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

Bregje Leyman, **Freddy Haesebrouck**, Filip Boyen, Alexander Van Parys, Elin Verbrugghe, Frank Pasmans

Faculty of Veterinary Medicine, Ghent University, Department of Pathology, Bacteriology and Avian Diseases
Salisburylaan 133, 9820 Merelbeke, Belgium; E-mail: *Bregje.Leyman@UGent.be*

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]. 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 Δ*rfbA*, Δ*rfaL* and Δ*rfaJ* but not Δ*rfaI*, Δ*rfaG* and Δ*rfaF* 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 10^7 CFU of one of the LPS mutant strains (either: Δ*rfbA*, Δ*rfaL*, Δ*rfaJ*, Δ*rfaI*, Δ*rfaG* or Δ*rfaF*) or with the wild type ***Salmonella*** **Typhimurium strain 112910a. Four weeks after immunization, all mice were challenged with 10^8 CFU of the virulent** ***Salmonella*** **Typhimurium strain NCTC12023^{Nal20} by the orogastric route. Mice were euthanized nine days post challenge.**

Conclusion:
Oral immunization of mice with ***Salmonella*** **Typhimurium strain 112910a, Δ*rfbA*, Δ*rfaL* or Δ*rfaJ* induced a significant ($P < 0.05$) protection against subsequent challenge with NCTC12023^{Nal20} in both spleen and liver compared to non immunized control animals. Results are shown in figure 1.**

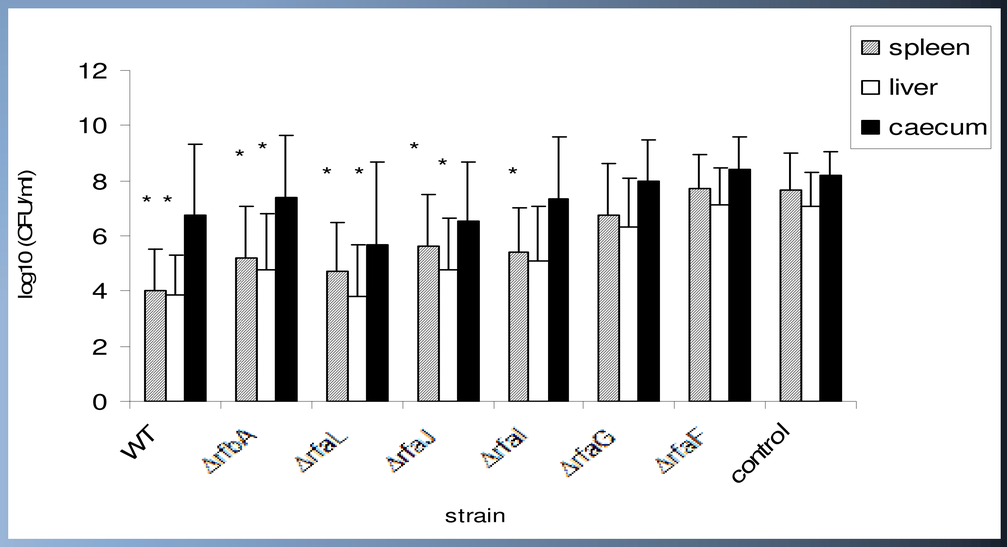

Figure 1:Recovery of *Salmonella* bacteria from various organs of mice immunized with either *Salmonella* Typhimurium, one of its isogenic LPS mutants or non immunized control animals and subsequently challenged with *Salmonella* Typhimurium strain NCTC12023Nal20. The mean of the log10 value of CFU per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

Pigs, immunized with the *rfaJ* 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 bacterins of either ***Salmonella*** **Typhimurium strain 112910a or one of its isogenic LPS mutant strains (Δ*rfaL* Δ*rfaJ*). 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, Δ*rfaJ* or Δ*rfaL*) in Freund's incomplete adjuvant. To obtain sera from** ***Salmonella*** **Typhimurium infected piglets, one experimental group (n = 3) was orally inoculated with approximately 2×10^7 CFU of** ***Salmonella*** **Typhimurium strain 112910a^{Nal20}.**

Conclusion:
Anti-*Salmonella*-antibody titers 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 Δ*rfaJ* or Δ*rfaL* strains and in sham-immuized 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 illustrate a clear differentiation between sera from piglets immunized with the Δ*rfaJ* strain or Δ*rfaL* 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 an in-house whole cell ELISA. Results are shown in figure 2.**

