Technology offer

A Salmonella serological markerstrain with reduced shedding for use as a DIVA vaccine

We offer technology for use in Salmonella vaccines for pigs and/or poultry to

Develop a Salmonella DIVA vaccine with

- *Salmonella* strains of which the humoral response elicited can easily be differentiated from the humoral response, elicited by a wildtype fieldstrain.
- the use of these markerstrains as a vaccine would not interfere with existing *Salmonella* control programs in which the classification is based on the serological status of the herd
- the novel strains can thus be used for vaccination in the existing *Salmonella* control programs as a control measure

and/or

Reduce shedding of Salmonella (vaccine) strains

- Increased cortisol levels after stress (eg induced by starvation and transport before slaughtering) lead to increased shedding of *Salmonella* and by consequence to increased contamination of carcasses.
- This increased recrudescence of Salmonella after stress can be significantly reduced when specific genes are knocked out.

Introduction

According to a study conducted by EFSA in 2008, approximately one out of ten Belgian pigs is *Salmonella* positive at slaughter. Besides affecting performance and health of pigs (Boyen et al. 2008, 2009 a and b), *Salmonella* Typhimurium is at present the most prevalent serovar in infections in humans in Western Europe. Next to the direct negative impact on public health, increasing multiple antimicrobial resistance associated with pork-related serotypes such as *Salmonella* Typhimurium may become a serious human health hazard in the near future. The fact that animals (fattening pigs or layers) can carry the bacterium for months without showing clinical symptoms (carrier animals), but can shed *Salmonella* in high numbers after a period of stress such as after transport to the slaughterhouse results in increased carcass contamination at slaughter.

Preventing the spread of *Salmonella* to the consumer requires special control measures during slaughter and processing but also on farm. The extra cost of these controls is increasingly being transferred back to the producer in the form of financial penalties or the loss of the market for contaminated animals

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or eggs. Currently, *Salmonella* monitoring programs aiming to reduce salmonellosis in humans are imposed all over the world. The European countries, however, are playing a leading role in this matter. In a substantial proportion of these monitoring programs, the amount of *Salmonella*-directed antibodies in the blood or meat juice of pigs is used as the sole tool to categorize pig farms as low-risk or high-risk farms regarding their *Salmonella* status.

The amount of *Salmonella*-directed antibodies in serum is measured using Enzyme linked immunosorbant assays (ELISA ; eg IDEXX Herdcheck or LDL Salmotype). Vaccination has proven to be an important tool to reduce *Salmonella* colonization in poultry, and it is expected that also in pigs, vaccination could contribute to the control of *Salmonella*. However, animals vaccinated with current vaccines cannot be distinguished from animals infected with *Salmonella* using the ELISA's. Moreover, increased shedding after stress should also be considered for vaccines based on attenuated *Salmonella* strains.

Therefore, in pig production farmers and veterinarians are reluctant to use vaccination, since vaccination would increase the chance that their farm would be considered as a high-risk farm with respect to *Salmonella*, while the actual infection status could be low. In poultry production bacteriological analyses are used to monitor the *Salmonella* status of a farm. These methods are however less reliable. Replacing them by serological monitoring would thus be an advantage.

For a good control and monitoring of *Salmonella* in pigs and poultry a vaccine strain is thus needed that

- induces a good immune response but that does not induce a high serological response in the ELISA's
- does not shed in high numbers after a period of stress such as after starvation and transport to the slaughterhouse

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Development of the DIVA-vaccine (Proof of Concept)

We have developed *Salmonella* strains that elicit a humoral immune response that is comparable to the immune response elicited by wild type strains (Leyman et al., 2011). The humoral response elicited by the novel strains however is significantly less detectable or even completely undetectable using a commercially available based ELISA (see Table 1). These strains can therefore be regarded as serological marker strains.

Table 1: Percentage OD that was obtained using different commercial ELISA's used in official European monitoring programs and an in house whole-cell ELISA for serum obtained from piglets vaccinated with the wild type strain (+ control) or the novel strains (strain 1 and strain 2) or phosphate buffered saline (PBS; - control).

