Veterinary Immunology and Immunopathology 174 (2016) 1–10

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Immune response of turkey poults exposed at 1 day of age to either attenuated or wild *Salmonella* strains

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ARTICLE INFO

Article history:
Received 23 June 2015
Received in revised form 1 April 2016
Accepted 4 April 2016

Keywords:
Turkey
Immune response
Immunisation

ABSTRACT

Salmonellosis is a foodborne zoonosis that is most often acquired by consuming poultry products such as eggs and poultry meat. Amongst other measures the vaccination of food-producing poultry is thought to contribute to a reduction in human salmonellosis. In the European Union (EU) in 2014 the licence of a commercially available *Salmonella* vaccine for chickens and ducks was extended to turkeys. In the present study, we examined the course of infection with a virulent *Salmonella enterica* ssp. *enterica* serovar Enteritidis (SE) strain, a virulent *S. enterica* ssp. *enterica* serovar Typhimurium (ST) strain, and the respective live vaccine containing attenuated strains of both serovars in turkey poults. Besides collecting microbiological data and detecting invading *Salmonella* in the caecal mucosa via immunohistochemistry, we also assessed immune reactions in terms of antibody production, influx of CD4-, CD8α- and CD28-positive cells into the caecal mucosa and the expression of four different immune-related proteins. We found that the attenuated strains were able to invade the caecum, but to a lower degree and for a shorter duration of time compared to virulent strains. Infections with virulent *Salmonellae* also caused an increase in CD4-, CD8α- and CD28-positive cells in the caecal mucosa and an increased transcription of iNOS, IL-8-like chemokines, and IFN-γ. In poults treated with attenuated bacteria we could not detect any evidence of immune responses. In conclusion, the vaccine showed a lower degree of caecal invasion and induced weaker immune reactions compared to the virulent *Salmonella* strains in turkeys. The efficiency of the vaccine has to be verified in future studies.

1. Introduction

Human salmonellosis is a disease of the gastrointestinal tract that may be especially dangerous in the immunosuppressed, children and the elderly (Gordon, 2008; Saphra and Winter, 1957). It is usually acquired by consuming food products of animal origin and is therefore a zoonotic infection (Rabsch et al., 2013). Mostly eggs, but also meat products contribute to human salmonellosis cases (Nørrung and Buncic, 2008). Turkey meat, too, has been discussed as a source of infection (European Food Safety Authority, 2008). The causative agents of the disease include various non-host-adapted serovars of *Salmonella enterica* ssp. *enterica*. For many years *S. Enteritidis* (SE) and *S.* Typhimurium (ST) had been the serovars most isolated from humans based on reports to authorities in Germany (Robert Koch Institute, 2010) and are also the subject of European legislation (Commission of the European Communities, 2012; Commission of the European Communities, 2003). Since salmonellosis is still one of the most important foodborne zoonoses within the EU, efforts have been made and are still being made to reduce the disease. In addition to improved hygiene regimes and management, vaccination of food animals is also considered to be a promising instrument for reducing salmonellosis. A recent decrease in human salmonellosis cases in several European countries might possibly be related to the introduction of the vaccination for chickens (O'Brien, 2013; Rabsch et al., 2013). Therefore, it stands to reason that a further decrease might be achieved by vaccinating turkeys. In contrast to the situation with laying hens, no *Salmonella* live vaccine had been available for turkeys in the European Union until recently.

However, various studies addressing the success of vaccination have yielded differing and sometimes conflicting results, whether vaccination can confer protection and which would be the most effective vaccination regime (Immerseel et al., 2005). Much of our

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http://dx.doi.org/10.1016/j.vetimm.2016.04.004
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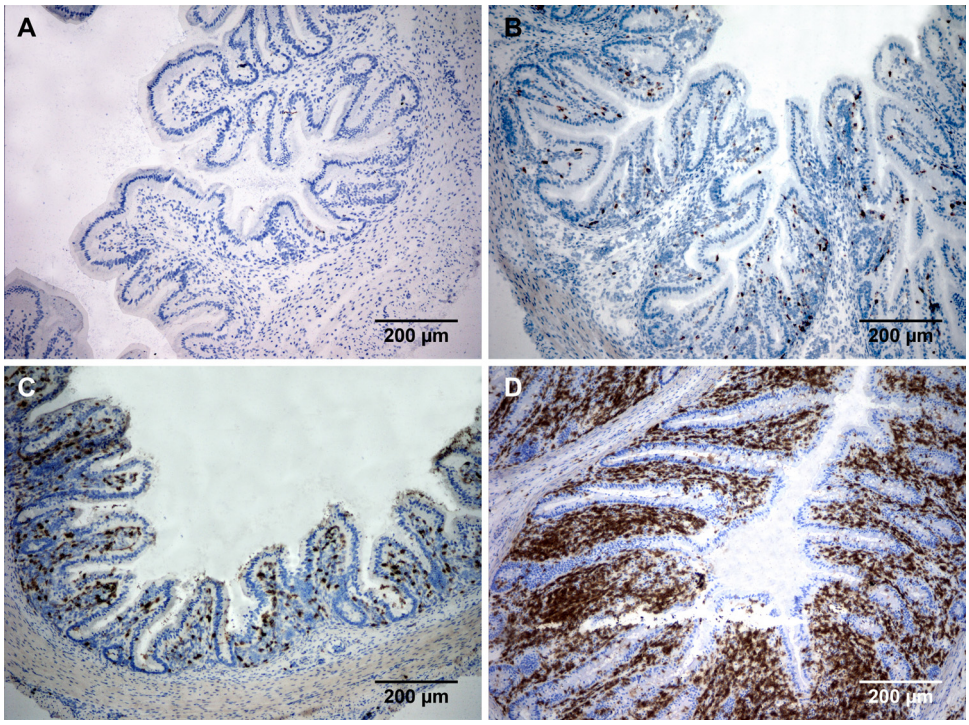

