Invasion inhibition effects and immunogenicity after vaccination of SPF chicks with a *Salmonella* Enteritidis live vaccine

Nachweis von Invasionshemmeffekten und der Immunogenität nach Impfung von SPF-Küken mit einer *Salmonella*-Enteritidis-Lebendvakzine

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ZUSAMMENFASSUNG

Gegenstand und Ziel Für den Eintrag von Salmonellen in die Lebensmittelkette des Menschen haben Infektionen der Hühner mit *Salmonella* Enteritidis, *Salmonella* Typhimurium und *Salmonella* Infantis große Bedeutung. Zur Bekämpfung von Infektionen werden, zusätzlich zu veterinärhygienischen Maßnahmen, inaktivierte Impfstoffe und Lebendimpfstoffe auf der Basis attenuierter Impfstämme eingesetzt. Neben einer adaptiven Immunantwort induzieren bestimmte Impfstämme angeborene Immunmechanismen, die eine Invasion des Erregers in innere Organe verhindern bzw. behindern. Es ist ungeklärt, ob sich diese Hemmungseffekte auch gegen andere *Salmonella*-Serovare richten. Weiterhin ist nicht bekannt, ob sich die adaptive Immunantwort nach Impfung mit einem *Salmonella*-Enteritidis-Phagentyp-4-Lebendimpfstoff auch auf andere Lysotypen von *Salmonella* Enteritidis und Typhimurium erstreckt.

Material und Methode Eintagsküken wurden mit einer *Salmonella*-Enteritidis-Lebendvakzine oral vakziniert. Zur Prüfung des Auftretens der Invasionshemmung wurden die Tiere am Tag darauf mit einem markierten *Salmonella*-Typhimurium- bzw. *Salmonella*-Infantis-Stamm infiziert. Zum Nachweis der adaptiven Immunantwort erfolgte nach der Impfung eine orale Infektionsbelastung mit markierten *Salmonella*-Enteritidis-Stämmen der Phagetypen 1, 8 und 21 am 63., 42. und 51. Lebenstag sowie einem monophasischen *Salmonella*-Typhimurium-Stamm („Definitive Type" 193) am 17. Lebenstag. Nach der Infektion wurde zu verschiedenen Zeitpunkten der Infektionsstammgehalt in Leber und Blinddarmgewebe ermittelt und mit den Werten einer nicht geimpften Kontrollgruppe verglichen.

Ergebnisse Die Impfung mit dem *Salmonella*-Enteritidis-Lebendimpfstoff führte zu einer signifikanten Reduzierung der Invasion in die Blinddarmschleimhaut und Leber nach früher Infektion mit *Salmonella* Typhimurium und *Salmonella* Infantis. Weiterhin induzierte die Impfung eine adaptive Immunität gegen die getesteten „Non-Phagentyp-4"-*Salmonella*-Enteritidis-Stämme und den monophasischen *Salmonella*-Typhimurium-Stamm.

Schlussfolgerung und klinische Relevanz Die Ergebnisse der Studie zeigen, dass eine Impfung mit dem *Salmonella*-Enteritidis-Phagentyp-4-Lebendimpfstoff eine frühe systemische Invasion mit *Salmonella* Typhimurium und *Salmonella* Infantis behindert. Gleichzeitig war eine adaptive Immunantwort gegen *Salmonella*-Enteritidis-Stämme, die nicht zum Phagentyp 4 gehören, und einen monophasischen *Salmonella* Typhimurium-Stamm nachweisbar.

ABSTRACT

Objective Meat and eggs from chickens infected with *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Infantis are considered to be an important source of *Salmonella* infections for humans. In order to control *Salmonella* infections in chickens, basic biosecurity measures are taken in combination with inactivated or attenuated live vaccines. Apart from an adaptive immune response, some live vaccines also induce innate immune mechanisms that prevent or inhibit systemic invasion with homologous *Salmonella* serovars. It is unknown whether these invasion inhibition effects are also directed against heterologous *Salmonella* serovars. Furthermore, it is unclear whether the adaptive immune response after vaccination with a *Salmonella* Enteritidis phage type 4 live vaccine is also directed against other phage types of *Salmonella* Enteritidis and Typhimurium.

