Efficacy of a *Salmonella* live vaccine for turkeys in different age groups and antibody response of vaccinated and non-vaccinated turkeys

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**Abstract**

**Objective:** Human Salmonellosis continues to be one of the most important foodborne zoonoses worldwide, although a decrease in case numbers has been noted in recent years. It is a foodborne zoonotic infection most commonly associated with the consumption of raw egg products but also with meat consumption including the consumption of poultry products. Turkey flocks in Europe have been reported to be affected by *Salmonella* infection, too. The present study examines the efficacy of a newly licensed *Salmonella* live vaccine in reducing infections with the *Salmonella* serovars Typhimurium and Enteritidis in turkeys. Turkeys were vaccinated the first day of life and at the age of 6 and 16 weeks. Groups of birds which had received different numbers of vaccinations were then submitted to challenge trials with either SE or ST.

**Results:** In vaccinated birds *Salmonella* counts in liver and spleen and, less effectively, in caecum were reduced compared to unvaccinated birds. In several groups serum antibody-titers were statistically significantly higher in vaccinated turkeys than in non-vaccinated ones at day seven post infection, but only in one out of six groups at day 14 post infection.

**Keywords:** *Salmonella*, Turkey, Immunization, Antibody response

**Introduction**

Non-host-adapted *Salmonellae* usually colonize the digestive tract of turkeys asymptomatically [1, 2]. Although in case of stress or at a very young age turkeys may develop severe clinical signs [1], the most important problem resulting from *Salmonella* infections lies in the transmission to humans. The main source of food-borne *Salmonella* outbreaks is the consumption of table eggs and egg products, but single samples of fresh turkey meat yielded the highest proportion of *Salmonella*-positive results [3, 4]. Control strategies focus on hygiene and management but also include vaccination [5, 6]. Despite a recent decrease of the prevalence of human Salmonellosis in several European countries it is still one of the most important food borne zoonoses in Europe [7, 8]. Vaccination of turkeys might help to reduce prevalence in turkey flocks and transmission to humans further. Barrow et al. [9] stress that the use of vaccines has been empirical and that immunological studies about *Salmonella* infections in turkeys are still scarce, although certain aspects of the humoral immune response have been studied before [10–12]. In two recent studies we examined a bivalent live *Salmonella* vaccine for its ability to induce primary immune reactions after vaccination of 1 day old turkeys [13] and for its protective efficacy in turkey poults against *Salmonella* challenge infections at the age of 3 weeks [14]. The latter study found lower *Salmonella* counts in liver, spleen and caecum of vaccinated turkey poults compared to unvaccinated poults in challenge trials at 3 weeks of age. No domination of either a T_h1-response...
or a Th2-response could be determined and no statistically significant difference regarding the IgG serum antibody response between vaccinated and non-vaccinated turkeys after challenge infection was found.

The aim of the present study was to examine the protective effect of the mentioned vaccine against *Salmonella Enteritidis* (SE) and *Salmonella Typhimurium* (ST) infections in turkeys in additional age groups and after a different number of vaccinations. The efficacy was determined by comparing bacterial counts in caeca, liver and spleen after challenge. Since it has been shown that turkeys do not produce antibodies from hatch it should also be determined if birds which were older than the birds in our former studies or which were vaccinated more often would produce a notable serum antibody-response.

**Main text**

**Materials and methods**

**Experimental design**

At day of hatch 320 turkeys were housed separately and divided randomly into two groups of 160 birds each. One group served as non-vaccinated control group whereas the other group was directly vaccinated with the *Salmonella* live vaccine. Booster immunizations were applied at the age of 6 and 16 weeks (Table 1). At 2, 6, 16 and 23 weeks of age challenge experiments were conducted (Table 1). For each challenge experiment 20 vaccinated and 20 non-vaccinated birds were infected with the virulent SE strain and 20 vaccinated and 20 non-vaccinated birds were infected with the virulent ST strain.

At day 7 and 14 post infection 10 individuals per group were sacrificed by exsanguination after they had been stupefied by manually applied blunt force trauma and samples were collected. For vaccinated birds which were infected with SE at 6 weeks of age only serum samples of four birds at 7 and 14 days post infection could be examined.

