

Because of some of the concerns associated with the use of undefined competitive exclusion products, studies were initiated in the 1980s to search for bacterial strains that possessed the colonization characteristics of *Salmonella* but not their virulence attributes (Barrow & Tucker, 1986). During this study one group of 1-day-old chicks was found to be completely refractory to infection with the challenge *S*. Typhimurium strain. This was as a result of the fact that the birds had become infected with a strain of *S*. Montevideo from the feed soon after hatching. This strain, isolated from the birds and administered to a new batch of newly hatched chicks, completely protected them against challenge 24 h later with the *S*. Typhimurium. In fact, it was found that an attenuated rough mutant of the *S*. Typhimurium strain also prevented establishment and colonization by the fully virulent, smooth parent strain. This effect was therefore studied further.

Initial studies revealed that the effect required live bacteria; a variety of killed preparations administered either orally or parenterally had no effect. The inhibition was therefore not the result of a novel rapid immune response stimulated by bacterial antigens in the gut. Neither was it the result of bacteriophage activity resulting from phage multiplying on the first strain. It was specific to related bacterial taxa. Thus strains of *E. coli*, *Citrobacter*, *Proteus* and other related bacteria had no effect against *Salmonella* but did inhibit colonization by organisms from their own genera. Among the *Salmonellae*, not all strains were equally inhibitory. The mechanism was studied using an *in vitro* system of stationary-phase broth cultures (Berchieri & Barrow, 1990, 1991) and it appeared to relate to the use and depletion of carbon sources and other nutrients available under the relevant redox conditions under which the organisms are growing (Zhang-Barber *et al*., 1997). However, the practical aspects of the effect were immediately apparent and warranted further investigation. This (Berchieri & Barrow, 1991; Martin *et al*., 2002) showed that the protective effect required high numbers of bacteria in the intestine and that, as the normal flora began to develop, the genus-specific exclusion reduced in efficacy. The effects were long lasting in terms of reduced faecal excretion and occurred in different chicken breeds, ducks (Barrow *et al*., 1999) and on different diets. The effect became apparent after 6 h or so but only became fully effective after 18 to 24 h. Some strains were more effective than others, although no strain was fully effective against all *Salmonella* strains (Iba *et al*., 1995; Martin *et al*., 2002) and there appeared to be a serovar-specific effect, but how far this was related to clonality, rather than serovar specificity, remains unclear. The most profound level of inhibition *in vivo* occurred between isogenic strains. The fact that the challenge strains did not colonize as a result of the inhibition also led to reduced invasion by them (Nógrády *et al*., 2003) and in the associated mortality induced by virulent challenge strains (Barrow & Lovell, unpublished results). These data suggested that it might be possible to administer live vaccine strains to newly hatched chicks such that they would colonize the gut extensively and rapidly before the normal flora became established, and that this should induce a profound resistance to colonization by strains that may be present in the poultry house or may also have arisen in the hatchery. A search was made for a strain of *Salmonella* with a wide spectrum of inhibition, capable of preventing colonization by an extensive selection of strains. A strain of *S*. Infantis (Berchieri & Barrow, 1990) and a strain of *S*. Hadar (Nógrády *et al*., 2003) were found to be more inhibitory than were other serovars. These serovars are characteristically poorly invasive but highly colonizing (Desmidt *et al*., 1998a) and it may be that this latter characteristic is related to the inhibitory activity, possibly through a wide variety of nutrients available to it (see mechanism of inhibition below).

