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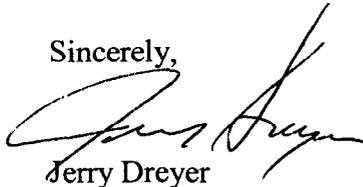
December 9, 2004

Division of Dockets Management
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Please find enclosed comments to Food and Drug Administration, 21 CFR Parts 16 and 118, Docket Nos. 1996P-0418, 1997P-0197, 1998P-0203 and 2000N-0504, RIN 0910-AC14 Prevention of *Salmonella* Enteritidis in Shell Eggs During Production.

Thank you for your attention.

Sincerely,



Jerry Dreyer

00N-0504

C285

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FORT DODGE

December 9, 2004

In response to the FDA Proposed Rule: Prevention of Salmonella enteritidis in Shell Eggs During Production, we would like to specifically address item M., page 56845, Response to Comments Related to On-Farm Prevention Measures, Comment 5, regarding the use of vaccines. This comment is in response to the statement, "*Vaccines are expensive and labor intensive, we estimate that vaccines cost 13.5 cents per layer, including labor*".

From a practical egg production standpoint, these costs are overstated. An optimal Salmonella vaccination program consists of two live vaccines and one inactivated vaccine during the pullet grow-out.

Program A	Program B	
\$0.01 - \$.016	\$0.01 - \$.016	2 Doses Live Vaccine
\$0.001	\$0.001	Live Vaccine Administration Costs
\$0.028		A. Salmonella/Newcastle/Bronchitis Inactivated
	\$0.046	B. Salmonella Inactivated
	\$0.05	Inactivated Vaccine Administration Costs
\$.039 - \$.045	\$.107 - \$.113	Total Program

Live vaccines are administered via the drinking water or coarse spray with a negligible labor cost (less than .001 cent per layer). Inactivated Salmonella vaccines range from \$.028 to \$.046 per dose. The lower price of \$.028 is the incremental cost using a combination Salmonella, Newcastle and Infectious Bronchitis product compared to a standard Newcastle and Infectious Bronchitis product. The administration cost of the inactivated vaccine is approximately \$.05 cents per layer. However in Program A, this additional labor cost is eliminated when administering the combination vaccine with Newcastle and Infectious Bronchitis. If a producer were not using the standard inactivated Newcastle and Infectious Bronchitis vaccine, the highest cost of adding a Salmonella program would reach \$.113 per bird. Therefore incremental cost of a complete vaccination program can range from \$.039 to \$.113 cents per bird.

Using these costs and layer numbers (218 million) in the proposed rule, total industry cost of a complete vaccination program would range from \$8.5 to \$24.6 million. It should be noted that a significant percentage of the industry is already vaccinating to some degree.

Respectfully,

Tim Davis

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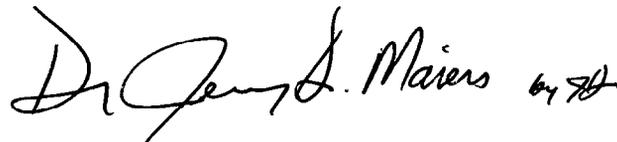
December 9, 2004

In response to the FDA Proposed Rule: Prevention of Salmonella enteritidis in Shell Eggs During Production, we would like to specifically address item M., page 56845, Response to Comments Related to On-Farm Prevention Measures, Comment 5, addressing the use of vaccines.

In response to the comment in the proposed rule that more information is needed regarding the effectiveness of vaccination, enclosed is an extensive literature review on the worldwide use of SE vaccines in commercial egg layers and meat type broiler breeders. Immunization of the laying hen has shown to significantly lower cecal shedding of SE, thus reducing both environmental and eggshell contamination. It has been recognized and stated in the proposed rule that the predominant route through which eggs become contaminated with SE is the "transovarian route". Studies have shown that immunization of the hen will reduce internal SE contamination of the egg as well as transfer protective SE antibodies into the egg yolk and albumen. The SE antibodies may provide an added safety factor by preventing growth of salmonella in the egg, should the egg be mishandled during processing and storage.

Fort Dodge Animal Health offers the following summary of vaccination studies, which includes a review of the scientific literature as well as data generated from our years of applied research and field trials. While we obviously have a vested interest in promoting vaccination as a crucial tool to help protect the nation's food supply, we are confident that the weight of the data supports our position.