Material and methods Specific pathogen-free day-old chicks were vaccinated orally with a commercially available *Salmonella* Enteritidis live vaccine. To test the invasion inhibition effect, the animals were challenged orally with a labelled *Salmonella* Typhimurium or *Salmonella* Infantis strain 1 day after vaccination. To demonstrate the adaptive immune response against non-phage type 4 *Salmonella* Enteritidis strains and a monophasic *Salmonella* Typhimurium strain, the chickens were challenged with *Salmonella* Enteritidis strains of phage types 1, 8 and 21 and a monophasic *Salmonella* Typhimurium strain (Definitive Type 193). After challenge, the abundance of the challenge strain in liver and cecal tissue was enumerated and compared with a corresponding control group.

Results Findings showed that the live *Salmonella* Enteritidis vaccine inhibits systemic invasion after early infection with *Salmonella* Typhimurium and *Salmonella* Infantis. Furthermore, adaptive immunity against the tested non-phage type 4 *Salmonella* Enteritidis strains and the monophasic *Salmonella* Typhimurium strain was demonstrated.

Conclusion and clinical relevance The results of this study demonstrate that vaccination with the *Salmonella* Enteritidis phage type 4 live vaccine significantly inhibits the invasion of *Salmonella* Typhimurium and Infantis. Furthermore, an adaptive immune response was also detected against non-phage type 4 *Salmonella* Enteritidis strains and a monophasic *Salmonella* Typhimurium strain.

Introduction

Despite intensive control measures to reduce *Salmonella* infection in poultry and humans in the last decades, 91 857 confirmed cases of salmonellosis in humans were reported in the EU countries in 2018 [1]. Although the prevalence of *Salmonella* in eggs and egg products has been markedly reduced, these are still the most common causes of *Salmonella* cases in humans. The 3 most commonly reported *Salmonella* serovars in humans were *Salmonella* (*S.*) Enteritidis, *S.* Typhimurium and monophasic *S.* Typhimurium (1,4,[5],12:i:-). *S.* Infantis was reported as the fourth most common serovar usually associated with broiler meat [1].

Historically, human infections with *S.* Enteritidis were predominantly phage type (PT) 4 [2]. However, other phage types like PT1, PT8, PT14b and PT21 (non-PT4 strains) have played an important role [2][3], as well.

In addition to hygiene measures at all levels of production, live attenuated and inactivated vaccines against *S.* Enteritidis and *S.* Typhimurium have proven to be effective in chicken flocks for many years. It is generally accepted that live attenuated vaccines are not only easier to administer but are also more effective due to the induction of local, humoral, and cell-mediated immune mechanisms [4].

Live vaccines are able to induce both an innate and an adaptive immune response. Braukmann et al. [5] were the first to show inhibition of the systemic invasion of a virulent *S.* Enteritidis strain (innate immunity) just 2 days after vaccination with a commercially available attenuated *S.* Enteritidis live vaccine (SE-LV). In contrast, the occurrence of an adaptive immune response has been shown several times for different vaccines by different authors [6][7][8][9][10].

The aim of our study was to test whether the homologous invasion-inhibition effect described for the SE-LV [5] can also be demonstrated against other serovars (*S.* Typhimurium and *S.* Infantis). In addition, we examined whether the SE-LV based on PT4 also induces immunogenicity against other non-PT4 strains and a monophasic *S.* Typhimurium strain.

Materials and methods

Chickens

Specific pathogen-free day-old White Leghorn chicks were hatched from eggs, which were obtained from VALO BioMedia GmbH (Osterholz-Scharmbeck, Germany). The vaccinated and control groups were kept in cages in separate rooms to avoid the spread of the vaccine strain to the control groups. During the rearing period, commercial feed for hens without feed additives (ssniff Special diets GmbH, Soest, Germany) and drinking water were available ad libitum. The experiments were performed in accordance with the German law of animal welfare (registration number IDT-A042-2015).

