A contribution to consumer protection: TAD Salmonella vac® E - A new live vaccine for chickens against Salmonella Enteritidis

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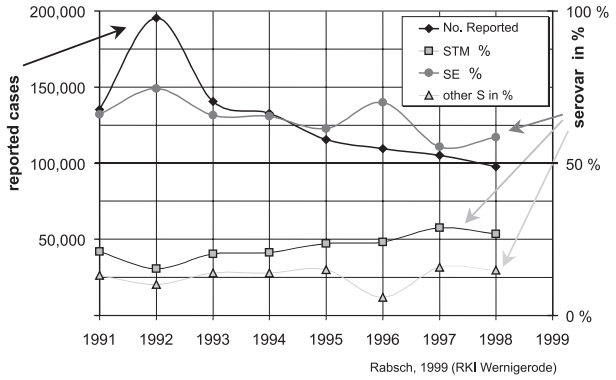
While the incidence of salmonellosis in humans has been declining in Germany, with recorded cases falling below 100,000 for the first time in 1998, Salmonella Enteritidis (S.E.) is the most commonly isolated serovar, accounting for 58 % of the total (Rabsch, 1999).

Figure 1: Incidence of Salmonella isolates in humans in Germany



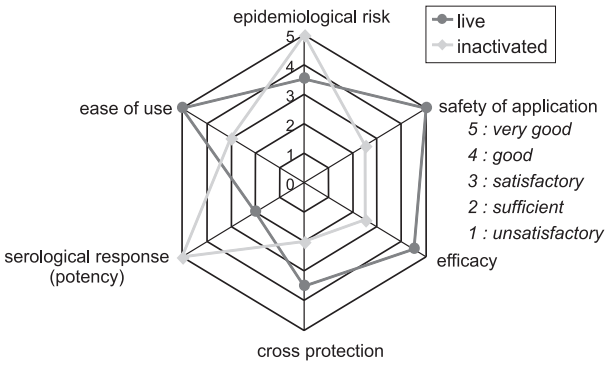
Human infections are associated with the consumption of dishes containing raw eggs which have either been improperly prepared or stored for too long or under inadequate conditions.

Paragraph 2 of the German Chicken Salmonella Order of 11 April 1994 (BGBl. I, p. 770) stipulates that all pullets in rearing establishments of more than 250 birds must be vaccinated against salmonella.

Live versus inactivated vaccines

The user has a choice of live or inactivated vaccines for salmonella vaccinations. The graph below shows a comparison of the two types of vaccines based on criteria of practical use.

Figure 2: Comparison plot: live and inactivated SE-vaccines



An inactivated vaccine, which contains killed antigen and an immune response-enhancing adjuvant, poses a lower epidemiological risk than live vaccines and has the potential for inducing high humoral antibody titres. But the latter are only partially correlated with efficacy in salmonella infections. Because of the intracellular parasitism of the causal agent effective vaccines must induce a sustained stimulation of cell-mediated immune reactions, which is best achieved with live vaccines (Selbitz et al., 1995).

This claim was proved in an efficacy trial where several inactivated vaccines were tested against a live vaccine.

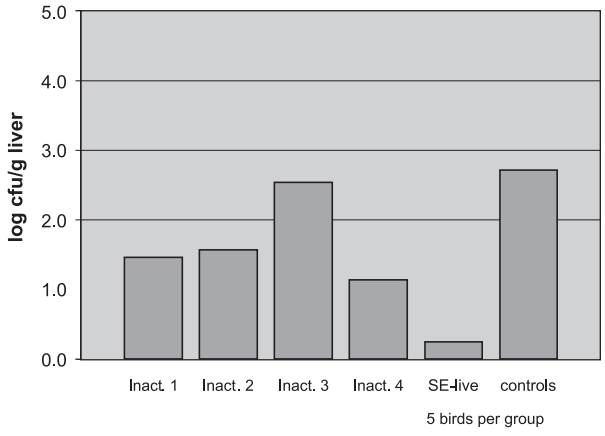
The results demonstrate that only the live vaccine prevents or reduces organ manifestations and excretion of the challenge strain when compared with unvaccinated controls (cf. table 1, fig. 3, 4, 5).

Table 1: Comparative efficacy of live and inactivated SE-vaccines

group	SE-vacczine	type	dose	application
1	inact. 1	commercial	0.1 ml	i.m.
2	inact. 2	commercial	0.1 ml	s.c.
3	inact. 3	experimental	0.1 ml	s.c.
4	inact. 4	autogenous	0.1 ml	s.c.
5	LAH first live	commercial	1 x 10^8 cfu	orally
controls	-	-	-	-

* SE Nal^{res} (K285/94) courtesy of Dr. U. Methner, Federal Institute for Consumer's Health Protection and Vet. Med.