**Experimental animals**

At the day of hatch commercially available female fattening turkeys type BUT Big 6 (MoorgutKartzfehn von Kameke GmbH&Co.KG, Germany) were housed. Continuous bacteriological and serological monitoring of the parent flock and of the poult upon arrival were conducted to ascertain that the birds were free of *Salmonella* at that stage of the study. The different groups were kept separately in isolation units accordant to their immunization or infection status. Cross contamination between the immunized group and the control group and between the four groups in the challenge experiments was effectively prevented by separate air conditioning, a separate feeding regime and the change of clothing as well as strict disinfection of the facilities. Commercial starter feed and water from the municipal water supply were offered ad libitum. No antibiotics were added to feed or drinking water. Water from the municipal water supply in Germany is suitable to be used as drinking water for humans.

**Bacterial strains and culture**

The vaccine and the challenge strains used in this study as well as the preparation of the inocula have been previously described [13]. Birds were immunized with a commercial live vaccine (“AviPro Duo”, Elanco Deutschland GmbH, Bad Homburg). The vaccine contains the metabolic drift mutant strains *Salmonella Typhimurium*-strain ST Nal2/Rif9/Rtt and the *Salmonella Enteritidis*-strain SE Sm24/Rif12/Ssq [15]. Each vaccine dose was prepared to contain $1 \times 10^8$ cfu of both strains per bird which was verified by decimal dilution series. For infection experiments nalidixic acid resistant mutants

| Table 1 Experimental design |
|-----------------------------|
| **Age at challenge** | **Vaccinations before challenge** | **Necropsy and examination** | **Group designation and number examined** |
| | **Vaccinated groups** | **Non-vaccinated groups** | **Day post infection** | **ST vacc./non vacc.** | **SE vacc./non vacc.** |
| 2 weeks | 1st d | – | 7 | 10/10 | 10/10 |
| 6 weeks | 1st d | – | 7 | 10/10 | 10/10 |
| 16 weeks | 1st d, 6 w | – | 14 | 10/10 | 10/10 |
| 23 weeks | 1st d, 6 w, 16 w | – | 7 | 10/10 | 10/10 |

*d day of life, w week of life
* Challenge with ST or SE, 20 turkeys per each vaccinated or non-vaccinated group


of the virulent strains SE K482/91 [16] and STm 27 NaFl of the phage type DT104 were used. The inocula of the challenge strains contained $1 \times 10^9$ cfu per dose and were administered with a buttoned cannula directly into the crop.

**Bacteriology**

Samples from caecum ingesta, liver and spleen were collected during necropsies and used for quantitative re-isolation of the challenge strains as described previously [13]. The samples and added phosphate buffered saline PBS were processed into a homogenous suspension with an Ultra-Thurrax® (IKA-Werke, Staufen, Germany) with dispersing tools (Omni-Tip, Omnilab, Bremen). The caecum ingesta were subjected to a decimal dilution. Two portions of every dilution step as well as the organ suspension diluted 1:4 were dispensed on agar plates selective for the antibiotic resistant challenge strains. The identification of the SE and ST challenge strains was confirmed by the affiliation to different serogroups using *Salmonella* Test-sera (REF ORND03 and REF ORNH03 by Dade Behring, Marburg, Germany).

**ELISA**

Blood samples of each individual were centrifuged and the serum stored at $-72{^\circ}C$. For antibody detection the ELISA Group B and Group D salmonella Combined Antibody Test Kit (BioChek, Reeuwijk) was used in accordance with manufacturer’s instructions. It discriminates between antibodies directed against LPS antigens belonging to the Salmonella serogroups B and D.

The absorption of the samples was measured in a microtitre plate reader at 405 nm wavelength and compared to the absorption of a positive control. The sample to positive ratio was determined and the cutoff for positive values was set at 0.5.

**Statistical analysis**

Statistical calculations were conducted with the computer program SigmaStat®, Version 3.1 (Jandel, Erkrath). To detect statistically significant differences between vaccinated and unvaccinated animals t-tests were carried out if data were normally distributed. If not, Mann–Whitney–Rank sum tests were employed. The significance level was set at $p < 0.05$ for all tests.

**Results and discussion**

The present study tested the effectiveness of a *Salmonella* live vaccine in turkeys in different age groups. To our knowledge this study is the first that assessed the effectiveness of live vaccination to prevent SE infection in turkeys over such a long period of time and in so many different age groups. In the literature we could not find reports on the use of live vaccines to prevent ST infections in turkeys.

In challenge experiments vaccinated and non-vaccinated turkey poultts were infected with either a virulent ST strain or a virulent SE strain. At day seven and 14 post infection caecum colonization as well as infection of liver and spleen were evaluated.