Respectfully submitted,



Dr. Jerry D. Maiers
Sr. Technical Services Specialist

INTRODUCTION

The Food and Drug Administration (FDA) recently proposed rules for American egg producers aimed at reducing the risk of *Salmonella enterica* serotype Enteritidis (SE) infection during production of shell eggs (Ref.1). The FDA modeled its proposal on the provisions of existing voluntary egg QA programs run by states or industry organizations. One such program was the PA Egg QA Program (PEQAP). FDA noted that during the mid 1990's, this program had reduced the incidence of flocks with environmental samples positive for SE at some time during flock life from about 50 percent of flocks to about 15 percent of flocks over a period of 3 to 4 years. The FDA also noted that the human SE isolation rate in the marketing area for eggs produced under the PEQAP program had decreased during the same time. Because voluntary egg QA programs covered only about 50 percent of shell egg production, the FDA tentatively suggests that its proposal for mandatory Federal rules "would exclude SE on the farm and, thus, remove sources of SE contamination of shell eggs."

The FDA proposal includes five measures:

- 1) Chicks and pullets must originate from breeder flocks that meet NPIP's standards for "U.S. S. Enteritidis Monitored" status or equivalent;
- 2) Farms must have a biosecurity program;
- 3) Farms must have a pest and rodent control program;
- 4) Farms must have a cleaning and disinfection program for poultry houses that have had an environmental sample or egg test positive for SE; and
- 5) There must be refrigerated on-farm storage of shell eggs.

Measure 1 provides for SE-free chickens entering the farm. Measures 2, 3, and 4 involve maintaining a clean environment to avoid introducing SE onto the farm or eliminate it once it has been introduced. Measure 5 seeks to minimize multiplication of SE in eggs should they be infected. Conspicuous by their absence from the FDA proposal are any measures aimed at strengthening the ability of chickens to resist SE exposure from the farm environment. Such measures would allow chickens to create a potential barrier against multiplication of salmonella in a flock after exposure. Also, these measures would attempt to minimize the transmission of SE into shell eggs by infected chickens. These are obvious critical control measures which, even though the FDA's proposals are not a HACCP program, ought to be part of an effective, complete program with the goal of reducing or eliminating the threat to humans of SE in shell eggs.

The FDA received comments from the public as part of its process to formulate the SE control proposal. Section III.M. of the FDA proposal (Ref. 1) states "Many comments addressed the use of vaccines for laying hens as an intervention against SE contamination of eggs".

Clearly, vaccination is the most obvious means of addressing the above-mentioned critical control points omitted by the FDA's proposed control program. Several comments were noted in that "flocks in the PEQAP program that were vaccinated against SE had significantly fewer environmental samples positive for SE than nonvaccinated

flocks. In addition, no SE-positive eggs from a vaccinated flock were found during the 3-year study period.” Also, “A few comments stated that vaccinating flocks against SE would have the most significant impact on SE prevention of any possible intervention”. In its response, the FDA admitted, “We agree that vaccines show promise in reducing the prevalence of SE in laying hens. The PEQAP data indicated that the SE bacterin vaccines used in that program were 70 percent effective in reducing SE-positive environmental samples in flocks...In addition, field trials in ME showed that vaccination significantly reduced the mean fecal counts of vaccinated birds compared to nonvaccinated birds”. The FDA also wrote that, “Conversely, other comments stated that the data from the PEQAP study were inconclusive because too few flocks were included in the study”. However, the latter statement is not the converse of the previous, because it did not deny or negate the superior SE performance of the vaccinated flocks. Nevertheless, the FDA took the position that “more information on the effectiveness of vaccines needs to be generated before we would mandate vaccination as an SE prevention measure”.

Section V.E.1.h. of the FDA proposal (Ref. 1) examines the costs and benefits of vaccination of flocks against SE as a potential prevention measure. The FDA’s benefit analysis concludes “The evidence regarding the efficacy of vaccines in reducing SE in laying hens is mixed”. The FDA’s basis for reaching that conclusion commands further dialogue as it appears to be based on only two cited vaccination studies, Gast et al. (Ref. 2) and Davison et al. (Ref.3). The FDA writes that “Gast, et al. showed in an experimental setting that vaccines do partially reduce the shed of SE from laying hens”, and that “by contrast, Davison et al. used a field experiment to show that vaccines are relatively ineffective in stopping the spread of SE on farms”. This analysis also requires further dialogue as it omits key considerations such as what vaccines were used; the vaccination program used; and most importantly, definition of the goal of vaccination. Is the goal of vaccination to reduce shedding of SE from laying hens, to stop the spread of SE on a farm, to reduce SE shedding into eggs, or is it an entirely different goal? Conspicuously missing is any reference or consideration of the voluminous world literature that supports the proven benefits of SE vaccination.