Figure 3: Persistence of challenge strain in the liver 5 days post infection



A further advantage of live vaccines is the facility for administration via the drinking water, which involves less stress for the birds and less work for the vaccinating personnel. Moreover, the oral route of administration simulates the natural port of infection.

Figure 4: Excretion of the Salmonella challenge strain in cloacal swabs

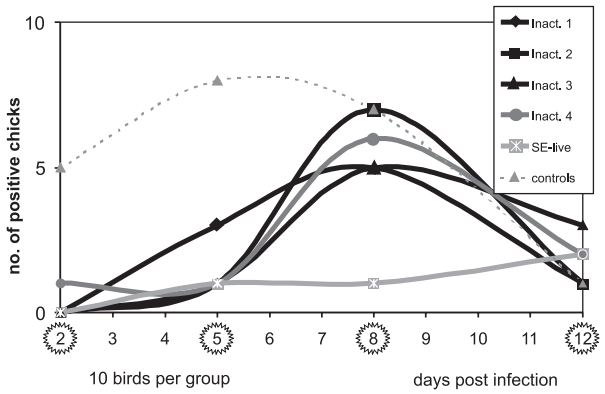
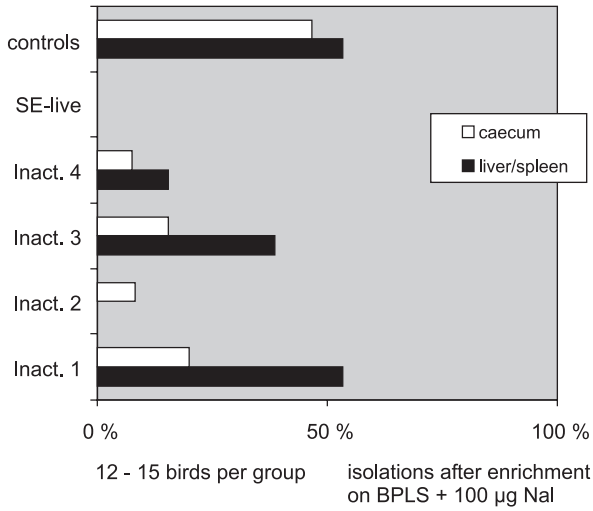


Figure 5: Persistence of challenge strain in organs 2 weeks post infection



The persistence of the vaccinal strain in the body induces several defensive mechanisms, thereby enabling live vaccines to promote cross-immunities. An S. typhimurium (S.Tm.) live vaccine from the USA for example has been reported to confer protection not only against S.tm. but also against S. Heidelberg and S.E. (Hassan and Curtiss III, 1994). Experiments of our own with TAD Salmonella vac® E (S.Tm. live vaccine) also demonstrate cross-protection against S.E., although this declines with increasing age of the vaccinated subjects and is less than the homologous protection.

TAD Salmonella vac® E

The basis of any live salmonella vaccine is a bacterial strain which combines extensive loss of virulence with good immunogenicity while maintaining viability.

Metabolic drift mutation

TAD Salmonella vac® was developed on the basis of the principle of metabolic drift mutation described by Linde et al. (1993). These are negative mutations in essential enzymes and metabolic control centres of the bacterium as a consequence of which the resulting metabolic processes lead to prolonged generation times and corresponding reductions in virulence. In the concrete case of TAD Salmonella vac® E the generation time increases from 22 min. for the initial strain to 28 min. for the vaccinal strain.
The fact that metabolic compartments are known to be sites of action for antibiotics can be exploited for therapeutic purposes. Structural changes due to mutation result not only in attenuation but have the concomitant side-effect of inducing antibiotic resistance associated with the loss of binding sites for antibiotics, a therapeutically safe phenomenon that is only used for vaccinal strain identification (Linde et al., 1996).

As well as metabolic drift mutation the vaccinal strain is also characterised by cell membrane mutation, which enhances its permeability for erythromycin and other antibiotics and noxious substances.

Diagnosis

The differentiation of vaccinal strains from wild strains is very easy by means of the built-in markers. Due to its metabolic drift markers (resistance markers) the vaccinal strain grows on media with rifampicin and streptomycin while exhibiting no growth on media containing erythromycin. The genetically unmodified wild strains behave in exactly the opposite way.