One reason for vaccination of domestic poultry is to reduce *Salmonella* prevalence in livestock, hence preventing the contamination of poultry products. A reduction of *Salmonella* in the intestine would be important for this purpose. Additionally, the prevention of systemic infection allegedly resulting in a diminished colonization of the reproductive tissues has been named as a goal of vaccination [17].

Although it has been argued that *Salmonellae* in the cecum lumen are not readily accessible for the humoral or cell-mediated immunity [18, 19], some studies found reduced cecal colonization or fecal shedding by the use of inactivated vaccines in turkeys [20–22]. Only in some of our challenge experiments presented here and in a previously published experiment [14] cecal colonization was reduced in vaccinated birds, too (Fig. 1).

At day seven post infection the re-isolation of ST from 22 weeks old vaccinated birds and the re-isolation of SE from 16 to 22-weeks-old vaccinated animals was significantly reduced compared with the non-vaccinated control group. In contrast, 14 dpi generally more colony forming units of SE were found in the vaccinated than in the non-vaccinated turkeys with significant differences at the age of 6 and 16 weeks.

Thus, the tested vaccine does reduce cecum colonization but not as reliably as desired.

In contrast to findings in the intestine, the systemic spread of *Salmonellae* was reduced considerably through vaccination (Fig. 1). The counts of virulent ST and SE in livers of vaccinated birds were reduced compared to the counts in livers of non-vaccinated birds in all age groups 7 days after challenge infection. This could be confirmed statistically in all groups but one. With the exception of

(See figure on next page.)

**Fig. 1** Re-isolation of virulent *Salmonella* strains from caecum, liver and spleen in different age groups. Figure bars represent the mean log 10 colony forming units/gram from 10 samples of caecum ingesta, liver or spleen, respectively. Error bars represent standard deviation. Statistically significant differences are indicated by different letters.
16 weeks old SE challenged birds; *Salmonellae* were completely eliminated from livers of vaccinated turkeys at day 14 post infection whereas the agent was still present in livers of non-vaccinated turkeys at a low level at this timepoint.

From spleens of vaccinated turkeys (Fig. 1) statistically significantly less ST were isolated than from spleens of non-vaccinated animals in all age groups at all timepoints. SE bacterial counts were statistically significantly lower at day 7 pi in spleens of vaccinated birds which had been infected at 2 and 22 weeks of age and at day 14 pi in vaccinated birds which had been infected at 22 weeks of age.

Thus the vaccine clearly reduced systemic spread in turkeys, which is in line with previous findings by our group for 3 weeks old turkeys. In contrast Krüger et al. [23] described the failure of a live attenuated vaccine to protect turkeys against *Salmonella* infection in a different setting. Generally studies addressing the success of vaccination against non-host-specific *Salmonella* serovars in poultry have yielded differing and sometimes conflicting results [5]. For chickens (reviewed by [24]) and ducks [25] live vaccines have been reported to confer protection against *Salmonella* infection.

In a previous study we could not detect antibody production of either vaccinated or non-vaccinated turkeys

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**Fig. 2** Antibody production after *Salmonella* infection in different age groups. Figure bars represent the mean log 10 colony forming units/gram from 10 samples of caecum ingesta, liver or spleen, respectively. Error bars represent standard deviation. Statistically significant differences are indicated by different letters.
after challenge at 3 weeks of age. The finding of IgG antibodies only at a very low level in young turkeys is in accordance with findings of others [26] who showed that turkeys started to produce humoral antibodies after vaccination with an inactivated Bordetella avium vaccine not before 28 days of age independently from the time of vaccination.

In the present study higher antibody titers were found in turkeys which were 6 weeks old at challenge compared to turkeys examined in our former study, which were only 3 weeks old at challenge (Fig. 2). We also found that 16- or 22-weeks old animals produced higher titers than 6 week old turkeys.

At day seven post infection vaccinated birds presented statistically significantly higher antibody titers compared to the control group with the exception of birds infected with SE at 6 weeks of age. At day 14 post infection titers generally had not changed much in vaccinated birds. In contrast antibody titers in non-vaccinated birds had risen to roughly the same level as in vaccinated birds or even higher. At day 14 post infection we could find statistically significantly higher antibody titers in vaccinated birds compared to non-vaccinated birds in the groups infected with ST at 6 weeks of age and SE at 16 weeks of age. For vaccinated birds infected at 22 weeks of age antibody titers were statistically significantly lower than in non-vaccinated birds.

