

DECREASED COLONIZATION WITH A HIGHLY VIRULENT *S. TYPHIMURIUM* DT104 AFTER VACCINATION WITH AN INVASIVE ATTENUATED *S. TYPHIMURIUM*-MUTANT.

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Introduction

Salmonellosis is one of the most important enteric infections in man as well as in livestock. Various *Salmonella* serotypes can cause self limiting gastroenteritis and systemic diseases. Besides *S. Enteritidis*, *S. Typhimurium* is the most frequent cause of food poisoning. Although poultry and pigs usually do not develop clinical salmonellosis, they become carriers and shedders (via meat and faeces) resulting in a substantial disease causing potential for humans (2).

Increasing interest can be observed in the use of vaccines to reduce the level of *Salmonella*-infections in livestock. Thereby, among the available approaches for triggering an efficient mucosal immunity, the use of live attenuated *Salmonella* strains is the best studied strategy (3). Several attenuation strategies have been employed to render salmonellae avirulent. Those include the generation of temperature-sensitive mutants, mutants deficient in the biosynthesis of aromatic amino acids (e.g. *aroA*, *aroC*, and *aroD* mutants) or purines (e.g. *purA* and *purE* mutants), mutants altered in the utilization or synthesis of carbohydrates (e.g. *galE* mutants), mutants altered in production of adenylate cyclase (*cya*) or of the cyclic AMP receptor protein (*crp*), or mutants with an affected global regulatory system (*phoP*). Additionally, different metabolic drift mutations of salmonellae were analyzed for their potency in immunization studies using experimental animals as well as livestock.

One of these metabolic drift mutants of *S. Typhimurium*, *S. Tm. Nal2/Rif9/Rtt* (*gyrA-cpxA-rpoB*), has been successfully used for immunoprophylaxis of latent *Salmonella* infections in chicken. Here, the prevention and reduction of *Salmonella* transmission between poultry and man is the main goal of vaccination strategies. The application of this vaccine leads to reduction of colonization by wild strains as well as to a reduced shedding period in infected laying hens.

The aim of this study was to characterize the efficacy and the humoral immune responses of a *S. Typhimurium gyrA-cpxA-rpoB*-mutant-based vaccination in pigs. Therefore, an oral infection model that results in a clinical infection and subsequently persistent shedding of *S. Typhimurium* in piglets was used (1). Here we demonstrate, that similar to the situation in chickens after homologous challenge infection, oral live vaccination of pigs revealed a significant reduction of the colonization of tissues and inner organs combined with a shortened shedding period as well as a prevention of clinical signs of salmonellosis (4).

Material and Methods

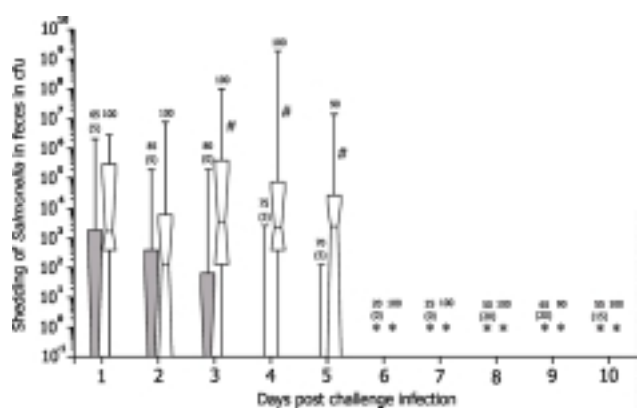
Twenty 4-week-old male hybrid SPF piglets were immunized orally with single inoculation of total 5×10^8 bacteria. A control group was treated with a placebo. The animals were challenged orally with a highly virulent *S. Typhimurium* DT104 strain three weeks post immunization. All pigs were controlled daily for clinical symptoms of salmonellosis. Additionally, fecal samples

were investigated daily for salmonellae. At day ten post vaccination, all pigs were euthanized. For *Salmonella* sp. examination, tissue samples (n=13) were collected aseptically and quantitatively and qualitatively examined. In addition, the systemic antibody response was investigated by ELISA.

Results

The clinical investigations revealed that the vaccination protected against symptoms of salmonellosis. While all placebo animals revealed moderate to severe clinical symptoms, the majority of vaccinated pigs did not develop disease. For instance, only 25 % of the immunized pigs showed slightly increased rectal temperatures (mean 39.5 °C), but 40 % of the placebo group had elevated rectal temperatures (40.0 – 41.3 °C, mean 39.9 °C). All animals (100 %) of the non-immunized group showed slight to moderate disturbed demeanour, while this was observed only in 10 % of the immunized pigs. A total of 60 % of the pigs in the placebo group, but only 5 % in the immunized group showed anorexia and diarrhoea.