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Attenuated live *Salmonella* Typhimurium and Enteritidis vaccines with certain metabolic pathway mutations (Vielitz *et al*., 1992; Meyer *et al*., 1993; Cooper *et al*., 1994a,b; Hahn, 2000; Springer *et al*., 2000; Feberwee *et al*., 2001a; Methner *et al*., 2001) or deletions in genes for *cya* and *crp* (Curtiss & Kelly, 1987) are immunogenic. However, it was also shown that these attenuated live *Salmonella* vaccines were generally not, or only briefly, able to inhibit intestinal colonization of homologous or heterologous *Salmonella* challenge organisms by the above exclusion mechanism (van Immerseel *et al*., 2002b; Methner *et al*., 1997). Thus, none of the currently available commercial live *Salmonella* vaccines is able to induce protection against *Salmonella* organisms by this exclusion or inhibition effect. There is therefore a need to identify live *Salmonella* strains that are sufficiently attenuated without affecting genes essential for colonization inhibition. Recent studies confirmed not only the high level of attenuation of *Salmonella* strains with deletions in *phoP*, but also demonstrated their colonization-inhibition ability (Methner *et al*., 2004).

Similar colonization-inhibition effects were also observed in the intestines of gnotobiotic pigs (Lovell & Barrow, 1999), suggesting that this is a general phenomenon not restricted to chickens. The occurrence of competition between related bacteria and its use in infection prevention has, in fact, been known for many years, although in most cases there is no understanding of its basis. It has been demonstrated between strains of *E. coli* in gnotobiotic mice, newborn infants and in pigs (Davidson & Hirsch, 1976; Duval-Iflah *et al*., 1983). Similar exclusion studies have been demonstrated between strains of *Campylobacter jejuni* (Barrow & Page, 2000), and work to determine whether the mechanism is similar in *Salmonella* and *Campylobacter* is underway.

The mechanism of colonization inhibition is also poorly understood and, although an early hypothesis arose from the observation that a similar inhibition could be demonstrated in stationary-phase nutrient broth cultures (Zhang-Barber *et al*., 1997), interactions with the host, either by competition for sites of adhesion or through stimulation of the innate immune system (van Immerseel *et al*., 2005), have by no means been discounted. Of these mechanistic explanations, neither explains completely the colonization-inhibition phenomenon, and both may be involved simultaneously.

The Host Response in Colonization Inhibition: A Role for Granulocytes?

From the above experimental studies there has been considerable argument as to how far the inhibitory effect was primarily a microbiological process or competition between related bacteria not involving a host response *per se*. However, other more recent studies have suggested that the host might be involved and have opened up further an area of infection-immune biology that also has considerable practical implications.

Since colonization inhibition is a process that rapidly induces resistance to infection, adaptive immune responses are thought not to play a significant role. Immune cells are attracted very rapidly to the site of the infection after inoculation of chickens with virulent and attenuated *Salmonella* strains (Kogut *et al*., 2003; van Immerseel *et al*., 2002b). These cells, comprising heterophilic granulocytes, macrophages, T lymphocytes and, to a lesser extent, B lymphocytes, infiltrate the caecal wall within 24 h post-vaccination in large numbers. These cells may play a role in colonization inhibition, since the caeca are known to be the predominant site for colonization and invasion by *Salmonella* in the chicken (Desmidt *et al*., 1997; Desmidt *et al*., 1998a). When birds were orally vaccinated with 10^8 CFU candidate vaccine strain *S.* Enteritidis *aroA* CVL30 immediately post-hatch and subsequently challenged with the virulent homologous *S.* Enteritidis strain 1 day later, colonization of the liver and spleen was strongly reduced during the first 5 days post-infection. However, on day 10 after infection no differences were seen in the number of challenge organisms in the liver and spleen. Caecal colonization by the challenge strain was only moderately suppressed in vaccinated birds compared with untreated controls (van Immerseel *et al*., 2002b). This observation suggested that this cellular infiltration was not likely to be the main cause of the colonization inhibition, although this was not conclusively proven—it did, however, demonstrate an interesting potential protective effect against virulent *Salmonella* invasion soon after hatching. The same experiment was repeated in animals that were depleted of heterophilic granulocytes by the well-established model of 5-fluorouracil depletion (Kogut *et al*., 1994; van Immerseel *et al*., 2003). In this experiment the protection against colonization of internal organs was completely lost, suggesting a central role for heterophilic granulocytes in protection against invasion and organ colonization (van Immerseel *et al*., 2003). This is consistent with previous studies in chickens assessing the role of PMN granulocytes in protection against organ colonization by *Salmonella*. The extent of heterophilic granulocytic depletion was proportionately related to increases in the number of *Salmonella* in internal organs, and increasing the number of circulating heterophilic granulocytes following administration of cytokines derived from stimulated T cells protected against organ colonization by *Salmonella* (Kogut *et al*., 1994; Tellez *et al*., 1993). Much older work had also shown that live vaccines can stimulate, within hours of inoculation, a high degree of protective immunity against homologous and heterologous bacterial challenge (Field *et al*., 1955; Smith, 1956; Wilson & Miles, 1964; Maskell *et al*., 1987), presumably through activation/priming of the innate immune system, once thought to be primarily macrophages (Kodama *et al*., 1976), but perhaps more likely to be PMN cells.