Bacterial strains and culture
Vaccine strain

In all trials, SPF chickens were orally vaccinated using a buttoned cannula with the minimum effective dose (1×10^8 cfu per animal)

Trial	Group	Animals (n)	Vaccination	Challenge	Quantitative determination of challenge bacteria
1	Vaccinated	12	Day 1	Day 2 (STm) 5×10^5 cfu/chicken	Days 6 and 10
	Control	12	–		
2	Vaccinated	12	Day 1	Day 2 (SI) 5×10^4 cfu/chicken	Days 6 and 9
	Control	12	–		

STm = *S.* Typhimurium, SI = *S.* Infantis, Day = day of age

Trial	Group	Animals (n)	Vaccination	Challenge	Quantitative determination of challenge bacteria
3	Vaccinated	8	Days 2 and 15	Day 42 (SE PT8) 1×10^9 cfu/chicken	Day 49
	Control	8	–		
4	Vaccinated	8	Days 2 and 15	Day 51 (SE PT21) 1×10^9 cfu/chicken	Day 58
	Control	8	–		
5	Vaccinated	8	Days 2 and 15	Day 63 (SE PT1) 1×10^9 cfu/chicken	Day 70
	Control	8	–		
6	Vaccinated	16	Day 1	Day 17 (mSTm) 1×10^9 cfu/chicken	Days 24 and 31
	Control	16	–		

SE = *S.* Enteritidis, PT = phage type, mSTm = monophasic *S.* Typhimurium strain, Day = day of age

of the attenuated and auxotrophic (adenine, histidine) *S.* Enteritidis PT4 strain no. 441/114 (Salmovac 440 [Salmovac SE], Ceva Santé Animale, Libourne, France) (SE-LV).

Challenge strains

To examine the heterologous invasion inhibition effect of the SE-LV, a nalidixic acid resistant *S.* Typhimurium strain Definitive Type (DT) 204 (FLI no. 421) and a multidrug-resistant *S.* Infantis strain (RKI no. 12–2357) were used.

The immunogenicity of the SE-LV against certain non-PT4 strains was examined by using spontaneously occurring nalidixic acid resistant *S.* Enteritidis strains: PT1 (RKI no. 06–7328), PT8 (RKI no. 04–7681) and PT21 (RKI no. 04–2049).

The monophasic *S.* Typhimurium strain (4,[5],12:i:-) DT 193 resistant to ampicillin, streptomycin, sulfamerazine, and oxytetracycline was used to demonstrate cross immunogenicity of the SE-LV against a monophasic *S.* Typhimurium strain. This strain was described in detail by Trüpschuch et al. [11].

Cultivation of the infection strains was carried out over 2 pre-cultures in STM 6/83 Medium (IDT Biologika GmbH, Dessau-Rosslau,

Germany). After cultivation the strains were washed, concentrated and stored at –20 °C until use.

Experimental design

All trials were designed as randomized, blinded and controlled studies.

Invasion inhibition trials

A total of 12 day-old chicks per group were vaccinated orally with the SE-LV at day 1 of age. A corresponding control group remained unvaccinated (▶ **Table 1**). On day 2 of age the chickens were challenged orally at a dose of 5×10^5 cfu *S.* Typhimurium no. 421/bird or at a dose of 5×10^4 cfu *S.* Infantis no. 12–2357/bird using a buttoned cannula. On days 6 and 9 (10) of age, the animals were euthanized and the number of challenge strain bacteria in liver and cecal tissue (complete cecum without ingesta) was determined.

Immunogenicity trials

For testing of the immunogenicity against *S.* Enteritidis non-PT4 strains 8 birds/group were vaccinated orally with the SE-LV on days

▶ **Table 3** Challenge strain content (mean log10 cfu/g) in liver and cecal tissue after challenge (testing of the invasion inhibition effect).

▶ **Tab. 3** Infektionsstammgehalte in Leber und Blinddarmgewebe nach der Infektion (Prüfung des Invasionshemmungseffekts).

Trial	Group	Challenge strain	Day post challenge	Challenge strain content (mean ± SD) in $\log_{10}$ cfu/g	
				Liver	Cecal tissue
1	Vaccinated	STm	4	0	4.72 ± 2.17[a]
	Control		4	1.09 ± 1.74	6.84 ± 1.32
	Vaccinated		8	0[a]	4.90 ± 0.87[a]
	Control		8	2.30 ± 0.76	7.24 ± 0.59
2	Vaccinated	SI	4	0	6.63 ± 0.45[a]
	Control		4	0.43 ± 0.78	7.72 ± 0.60
	Vaccinated		7	0[a]	4.48 ± 0.45[a]
	Control		7	0.67 ± 0.67	7.27 ± 0.17