The FDA determined the cost of vaccination at 13.5 cents per layer, but remained “uncertain” about the estimated benefits of vaccination, so was unable to neither expand upon a benefit analysis of vaccination nor draw any conclusions regarding the benefit of vaccination as a potential SE reduction measure. The absence of analysis was validated by stating “the need for more information on the effectiveness of vaccines”.

Four years before publishing the present proposed rule, the FDA held a Public Meeting on *Salmonella Enteritidis* Research, of which the proceedings (Ref. 4) are available. At that hearing the FDA was given a “heads up” on the importance of vaccination in SE control. At that meeting, Dr. Peter Holt of the ARS, USDA, reviewed his study of a live vaccine used in combination with molting. Dr. Holt said that live vaccination reduced the number of SE positive hens post-molt and that vaccinates that were SE infected shed SE at much lower numbers than did nonvaccinates (p. 7). In discussing SE vaccines in general, Dr. Holt stated that inactivated SE vaccines were shown to: reduce clinical signs

and pathology; reduce shedding and organ positivity, and reduced egg positivity. Dr. Holt further stated that inactivated vaccines reduced SE growth in egg contents; albeit that vaccination should be used in combination with good management practices to help eliminate SE problems in the flock. He went on to say that the PEQAP results showed reduction in positive environmental samples and in positive eggs from vaccination, and that in England use of SE bacterins was felt to play a “substantial role” in producing a “substantial drop” in SE cases in humans. Dr. Holt pointed out that he believed live SE vaccines “are a very important mechanism for helping to eliminate the SE problem” (pp. 10-11).

Dr. Charlie Beard, U.S. Poultry & Egg Association, another speaker at the meeting, said that many SE vaccine users relied on them quite heavily and that in some industry situations “vaccine is probably their only hope.” He called for independent evaluation of vaccines in field situations (p. 54).

Later in the same public meeting (Ref. 4) the FDA moderator called on the panel of speakers for their ideas on top research priorities. Vaccination was one of the ideas brought up (pp. 74-85). Dr. Beard stated “Some countries like Germany are already requiring immunization of layer flocks, so there must be some rationale for that.” Dr. Beard called again for third-party evaluation of SE vaccines. Discussion regarding laboratory versus field research on SE vaccination followed. Dr. Beard said that the criterion for evaluating SE vaccines should be rate of egg contamination, and that since many egg-producing companies were using vaccine, research in those field situations was an opportunity that should not be missed. Robert Brackett, FDA, who presided at the meeting specifically recognized from the panel of speakers that the vaccine development and intervention strategy was a top priority, and asked who should provide funding for that (p. 81). Dr. Beard, administrator of the grants program for the U.S. Poultry & Egg Association, pointed out that the industry already was funding a lot of SE research, including that of USDA researchers. Dr. Jean Guard-Petter, ARS, USDA, concurred, as did Dr. Gast, who said that SE vaccination will be one of the major intervention strategies.

VACCINATION AS A TOOL IN A SALMONELLA CONTROL PROGRAM

In table egg layers, different vaccination programs may be used. These programs may include vaccination with a killed bacterin, or vaccination with one or more applications of live vaccines, or a combination of both. There are differences in efficacy depending on the vaccination program.

Bacterins

A laboratory study (Ref. 5) evaluated an experimental inactivated SE PT4 bacterin, containing 10^{11} CFU/ml and 50% oil adjuvant, given subcutaneously to SPF chickens at 3 weeks of age or at 3 and 6 weeks of age, then challenged two weeks later with virulent SE PT4 at 10^9 (5 weeks) or 10^8 (8 weeks) CFU per dose given intramuscularly or intravenously. **It was concluded that the vaccinations protected the chickens against the massive challenges at either age.**

The Agricultural Research Service (ARS) of USDA reported a study (Ref. 6) using an acetone-killed oil-emulsion bacterin made from 10^{10} cells/dose of phage type 13a SE, a common egg-related isolate, to vaccinate SPF leghorn hens. Hens housed in individual cages were vaccinated, every other one, at 23 weeks of age in one experiment and at 45 weeks of age in another. The bacterin was administered again 6 weeks later in both trials. Three weeks following the second vaccination, vaccinates and nonvaccinated controls were challenged orally with approximately 10^9 cells of a highly invasive heterologous phage type 14b SE. Serum antibodies measured by a microagglutination test 2 weeks following initial vaccination reached 1:2389 in trial 1 and 1:2198 in trial 2. At 9 weeks post vaccination, antibody titers of vaccinates remained significantly higher than those of controls. **In both trials, SE was isolated from fewer internal organs (spleens, ovaries, and oviducts) and pools of egg contents from vaccinates than from controls. For all sampling dates in both trials, no SE was isolated from any ovary or oviduct samples from vaccinated hens, but SE was isolated from a significantly higher percentage of ovaries (37.5%, $P < 0.001$) and oviducts (25.0%, $P < 0.01$) from control hens.** It was concluded that immunization of chickens with SE bacterins reduced but did not eliminate the frequency of SE isolation from internal organs and egg contents. It was further concluded that bacterin vaccination should be part of a comprehensive program to reduce SE incidence in laying flocks, along with appropriate cleaning and disinfection, rodent control, and bacteriological monitoring.

