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Immune response to *Salmonella* infections in vaccinated and non-vaccinated turkeys

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ABSTRACT

Vaccination has been widely used to reduce the *Salmonella* burden in poultry and subsequently the transmission to humans. Concerning turkey, there is little knowledge on the immune response to colonization and invasion by *Salmonella* species or about efficacy of vaccination and involved immune mechanisms. In the present study, turkeys were vaccinated at the day of hatch and infected with *Salmonella* Typhimurium (ST) or Enteritidis (SE) field strains three weeks later. A control group was kept uninfected. After challenge infection, bacterial counts in the cecal content, liver and spleen were determined 7 and 14 days post infection. They were often statistically significantly lower in vaccinated poults than in non-vaccinated ones. Production of iNOS, and the cytokines IL-8, IL-10 and IFN-γ were reduced in vaccinated birds. However, neither the influx of CD4 +, CD8α + and CD28 + cells into cecal mucosa after infection nor the antibody response were statistically significantly altered in vaccinated birds.

1. Introduction

Salmonellosis continues to be one of the most important foodborne zoonoses in humans worldwide. The World Health Organization estimates that in 2010 worldwide approximately 78 million diarrheal illnesses and 29,000 deaths due to diarrheal illnesses were caused by non-typhoidal *Salmonella* (World Health Organization, 2015). Turkey meat is considered to contribute to human Salmonellosis cases (European Food Safety Authority, 2008) in addition to eggs, egg products and other poultry meat. A European Union-wide baseline survey carried out between October 2006 and September 2007 found the prevalence of all *Salmonella* serovars in fattening turkey flocks in holdings with at least 500 birds to be 30.7% although the prevalence varied widely among the Member States, from 0% to 78.5%. The Member State-specific observed flock prevalence of *S.* Enteritidis and/or *S.* Typhimurium varied from 0% to 18.4% in fattening turkeys (European Food Safety Authority, 2008).

The infection can spread within and between flocks without clinical signs of salmonellosis (Dhillon et al., 1999). Subsequently, *Salmonella* can be introduced into the food chain via cross-contamination in slaughterhouses and meat-processing plants (Lillard, 1990; Olsen et al., 2003; Rasschaert et al., 2007).

The non-host-adapted serovars *S.* Enteritidis (SE) and *S.* Typhimurium (ST) have been most frequently reported by the authorities in Germany as being the cause of human infections in the last years (Robert-Koch-Institute, 2014). The European legislation has dedicated special regulations to these *Salmonella* serovars (Commission of the European Communities, 2003, 2012). Beyond being a hazard to human health ST is also an important bacterial pathogen in the turkey breeder sector. ST may cause high losses among turkey poults during the first month after hatching which might be prevented by vaccination (Hafez, 2013).

Different live attenuated and killed vaccines for poultry have been examined in a variety of vaccination and challenge schemes. Previous studies demonstrated that vaccinations protected poultry against infections with non-host-adapted *Salmonellae* (Charles et al., 1993; Hafez and Jodas, 2000). Generally, it is assumed that live vaccines are more effective than killed vaccines because the former stimulate both cell-mediated and humoral immunity (Immerseel et al., 2005; Sharma, 1999). For turkey the effectiveness of *Salmonella* vaccination has been reported mostly for the usage of killed vaccines, including autogenous bacterins and outer membrane preparations (Hafez and Jodas, 2000).

Krüger et al. (2008) described the use of a commercially available *S.* Enteritidis live vaccine in fattening turkeys. Administering a spray

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vaccination at the first day of life was followed by booster immunization via drinking water at 6 and 11 weeks of age. In two challenge trials with SE at the age of 7 weeks and 16 weeks, however, shedding and colonization of internal organs were not reduced in vaccinated compared to non-vaccinated turkeys 11 days post infection. The author concluded that this vaccine is not suitable for *Salmonella* control in turkey.

Besides the effectiveness of vaccines, the mechanisms involved in immune responses to *Salmonella* infections in primed or immunologically naïve turkey have also been the subject of discussion. Especially the relative significance of cell-mediated and humoral immune response to infection have been controversially discussed (Zhang-Barber et al., 1999). In more recent publications the importance of the cellular immune response in chicken after primary *Salmonella* infection has been emphasized (Beal et al., 2006; Berthelot-Herault et al., 2003). It has been proposed that the cellular immune response is only crucial for eliminating primary infections with attenuated *Salmonella* strains, whereas the humoral immune response is responsible for the clearance of secondary infections with virulent strains (McSorley and Jenkins, 2000). Little is known about immune response against primary or secondary *Salmonella* infections in turkey (Barrow et al., 2012). Until recently, detailed immunological investigations in turkey were hindered by a lack of knowledge about gene sequences and specific immune markers for turkey cells. The most thoroughly studied immune parameter in turkey is that of antibody production. The onset of antibody-production on day 21 has been described after using an oral life attenuated SE-vectored vaccine (Kremer et al., 2011). Additionally, the transfer of maternal antibodies to the offspring after vaccinating the parents has been documented (Thain et al., 1984). Other authors stated that antibody production is not necessarily correlated with protection (Beal et al., 2006; Berthelot-Herault et al., 2003). In a previous study by the present authors no increase in antibody titers, nor in immune cell scores in cecal mucosa or transcription of different immune-related proteins after vaccination of day-old turkeys could be observed (Hesse et al., 2016), although infection with virulent *Salmonellae* caused elevated transcription levels of IFN-γ, iNOS and IL-8 compared to uninfected turkeys and SE-infection also higher scores of CD4-,CD8- and CD28-positive cells in the cecal mucosa.

