

Development of a third generation vaccine to prevent *Salmonella* infections in commercial poultry flocks

**Nathalie Desloges, Ilka Schröder and Sarah Schwefer
Lohmann Animal Health, Cuxhaven**

Introduction

Even after more than a decade of combating *Salmonella* infections, this organism still represents an important cause of human disease (EFSA, 2009, Newell *et al.*, 2010). Recent studies estimate 80.3 million annual cases of food-borne disease related to *Salmonella* worldwide (Majowicz *et al.*, 2010). Within the European Union *Salmonella* is the second most important cause of food-borne infections (EFSA, 2007) with *Salmonella* Enteritidis still representing the most commonly isolated *Salmonella* serovar in human salmonellosis (EFSA, 2007; WHO, 2006). Even today contaminated eggs from infected layers remain the major source of *Salmonella* Enteritidis infection (Delmas *et al.*, 2006; EFSA, 2007; Korsgaard *et al.*, 2009; Stevens *et al.*, 2009). As a consequence all member states of the European Union have to implement Regulation EC No. 2160 from 2003 on a national basis in order to control *Salmonella* and other zoonotic agents of significance for public health in farm animals. The prevention of *Salmonella* infections in laying hens and the control of the pathogen at farm level is the key to producing safe egg products and to being in line with Regulation EC No. 2160/2003. As described recently by Carrique-Mas and Davies (2008) the member states of the EU have to invest more in the prevention, detection and control of *Salmonella* infections in laying hens.

Vaccination plays an important role in the overall biosecurity system on chicken farms to prevent *Salmonella* infections (Temelli *et al.*, 2010). When vaccination first arose as a method of combating this organism, inactivated vaccines were developed by various companies. Due to many reasons, such as ease of application, animal welfare, and especially efficacy, attenuated live vaccines entered the market with great success a short time later. These live, attenuated vaccines were homologous vaccines against either *Salmonella* Enteritidis or *Salmonella* Typhimurium. Scientific evidence shows that serovar overlapping effects exist, but homologous vaccines offer the best protection against infection (Springer *et al.*, 2000; Martin *et al.*, 1996; Chacana *et al.*, 2006).

In this paper results on the safety and efficacy of a new combined homologous vaccine against *Salmonella* Enteritidis and *Salmonella* Typhimurium (Lohmann testing vaccine) are discussed.

Safety of the vaccine

The Lohmann testing vaccine is safe for day-old Specific Pathogen Free (SPF) chicks, the most susceptible chickens for infection with *Salmonella*, when administered with a single, repeated or 10-fold dose. The dissemination of the vaccine strains is limited and the strains do not persist in internal organs for a long period of time. The vaccine is also not transmitted on or into the eggs in vaccinated birds.

Safety trials with day-old Peking ducklings showed that oral application of the vaccine is completely safe to use. The Lohmann testing vaccine also represents no health hazard to turkeys or any other tested species (Lohmann Animal Health, data on file).

Oral application via drinking water of the Lohmann testing vaccine to 25.000 day-old commercial layers under field conditions was proven to be safe. Repeated vaccination on day one, week 6 and week 16 of life was observed under field conditions and no safety issues caused by the vaccination were observed (Table 1).

Table 1: Safety of repeated oral application of the Lohmann testing vaccine under field conditions in two flocks, each with 12.500 commercial layers (Hy-Line Brown) (Lohmann Animal Health, data on file)

Mortality rate	not affected
Dissemination into organs (liver, bile, caecum, tonsils, ovary)	no positive findings
Disseminations to eggs	no positive findings
Spread to humans	no positive findings
Egg production	not affected
Spread to environmental samples including birds and other animals	no positive findings

Table 2: Vaccination scheme

Group	1 st day of life	6 th week of life	16 th week of life
A	min. dose of Lohmann testing vaccine, orally	min. dose of Lohmann testing vaccine, orally	min. dose of Lohmann testing vaccine, orally
B	AviPro® Salmonella Vac E, orally	AviPro® Salmonella Vac E, orally	AviPro® Salmonella Vac E, orally
C	AviPro® Salmonella Vac T, orally	AviPro® Salmonella Vac T, orally	AviPro® Salmonella Vac T, orally
Unvaccinated control	-	-	-

