Technology offer

Safe and effective Salmonella vaccines for poultry

Attenuated vaccines based on *Salmonella* deletion mutant strains (with defective multi drug resistance (MDR) efflux pump systems)

Target market and value

The two major zoonotic *Salmonella* serotypes are Enteritidis and Typhimurium, of which the first is typically associated with layers and eggs, while both serotypes are colonizing broilers. Because of trade issues of eggs and meat when flocks are contaminated, these infections can result in severe economical losses (in addition to health care costs for humans).

Another serotype that is economically important worldwide is *Salmonella* Gallinarum. This serotype causes fowl typhoid, a severe septicaemic disease in layers that is still a major problem in the middle East, Asia (including China), South America and Africa (and still occasionally in the EU and US). It can induce high mortality in both chicks and adult hens, with mortality rates above 50%.

Salmonella vaccines have been developed, but these are mostly strains carrying undefined mutations. As an example, the dominant commercial *Salmonella* Enteritidis and Typhimurium vaccines used in the EU are Avipro VacE and VacT (and the combined Vac DUO) are so-called metabolic drift mutants. Another example is the dominant *Salmonella* Gallinarum vaccine used worldwide, SG9R. In countries with a low prevalence of fowl typhoid the use of this vaccine strain has caused some outbreaks due to reversion of the vaccine strain to a virulent phenotype (Van Immerseel *et al*., 2013). This clearly highlights the importance of using well defined gene deletion mutants as vaccines in the future instead of undefined strains with unknown mutations.

With our technology we can target the following market segments:

- Food safety :
  - Vaccination of layers to reduce contamination of eggs with zoonotic *Salmonella* serotypes Enteritidis
  - Colonization inhibition to reduce contamination of meat with zoonotic *Salmonella* serotypes Enteritidis and Typhimurium
- Animal health: vaccination of layers to protect them against fowl typhoid *Salmonella* Gallinarum generating huge economic losses due to high mortality.

While *Salmonella* Gallinarum is a major problem in regions like the middle East, Asia (including China), South America and Africa (and still occasionally in the EU and US) the control of *Salmonella* as a zoonotic pathogen in poultry is a global issue.

Salmonellosis is one of the most prevalent zoonotic diseases in humans. In Europe alone over 100,000 cases are reported each year and in the United States there are approximately 40,000 cases reported annually. Since many

milder cases are not diagnosed or reported, the actual number of infections may be over thirty times higher. The most important serovars found are *Salmonella* Enteritidis and *Salmonella* Typhimurium, both of which are often linked back to poultry products. Examples include the 2010 massive egg recall in the US due to *Salmonella* Enteritidis-contaminated eggs, and more recently, the *Salmonella* Heidelberg contamination of ground turkey meat in the US. These incidents prove that *Salmonella* control in poultry remains important to guarantee safer food production for consumers.

The poultry industry and regulatory bodies however do not respond to it in the same manner everywhere. In Western as well as exporting countries control measures have been implemented, sometimes voluntary by the poultry industry, sometimes forced by a governmental regulation.

The importance of controlling *Salmonella* in poultry is gaining more interest and is mainly driven by:

- Increased regulation for the control of *Salmonella* such as in the EU and US
- Increased export regulations and barriers, making control of *Salmonella* vital for broiler integrators exporting broiler meat
- Increased branding of poultry meat and eggs makes that *Salmonella* contamination can do great damage to the brand and the company, affecting future sales
- Human salmonellosis outbreaks are the trigger for strong regulations and control measures.

Technology

Our are key targets for the development of safe live attenuated vaccine strains are the multi drug resistance efflux pump systems (MDR). **Safe** and **effective** live vaccines are obtained by the generation of deletion mutants (MDR genes). Indeed, these deletion mutant strains

- cannot survive in a harmful environment, such as litter, in tissues of the vaccinated birds and in eggs
- cannot revert to virulent phenotypes

The technology can be applied to several *Salmonella* serotypes, yielding live attenuated vaccines for different applications.