Fig. 1. Categories of immune cell invasion into the caecal mucosa. By means of immunohistochemistry we determined invasion of *immune cells* (brown stained) into caecal mucosa. For classification, we used the categories 0–3, 0 meaning no positive cells were observed at all (panel A), 1 meaning marginal invasion of immune cells was detected (panel B), 2 meaning moderate invasion (panel C) and 3 meaning the maximum level of immune cell invasion (panel D) was detected.

current knowledge about the biology of *Salmonella* infection and the immune reactions towards it, derives from studies conducted in mice, which unlike birds develop a typhoid-like disease (Barrow, 2007). In more recent studies chickens have been used. Little information is available on immune reaction of turkeys after *Salmonella* infection (Barrow et al., 2012) and this mostly focuses on the innate immunity (He et al., 2008; Kogut et al., 1998; Lowry et al., 1997; Stabler et al., 1994) or on the antibody response (Kremer et al., 2011).

Non-host-adapted *Salmonellae* usually colonise the gut of their hosts. In chickens they preferentially settle in the caecum (Barrow et al., 1988; Fanelli et al., 1971; Turnbull and Snoeyenbos, 1974), which is also the point where the invasion of the gut mucosa takes place (Turnbull and Snoeyenbos, 1974) and the spread to internal organs is initiated. After crossing the epithelial barrier *Salmonellae* meet the cells of the innate immune system. For chickens it has been shown that epithelial cells start to recruit heterophils via release of IL-8 after contact with *Salmonellae* (Chappell et al., 2009; Henderson et al., 1999; Immerseel et al., 2002; Kogut, 2002; Kogut et al., 1994). Also, turkey heterophils have been reported to be activated by immune lymphokines produced after *Salmonella* infection (Kogut et al., 1998) and to phagocytise and kill the agent (He et al., 2008; Lowry et al., 1997; Stabler et al., 1994).

Macrophages belong to the first immune cells that reach the site of infection (Korver, 2006). Both macrophages of chicken and turkey origin have been shown to kill *Salmonellae* effectively by reactive oxygen species formed by the inducible nitric oxide synthetase (iNOS) (Dil and Qureshi, 2002; Thain et al., 1984). However, the bacteria are also capable of resisting the immune cell's killing mechanisms (Holt et al., 1995; Kogut, 2002). Within chicken macrophages they can even cross the epithelial barrier and travel through the blood circulation and settle down in other organs, preferentially liver and spleen (Chappell et al., 2009). Whereas, a certain amount is known about innate immune responses of turkeys after *Salmonella* infection; no detailed investigations of the adaptive immune response exist for this poultry species. The cellular immune response within the caecal mucosa has not been studied at all. Only certain aspects of the humoral immune response like the protective effect of maternal antibodies (Thain et al., 1984) have been investigated. Examinations of cytokine-expression patterns associated with *Salmonella* infection had been hindered by the lack of specific tools for turkeys until recently. In contrast, some studies concerning chickens are reported. They implicate that the T_H1-response is significant after *Salmonella* infection (Cheeseman et al., 2007; Withanage et al., 2005).

An involvement of the humoral arm of the immune system has been discussed. Nonetheless, it has been suggested that bacteria in the gut lumen in general are not readily available for the host immune response, except for IgA antibodies that are secreted from mucosal surfaces (Barrow and Wallis, 2000).

In day old chicken poults an increase in the number of CD8-positive γδ T cells in the gut after *Salmonella* Typhimurium infection was observed (Berndt and Methner, 2001). CD4-positive T-helper cells are needed for activating naïve immune cells and induce cellular or humoral immune responses (Erf, 2004; Jankovic et al., 2001). Binding of CD28, a co-stimulatory molecule expressed on different T-cell types, plays a central role in T-helper cell activation (Bernard et al., 2007) and the generation of effector T-cells in chickens and mammals (Liu et al., 1997; Malek et al., 2004). The aim of the present study was to determine the course of infection of a commercially available live vaccine containing an attenuated SE and an attenuated ST-strain in turkeys and the type of immune response triggered by this infection. The immune response was compared to immune reactions caused by inoculation with virulent strains to evaluate the potential effect of the vaccine on the development of immune protection and its influence on the health of turkeys.