In summary vaccinated birds produced antibodies earlier than non-vaccinated birds. High titers of circulating antibodies have been associated with protection against systemic infections and could therefore explain the reduced numbers of colony forming units in livers and spleens of vaccinated birds [27, 28]. Higher counts of Salmonellae in internal organs may then have induced a stronger antibody response in non-vaccinated turkeys until day 14 post challenge. Similar results of earlier antibody production in vaccinated individuals have been reported for Salmonella vaccination before [29]. However, antibody-production does not always correlate with Salmonella resistance [30–32] and in the present study we could not find a consistent relationship between antibody titers and cecum colonization or infection of internal organs.

Limitations
For the immune response against bacteria in the gut lumen IgA antibody titers in the bile would possibly be even more interesting but were not addressed in this study.

Due to the experimental design the present study cannot determine if there is a causal relationship between antibody response and protection against Salmonella challenge infections.

Abbreviations
T1,1-response: T helper type 1 response; T1,2-response: T helper type 2 response; SE: Salmonella Enteritidis; ST: Salmonella Typhimuriumdpi day(s) post infection.

Authors’ contributions
MH, AS, GG and RW conceived and designed the experiments, MH and AS performed the experiments; MH, AS, and GG analyzed the data, MH and GG wrote the paper. All authors read and approved the final manuscript.

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Competing interests
Andreas Stamm received a scholarship from Lohmann Animal Health for work that was not directly related to the present study. Aside from that the authors declare to have no competing interests.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The use of animals in this study was reviewed by the animal welfare officer of the University of Veterinary Medicine Hannover, which includes the scrutiny of animal welfare, ethics and handling, and then announced to the Lower Saxony State Office for Consumer Protection and Food Safety according to §8a(1,2) of the German Animal Health and Welfare Act. Work on this study was approved under file number 33.9-42502-05-11A153 of the competent authority.

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References
1. Hafez HM, Jodas S. Salmonella Infections in Turkeys. In: Wray C, Wray A, editors. Salmonella in domestic animals. New York: CABI Publishing; 2000.
2. Gast RK. Salmonella Infections. In: Safi YH, editor. Diseases of poultry. 1st ed. Ames: Iowa State University Press; 2003. p. 567–8.
3. European Food Safety Authority. Report of the task force on zoonoses data collection on the analysis of the baseline survey on the prevalence of Salmonella in turkey flocks, Part B. EFSA J. 2008;198:1–124.
4. Bundesinstitut für Risikobewertung. Grundlagenstudi...Prävalenz von Salmonellen in Truthühnerbeständen. 2008. http://www.bfr.bund.de/cm/208/grundlagenstudi...von_salmonellen_in_tru...a). Accessed 16 Feb 2018.

5. Immerseel FV, Metnher U, Rychlik I, Nagy B, Velge P, Martin G, et al. Vaccination and early protection against non-host-specific Salmonella serotypes in poultry: exploitation of innate immunity and microbial activity. Epidemiol Infect. 2005;133:959–78.

6. O’Brien SJ. The ‘decline and fall’ of nontyphoidal Salmonella in the United Kingdom. Clin Infect Dis. 2013;56:705–10.

7. EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA J. 2015;13:4329.

8. Robert Koch-Institut. Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2015. Berlin: Robert Koch-Institut. 2016.

9. Barrow PA, Jones MA, Smith AL, Wigley P. The long view: salmonella—the last forty years. Avian Pathol. 2012;41:413–20.

10. Kremer CJ, O’Meera KM, Layton SL, Hargis BM, Cole K. Evaluation of recombinant Salmonella expressing the flagellar protein flic for persistence and enhanced antibody response in commercial turkeys. Poult Sci. 2011;90:752–8.

11. Thain JA, Baxter-Jones C, Wilding GP, Cullen GA. Serological response of turkey hens to vaccination with Salmonella hadar and its effect on their subsequently challenged embryos and pouls. Res Vet Sci. 1984;36:320–5.

12. Tenk I, Gyovary I, Erdeli P, Szabo Z, Kostyak A, Matray D. Effect on Salmonella shedding in breeding turkey flocks of vaccine (Salenvac) against Salmonella Enteritidis. Magy. Allatorvosok Lapja. 2000;122:737–41.

13. Hesse M, Stamm A, Weber R, Glünder G, Berndt A. Immune response following immunisation of turkey pouls exposed at 1 day of age to either attenuated or wild Salmonella strains. Vet Immunol Immunopathol. 2016;174:1–10.

14. Hesse M, Stamm A, Berndt A, Glünder G, Weber R. Immune response to Salmonella infections in vaccinated and non-vaccinated turkeys. Res Vet Sci. 2017;115:165–73.

