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Immunisation of chickens with live *Salmonella* vaccines – Role of booster vaccination

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ABSTRACT

It is accepted that booster vaccinations of chickens with live *Salmonella* vaccines are essential part of vaccinations schemes to induce an effective adaptive immune response. As manufacturer of registered live *Salmonella* vaccines recommend different times of booster the question raises whether the duration between the first and second immunisation might influence the protective effect against *Salmonella* exposure. Chickens were immunised with a live *Salmonella* Enteritidis vaccine on day 1 of age followed by a booster vaccination at different intervals (day 28, 35 or 42 of age) to study the effects on the colonisation and invasion of the *Salmonella* vaccine strain, the humoral immune response and the efficacy against infection with *Salmonella* Enteritidis on day 56 of age. Immunisation of all groups resulted in a very effective adaptive immune response and a high degree of protection against severe *Salmonella* exposure, however, the time of booster had only an unverifiable influence on either the colonisation of the vaccine strain, the development of the humoral immune response or the colonisation of the *Salmonella* challenge strain. Therefore, the first oral immunisation of the chicks on day 1 of age seems to be of special importance and prerequisite for the development of the effective immune response. A booster immunisation should be carried out, however, the time of booster may vary between week 3 and week 7 of age of the chickens without adversely impact on the efficacy of the adaptive immune response or the protective effects.

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1. Introduction

After implementing regulation (EC) No 2160/2003 [1] of the European Parliament on the control of *Salmonella* and other specified food-borne zoonotic agents, the prevalence of *Salmonella* organisms in poultry decreased considerably in most European countries. The reduction of *Salmonella* Enteritidis in the whole egg production pyramid during the last decade [2] contributed to the decline of human cases of salmonellosis in the EU. However, the observed decrease of *Salmonella* infections in men between 2011 and 2014 did not continue in 2015. Also the proportion of *Salmonella* Enteritidis, the now as ever most prevalent serovar for human infections caused by contaminated eggs and egg products, has increased in 2015 [3]. Consequently, there is still a need to continue the proven control strategy by using both effective management and hygiene regimes along the production chain and measures to increase the resistance of chickens against *Salmonella* exposure by vaccination. The special role of vaccination with both live and inactivated vaccines as effective tool to control *Salmonella* infections [4] is underlined also by the regulation (EC) No 1177/2006 [5] which obliges the use of vaccination programmes against *Salmonella* Enteritidis in Member States as long as they did not demonstrate a prevalence below 10%. Numerous studies on the special characteristics and protective effects of commercial and potential *Salmonella* vaccines have been carried out [6–8,4,9–15]. Apart from inducing protection by colonisation and invasion inhibition effects already very shortly after administration of live *Salmonella* vaccines [7,11–13], the development of a protective adaptive immunity is needed in older and adult birds. It is assumed that booster vaccinations are an essential element of schemes aiming to develop an adaptive immune response [4,8]. However, as the manufacturer of the registered live *Salmonella* vaccines recommend different times of booster vaccination [16–21], the question raises, whether the duration between the first and second immunisation might influence the efficacy of the adaptive immune response of the chickens. The aim of the present study was to examine the effect of different times between the first application of the live *Salmonella* vaccine at day of hatch of the chicks followed by a booster immunisation at different intervals on both the humoral immune response and the protective effect against infection with *Salmonella* Enteritidis.

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2. Materials and methods

2.1. Chickens

Specific pathogen-free White Leghorn chickens were hatched at the facilities of the Friedrich-Loeffler-Institute from eggs obtained from Charles River Deutschland GmbH. Experimental and control groups were kept in cages in separate negative pressure rooms. Commercial feed (coarse meal without antibacterial additives) and public drinking water were both available *ad libitum*. The single groups were managed separately (including cleaning and feeding regimes) to prevent cross-contamination between the groups effectively throughout the trials. Animal experiments were performed in accordance with the German Animal Protection Act and approved by an ethical committee (Animal Ethics approval number: 03-006/06).

2.2. Bacterial strains and culture

Salmovac SE (IDT Biologika, Dessau-Roβlau, Germany), a registered *Salmonella* Enteritidis live vaccine (SE-LV) strain (phage type PT4) was used for oral immunisation (PO) of the chickens. To facilitate accurate enumeration of the vaccine strain in caecal contents and liver, a spontaneous nalidixic acid-resistant (N) mutant was produced for immunisation [22]. The resistance has no perceptible impact on the in vivo results [23,11,12]. This assumption was confirmed using an in vitro cell culture model for adhesion and invasion [24] and in vivo studies on the combined administration of the nalidixic acid-resistant SE-LV with a competitive exclusion culture [7]. The viable count of the attenuated SE-LV administered PO via crop instillation was 2×10^8 colony forming units (cfu) per bird. Oral infection of the chicken was carried out with a rifampicin (R) resistant variant [7,13] of the comprehensively characterised strain *Salmonella enterica* subspecies *enterica* serovar Enteritidis 147 (SE 147, phage type PT4) [11,12] at a dose of 2×10^8 cfu/bird. All strains had been stored in a Cryobank system (Mast Diagnostica) at −20 °C.

