Application of the DIVA principle to Salmonella Typhimurium vaccines in pigs avoids interference with serosurveillance programmes

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Abstract

Salmonellosis is one of the most important bacterial zoonotic diseases in humans and Salmonella infections are often linked with the consumption of contaminated pork. In order to reduce Salmonella Typhimurium infections in humans, minimization of the Salmonella intake into the food chain is important. Vaccination has been proposed to control Salmonella infections in pigs. However, pigs vaccinated with the current vaccines cannot be discriminated from infected pigs with the lipopolysaccharide (LPS) -based serological tests used in European serosurveillance programmes. We therefore examined which LPS encoding genes of Salmonella Typhimurium can be deleted to allow differentiation of infected and vaccinated pigs, without affecting the vaccine strain's protective capacity. For this purpose, deletion mutants in Salmonella strain 112910a, used as vaccine strain, were constructed in the LPS encoding genes: ΔrfbA, ΔrfaL, ΔrfaJ, ΔrfaI, ΔrfaG and ΔrfaF. Inoculation of BALB/c mice with the parent strain, ΔrfaL, ΔrfbA or ΔrfaJ strains but not the ΔrfaG, ΔrfaF or ΔrfaI strains protected significantly against subsequent infection with the virulent Salmonella Typhimurium strain NCTC12023. Immunization of piglets with the ΔrfaJ or ΔrfaL mutants resulted in the induction of a serological response lacking detectable antibodies against LPS. This allowed a differentiation between sera from pigs immunized with the ΔrfaJ or ΔrfaL strains and sera from pigs infected with their isogenic wild type strain.

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 [1] [3] [4] and has already proven to be efficient in laying hens, reducing faecal shedding and internal egg contamination [5] [6]. Currently, one licensed Salmonella Typhimurium live vaccine for pigs is commercially available in Europe [7]. The use of this vaccine is limited due to interference with European Salmonella serosurveillance programmes based on the detection of antibodies against the lipopolysaccharides (LPS) of Salmonella [8]. It was therefore the aim of this study to develop a DIVA-vaccine strain (Differentiation of Infected and Vaccinated Animals), without attenuating the vaccine strain, which would not interfere with current LPS-ELISA based serosurveillance programmes.

Material and Methods

Salmonella Typhimurium strain 112910a, phage type 120/ad, isolated from a pig stool sample and characterized previously [3], was used as the wild type background to construct several isogenic LPS knock-out mutants: ΔrfbA, ΔrfaL, ΔrfaJ, ΔrfaI, ΔrfaG and ΔrfaF. A commercially available enzyme-linked immunosorbent assay (ELISA) (HerdChek Salmonella; IDEXX Laboratories, Schiphol-Rijk, Noord-Holland, The Netherlands) for the detection of porcine antibodies against the LPS of Salmonella was used as a reference according to the manufacturer's instructions. Besides, an in-house Salmonella Typhimurium strain 112910a whole cell ELISA to detect porcine anti Salmonella Typhimurium antibodies, was prepared as described before [9]. In a mouse model, we tested whether the LPS mutants affect the protective capacity of Salmonella Typhimurium strain 112910a against a subsequent challenge with a highly virulent strain. For that purpose, seven groups of ten mice were inoculated first via the orogastric route with 2×10^7 CFU/ml of one of the LPS mutant strains (either: ΔrfbA, ΔrfaL, ΔrfaJ, ΔrfaI, ΔrfaG or ΔrfaF) or with the wild type Salmonella Typhimurium strain 112910a. Four weeks after primary inoculation, all mice were challenged with 108 CFU of the virulent Salmonella Typhimurium strain NCTC-12023Nal20 by the orogastric route. In a second in vivo study, we examined whether it was possible to discriminate between the serological response induced after immunization of pigs with either Salmonella Typhimurium strain 112910a or one of its isogenic strains (ΔrfaL ΔrfaJ) on the one hand and after infection of pigs with Salmonella Typhimurium strain

112910a on the other hand. Therefore, 14 piglets were randomly allocated to three vaccinated groups (n = 12) and one sham-vaccinated control group (n = 2). Vaccinated animals were intramuscularly immunized (2x) with one of the formalin-inactivated Salmonella strains (either: Salmonella Typhimurium strain 112910a, ΔrfaJ 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 x 107 CFU of Salmonella Typhimurium strain 112910aNal20.