The aim of our present study was to test the protective effect of a bivalent *Salmonella*-Enteritidis/Typhimurium-live vaccine in turkey. Determination of *Salmonella* counts in cecal contents, the primary colonization site, and liver and spleen should provide information on the course of infection. Furthermore, the influx of immune cells into the cecal mucosa as well as the expression of cytokines and iNOS and the production of humoral antibodies should open up new insights into immune mechanisms involved in secondary immune response to *Salmonella* infections in turkey.

2. Materials and methods

2.1. Experimental design, sample collection and preparation

The protective effect of the vaccine and the immune response of vaccinated turkeys were examined after the challenge infection of previously vaccinated turkeys in comparison to non-vaccinated birds. No official guidelines to test the effectiveness for SE and ST vaccines in turkey exist. The present experimental design is adapted from the design described in the monographs of the European Directorate for the Quality of Medicine and Healthcare on testing SE and ST vaccines in chickens (EDQM, 2009a, 2009b). The monographs mandate challenging 20 vaccinated and 20 unvaccinated birds with virulent *Salmonella* and to examine samples of cecum ingesta, liver and spleen from 10 birds per group at day 7 and 14 post infection, respectively. Accordingly, on the day of hatch poults were randomly divided into two groups. One group was vaccinated with the bivalent SE/ST vaccine, whereas the other group remained untreated as control group. Three weeks later half of the individuals from each group was infected with the SE- challenge strain while the other half were infected with the ST-challenge strain Consequently, four groups were formed: i: vaccinated and SE-infected (vacc-SE), ii: non-vaccinated and SE-infected (non-vacc-SE), iii: vaccinated and ST-infected (vacc-ST) and iv: non-vaccinated and ST-infected (non-vacc-ST) (see Table 1). Each bird received the respective dose of vaccine or *Salmonella* suspension at a volume of 0.5 mL with a buttoned cannula directly into the crop. At days 7 and 14 post infection, 10 birds per group were sacrificed to collect cecal contents, spleen and liver. These samples were then examined bacteriologically for the presence of *Salmonellae*.

Table 1
Experimental design.

Day of life	dpi	Action	Groups			
			Vaccinated (n = 58)		Non-vaccinated Control (n = 58)	
1		Vaccination	Yes		No	
22		Challenge infection	SE n = 29	ST n = 29	SE n = 29	ST n = 29
23	1	Immunol.	n = 3	n = 3	n = 3	n = 3
25	3	Immunol.	n = 3	n = 3	n = 3	n = 3
29	7	Bacteriol. immunol.	n = 10, 3	n = 10, 3	n = 10, 3	n = 10, 3
32	10	Immunol.	n = 3	n = 3	n = 3	n = 3
36	14	Bacteriol.	n = 10	n = 10	n = 10	n = 10

SE = *S.* Enteritidis; ST = *S.* Typhimurium; dpi = days post infection; bacteriol. = bacteriological re-isolation from liver, spleen and cecum; immunol. = immune cell migration, cytokine- and iNOS-expression, antibody production at day 7 post infection 10 birds per group were sacrificed, samples of all 10 birds were used for bacteriological re-isolation and samples of 3 out of the same 10 birds were used for immunological examination.

In order to investigate the course of the immune response to *Salmonella* infections samples from 3 birds per group were collected at 1, 3, 7 and 10 days post infection so that nine additional birds per group were needed. The number of samples for immunological investigations per date and group were chosen on the basis of similar experiments in chickens (Carvajal et al., 2008). These samples included samples for sections of the cecum for immunohistochemical studies and for quantitative real-time RT-PCR and serum samples for antibody detection via ELISA. Sample collection and storage have been previously described in detail (Hesse et al., 2016) and are therefore only mentioned briefly here. In total 20 birds per each of the four groups were needed for the microbiological investigations: 10 birds at day 7 and 10 birds at day 14 post infection. At day 7 post infection samples for immunological investigations could be collected from 3 animals out of the group of 10 which were also used for microbiological investigations so that only three additional birds per immunological investigations at days 1, 3 and 10 post infection were needed. Altogether 116 birds were used in this experiment, 29 birds per each of the four groups.

2.2. Experimental animals

Commercially available female fattening turkeys, type BUT Big 6 (Moorgut Kartzfehn von Kameke GmbH & Co. KG, Germany), were used for the experiments. Bacteriological control of the parent flock and serological examination of the newly hatched poults proved the *Salmonella* free status.

The different groups were kept in separate isolation units in different buildings with separate air conditioning. A separate feeding regime and clothing as well as cleaning and disinfection of the facilities were implemented to prevent cross contamination. Commercial starter and fattening feed as well as water from the municipal water supply were offered ad libitum.

The animal experiment in this study was controlled by the Animal