Elisa	+ control	strain 1	strain 2	- control
Whole cell ELISA	1.1 ± 0.03	1.1 ± 0.08	1.1 ± 0.07	0.1 ± 0.02
Idexx Herdcheck (%OD)	23.0 ± 13.0	-1.8 ± 0.6	5.1 ± 1.5	-2.4 ± 0.3
LDL Salmotype (%OD)	53.7 ± 16.7	14.2 ± 11.5	63.0 ± 8.7	3.0 ± 6.5

We have shown in *in vivo* experiments in pigs that a double intramuscular injection of pigs with the inactivated novel strains, supplemented with Freund's incomplete adjuvant, elicits a humoral immune response that is comparable to the immune response elicited by a double intramuscular injection of an inactivated wild type *Salmonella* strain supplemented with Freund's incomplete adjuvant using an in-house whole cell ELISA (Table 1). These experiments show that the novel strains generate the production of *Salmonella*-specific antibodies.

Moreover we demonstrated in *in vivo* experiments in pigs that the humoral immune response elicited by the double intramuscular injection of the formalin inactivated novel strains supplemented with Freund's incomplete adjuvant can not be detected by commercial ELISA's that are currently used in official national *Salmonella* monitoring programs (Table 1). On the contrary these ELISA's detect high levels of antibodies in pigs, injected with the formalin inactivated wild type strain (Table 1). This demonstrates that the humoral immune response elicited in pigs by our novel strains does not interfere with the current *Salmonella* pig monitoring programs.

To examine the effect of the strains on the protective capacity of a live *Salmonella* strain, BALB\c mice were intragastrically vaccinated with either the wild type strain (+ control), the novel strains or phosphate buffered saline (PBS; - control). Four weeks after vaccination, these mice were orally challenged with the highly virulent *Salmonella* Typhimurium strain NCTC 12023. In the group of mice sham-vaccinated with PBS, all mice died from systemic salmonellosis within 10 days after inoculation, showing high numbers of bacteria in all internal organs. In the groups of mice vaccinated with either the wild type strain (+ control) or the novel strains, the majority of