Efficacy and duration of immunity

An optimum vaccine should protect against *Salmonella* infection throughout the laying period. In order to test efficacy of the immune response at the beginning and at the end of the laying period, SPF (Lohmann Selected Leghorn) birds were vaccinated orally either with a minimum dose of the Lohmann testing vaccine, AviPro® Salmonella Vac E or AviPro® Salmonella Vac T on the first day of life, in week 6 and in week 16 (Table 2). Vaccinated birds were kept throughout the laying period.

At the beginning of production (week 21 or 22 of life) birds from each group were challenged orally with either 2×10^9 cfu of *Salmonella* Enteritidis NaI^{res} or 3×10^9 cfu of *Salmonella* Typhimurium K284/93 NaI^{res} per bird. Seven days post challenge the caeca and liver of all birds were investigated bacteriologically for the presence of the challenge strain. The liver and caeca of the chickens vaccinated with either the monovalent vaccines (AviPro® Salmonella Vac E (Figure 1) or AviPro® Salmonella Vac T (Figure 2)) or Lohmann Testing Vaccine showed a reduction in their colonisation by the *Salmonella* field strains as compared to unvaccinated chickens.

At an age of 68 weeks 10 birds were challenged with 2×10^9 cfu of *Salmonella* Enteritidis NaI^{res} per bird and analysed in comparison to an unvaccinated, challenged control group of the same hatch that was kept under the same conditions. Seven days post challenge the caeca and liver of all birds were examined bacteriologically for the presence of the challenge strain. The unvaccinated control birds had diarrhoea starting 4 days post challenge. The vaccinated birds showed no clinical signs. Bacteriological analysis revealed that the titre of the virulent strain *Salmonella* Enteritidis NaI^{res} was significantly reduced in the liver and caeca of vaccinated birds as compared to organs of unvaccinated control birds (Figure 3). Persistence of the challenge strain in the liver was completely inhibited in the vaccinated birds.

Figure 1: Persistence of the challenge strain *Salmonella* Enteritidis NaI^{res} in internal organs 7 days post infection in week 21

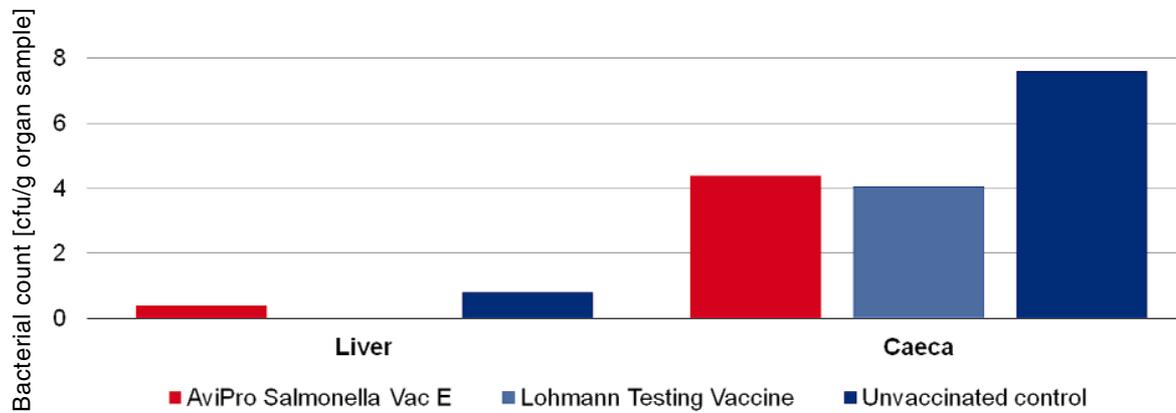


Figure 2: Persistence of the challenge strain *Salmonella* Typhimurium K284/93 NaI^{res} in internal organs 7 days post infection in week 22