[a] Mann-Whitney test (one sided) $p < 0.05$, STm = *S.* Typhimurium, SI = *S.* Infantis

2 and 15 of age. Corresponding control groups were not vaccinated (▶ **Table 2**). Both the vaccinated and non-vaccinated control groups were challenged orally using a buttoned cannula with *S.* Enteritidis PT8 on day 42, with *S.* Enteritidis PT21 on day 51 and with *S.* Enteritidis PT1 on day 63 of age. The infection dose was 1×10^9 cfu per animal. The animals were stunned by head impact followed by exsanguination 7 days (trials 3–5) post challenge (dpc). Liver and cecal mucosa were taken from each animal for determination of the number of the challenge strain bacteria. Blood samples were taken for serological testing for antibodies against *S.* Enteritidis on the day of challenge and necropsy, respectively.

To prove the immunogenicity against monophasic *S.* Typhimurium (DT 193), only a single oral vaccination was carried out on day 1 of age (trial 6). The animals of the vaccinated group (n = 16) and an unvaccinated control group (n = 16) were orally infected with 1×10^9 cfu per animal on day 17 of age (▶ **Table 2**). The quantification of the *Salmonella* challenge strain in the liver and cecal tissue (complete cecum without ingesta) was performed on days 24 and 31.

Quantitative determination of the challenge strain content in liver and cecal mucosa

The quantification of the *Salmonella* challenge strains ($\log_{10}$ cfu/g) in the liver and cecal tissue was carried out using the plate counting method as previously described by Methner et al. [12]. In brief, the intestinal content was removed from the ceca. The livers, ceca (trials 1, 2 and 6) or cecal mucosa (trials 3–5) were respectively weighed and subsequently homogenized (Ultra-Turrax T25, Janke & Kunkel, IKA®-Labortechnik, Staufen, Germany). A 10-fold dilution series was prepared for enumeration of the *Salmonella* challenge strain of the processed organs. The number of bacteria was then estimated by spreading out the homogenate on deoxycholate citrate agar according to Leifson (Merck, Darmstadt, Germany) supplemented with antibiotics (50 µg/l nalidixic acid [*S.* Typhimurium 421, *S.* Enteritidis PT1, 8 and 21]; 500 µg/l streptomycin [*S.* Infantis]; 50 mg/l ampicillin, 500 µg/l streptomycin, 100 mg/l sulfamera-

zine and 10 mg oxytetracycline [monophasic *S.* Typhimurium]). The incubation was performed at $37 \pm 1\,°C$ for 24 hours under aerobic conditions. Samples which failed to grow were enriched in Rappaport Vassiliadis Broth (Merck, Darmstadt, Germany) at $37 \pm 1\,°C$ for 24 hours and spread on the above-mentioned agar. Samples that were only positive after enrichment received a $\log_{10} = 1$. Samples that showed no growth received a $\log_{10} = 0$. The mean and standard deviation were calculated for each group. Statistical evaluation was performed using the Mann-Whitney U-test (level of significance $p < 0.05$) (GraphPad Prism version 8.4.3 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com).

Serological testing

Serum was obtained after centrifugation of blood samples for 10 minutes and $3500 \times g$ at 2–8 °C. For the detection of *Salmonella*-specific antibodies (group D), serum samples were tested using a commercially available ELISA kit (Chicken *Salmonella* Antibody Test Kit Group D, BioChek, Reeuwijk, The Netherlands) according to the manufacturer's instructions. Results were recorded as sample to positive (S/P) ratios determined by the ratio between the mean optical density (OD) of each sample and the mean OD of the positive control.

Results

Invasion inhibition effect

Post infection, the challenge strains were isolated in the liver and cecal tissue of the control groups. In the vaccinated groups, the corresponding challenge strain was only detected in the cecal tissue. However, clinical signs were not observed in any chicken. The results of quantitative bacteriological examination of the liver and cecal tissue of the vaccine and control group animals are shown in ▶ **Table 3**. After infection with *S.* Typhimurium, significantly lower bacterial counts were found in the livers of the vaccine group animals compared to the control group chicks (8 dpc, $p = 0.0011$) in addition to the cecal tissue (4 dpc, $p = 0.0465$; 8 dpc,

▶ **Table 4** Challenge strain content (mean log10 cfu/g) in the liver and cecal tissue after challenge (testing of the immunogenicity).