These data strongly suggest a role for heterophilic granulocytes in protection against internal organ colonization by *Salmonella* in chickens, and also suggest that this is inducible by oral inoculation with live, attenuated *Salmonella* vaccines. This has considerable practical implications for poultry. The bacterial factors that are responsible for this effect have not been fully elucidated (see above). Similar results have also been found in mammals. A strain of *S*. Infantis was found to have a wide spectrum of colonization inhibition against different *Salmonella* strains in newly hatched chicks (Berchieri & Barrow, 1990). This strain was also tested in gnotobiotic pigs to determine whether it would be similarly inhibitory against other serovars in young milk-fed mammals. This was found not to be the case. Although

the *S*. Infantis strain was completely avirulent for 1-week-old pigs, it did not show colonization inhibition against a fully virulent *S*. Typhimurium strain. However, the pigs pre-inoculated with the *S.* Infantis and challenged with the *S*. Typhimurium remained perfectly healthy (Barrow *et al*., 1997), whereas pigs inoculated with only the *S*. Typhimurium developed severe enteritis and required humane killing. Similar results were found with a second *S*. Typhimurium challenge strain and *S*. Choleraesuis, and also when the experiments were carried out in gnotobiotic calves (Foster *et al*., 2003). Of the cell types studied, only PMN cells were observed in high number in the villi of the gut in the vaccinated groups. A more detailed study of this effect (Foster *et al*., 2003b, 2005) concluded that the *S*. Infantis strains was sufficiently invasive to induce infiltration of large numbers of primed and activated PMN granulocytes into the intestinal mucosa, which themselves did not induce any pathological changes, but which were highly antibacterial to the virulent *S*. Typhimurium strain inoculated 1 day later. In this context, pre-inoculation with attenuated *Salmonella*e may act similarly to commercially available Biostim (Roch-Arvellier *et al*., 1987). Biostim is a glycoprotein derived from *Klebsiella pneumoniae* that has been shown to reduce the duration and rate of bacterial infection in the airways. The drug stimulates increased C3b and C3bi receptor expression in neutrophils (Capsoni *et al*., 1991), increases neutrophil phagocytic capacity (Minonzio *et al*., 1991) and increases neutrophil oxidative metabolism (Idohou *et al*., 1993).

These mechanisms appear superficially to be separate and distinct phenomena, two microbiological and the other involving the innate immune system, but both with practical implications for the use of live vaccines in young animals, including poultry. As indicated above it may be that these effects may operate simultaneously. However, the obvious differences may conceal a common thread that merits further exploration; namely that, during colonization of the chicken caeca by *Salmonella*, these micro-organisms come into close contact with the mucosa, particularly in the region of the caecal tonsil An assumption was made in early studies that intestinal colonization was primarily a reflection of bacterial metabolism, of whether or not the bacteria involved were able to exploit the nutritional and other physiological conditions present in the gut (Turner *et al*., 1998). There is increasing evidence that this is not the case and that an interaction between colonizing bacteria and the host is required as a component of colonization, whether or not this leads to extensive invasion and systemic disease. Colonization inhibition may require a combination of mechanisms for full inhibition. The microbiological studies suggest establishment in the gut through appropriate metabolism and a failure to do this would prevent any interaction with the host, which may then take the form of a competition for adhesion sites or, where invasion takes place, involving heterophil activity, which may occur in or close to the lumen in the caecal tonsil. Thus, studies on these effects may also ultimately tell us a great deal about the mechanism of colonization and the extent to which host–pathogen interactions may be involved in this aspect of infection, which is central to food-poisoning.