- *Salmonella* Enteritidis mutants as live vaccines for laying hens to control egg contamination
- *Salmonella* Enteritidis/Typhimurium mutant combination vaccine to reduce shedding and colonization of *Salmonella* in the broiler gut. This concept of vaccinating broilers is not based on adaptive immunity induction, but on a colonization-inhibition phenomenon.
- Attenuated *Salmonella* Gallinarum mutant vaccines with increased safety to control fowl typhoid

Proof of concept and development status

PoC1: Prevention of egg contamination by *Salmonella* Enteritidis after oral vaccination of laying hens with *Salmonella* Enteritidis mutants

Two different groups (n=30) were orally immunized at day of birth, at 6 weeks of age and at 16 weeks of age through crop instillation of 0.5 ml containing 10^8 cfu of *Salmonella* Enteritidis 147 mutant 1 (single deletion ; group 1) or *Salmonella* Enteritidis 147 mutant 2 (multiple deletion ; group 2). A third group of birds (n=30) was kept as non-immunized but *Salmonella* challenged positive controls (group 4). At 24 weeks of age, all the animals were intravenously inoculated in the wing vein with 0.5 ml containing 5 x 10^7 cfu of the *Salmonella* Enteritidis strain S1400/94.

Table 1: The percentage of egg content batches positive for the challenge strain during the two weeks following infection. Results between brackets show the percentage of batches positive after enrichment in tetrathionate brilliant green broth (37°C, overnight). Different superscripts indicate significant differences between the groups ($p<0.05$).

Group	Week 1	Week 2
Non-vaccinated	$70^a(74^a)$	$0(17)^a$
single deletion	$0^c(0)^c$	$0(0)^c$
multiple deletion	$0^c(0)^c$	$0(0)^c$

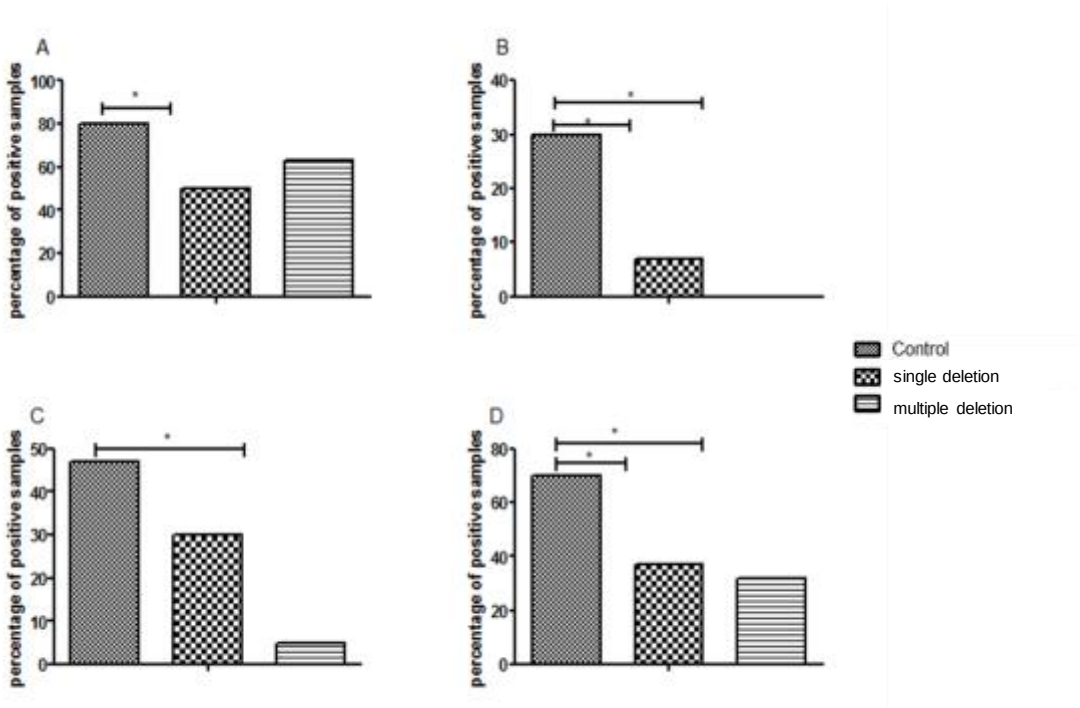

Figure 1: The percentage of *Salmonella* positive samples in spleen (A), caeca (B), oviduct (C) and ovary (D) in non-vaccinated and vaccinated animals, after enrichment. Statistical

significant differences ($p<0.05$) in percentage of positive organ samples between vaccinated groups and the non-vaccinated control group are marked with an asterix.

