Investigation of the efficacy of a genetically-stabile live *Salmonella typhimurium* vaccine for use in swine

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Abstract: Hybrid swine were immunized twice at an interval of 3 weeks to evaluate the efficacy of a live *S. typhimurium* vaccine. The animals and a control group were challenged at the age of 8-10 weeks by oral test infection with a labelled *S. typhimurium* DT 104 strain. An ELISA was used to establish the presence of antibodies to *S. typhimurium* in serum samples. The presence of the challenge strain in the ileal and caecal mucosa and in the ileocolic lymph nodes was investigated quantitatively using the Koch plating method. The vaccinated animals had significantly higher antibody titres after the second vaccination than the unvaccinated animals. The vaccinated animals had a significantly lower ($p < 0.05$) colonization of the ileal and caecal mucosa as well as the ileocolic lymph nodes than the unvaccinated animals.

Key words: *Salmonella typhimurium*, live vaccine, efficacy, oral test infection

Introduction: Live vaccines are particularly important as salmonella are facultative intracellular parasites and the use of such vaccines can result in optimal induction of cell-mediated defence mechanisms. The aim of the study presented here was to investigate the efficacy of a *S. typhimurium* live vaccine using an infection model which primarily results in sub-clinical infection.

Materials and Methods: Hybrid swine (Landrace x Pietrain) aged 3-4 weeks were immunized twice using the *S. typhimurium* live vaccine SALMOPORC®. All animals were derived from an experimental animal population monitored for salmonella. The animals were kept on tenderfoot flooring and were given a complete commercial feed. All pigs were challenged with a *S. typhimurium* DT 104 strain (No. 958/96) which was nalidixic acid-resistant. The vaccination and challenge parameters are presented in Table 1. Following challenge of the animals their general well-being, feeding habits, incidence of diarrhoea and vomiting were monitored, and the rectal body temperature measured. Seroconversion was investigated using an ELISA established for use with meat juices in Denmark (Nielsen, B. et al., 1998, Steinbach, G. et al., 2000). The almost normally-

distributed logarithms of extinction values were used for statistical analysis of the data (2-factorial variance analysis, Statgraphics Inc., Rockville, Maryland, USA).

Tab. 1: No. of animals, times of vaccination and challenge with doses and time of quantitative investigation for the individual studies

Exper-iment	Group	No. of animals	Vaccination Dose in cfu/animal	Challenge (cfu/animal)	Quantitative investigation
1	Vaccinated	6*	Days 0/21 ($5x10^8$ each)	Days 34/35 ($5x10^{10}$)	Day 41
	Control	6*	-		
2	Vaccinated	6	Days 0/21 ($5x10^8$ each)	Days 41/42 ($5x10^{10}$)	Day 51
	Control	6	-		
3	Vaccinated	6*	Days 0 ($5x10^8$ oral)/22 ($2x10^7$ i.m.)	Days 41/42 ($5x10^{10}$)	Day 49
	Control	6*	-		

* Challenge infection in only 4 animals

After slaughtering of the animals the number of challenge strain organisms per g of ileal and caecal mucosa and per g of ileocolic lymph nodes was determined using the Koch plating method, modified similarly to the method of Methner et al. (1995). Statistical evaluation of the differences between the vaccinated and unvaccinated groups was carried out using the Mann-Whitney U test (one-tailed) using SPSS 7.5.2. for Windows.

Results : The results of quantitative investigation of the challenge strain in the ileal and caecal mucosa and the ileocolic lymph nodes of the vaccinated groups and unvaccinated control groups are presented in Table 2. The results for the investigation of sera for the presence of antibodies before and after vaccination / challenge are presented for all experiments in Table 3. Analysis of variance for all 3 experiments showed that the vaccinated animals had a significant increase in antibody titre after the second immunization before experimental challenge ($\alpha < 0.01$; $\alpha < 0.01$; $\alpha < 0.05$). The challenge resulted in a further significant rise in antibody titre in all 3 experiments both for the immunized and the control animals ($\alpha < 0.001$; $\alpha < 0.001$).