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Non-English Abstracts

Salmonella infections: immune and non-immune protection with vaccines

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Infections salmonelliques: protection non immune ou immune avec des vaccins

Salmonella enterica chez les volailles demeure un problème politique majeur. *S.* serovar Enteritidis, particulièrement, demeure un problème mondial. Le contrôle chez les volailles par l'immunité soit acquise soit innée, est un moyen possible de maîtriser le problème. L'utilisation, à grande échelle, des antibiotiques a conduit à l'émergence de bactéries multirésistantes aux antibiotiques. Ce problème a mis en évidence un besoin croissant de vaccins efficaces pour contrôler cette importante zoonose.

Dans cette revue, on a essayé d'expliquer le succès relativement faible de l'immunisation des animaux contre les sérotypes de salmonelles non spécifiques d'hôte qui entraînent généralement des intoxications alimentaires, comparé au succès obtenu avec le petit nombre de sérotypes qui plus typiquement produisent des maladies systémiques de type "typhoïde". Des examens nouveaux concernant de vieux problèmes tels que le statut de porteur et la transmission verticale, observés avec *S*. Pullorum sont générateurs de nouvelles informations en rapport avec l'immunité. Des méthodes plus récentes d'atténuation ont été développées. Les vaccins vivants, s'ils sont administrés par voie orale, démontrent une protection rapide et non spécifique contre l'infection, ce qui a un intérêt biologique et pratique. Cependant, du point de vue de la santé du consommateur, il y a une école de pensées qui considère que les vaccins sous-unitaires ou inactivés présentent la meilleure innocuité. Les bénéfices pour le développement de vaccins sous-unitaires ou inactivés efficaces par rapport à l'utilisation de vaccins vivants sont importants. Récemment, il y a eu des progrès significatifs dans le développement des adjuvants, par exemple les microsphères, qui sont capables de puissante stimulation immunitaire ciblant différentes branches du système immunitaire. L'exploitation de telle technologie conjointement avec les développements en cours concernant l'identification des déterminants clefs de la virulence des salmonelles devraient constituer la prochaine génération de vaccins salmonelle sous-unitaires pour la maîtrise de ce groupe important d'agents pathogènes. Il existe par ailleurs d'autres domaines de préoccupation, en relation avec l'utilisation de vaccins vivants, particulièrement s'ils sont issus de manipulation génétique.

Salmonelleninfektionen: spezifische und unspezifische Schutzwirkung durch Vakzine

Salmonella enterica beim Geflügel ist immer noch ein großes politisches Thema, wobei insbesondere *S*. serovar Enteritidis weiterhin ein weltweites Problem darstellt. Ein mögliches Mittel, das Problem zu kontrollieren, ist beim Geflügel die Bekämpfung mittels Induzierung einer erworbenen oder angeborenen Immunität. Der weitverbreitete Einsatz von Antibiotika hat zur Entstehung von Bakterienstämmen mit multipler Antibiotikaresistenz geführt. Dieses Problem weist auf einen zunehmenden Bedarf nach effektiven Vakzinen zur Bekämpfung dieser bedeutenden Zoonose hin.