Table 2: Differentiation between TAD Salmonella vac® E and field strains

Antibiotics	Concentration	Vaccine strain*	Field strains
Streptomycin	100µg/ml	resistant	sensitive
Rifampicin	100µg/ml	resistant	sensitive
Erythromycin	15µg/ml	sensitive	resistant

* SE Nal^{res} Sm24 / Rif12 / SSq

It should be noted that due to the cell membrane mutation the vaccinal strain does not grow on all selective culture media commonly used in salmonella diagnosis. But no growth inhibition occurs in conventional culture media used in veterinary medicine such as BPLS, Gaßner and XLD agar.

Genetic stability

As a result of three independent mutations TAD Salmonella vac® E possesses complete stability, with statistical unbiasedness, against the risk of reversion to the wild strain under field conditions. As the total stability of all three markers can be calculated as the product of the stabilities of the individual markers (minimum 10^{-8}, Kaplan, 1969), the former is therefore 10^{-24}.

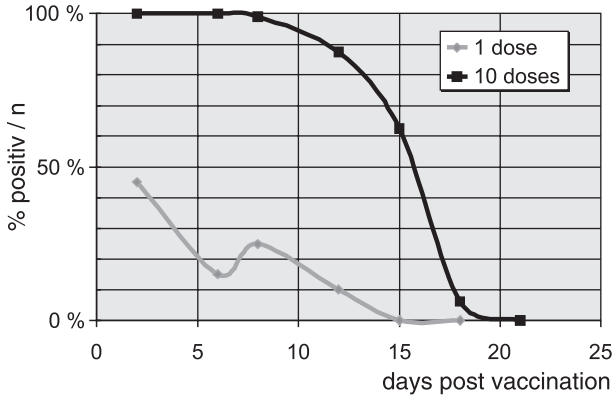
In order to deliver the key benefits of a live vaccine the vaccinal strain must propagate in the body of the vaccinated animal for a certain time. This phenomenon, which is occasionally and somewhat misleadingly referred to as residual virulence, is closely correlated with immunity. The correct ratio of these two characteristics determines not only the efficacy but also the safety of a vaccine.

The duration of excretion of the vaccinal strain was

studied by bacteriological tests of cloacal swabs. The results were dose-dependent: two weeks post immunisation the vaccinal strain was only demonstrable in animals vaccinated with 10 times the immunising dose. After 21 days the excretion in this group had also fallen to 0 %.

Another study was concerned with the question whether

Figure 6: Shedding of vaccine strain via cloacal swabs



Animals	SPF day-old chicks
Vaccination	day 1, orally, one dose or 10 doses

the vaccinal strain is transmitted to the egg. 40 SPF hens already in lay were orally immunised with the recommended dose or a 10-fold overdose of TAD Salmonella vac® E.

641 eggs were bacteriologically tested for a period of three weeks post immunisation (separate enrichment of egg shell, egg white and yolk). All samples were negative for the vaccinal strain. The prescribed withdrawal period between immunisation and slaughter or sale of the eggs is 21 days including a safety margin.

Safety

In field trials with 795,000 chickens TAD Salmonella vac® E administered orally via the drinking water proved to be entirely safe. In addition, each batch is tested at 10 times the recommended dose in day-old chicks, the most sensitive species for salmonella.

In view of the possibility that TAD Salmonella vac® E might be accidentally ingested by other species, tolerance studies were conducted in ducks, turkeys, calves and pigs. The vaccine caused no ill effects in these animals.

Efficacy and duration of immunity

As the vaccination is supposed to protect chickens against invasion by S.E. strains throughout the entire laying period the duration of immunity was studied in the following experiment (cf. table 3, fig. 7, 8, 9).

The results demonstrate the extent to which the vaccinated birds were protected against experimental S.E. challenge on completion of the laying period (60 weeks of age): persistence of the challenge strain in the liver was largely prevented and excretion was reduced by about two powers of ten compared with the control birds.

Table 3: Efficacy and Duration of Immunity Studies with TAD Salmonella vac® E

Vaccination schedule:

group	day 1	week 6	week 16
1	SE-live orally	SE-live orally	SE-live s.c.
2	SE-live orally	SE-live orally	SE-inact. i.m.
controls	-	-	-

Animals: day-old broiler breeders, 18 per group
Challenge: at 60 weeks (= 44 weeks post 3rd vaccination) orally SE, 1 x 10^{10} cfu*

* SE Nal^{res} (K285/94) courtesy of Dr. U. Methner, Federal Institute for Consumer's Health Protection and Vet. Med.