Results

Vaccination of mice with ΔrfbA, ΔrfaL and ΔrfaJ but not ΔrfaI, ΔrfaG and ΔrfaF protects mice against a Salmonella Typhimurium infection:

Oral immunization of mice with Salmonella Typhimurium strain 112910a, ΔrfbA, ΔrfaL or ΔrfaJ induced a significant ($P < 0.05$) protection against subsequent challenge with NCTC12023Nal20 in both spleen and liver compared to non immunized control animals. Results are shown in figure 1.

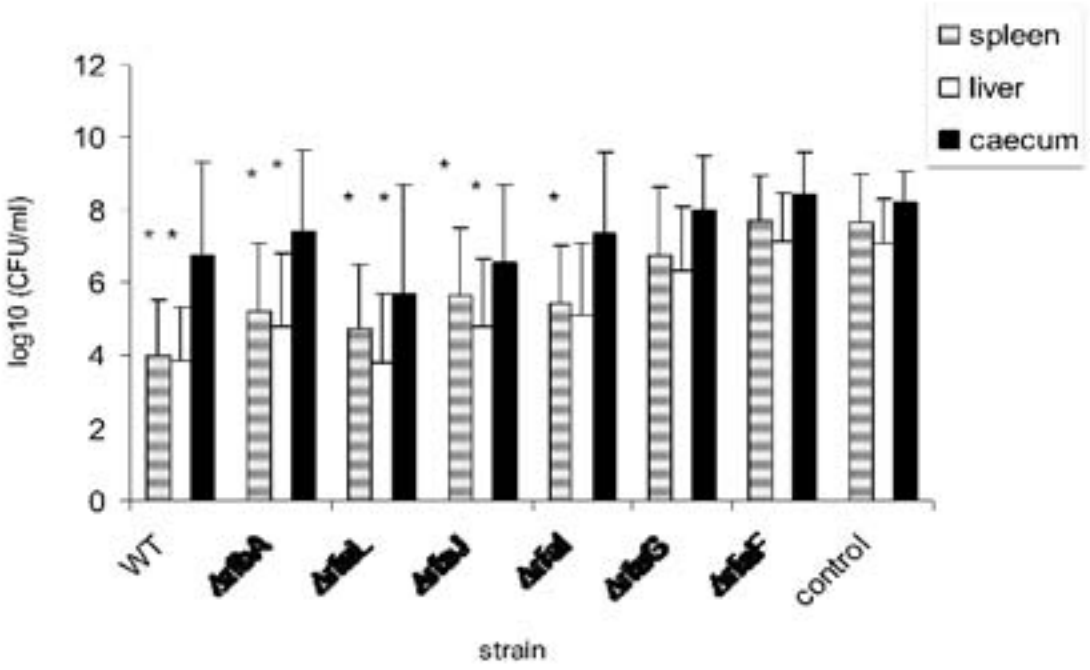

Figure 1: Recovery of Salmonella bacteria from various organs of mice immunized with either Salmonella Typhimurium, one of its isogenic LPS mutants or non immunized control animals and subsequently challenged with Salmonella Typhimurium strain NCTC12023Nal20. The log10 value of the ratio of CFU per gram sample and standard deviations are given. An asterisk refers to a significant difference with the control group (P < 0.05).

Pigs, immunized with the ΔrfaL or ΔrfaJ mutant, can be serologically differentiated from Salmonella infected animals:

Results showed no significant seroconversion ($P > 0.05$) in animals immunized with inactivated ΔrfaJ or ΔrfaL strains and in sham-vaccinated 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 112910a. Results also illustrate a clear differentiation between sera from piglets immunized with the ΔrfaJ strain or ΔrfaL strain and sera of pigs infected with their isogenic wild type strain. Anti-Salmonella-antibody titers were detected in the serum of all immunized and infected animals, when using the in-house whole cell ELISA. Results are illustrated in figure 2.