Immunisierungen gegen die geringe Anzahl von *Salmonella*-Serotypen, die typischerweise systemische "Typhus-ähnliche" Erkrankungen verursachen, waren recht erfolgreich. In diesem Übersichtsreferat wird der Versuch gemacht, den im Vergleich dazu relativ geringen Erfolg bei der Immunisierung Lebensmittel produzierender Tiere gegen die nicht wirtsspezifischen *Salmonella*-Serotypen, die gewöhnlich zu Lebensmittelvergiftungen führen, zu erklären. Neue Untersuchungen alter Probleme wie den Trägerstatus und die vertikale Übertragung im Zusammenhang mit *S*. Pullorum erbringen neue Informationen über die Bedeutung der Immunität. Neuere Attenuierungsmethoden werden entwickelt. Oral verabreichte Lebendvakzine induzieren einen unspezifischen und schnellen Schutz gegen die Infektion, was sowohl von biologischem als auch praktischem Interesse ist. Aus Sicht der Verbrauchersicherheit wird jedoch die der Standpunkt vertreten, dass inaktivierte oder Subunitvakzinen die sichersten Impfstoffe sind. Der Vorteil der Entwicklung wirksamer Tod- oder Subunitvakzinen ist gegenüber der Verwendung von Lebendvakzinen enorm. Kürzlich sind bedeutende Fortschritte in der Adjuvansentwicklung zum Beispiel mit den Mikrosphären, die eine starke Immunstimulation in verschiedenen Bereichen des Immunsystems auslösen können,

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gemacht worden. Die Ausnutzung einer derartigen Technologie in Verbindung mit der weitergehenden Forschung hinsichtlich der Identifizierung der Schlüsseldeterminanten der Salmonella-Virulenz sollte die nächste Generation von Salmonella-Subunitvazinen zur Kontrolle dieser wichtigen Erregergruppe gestalten. Es existiert aber weiterhin auch das Interesse an der Verwendung von Lebendvakzinen, insbesondere an solchen, die durch Genmanipulation optimiert werden.

Infecciones por Salmonella: protección inmune y no inmune mediante vacunas

Salmonella enterica continua siendo en avicultura uno de los mayores temas con repercusión política. En concreto *S.* serovar Enteritidis, sigue siendo un problema a nivel mundial. El control en las aves a través de la inmunidad, bien sea innata o adquirida, es probablemente una manera de contener el problema. El uso indiscriminado de antibióticos ha permitido la aparición de bacterias multirresistentes. Este problema ha mostrado la necesidad creciente de vacunas efectivas para el control de esta importante infección zoonótica.

Esta revisión pretende explicar el poco éxito relativo de la inmunización de animales de abasto frente a los serotipos de *Salmonella* no específicos de huésped, causantes de las toxiinfecciones alimentarias, en comparación con el éxito obtenido con el bajo número de serotipos que de manera clásica producen las enfermedades de "tifoideas". La revisión de viejos problemas como el estatus portador y la transmisión vertical, observados en *S. pullorum*, genera información nueva de relevancia para la inmunidad. Se están desarrollando nuevos métodos de atenuación. Las vacunas vivas, si se administran oralmente muestran una protección rápida y no específica de gran interés biológico y práctico frente a la infección. Sin embargo, desde el punto de vista de la seguridad del consumidor, hay una escuela de pensamiento que considera más seguras las vacunas inactivadas o de subunidades . Los beneficios del desarrollo de vacunas muertas o de subunidades efectivas frente al uso de vacunas vivas son enormes. Recientemente, se han obtenido grandes avances en el desarrollo de adyuvantes, por ejemplo microesferas, que son capaces de potenciar inmunoestimulación, actuando en diferentes vías del sistema inmune. La explotación de esta tecnología junto con los actuales avances en la identificación de los determinantes de la virulencia de *Salmonella* debería ser la próxima generación de vacunas de subunidades de *Salmonella* para el control de este grupo tan importante de patógenos. Hay otros puntos de preocupación asociados al uso de vacunas vivas, principalmente si éstas se han generado mediante manipulación genética.

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