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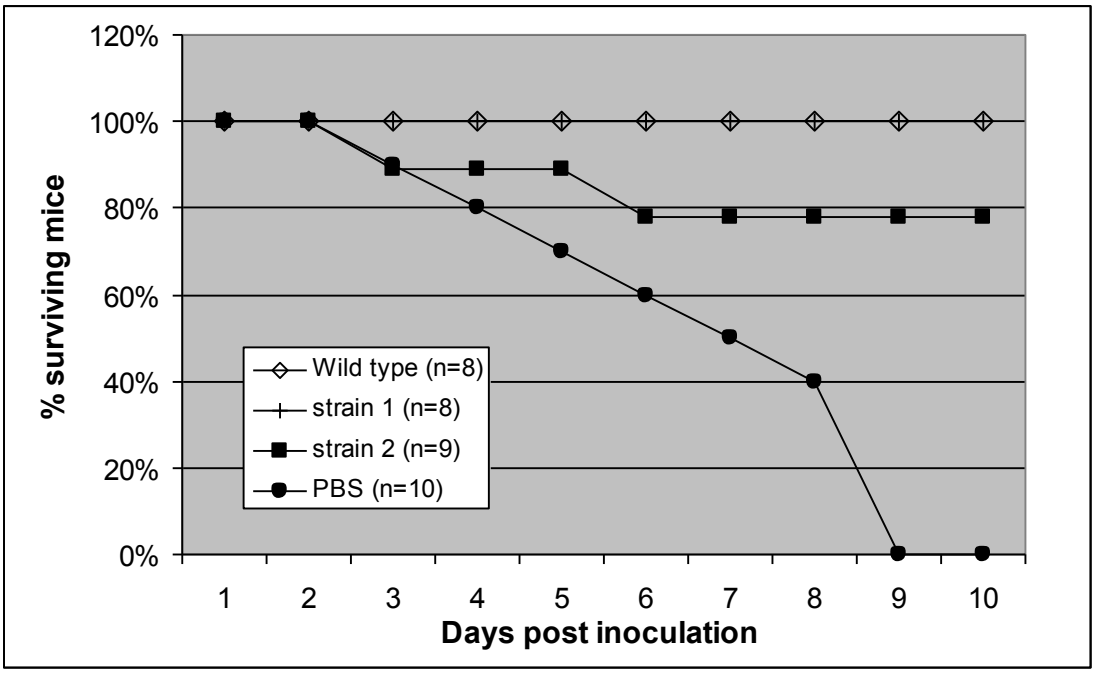
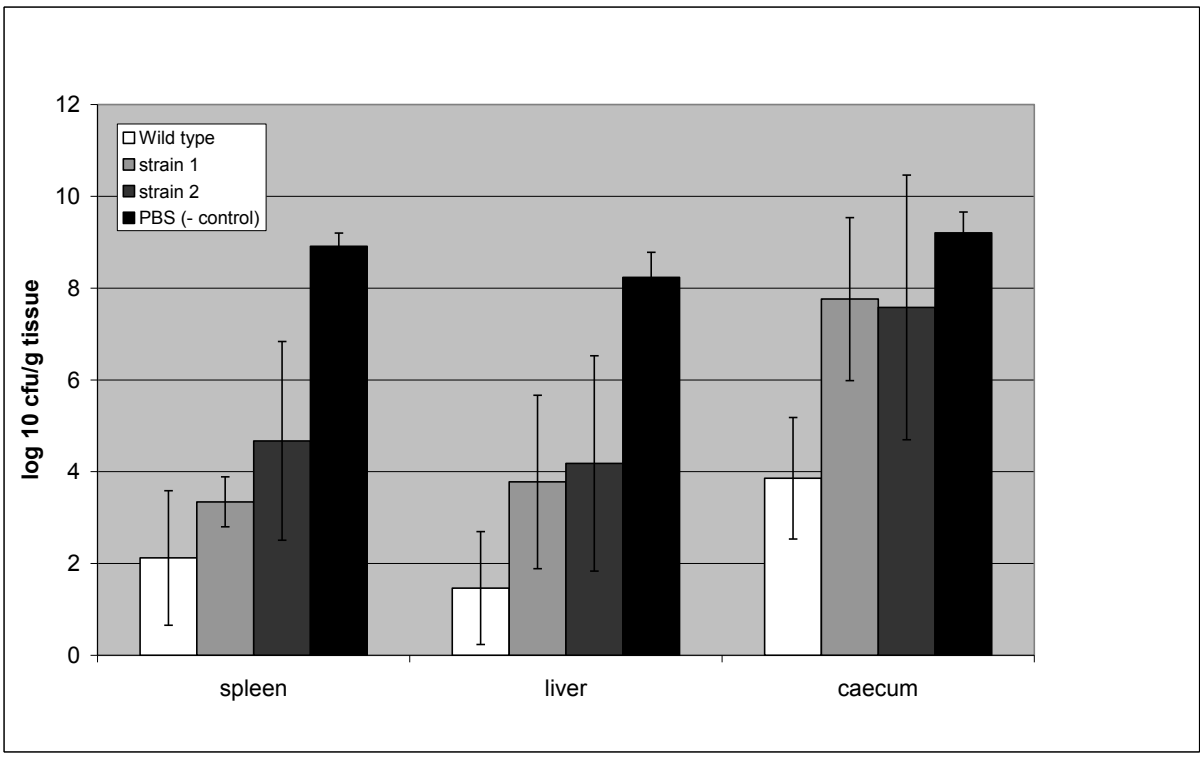
mice were alive 10 days after inoculation and all living mice were in good clinical condition. These results are shown in Figure 2.

Figure 2: Percentages of living mice at days one to 10 after inoculation. BALB\c mice were intragastrically vaccinated with either the wild type strain (+ control), the novel strains or phosphate buffered saline (PBS; - control). Four weeks after vaccination, these mice were orally challenged with the highly virulent *Salmonella* Typhimurium strain NCTC 12023.



The results of the quantitative bacteriological examination of the internal organs are shown in Figure 3.



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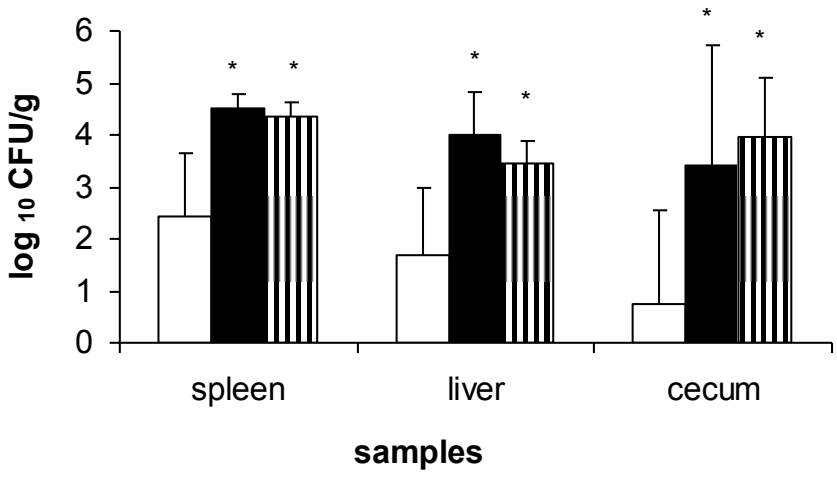
These results confirm the clinical protective capacities of the novel strains in a mouse model.