▶ **Tab. 4** Infektionsstammgehalte in Leber und Blinddarmgewebe nach der Infektion (Prüfung der Immunogenität).

Trial	Group	Challenge strain	Day post challenge	Challenge strain content (mean ± SD) in $\log_{10}$ cfu/g	
				Liver	**Cecal tissue**
3	Vaccinated	SE PT8	7	1.20 ± 0.77 [a]	6.05 ± 0.42 [a]
	Control		7	2.85 ± 0.12	6.96 ± 0.50
4	Vaccinated	SE PT21	7	0.46 ± 0.66 [a]	5.41 ± 0.71 [a]
	Control		7	1.48 ± 0.66	6.26 ± 0.63
5	Vaccinated	SE PT1	7	0.94 ± 0.38 [a]	5.42 ± 0.84 [a]
	Control		7	2.34 ± 0.45	6.19 ± 0.52
6	Vaccinated	mSTM	7	1.19 ± 0.59 [a]	6.78 ± 0.28 [a]
	Control		7	2.06 ± 0.26	7.27 ± 0.54
	Vaccinated		14	0.12 ± 0.35 [a]	5.51 ± 0.76 [a]
	Control		14	0.75 ± 0.46	6.11 ± 0.31

[a] Mann-Whitney test (one sided) p < 0.05, SE = *S.* Enteritidis, mSTm = monophasic *S.* Typhimurium

p = 0.0022). Significantly lower invasion of the cecal tissue was also observed after infection with *S.* Infantis on 4 dpc (p = 0.0055) and in both the liver and cecal tissue on 7 dpc (liver: p = 0.0350, ceca: p = 0.0006).

Immunity against non-PT4 *S.* Enteritidis strains and monophasic *S.* Typhimurium

After infection with the different phage types of *S.* Enteritidis and the monophasic *S.* Typhimurium strain, no symptoms associated with the infection were detected in the chickens.

The results of the mean $\log_{10}$ cfu/g of the challenge strain in liver and cecal tissue of the vaccine groups compared to the control groups after infection with the different *S.* Enteritidis phage types and the monophasic *S.* Typhimurium strain are shown in ▶ **Table 4**.

The mean $\log_{10}$ cfu/g of the challenge strain in liver and cecal tissue of the vaccinated animals was significantly lower after infection with *S.* Enteritidis PT8 (liver: reduction 1.65 $\log_{10}$, p < 0.0001, cecal tissue: reduction 0.91 $\log_{10}$, p = 0.0003), PT21 (liver: reduction 1.02 $\log_{10}$, p = 0.0048, cecal tissue: reduction 0.85 $\log_{10}$, p = 0.0190) and PT1 (liver: reduction 1.40 $\log_{10}$, p < 0.0001, cecal tissue: reduction 0.77 $\log_{10}$, p = 0.0325) when compared to the controls.

After infection with the monophasic *S.* Typhimurium strain, significant differences in the number of the challenge strain bacteria in liver and cecal tissue were found in vaccinated chickens compared to the controls on both 7 dpc (liver: reduction 0.87 $\log_{10}$, p < 0.001, cecal tissue: reduction 0.49 $\log_{10}$, p = 0.023) and 14 dpc (liver: reduction 0.63 $\log_{10}$, p = 0.020, cecal tissue: reduction 0.60 $\log_{10}$, p = 0.032).

The results of the serological examination after vaccination and challenge with *S.* Enteritidis PTs 1, 8 and 21 are shown in ▶ **Fig. 1**. Immediately before the challenge and on day 7 after the challenge (day of necropsy), higher antibody titers occurred in the vaccine groups than in the corresponding control groups. Before challenge with *S.* Enteritidis PT1 (trial 5), the difference was found to be signif-

icant (p = 0.0065). At necropsy significant differences (p = 0.0047) occurred after challenge with *S.* Enteritidis PT8 (trial 3). Overall, the vaccination only resulted in a negligible increase in antibody titers. A total of only 2 out of 48 vaccinated animals (all 3 trials together) showed antibodies above the cut off (SP ≥ 50 %) before the challenge.