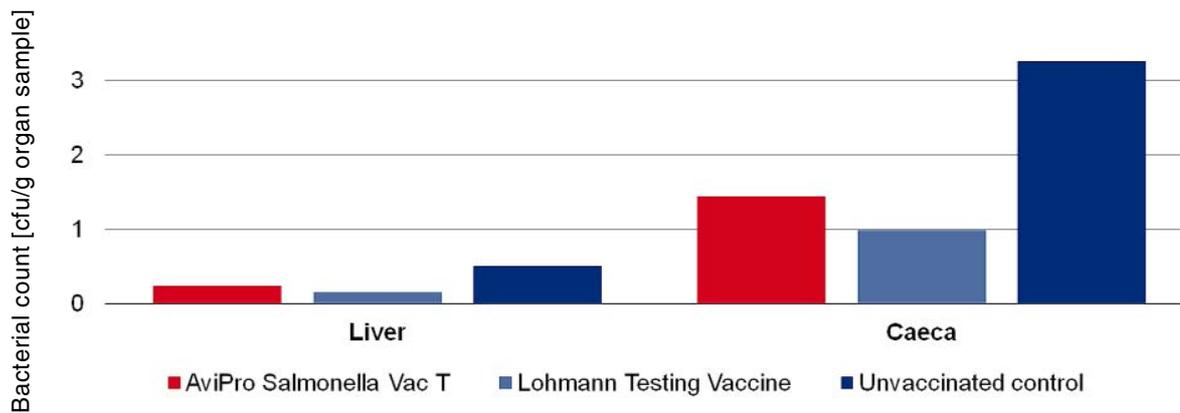


Figure 3: Persistence of the challenge strain *Salmonella* Enteritidis NaI^{res} in internal organs 7 days post infection in week 68

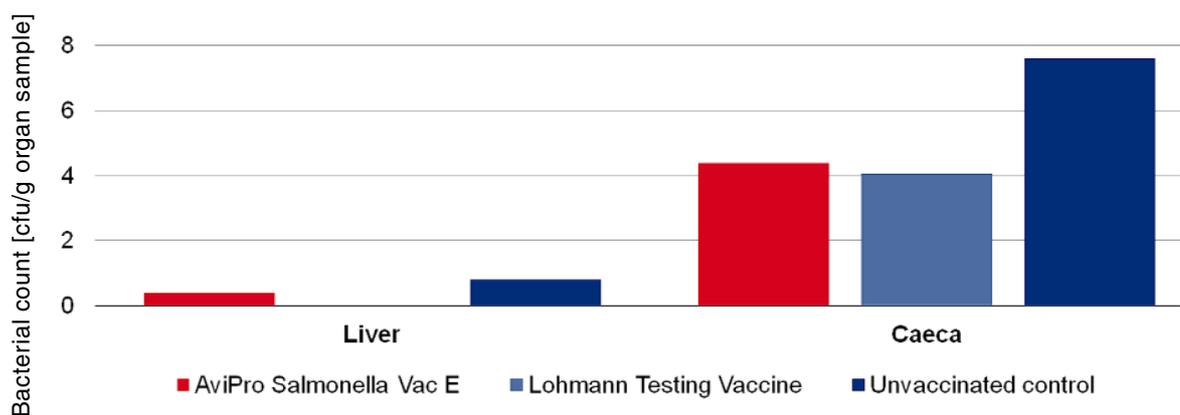
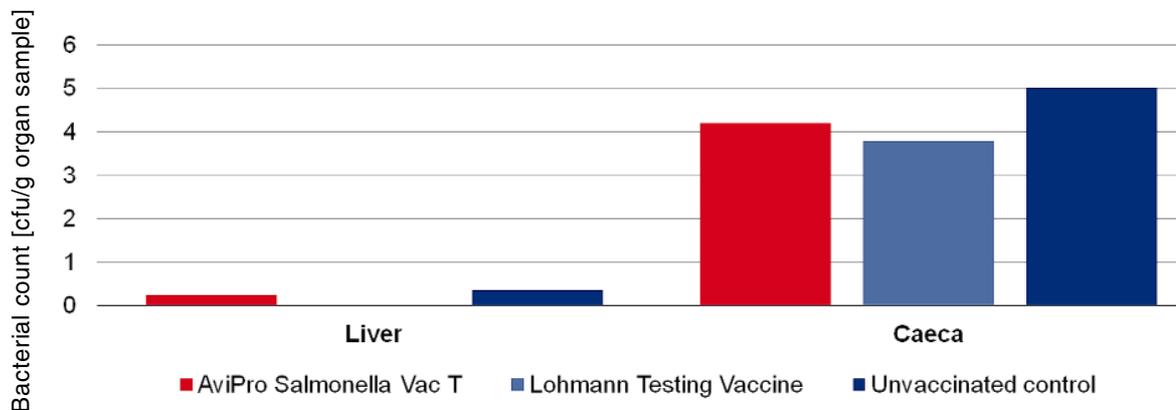