A 1993 laboratory study (Ref. 7) evaluated six formulations of SE bacterins, each containing PT8, 13a, and 23 in white leghorn pullets vaccinated subcutaneously at 3.5 and again at 4.5 months, and orally challenged at 5.5 months with 2.7×10^7 CFU per dose of each of SE PT8, 13a, and 23. **Protection against shedding and cecal harboring was evaluated via anal swabs and cecal swabs respectively, and seroconversion was evaluated by ELISA.** Post challenge, PT8 was most frequently isolated, followed by 13a and 23. **Based on these results, a bacterin containing SE inactivated by acetone and made with modified Freund's incomplete adjuvant performed the best.**

The Agricultural Research Service (ARS) of USDA reported a study (Ref. 2) comparing the efficacy of two oil-emulsion SE bacterins; one experimentally-prepared from acetone-killed phage type 13a SE and the second a U.S. commercial vaccine, Layermune SE (Biomune, Lenexa, KS). Each vaccine was administered twice subcutaneously, 4 weeks apart, in two trials, to SPF leghorns at 22 weeks of age in trial 1 and 41 weeks of age in trial 2 at the time of the first vaccination. Chickens were challenged orally 2 weeks following the second vaccination with 10^8 phage type 14b cells/dose. Hens were housed

in individual cages in sets of 3 cages, the first given the experimental bacterin, the second given the commercial bacterin, and the third being a nonvaccinated control. Both bacterins elicited significantly higher ($P<0.005$) antibody responses as compared to then control chickens. In these trials, protection was evaluated only by isolation and enumeration of SE from fecal samples. **Both vaccines significantly reduced the incidence of intestinal colonization ($P<0.05$) and the mean number of SE cells shed in feces ($P<0.01$) at one-week post challenge compared to nonvaccinated controls.** It was concluded that protection was only partial, as more than half (57%) of vaccinates still were intestinally colonized and shed substantial numbers of SE (1.6 to 1.8 \log_{10} cells/gram of feces). This was the only study singled out and chosen by FDA to support vaccination in the cost benefit analysis used in its proposed rule (Ref. 1).

A Japanese study (Ref. 8) evaluated the efficacy of a formalin-inactivated, oil-emulsion phage type 4 SE bacterin against SE challenge at a dose simulating natural infection (Trial 1) and at a very high dose (Trial 2). SPF leghorn chickens housed in individual cages were vaccinated twice, 4 weeks apart, in both trials with the first vaccination given at 14 weeks of age in trial 1 and 8 weeks of age in trial 2. Challenge SE was administered orally 6 weeks following the second vaccination in trial 1 (10^6 or 10^3 cells per dose) and 4 weeks following second vaccination in trial 2 (10^9 cells/ dose). Antibody titers were evaluated for specific O antibodies in a tube agglutination test using specific antigen. Antibody titers in vaccinates peaked 2 weeks following primary vaccination and remained high until and beyond time of challenge. Vaccine efficacy was evaluated by enumerating SE shedding in cecal droppings. **In hens given 10^6 cells/challenge dose, SE was recovered significantly less frequently and in lower amounts from cecal droppings of vaccinates from days 6 to 12 and on day 21 post challenge than from controls. In hens given 10^3 cells/challenge dose, SE was recovered less frequently and in lower amounts between days 2 and 15 post challenge than from controls, with no SE detected from vaccinate cecal droppings on days 3, 6, 9, and 12 post challenge.** Stress, in the form of feed and water deprivation at 41 or 26 days after challenge, led to similar low rates of recovery of SE from cecal droppings of both vaccinates and controls.