Reduce recrudescence and shedding (Proof of Concept)

We demonstrated that stress related re-excretion of *Salmonella* is linked to increased serum cortisol levels (Verbrugghe et al., 2011).

In a mouse model (DBA/2J mice) a subcutaneous injection of dexamethasone (100mg/kg) is capable to induce recrudescence of *Salmonella* Typhimurium strain 112910aNal^{20} : *Salmonella* infected DBA/2J mice, subsequently injected with dexamethasone had a significantly ($P < 0.05$) higher number of *Salmonella* Typhimurium bacteria in the spleen, the liver and the cecum, compared to DBA/2J mice that were injected with a saline solution.

Figure 4: Recovery of Salmonella Typhimurium 112910aNal20 from various organs from DBA/2J mice 14 days post infection. Black bars represent infected mice that received a sc injection of dexamethasone (100 mg/kg) 24h before euthanasia and white bars represent infected mice that received an subcutaneous injection of HBSS (control group). Striped bars represent DBA/2J mice that received 25 mg/kg dexamethasone 24h and 21h before euthanasia. The mean log10 values of the number of CFU per gram sample with their standard deviations are given. An asterisk (*) refers to a significant difference (P < 0.05) between the control group and the dexamethasone group.



A deletion selected genes in *Salmonella* Typhimurium strain 112910a could abolish stress induced recrudescence of that strain (Figure 5). Consequently, applying this deletion promotes the safety of live *Salmonella* vaccines.

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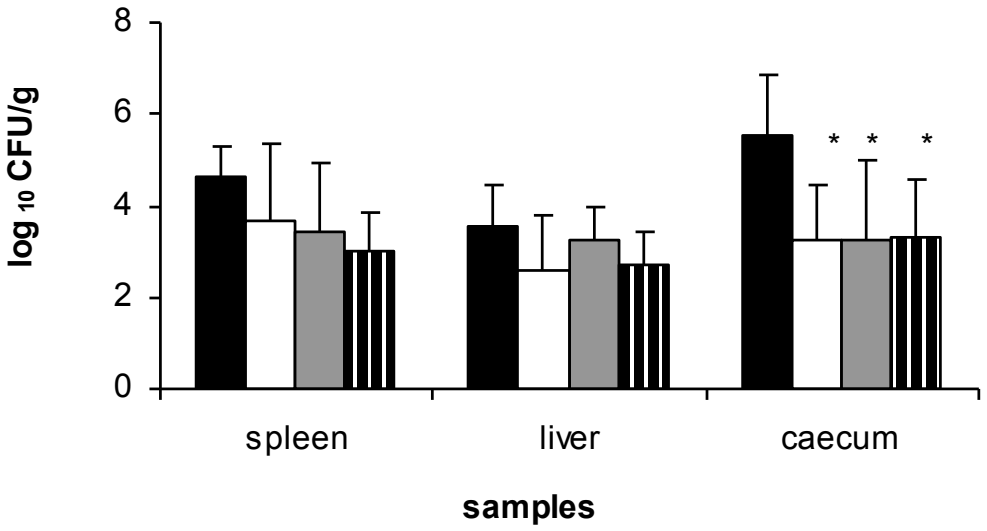
Figure 5: Recovery of Salmonella Typhimurium 112910a (WT) and its isogenic knock-out (KO) mutant from various organs from mice 14 days post infection. Black bars represent WT infected DBA/2J mice that received a subcutaneous injection of dexamethasone (100 mg/kg) 24h before euthanasia. White bars represent WT infected mice that received a subcutaneous injection of HBSS. Gray bars represent KO infected DBA/2J mice that received a subcutaneous injection of dexamethasone (100 mg/kg) 24h before euthanasia. Striped bars represent KO infected mice that received a subcutaneous injection of HBSS. The mean log10 values of the number of CFU per gram sample with their standard deviations are given. An asterisk (*) refers to a significant difference ($P < 0.05$) with the WT dexamethasone group.



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IP-position

Patent application : *Salmonella* vaccine

Patent application : Prevention of *Salmonella* recrudescence

Collaboration type

We are looking for a partner that is interested in the commercialization of a safe *Salmonella* DIVA-vaccine.

References

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