PoC2: *Salmonella* Enteritidis and *Salmonella* Typhimurium deletion mutant strains are safe for use in practice and protect broilers against Salmonella infection

Forty one-day-old chicks were divided into 4 groups of 10. Two groups were given 0.5 ml of a mixture containing 2 x 10^8 CFU/ml of the *Salmonella* Enteritidis mutant strain and 2 x 10^8 CFU/ml of the *Salmonella* Typhimurium mutant strain by oral gavage on day 1 of the experiment. The two other groups were given 0.5 ml of Hank's Balanced Salt Solution by oral gavage as a control on day one of the experiment. On day two of the experiment, one control group and one group treated with the mixture were given 0.5 ml of a solution containing 2 x 10^5 CFU/ml of the *Salmonella* Enteritidis 76Sa88 nal^R challenge strain by oral gavage, while the other two groups were challenged by administering 0.5 ml of a solution containing 2 x 10^5 CFU/ml of the *Salmonella* Typhimurium MB2136 $strept^R$ challenge strain by oral gavage. To evaluate colonization by the challenge strains, their numbers in caecum and spleen were determined at day 7 of the experiment. Shedding of the challenge strains was evaluated by bacteriological analysis of cloacal swabs taken on days 3 and 7.

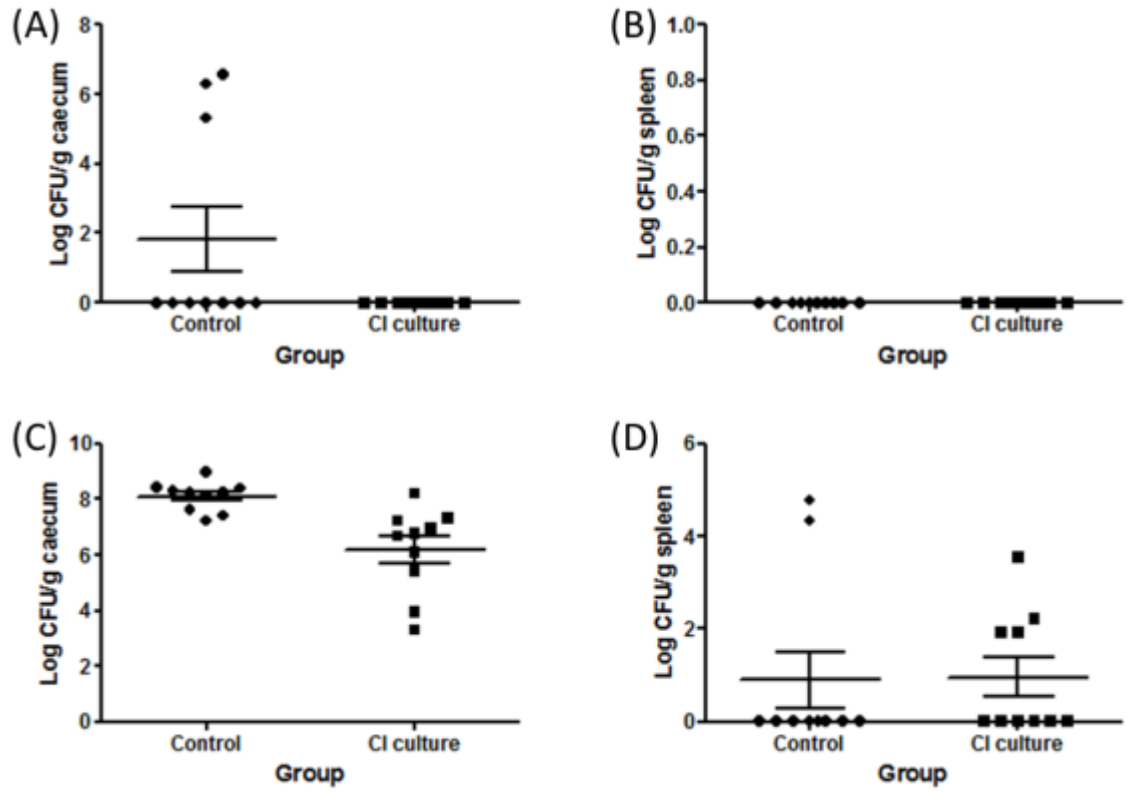

Figure 2: Caecal (A & C) and spleen (B & D) colonization by *Salmonella* Enteritidis (A & B) or *Salmonella* Typhimurium (C & D) wild-type strains on day 7 of age after experimental infection of broiler chickens treated with a CI culture. The values shown represent log10 of the CFU/g sample. The horizontal lines represent the mean, the error bars represent the standard error of mean (SEM). The number of samples equals 10 in all groups.