Figure 4: Persistence of the challenge strain *Salmonella* Typhimurium K284/93 NaI^{res} in internal organs 7 days post infection in week 62



In order to determine the protection against infection with *Salmonella* Typhimurium 10 birds were challenged with 3×10^9 cfu of *Salmonella* Typhimurium K284/93 NaI^{res} per bird and compared with birds from the unvaccinated, challenged control group. Seven days post challenge infection the liver and caeca of the vaccinated birds contained significantly less of the challenge strain than the unvaccinated control birds (Figure 4).

Diagnostics

According to the European regulations (EC No. 213/2009 and EC No. 1168/2006) samples taken from layer and breeder flocks are supposed to be tested for the presence of *Salmonella* by following EN ISO 6579:2002. This method includes the use of modified semi-solid Rappaport-Vassiladis medium (MSRV) as selective growth medium. Since growth of both *Salmonella* strains of the Lohmann testing vaccine is rather poor on this medium, this method cannot be recommended for the determination of the presence of the vaccine strains. Both strains can be detected as described previously in the literature (Schröder *et al.*, 2004). In addition the AviPro® Plate, a susceptibility microdilution test, allows for a standardized test to detect and differentiate between the vaccine strain and *Salmonella* field strains.

Conclusion

The Lohmann testing vaccine was proven to be safe and efficacious after challenge with high doses of *Salmonella* Enteritidis as well as *Salmonella* Typhimurium, the most common *Salmonella* serovars in poultry. Therefore, this new vaccine represents a new potent tool in the prevention of *Salmonella* infections in poultry flocks and contributes highly towards establishing consumer confidence in safe poultry products. The novel and unique combination of two live *Salmonella* strains in one vaccine add to the development of user-friendly products in *Salmonella* prevention at farm level. In general, vaccination alone cannot keep flocks free of *Salmonella*. Only a combination of high standards in biosecurity and hygiene as well as proper vaccination with homologous vaccines can protect poultry flocks against infection with *Salmonella* Enteritidis and *Salmonella* Typhimurium.

Summary

Salmonella is one of the major sources of food-borne disease in humans. Contaminated eggs still cause high numbers of cases of human salmonellosis. Vaccination of poultry flocks against *Salmonella* Enteritidis and *Salmonella* Typhimurium is an important part of the biosecurity and hygiene programs to prevent infection in the first place. The new combined homologous vaccine tested in this study was proven to be safe and efficacious after challenge infection with virulent strains of both serovars.

Zusammenfassung

Salmonellen sind eine der Hauptursachen von Lebensmittel-bedingten Erkrankungen beim Menschen. Noch immer sind kontaminierte Eier die Ursache für eine Vielzahl von Salmonellosen. Die Impfung von Geflügelbeständen gegen *Salmonella* Enteritidis und *Salmonella* Typhimurium ist ein wichtiger Bestandteil der Hygiene- und Biosicherheitsmaßnahmen, um eine Infektion in den Beständen zu vermeiden. Der im Rahmen dieser Untersuchungen getestete homologe Kombinationsimpfstoff war absolut unbedenklich und wirksam nach einer Belastungsinfektion mit virulenten Stämmen beider Serovare.