Fig. 1: Quantitative isolation of *Salmonella* in faeces from immunized (grey notch boxes) and placebo-treated (white notch boxes) pigs. The percentages of animals tested bacteriologically positive for *S. Typhimurium* DT104 using pre-enrichment procedures (qualitatively detection) is depicted separately in numbers. The percentage of animals tested positive for immunization strain *S. tm. Nal2/Rif9/Rtt* is shown in parentheses. (#), indicates significant differences ($P < 0.05$) between immunized pigs and those of the placebo-treated group.

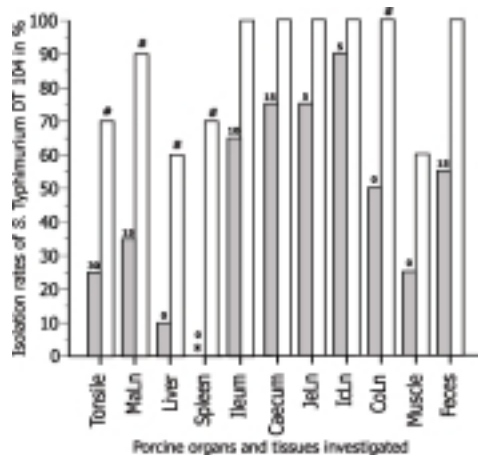


Furthermore, the bacteriological investigation showed also marked effects of vaccination.

Significant differences could be observed between the immunized group and the placebo group at shedding of the challenge strain (Figure 1). Beginning at day 3 post infection the group of immunized pigs shed *S. Typhimurium* DT104 to a significant lesser extent ($P < 0.05$) than non-immunized pigs. Additionally, it could be demonstrated that vaccine strain invaded the gut and

gut-associated lymph nodes. Furthermore, vaccinated pigs showed a significantly decreased rate of colonization (42.5 % versus 87.5 % at the placebos) of the inner organs (Figure 2). Particularly in organs, which were used for human consumption (muscle, liver and spleen), the rate of colonizing was significantly increased. The challenge strain could not be detected in liver, spleen, and muscle.

Fig. 2: Rate of colonization of different tissues and inner organs by *S. Typhimurium* DT104 in immunized (grey boxes) and placebo-treated pigs (white boxes). MaLn = mandibular lymph node, JeLn = jejunal lymph node, IcLn = ileocolic lymph node, CoLn = colic lymph node. The percentage of animals tested positive for immunization strain *S. Tm. Nal2/Rif9/Rtt* is given separately in numbers. Asterisks (*) indicate, that *S. Typhimurium* DT104 could not be cultured from the samples. (#), indicates significant differences ($P < 0.05$) between immunized pigs and those of the placebo-treated group.



Significant differences could be observed between the immunized group and the placebo group (Figure 3) at shedding of the challenge strain. Beginning at day 3 post infection the group of immunized pigs shed *S. Typhimurium* DT104 to a significant lesser extent ($P < 0.05$) than non-immunized pigs. Additionally, it could be demonstrated that vaccine strain invaded the gut and gut-associated lymph nodes. Furthermore, vaccinated pigs showed a significantly decreased rate of colonization (42.5 % versus 87.5 % at the placebos) of the inner organs. Particularly in organs, which were used for human consumption (muscle, liver and spleen), the rate of colonizing was significantly increased. The challenge strain could not be detected in liver, spleen, and muscle.

Serological investigation shows that in comparison to non-immunized pigs, immunized animals revealed a markedly higher IgA antibody activity in the serum.

Additionally, a significant discrimination ($P < 0.05$) between vaccinated and non vaccinated pigs was observed when IgM was measured, but, here the non-immunized animals had an elevated humoral antibody response.

Discussion

As in poultry, the aim of vaccination in pigs is the protection of the human consumer from a *Salmonella*-infection by infected or contaminated meat (2,3,6,8,10). Therefore, vaccination of pigs against *Salmonella* ssp. should decrease the number of viable salmonellae in both meat (muscle, liver, spleen) and faeces. This was achieved by vaccination as presented here. Vaccinated animals shed substantially smaller amounts of the challenge strain and for a shorter period of time. Therefore, it can safely be assumed that vaccination of pigs reduce the transmission of *S. Typhimurium* on the herd level.

In further studies, it has to be investigated if repeated ("booster") immunizations will further reduce the colonization rate in the inner organs.

Conclusion

The findings underline the potency of the vaccine tested to prevent clinical symptoms of salmonellosis and to significantly reduce the colonization of inner organs as well as the shedding of *Salmonella* Typhimurium, which both contributes to an increased consumer protection.

Acknowledgements

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