Discussion

After infection with *Salmonella* wild-type strains, phagocytes of the intestinal mucosa release reactive oxygen intermediates (ROI). According to Sterzenbach et al. [13] these processes are directed against the invasion of the pathogen and are an expression of innate immunity. Optimally, live vaccines should also induce ROI, which are directed against invasion of infectious strains after early infection. However, studies confirming these assumptions are currently lacking.

Braukmann et al. [5] showed an influx of neutrophils and macrophages into the lamina propria of the cecal mucosa after vaccination of day-old chicks with an attenuated SE-LV (Salmovac SE, Ceva Santé Animale, Libourne, France). This was associated with a strong elevated transcription of the pro-inflammatory mediators iNOS, IL-1β, LITAF, and IL12 which led to an inhibition of invasion and short-term inhibition of colonization after challenge with a *S.* Enteritidis wild-type strain.

In the present study, the invasion-inhibition effect could also be demonstrated after infection with *S.* Typhimurium and *S.* Infantis. Eeckhaut et al. [14] showed an invasion-inhibition effect against *S.* Enteritidis and *S.* Infantis after vaccination with a combined *S.* Enteritidis/*S.* Typhimurium live vaccine. After vaccination with a *S.* Enteritidis live vaccine alone, this effect was not present. Only both vaccines in combination showed a significant reduction of colonization of ceca (tissue and content) after infection with *S.* Enteritidis but not after infection with *S.* Infantis. In comparison to the re-
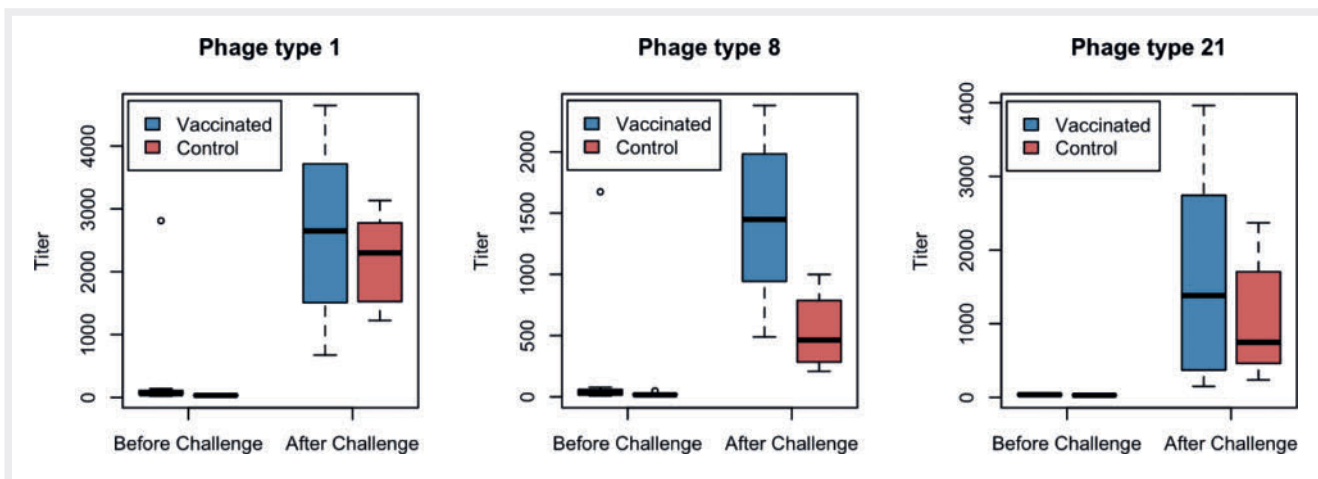
▶ **Fig. 1** Results of the investigation of *Salmonella* group D specific antibody titers of the vaccinated (blue bars) and the control animals (red bars) before and after challenge with *S.* Enteritidis PT1, PT8 and PT21 (bold black lines indicate the median). Source: © S. Springer.

▶ **Abb. 1** Ergebnisse der Untersuchung von *Salmonella*-Gruppe-D-spezifischen Antikörpertitern geimpfter Tiere (blaue Balken) und der Kontrolltiere (rote Balken) vor und nach Testinfektion mit *S.* Enteritidis PT1, PT8 und PT21 (die dicken schwarze Linien geben den Median an). Quelle: © S. Springer.

sults of Eeckhaut et al. [14] the results of this study imply that the extent of attenuation of the vaccine strain and possibly synergistic effects (live vaccines containing different vaccine strains) may have an influence on the invasion of *S.* Enteritidis and other *Salmonella* serovars. It is generally accepted that the reduction of colonization of the cecal mucosa is best achieved between isogenic *Salmonella* strains or *Salmonella* strains of the same serovar [15][16].