15. Hahn I. A contribution to consumer protection. TAD Salmonella Vac E-A: a new live vaccine for chickens against salmonella enteritis. Lohmann Immun. 2000;23:29–32.

16. Ludwig HJ, Calson P. Prevention of Salmonella infections in laying hens by vaccination. Berl Munch Tierarztl Wochenschr. 1992;105:96–8.

17. Barrow PA, Metnher U. Vaccination against Salmonella infections in food animals: rationale, theoretical basis and practical application. In: Barrow PA, Metnher U, editors. Salmonella in domestic animals. 2nd ed. Oxfordshire: CABI Publishing; 2000.

18. Barrow PA, Wallis TS. Vaccination against Salmonella in food animals: rationale, theoretical basis and practical application. In: Wray C, Wray A, editors. Salmonella in domestic animals. New York: CABi Publishing; 2000.

19. Barrow PA. Immunological control of Salmonella in poultry. In: Blanken-ship LC, editor. Colonization control of human bacterial enteropathol...enthids vaccine (Salenvac®) in turkey breeder flocks. In: Ha¥ez HM, editor. Proceedings of 4th international symposium on turkey diseases. Berlin, 2002. p. 259–70.

20. Charles SD, Nagaraja KV, Sivanandan V. A lipid-conjugated immunostimulating complex subunit vaccine against Salmonella infection in turkeys. Avian Dis. 1993;37:477–84.

21. Jodis S, Ha¥ez HM. Field investigations on the efficacy of inactivated Salmonella enteritidis vaccine (Salenvac®) in turkey breeder flocks. In: Ha¥ez HM, editor. Proceedings of 4th international symposium on turkey diseases. Berlin, 2002. p. 259–70.

22. Nagaraja KV, Kim CJ, Pomeroy BS. Prophylactic vaccines for the control and reduction of Salmonella in turkeys. In: Proceedings 92nd annual meet. US Animal Health Association. 1988. p. 347–8.

23. Krüger A, Redmann T, Krajewska V. Field investigations on the efficacy of a live vaccine of Salmonella Enteritidis in Meat Turkeys. In: 7th international symposium on turkey diseases. Berlin: German Veterinary Medical Society, 2008.

24. Shivaprasad HL, Metnher U, Barrow PA. Salmonella infections in the domestic fowl. In: Barrow PA, Metnher U, editors. Salmonella in domestic animals. 2nd ed. Oxfordshire: CABi Publishing, 2013. p. 162–92.

25. Tang T, Gao Q, Barrow P, Wang M, Cheng A, Jia R, et al. Development and evaluation of live attenuated Salmonella vaccines in newly hatched ducklings. Vaccine. 2015;33:5564–71.

26. Foulman A, Glünder G. Humoral immune response following immunisation of turkey pouls with an inactivated Bordetella avium vaccine. In: 3rd International symposium on turkey diseases. Berlin, 2000.

27. Clifton-Hadley FA, Breslin M, Venable LM. A laboratory study of an inactivated bivalent iron restricted Salmonella enterica serovars Enteritidis and Typhimurium dual vaccine against Typhimurium challenge in chickens. Vet Microbiol. 2002;89:167–79.

28. Woodward MJ, Gittinby G, Breslin MF, Corkish JD, Houghton S. Theeficiary of Salmonel a, a Salmonella enterica subspp. enterica serotype Enteritidis-restricted bacterin vaccine, in laying chickens. Avian Pathol. 2002;31:383–92.

29. Hassan JO, Mockett AP, Catty D, Barrow PA. Infection and re-infection of chickens with Salmonella Typhimurium: bacteriology and immune responses. Avian Dis. 1991;35:809–19.

30. Beal RK, Wigley P, Powers C, Barrow PA, Smith AL. Cross-reactive cellular and humoral immune responses to Salmonella enterica serovars Typhi- mumirum and Enteritidis are associated with protection to heterologous re-challenge. Vet Immunol Immunopathol. 2006;114:84–93.

31. Berthelot-Herault F, Mompart F, Zygmunt MS, Dubray G, Duchet-Suchaux M. Antibody responses in the serum and gut of chicken lines differing in cecal carriage of Salmonella enteritis. Vet Immunol Immunopathol. 2003;96:43–52.

32. Beal RK, Smith AL. Antibody response to Salmonella: its induction and role in protection against avian enteric salmonellosis. Expert Rev Anti Infect Ther. 2007;5:873–81.