PoC3: Safety of a *Salmonella* Gallinarum mutant strains as a potential live vaccine strain for use in laying hens

Forty one-day-old chicks were divided into 2 groups of 20. On day 35 of age, one group was given 1 ml of a mixture containing 10^6 CFU/ml of the *Salmonella* Gallinarum SG9R strain by oral gavage as a control, while the other group was given 1 ml of a solution containing 10^6 CFU/ml of the *Salmonella* Gallinarum mutant strain. After inoculation, the weight of chickens was determined daily.

There was no difference in weight between the group treated with the SG9R strain and the group treated with the *Salmonella* Gallinarum mutant strain, indicating that the *Salmonella* Gallinarum mutant strain does not cause a growth depression when compared to the SG9R strain that is currently frequently used in practice.

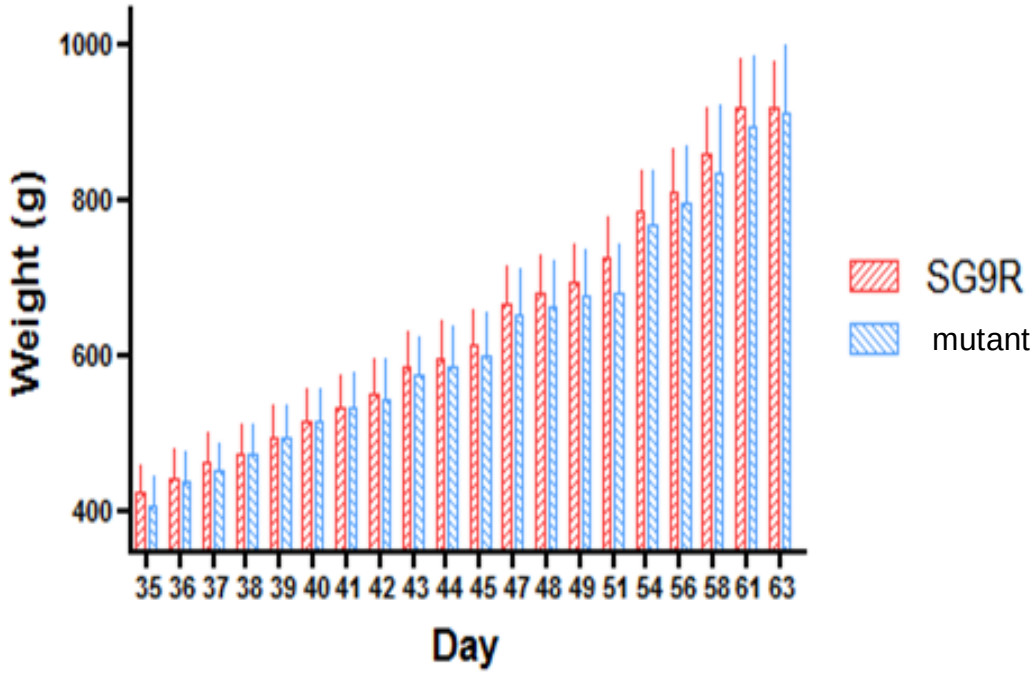

Figure 3: Average weight of 20 laying hens orally inoculated with either 10^6 CFU of a SG9R strain or 10^6 CFU of a *Salmonella* Gallinarum mutant strain.

Technology offer

IP-position

A patent application has been filed and is currently pending: application number EP16171540.4.

Partnering

We are looking for partners that are interested to use the technology for the development and marketing of commercial vaccines and we are open to assist during the transfer of the technology and during the development phase.

We can offer

- a challenge model for *Salmonella* (broilers and layers ; *Salmonella* Enteritidis, Typhimurium and Gallinarum)
- diagnostics for monitoring *Salmonella* on farm and in infected animals (isolation, quantification)
- diagnostics to evaluate immune responses to vaccination/infection

References

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- Desin TS, Koster W, Potter AA. *Salmonella* vaccines in poultry: past, present and future. Expert Rev Vaccines 2013;12:87-96.
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