The present study demonstrates that a reduction of invasion of the cecal mucosa by *S.* Typhimurium and *S.* Infantis wild-type strains occurs also after immunization with SE-LV. The challenge strain used [16] and certainly the vaccination and infection doses can influence these effects. To compare our results with those of Braukmann et al. [5], how long these effects last and what significance they have for the field should be further examined.

The evidence of an adaptive immune response against *S.* Enteritidis PT4 after vaccination with a SE-LV (Salmovac SE, Salmovac 440, both Ceva Santé Animale, Libourne, France) was demonstrated with quantitative challenge models by Martin et al. [17], Springer et al. [9][18], Atterbury et al. [19] and Methner [7]. Furthermore, Theuß et al. [10] showed the efficacy of the vaccine after challenge with a *S.* Enteritidis PT4 strain according to the qualitative method described in the European Pharmacopoeia monograph 04/2013:2520.

In this study, immunity after vaccination with the SE-LV was also shown against PT1, 8 and 21 of *S.* Enteritidis. The differences in the quantification of the challenge strain in liver and cecal tissue between the vaccinated and the control group correspond to the results of Springer et al. [18] after infection with virulent *S.* Enteritidis PT4 strains.

Effects of cross-protection against *S.* Typhimurium have been demonstrated by Springer et al. [9] and for another SE-LV by Nandre et al. [20]. Results of this study show that a significant reduction of invasion of cecal tissue and liver also occurred after infection with a monophasic *S.* Typhimurium strain (DT 193). A direct

comparison with the above-mentioned examinations is not possible, as the trials differ in the number of vaccinations and/or the infection model used.

Arnold et al. [21] demonstrated a reduction of eggshell contamination after infection with *S.* Enteritidis (PT14b), a biphasic *S.* Typhimurium strain (DT 8) and monophasic *S.* Typhimurium strains after vaccination with inactivated and live vaccines based on *S.* Enteritidis and *S.* Typhimurium, as well as by combinations of live and inactivated vaccines. However, they could not show a significantly reduced excretion of the challenge strains, measured by the number of *Salmonella* positive cloacal swabs, independently from the different vaccination regimen. This contrasts with the results of Theuß et al. [10], showing a significant and relevant reduction of the number of positive cloacal swabs after threefold vaccination with a SE-LV.

An unequivocal conclusion to this question is not possible because so many factors can influence the excretion rate including the vaccine and vaccination schedule, the challenge strain, the challenge dose, and individual animal factors. Nevertheless, Arnold et al. [21] recommend the use of vaccination against *S.* Enteritidis, *S.* Typhimurium and monophasic *S.* Typhimurium strains. As the contamination of the eggshells also depends on other factors [22] the use of vaccines should always be combined with veterinary hygiene measures like cleaning and disinfection in addition to rodent control.

The animals used to demonstrate immunogenicity against the *S.* Enteritidis non-PT strains in this trial were *Salmonella* negative and kept under isolated conditions. Thus it can be assumed that the *Salmonella* group D specific antibodies detected are antibodies against *S.* Enteritidis. The increase of the specific antibody titers after vaccination with the SE-LV and subsequent challenge with virulent *S.* Enteritidis strains corresponds to reports from Methner [7], Springer et al. [9] and Theuß et al. [10]. The increase after vaccination is usually much lower than after infection. This is most likely related to the attenuation of the vaccine strain.

CONCLUSION FOR PRACTICE

As chicks are hatched without any substantial gut flora, they are very susceptible to *Salmonella* infections in their first few days of life. The results of this study demonstrate that vaccination with a *Salmonella* Enteritidis live vaccine provides early and not exclusively homologous protective effects. As *Salmonella* Enteritidis non phage type 4 strains and monophasic *Salmonella* Typhimurium strains are increasingly important in laying hen farms, the demonstration of immunogenicity against these variants after vaccination with a live *Salmonella* Enteritidis phage type 4 vaccine is of increased relevance for the field.

Conflict of interest statement

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