Figure 7: Persistence of challenge strain in the liver 5 days post infection

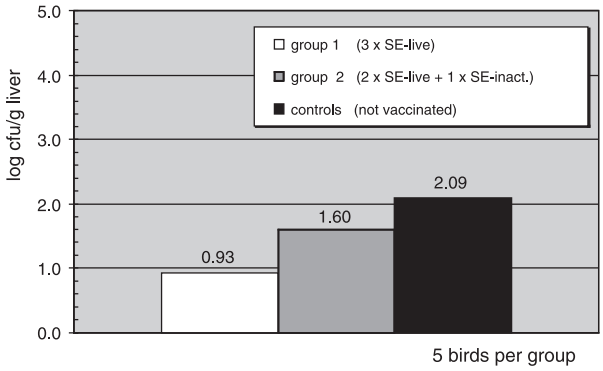
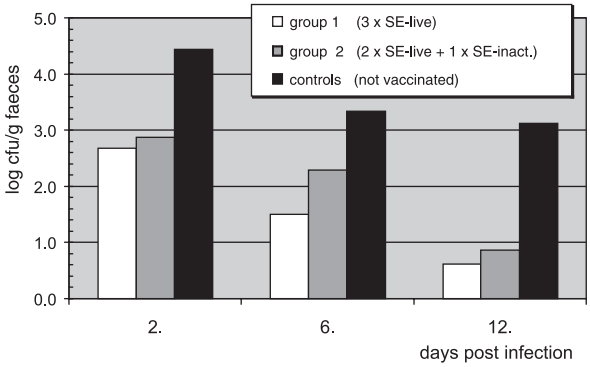


Figure 8: Shedding of challenge strain via faeces

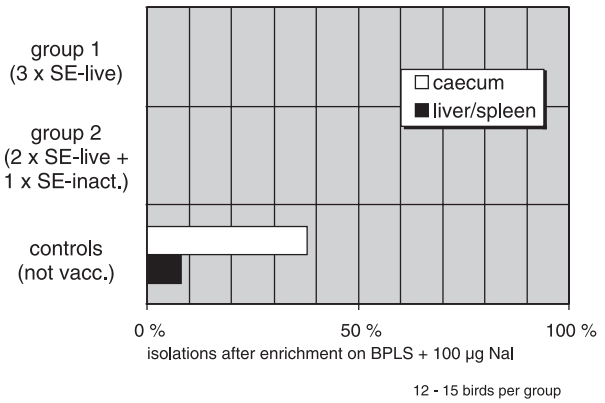


According to Dorn this meets a key requirement for the effectiveness of salmonella control measures. Reduced persistence and bacterial shedding in an immunised flock ultimately leads to disruption of the infectious chains, thereby minimising the contamination of animal-derived foods with salmonella.

The widespread practice in poultry production, especially in replacement flocks, of combining live and inactivated vaccines is therefore also effective in salmonella vaccinations (Vielitz et al., 1995).

Vaccination programme

Figure 9: Persistence of challenge strain in organs 2 weeks post infection



Based on the trials carried out the recommended vaccination schedule shown below has been elaborated. Vaccination at the earliest possible time after hatching is of paramount importance to prevent the establishment of field strains.

Table 4: Vaccination schedule - recommendations for vaccinations against S.E.

Age	Layers	Breeders
Day-old	TAD Salmonella vac® E oral	TAD Salmonella vac® E oral
6-8 weeks	TAD Salmonella vac® E oral	TAD Salmonella vac® E oral
	TAD Salmonella vac® E oral	TAD Salmonella vac® E
16-18 weeks	TAD Salmonella vac® E oral	Inactivated S.E. vaccine i.m.

Conclusions

TAD Salmonella vac® E proved to be genetically stable, safe and effective in experimental studies. Based on these results the Paul-Ehrlich Institute granted approval of the vaccine for the Federal Republic of Germany in July 1999.

This has provided the poultry industry with another important tool for salmonella control. Of particular interest is the homologous protection against S. Enteritidis, the predominant serovar in human infections.

It should be emphasised that successful vaccination is closely correlated with optimal husbandry conditions and the maintenance of high sanitary standards.

The overall salmonella burden of a chicken population can only be reduced by long-term, comprehensive vaccination of flocks, which will ultimately minimise contamination of foods of animal origin with salmonella. As well as safeguarding animal health, TAD Salmonella vac® E thus makes a useful contribution to consumer protection.

References

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