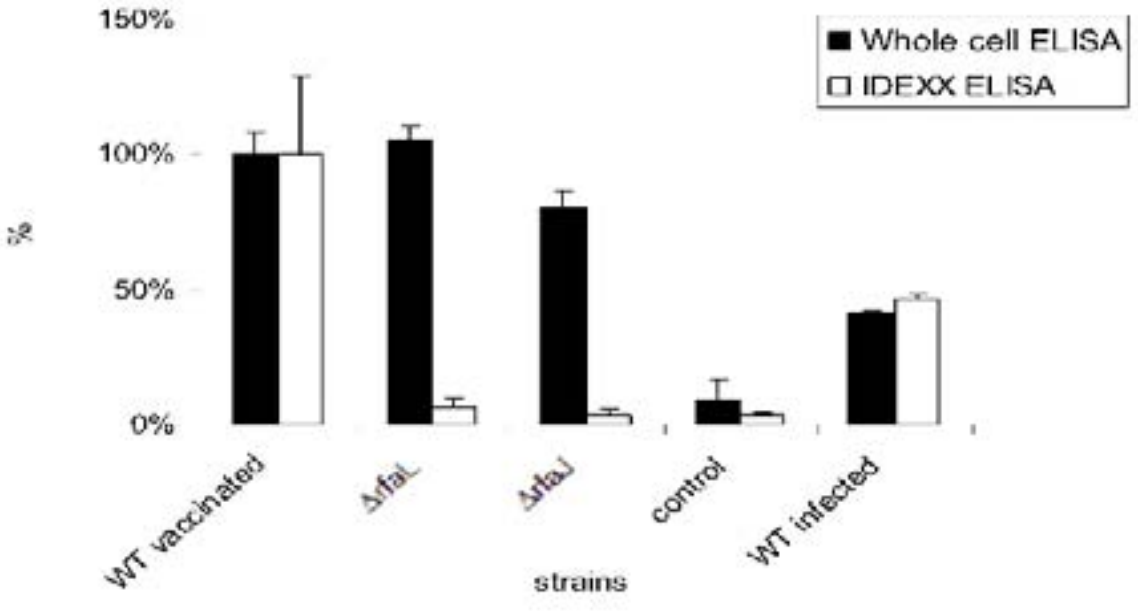

Figure 2: Serological results of pigs immunized with ΔrfaL, ΔrfaJ or Salmonella Typhimurium strain 112910a, control pigs (animals that were not immunized and not infected) and pigs infected with Salmonella Typhimurium strain 112910a Nal20. Values are represented as a percentage compared to the wild type vaccinated group. We emphasize that these results are based on a small sample size.

Discussion

DIVA vaccines are a recent advance in vaccinology enabling distinction between an animal that is seropositive to a particular infectious agent because it has been vaccinated, and one that is seropositive because it has been infected with virulent field organisms [10]. Because current Salmonella serosurveillance programmes are generally based on detection of antibodies against LPS antigens, we selected six LPS genes that might be suitable markers to develop a LPS based DIVA-vaccine. In a mouse in vivo experiment we showed that the rfaG and rfaF mutant strains were not able to protect BALB/c mice against a subsequent infection with Salmonella Typhimurium NCT12023Nal20 and that the Δrfal strain was only able to significantly reduce bacterial counts in the spleen of mice. Conversely, ΔrfbA, ΔrfaL and ΔrfaJ strains, with less truncated LPS, were able to successfully protect BALB/c mice against a Salmonella Typhimurium infection and their protective capacity was not impaired compared to their isogenic wild type strain. These results strongly suggest that a confined truncation of LPS is essential to maintain protection against challenge with the virulent strain Salmonella Typhimurium NCTC12023Nal20 in mice. The ultimate goal of this study was to verify whether LPS mutant strains were able to elicit a DIVA humoral immune response in pigs. Our results illustrate that both the ΔrfaL and the ΔrfaJ strain gave no seroconversion when using a LPS based ELISA, while a clear-cut seroconversion was observed when using an in-house Salmonella Typhimurium strain112910a whole cell ELISA. Besides, immunization of piglets with the ΔrfaJ or ΔrfaL mutants resulted in the induction of a serological response allowing clear differentiation between sera from piglets immunized with the ΔrfaJ or ΔrfaL strains and sera of pigs infected with their isogenic wild type strain when using a LPS based ELISA.

Conclusion

In conclusion, applying deletions in the rfaJ 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.

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