2.3. Experimental design and bacteriology

In experiment 1, three groups (A–C) of chickens were immunised PO via crop instillation with the SE-LV at a dose of 2×10^8 cfu/bird on day 1 of life (Table 1). Birds of these groups received a booster vaccination, group A on day 28, group B on day 35 and group C on day 42 of life, each with the SE-LV at a dose of 2×10^8 cfu/bird, chicks in group D were used as non-immunised controls. SE-LV was enumerated in caecal contents and in liver at days 7, 14, 21, 28, 35, 42, 49 and 56 of life from 4 birds/group, respectively, by a standard plating method [7,11]. Homogenised organ samples were diluted and plated on brilliant-green phenolred agar (SIFIN) with sodium nalidixate (50 μg/ml) and incubated at 37 °C for 18–24 h. Caecal contents and liver samples from all birds in groups A-D (Table 1) were also pre-enriched in buffered peptone water (SIFIN), incubated at 37 °C for 18–24 h and streaked onto brilliant-green phenolred agar with sodium nalidixate (SIFIN).

In experiment 2, all immunised groups A–C and the non-immunised control group D were challenged orally with SE 147R administered at a dose of 7×10^8 cfu/bird PO on day 56 of life (Table 3). The challenge strain was enumerated in caecal contents and liver from 4 birds/group at days 59, 63, 66, 70 of age using a standard method described above. To detect the challenge strain SE 147R organ samples were plated on deoxycholate-citrate agar (SIFIN) supplemented with rifampicin (100 μg/ml) and incubated at 37 °C for 18–24 h. Samples were pre-enriched in buffered peptone water (SIFIN), incubated at 37 °C for 18–24 h and streaked onto deoxycholate-citrate agar with rifampicin. Additionally, blood from each animal in experiments 1 and 2 was gained on all days of bacterial examination, the sera were frozen at −20 °C until use for serology.

2.4. Serological analysis

The *Salmonella* antibody response was measured as optical density (OD) after immunisation and/or infection of the chickens in experiments 1 (Table 2) and 2 (Table 4) with a commercial ELISA-system (Flocktype® *Salmonella* Ab, Qiagen). Additionally to the instructions of the manufacturer the serum samples were diluted not only 1:500 before use but also 1:100. The evaluation of the results was performed by calculating the ratio of the optical density between serum sample and positive control according to the instructions of the manufacturer.

2.5. Statistical analysis

Viable bacterial counts were converted into logarithmic form. For statistical purposes a viable count of $\log_{10} < 1.47$ (the limit for direct plate detection) from a sample detected positive only after enrichment was rated as $\log_{10} = 1.0$. A sample which yielded

Table 1
Number (mean $\log_{10}$ cfu/g of 4 birds) of *Salmonella* Enteritidis live vaccine (SE-LV) in liver and caecal contents of chickens after oral administration of 2×10^8 cfu/bird at day 1 of age followed by booster immunisation at different times (day 28, 35 or 42 of age) (experiment 1).

Day of age	Group A		Group B		Group C		Group D	
	Liver	Caecal contents	Liver	Caecal contents	Liver	Caecal contents	Liver	Caecal contents
1	SE-LV		SE-LV		SE-LV		–	
7	3.2	7.5	3.3	7.4	3.1	7.3	–	–
14	1.6	5.0	1.9	4.9	1.1	4.6	–	–
21	0.5	4.5	0	3.8	0	3.5	–	–
28	0	4.6	0	4.1	0	3.4	–	–
	SE-LV		–		–		–	
35	0	3.6	0	3.0	0	3.3	–	–
	–		SE-LV		–		–	
42	0	4.1	0	3.1	0	3.9	–	–
	–		–		SE-LV		–	
49	0	2.4	0	3.1	0	3.6	–	–
56	0	2.1	0	1.7	0	2.2	–	–

Standard error: liver: 0.169, caecal contents: 0.948.
[a] Significantly lower than group A.
[b] Significantly lower than group B.
[c] Significantly lower than group C.

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