Introduction

Salmonella infections in humans are often linked with the consumption of contaminated pork [1] [2]. Vaccination has been proposed to control Salmonella infections in pigs [3] [4] and has already proven to be efficient in laying hens, reducing faecal shedding and internal egg contamination [5] [6]. The use of vaccines in pigs is currently limited due to interference with European Salmonella serosurveillance programmes based on the detection of antibodies against the lipopolysaccharides (LPS) of Salmonella [7]. It was therefore the aim of this study to develop a DIVA-vaccine marker (Differentiation of Infected and Vaccinated Animals), without affecting immunization capacity of this strain, that would avoid interference with current LPS-ELISA based serosurveillance programmes.

Experimental objectives, methods and results

Vaccination of mice with ΔrfbA, Δrfal and Δrfal but not Δrfal, Δrfag and Δrfaf induces protection in mice against a Salmonella Typhimurium infection

We tested whether mutations in the LPS of Salmonella Typhimurium strain 112910a affect its protective capacity against a subsequent challenge with a highly virulent strain.

For that purpose, seven groups of ten mice were immunized via the orogastric route with 10⁷ CFU of one of the LPS mutant strains (either ΔrfbA, Δrfal, Δrfal, Δrfag or Δrfaf) or with the wild type Salmonella Typhimurium strain 112910a. Four weeks after immunization, all mice were challenged with 10⁶ CFU of the virulent Salmonella Typhimurium strain NCTC12023Δrfal by the orogastric route. Mice were euthanized nine days post challenge.

Conclusion: Oral immunization of mice with Salmonella Typhimurium strain 112910a, ΔrfbA, Δrfal or Δrfal induced a significant (P < 0.05) protection against subsequent challenge with NCTC12023Δrfal in both spleen and liver compared to non immunized control animals. Results are shown in figure 1.

Pigs, immunized with the rfal or rfal mutant, can be serologically differentiated from Salmonella infected animals

We examined if it was possible to discriminate between the serological response induced after vaccination of pigs with adjuvanted bacteria of either Salmonella Typhimurium strain 112910a or one of its isogenic LPS mutant strains (Δrfal, Δrfaf). Secondly we compared this with the serological response of pigs after infection with Salmonella Typhimurium strain 112910a.

Therefore, 14 piglets were randomly allocated to three vaccination groups (n = 12) and one sham-immunized control group (n = 2). The animals were intramuscularly immunized with one of the formalin-inactivated Salmonella strains (either: Salmonella Typhimurium strain 112910a, Δrfal or Δrfal) in Freund’s incomplete adjuvant. To obtain sera from Salmonella Typhimurium infected piglets, one experimental group (n = 3) was orally inoculated with approximately 2×10⁷ CFU of Salmonella Typhimurium strain 112910aΔrfal.

Conclusion: Anti-Salmonella-antibody titres were detected in the serum of all immunized and infected animals, when using an in-house whole cell ELISA. No significant seroconversion was seen (P > 0.05) in animals immunized with inactivated Δrfal or Δrfaf strains and in sham-immunized control animals (non immunized and non infected animals), when using the commercial IDEXX ELISA. Conversely, marked seroconversion occurred in pigs immunized with the inactivated Salmonella Typhimurium strain 112910aΔrfal. Results illustrate a clear differentiation between sera from piglets immunized with the Δrfal strain or Δrfal strain and sera of pigs infected with their isogenic wild type strain. Anti-Salmonella-antibody titres were detected in the serum of all immunized and infected animals, when using an in-house whole cell ELISA. Results are shown in figure 2.

General conclusion

In conclusion, we provide proof of concept that deletions in the rfal or the rfal gene in Salmonella Typhimurium strain 112910a allows differentiation of infected and vaccinated pigs in an LPS based ELISA without reducing the strain’s protective capacities in mice. Further research in pigs is underway.