Discussion: The use of the *S. typhimurium* live vaccine resulted in a significantly lower colonization of the ileal and caecal mucosa by the challenge strain after both oral/oral and oral/parenteral administration. Significant differences in the colonization of the ileocolic lymph nodes between the vaccinated group and the control group were only evident from day 10 post challenge.

Tab. 2: Results of quantitative determination of the challenge strain per g ileal/caecal mucosa and per g ileocolic lymph node

Experiment	Group	Challenge strain content ($\bar{x}$ ± SD) in log cfu/g		
		Ileum	Caecum	Ileocolic lymphn.
1	Vaccinated	1.78 ± 1.20*	1.50 ± 0.60*	2.50 ± 0.93
	Control	4.28 ± 0.09	4.16 ± 0.14	2.80 ± 0.74
2	Vaccinated	2.41 ± 0.63*	1.95 ± 0.33*	2.19 ± 0.32*
	Control	3.54 ± 1.56	2.89 ± 1.34	2.62 ± 0.57
3	Vaccinated	2.15 ± 0.80*	2.10 ± 0.56*	2.75 ± 0.41
	Control	3.63 ± 0.44	3.54 ± 0.63	2.90 ± 0.40

* = $p < 0.05$ (Mann-Whitney U Test, one-tailed)

Tab. 3: Results of antibody determination ($\bar{x}$ ± SD), expressed as the logarithm of the extinction value and as antibody percent after vaccination and challenge for the vaccinated and control groups

Exper-iment	Time (Day)	n	$\bar{x}$ ± SD as log of extinction value		$\bar{x}$ ± SD in antibody percent	
			Vaccinated	Control	Vaccinated	Control
1	- 7	6	-1.71 0.14	-1.86 0.40	0.64 ± 0.17	0.60 ± 0.51
	21	6	-0.76 0.64	-1.60 0.21	11.02 ± 10.06	0.87 ± 0.41
	34	6	-0.62 0.54	-1.44 0.19	11.70 ± 9.38	1.25 ± 0.56
	41	4	0.02 0.24	0.06 0.20	37.30 ± 21.35	38.90 ± 14.70
2	0	6	-1.43 0.13	-1.53 0.16	2.40 ± 0.76	1.88 ± 0.73
	21	6	-1.22 0.26	-1.65 0.06	4.26 ± 2.18	1.32 ± 0.23
	41	6	-1.00 0.28	-1.62 0.15	7.41 ± 4.96	1.50 ± 0.53
	51	6	0.02 0.27	0.01 0.13	74.20 ± 38.67	63.68 ± 17.00
3	0	6	-0.77 0.15	-1.00 0.18	5.37 ± 1.80	3.12 ± 1.11
	21	6	-0.79 0.24	-1.15 0.10	5.49 ± 3.13	2.18 ± 0.54
	41	6	-0.23 0.38	-0.99 0.11	23.88 ± 20.40	3.10 ± 0.73
	49	4	0.10 0.29	-0.01 0.11	49.74 ± 20.85	29.64 ± 7.02

This may be attributable to a relatively late activation of cell-mediated immune mechanisms, as has been demonstrated for Balb/c mices after infection with *S. enteritidis* (Lehmann et al., 1999). The vaccinated animals in all 3 experiments had significantly higher antibody titres after the second vaccination than the unvaccinated animals. Despite the demonstrable immunological reaction of the animals to oral vaccination, no adverse effect on the serological diagnostics used in the course of eradication measures is to be expected from the vaccination procedure. In summary it can be said that vaccination twice by the oral route or the

oral/parenteral route with the *S. typhimurium* live vaccine results in a reduction in the level of shedding and persistence of the pathogen which is epidemiologically-relevant.

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