References

- Carrique-Mas, J. J. and R. H. Davies (2008): *Salmonella* Enteritidis in commercial layer flocks in Europe: Legislative background, on-farm sampling and main challenges. *Braz. J. Poult. Sci.* 10:1-9.
- Chacana, P. A. and H. R. Terzolo (2006): Protection Conferred by a Live *Salmonella* Enteritidis Vaccine Against Fowl Typhoid in Laying Hens. *Avian Diseases* 50:280–283.
- EFSA (2007): Report of the task force on zoonoses data collection on the analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying flocks of *Gallus gallus*. *EFSA J.* 97:1-84.
- EFSA (2009): The Community Summary Report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007. *EFSA J.* 223:1-320.
- Delmas, G., A. Gallay, E. Espié, S. Haeghebaert, N. Pihier, F. X. Weill, H. De Valk, V. Vaillant, and J. C. Désenclos. (2006): Food-borne disease outbreaks in France between 1996 and 2005. *B. E. H.* 51/52:418-422.
- Korsgaard H., M. Madsen, N. C. Feld, J. Mygind, and T. Hald. (2009): The effects, costs and benefits of *Salmonella* control in the Danish table-egg sector. *Epidemiol. Infect.* 137:828-836.
- Linde K., I. Hahn, and E. Vielitz (1996): Entwicklung von optimal an das Huhn attenuierten *Salmonella*-Lebendimpfstoffen. *Tierärztl. Umschau* 51:23-31.
- Majowicz, S. E., J. Musto, E. Scallan, F. J. Angulo, M. Kirk, S. J. O'Brien, T. F. Jones, A. Fazil, and R. M. Hoekstra. (2010): The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin. Infect. Dis.* 50:882-889.
- Martin G., I. Hänel, R. Helmuth, A. Schroeter, W. Erler, and H. Meyer (1996): Investigations about the immunisation with potential *Salmonella* Enteritidis mutants – 1. production and characterization in vitro. *Berl. Münch. Tierärztl. Wschr.* 109:325-329.
- Newell, D. G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, H. Sprong, M. Opsteegh, M. Langelaar, J. Threlfall, F. Scheutz, J. V. van der Giessen, and H. Kruse. (2010): Food-borne diseases – The challenges of 20 years ago still persist while new ones continue to emerge. *Int. J. Food Microbiol.* 139(Suppl. 1): 3-15.
- Schröder I., M. Iburg, and D. E. Rebeski (2004): Anwendung von TAD *Salmonella* vac® T und TAD *Salmonella* vac® E in der Geflügelpraxis unter besonderer Berücksichtigung der Impfstoffapplikation und -kontrolle. *Lohmann Information* 4/2004:1-4.
- Springer S., J. Lehmann, Th. Lindner, J. Thielebeln, G. Alber, and H.-J. Selbitz (2000): A new live *Salmonella* Enteritidis vaccine for chicken – experimental evidence of its safety and efficacy. *Berl. Münch. Tierärztl. Wschr.* 113:246-252.
- Stevens M. P., T. J. Humphrey, and D. J. Maskell (2009): Molecular insights into farm animal and zoonotic *Salmonella* infections. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364:2709-2723.
- Temelli, S., S. Kahya, A. Eyigor, and K. T. Carli (2010): Incidence of *Salmonella* Enteritidis in chicken layer flocks in Turkey: Results by real-time polymerase chain reaction and International Organization for Standardization culture methods. *Poult. Sci.* 89:1406-1410.
- WHO. (2006): Progress Report (2000-2005): Building capacity for laboratory-based foodborne disease surveillance and outbreak detection and response/WHO Global Salm-Surv. <http://www.who.int/gfn/links/GSSProgressReport2005.pdf>

Corresponding author:

Dr. Sarah Schwefer
Lohmann Animal Health GmbH & Co. KG
Heinz-Lohmann-Str. 4, 27472 Cuxhaven, Germany
sarah.schwefer@lohmann.de