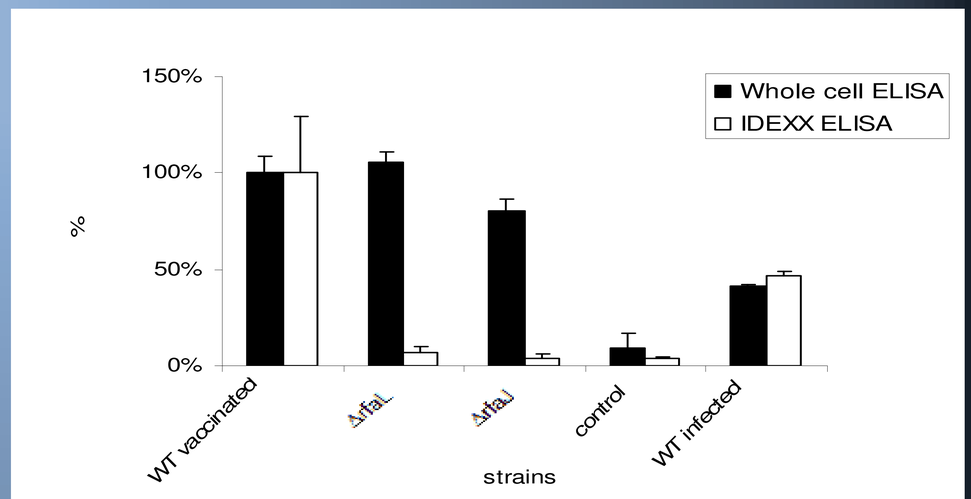

Figure 2: Serological results of pigs immunized with Δ*rfaL*, Δ*rfaJ* or *Salmonella* Typhimurium strain 112910a, control pigs (animals that were not immunized and not infected) and pigs infected with *Salmonella* Typhimurium strain 112910a Nal20. Values are represented as a percentage compared to the wild type vaccinated group.

General conclusion

In conclusion, we provide proof of concept that 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. Further research in pigs is underway.**

[1] Boyen F., Haesebrouck F., Maes D., Van Immerseel F., Ducatelle R., Pasmans F., Non-typhoidal *Salmonella* infections in pigs: A closer look at epidemiology, pathogenesis and control., Vet Microbiol, 130, 2008, 1-19.
[2] Majowicz S.E., Musto J., Scallan E., Angulo F.J., Kirk M., O'Brien S.J., Jones T.F., Fazil A., Hoekstra R.M., The global burden of nontyphoidal *Salmonella* gastroenteritis., Clin Infect Dis, 50, 2010, 882-889.
[3] Boyen F., Pasmans F., Van Immerseel F., Donne E., Morgan E., Ducatelle R., Haesebrouck F., Porcine *in vitro* and *in vivo* models to assess the virulence of *Salmonella enterica* serovar Typhimurium for pigs., Lab Anim, 2009, 43, 46-52.
[4] Denagamage T.N., O'Connor A.M., Sargeant J.M., Rajić A., McKean J.D., Efficacy of vaccination to reduce *Salmonella* prevalence in swine: a systematic review of literature from 1979 to 2007. Foodborne Pathog Dis, 2007, 4, 539-49.
[5] Collard J.M., Bertrand S., Dierick K., Godard C., Wildemauwe C., Vermeersch K., Duculot J., Van Immerseel F., Pasmans F., Imberechts H., Quinet C., Drastic decrease of *Salmonella* Enteritidis isolated from humans in Belgium in 2005, shift in phage types and influence on foodborne outbreaks., Epidemiol Infect, 2008, 136, 771-81.
[6] Gantois I., Ducatelle R., Timbermont L., Boyen F., Bohez L., Haesebrouck F., Pasmans F., Van Immerseel F., Oral immunisation of laying hens with the live vaccine strains of TAD *Salmonella* vac E and TAD *Salmonella* vac T reduces internal egg contamination with *Salmonella* Enteritidis., Vaccine, 2006, 24, 6250-6255.
[[7] Cortinas Abrahantes J., Bollaerts K., Aerts M., Ogunsanya V., Van der Stede Y., *Salmonella* serosurveillance: different statistical methods to categorise pig herds based on serological data., Prev Vet Med, 2009, 89, 59-66.