A 1994 study (Ref. 9) of an inactivated oil-adjuvanted SE PT4 bacterin reported on both laboratory (SPF chickens) and field trial (Rhode Island Red layers) results in chickens vaccinated subcutaneously at one day old and again at 4 weeks of age. Subgroups of birds were massively challenged by intravenous administration of 10^8 CFU of virulent SE PT4 at 8, 12, and 16 weeks of age and assessed by observation of clinical signs and mortality for 3 weeks following challenge and postmortem lesions and challenge organism recovery from organs. Based on those criteria, mean protective indices in the laboratory trial ranged from 28 to 80 and in the field trial from 38 to 70 at the various challenge ages. **It was concluded that the vaccine provided protection against overwhelming challenge for up to 12 weeks post vaccination in SPF and layer chickens.**

A Japanese study (Ref. 10) evaluated the efficacy of a commercial oil-emulsion SE bacterin (Layermune SE, Biomune, Lenexa, KS). Dekalb-TX45 hens were vaccinated at 38 and 42 weeks of age then challenged at 44 weeks of age intravaginally with 10^7 CFU/dose of phage type 4 SE. Serum antibody levels of SE-specific IgA, IgG, and IgM measured by ELISA all were significantly ($P < 0.05$ only for IgG at 1 week post vaccination, or $P < 0.01$) higher than in controls. **In oviduct washings, IgA, IgG, and IgM were usually significantly ($P < 0.05$ and $P < 0.01$) greater than in controls at 7 and at 14 days post challenge. Significantly ($P < 0.01$) fewer eggs taken following challenge from vaccinates were positive for SE in any portion of the egg (19.0%) than eggs from nonvaccinated controls (37.0%). SE was recovered from liver, spleen, and cecum at significantly ($P < 0.05$) lower rates from tissue samples of vaccinates than from nonvaccinates, and tissue invasion was partially reduced.** It was concluded that the bacterin induced secretion of statistically significant amounts of SE-specific antibodies of all subclasses in both serum and oviduct compartments. However, the oviduct antibodies did not completely prevent production of contaminated eggs. It was concluded that humoral immunity alone was unlikely to protect fully against SE, as SE is facultatively intracellular. It was further concluded that total protection against SE requires the induction of humoral and cellular immunity as well as other nonspecific immunities.

A field study (Ref. 11) of Japanese commercial layer flocks evaluated whether administration of an SE bacterin (Layermune SE) to pullets affected the incidence of SE contamination of commercial batches of liquid eggs from those flocks. Three farms, labeled S, T, and B were studied because they had previously had SE contamination of liquid egg samples. The farms were SE-contaminated and had been demonstrated to have horizontal transmission of SE among flocks on the farm. Flocks vaccinated with the bacterin and nonvaccinated control flocks of similar ages were compared on each farm. The SE incidence from vaccinated and control flocks respectively showed negative and < 2 most probable number (MPN)/100 ml for Farm B, < 2 and > 1600 MPN/100 ml for Farm S, and negative and > 1600 MPN/100 ml for Farm T. **It was concluded that the SE bacterin was effective in reducing the incidence of SE in shell eggs produced by flocks on SE-contaminated farms.**

A study (Ref. 3) of 11 commercial layer flocks vaccinated with autogenous (Maine Biological Laboratories, Waterville, ME) or a commercial SE bacterin (Layermune SE, Biomune Co., Lenexa, KS) evaluated bacterin efficacy in the field. The evaluation criteria included presence or absence of SE in the environment, in bird organs, and in eggs. The flocks varied in number of vaccinations, in dosage used and in having other nonvaccinated flocks on the same premises. All vaccinated flocks had birds with SE-positive organ cultures. Four flocks had environmentally negative culture results, but all had SE-positive mice. When there were other nonvaccinated flocks on the premises, their culture results were similar to those of the vaccinated flocks. **Only one sample of pooled eggs, however, was positive for SE in this study. That pool came from birds coming back into production after molt and consisted of eggs pooled from vaccinated and nonvaccinated portions of the flock.** This study was one of two chosen by FDA to use in the cost benefit analysis of vaccination for its proposed rule (Ref. 1).

A Dutch vaccination field trial evaluated adding SE vaccination to a certified standardized biosecurity program in broiler breeder flocks considered to have increased risk of infection due to being previously infected and treated or for other reasons (Ref. 12). The study, between August, 1995, and December, 1997, included flocks in 3 groups: Group A comprised 15 flocks vaccinated with VAC-T, an attenuated live ST vaccine given by drinking water at 1 day of age and at 7 weeks of age, plus TALOVAC logSE, an inactivated oil-emulsion bacterin given subcutaneously at 16 weeks of age; Group B comprised 49 flocks vaccinated with SALENVAC, an inactivated aluminum hydroxide-adjuvanted bacterin given intramuscularly at 12 and at 16 weeks of age; Group C comprised 608 nonvaccinated flocks. Groups A and B were examined bacteriologically (150 cecal droppings pooled to 6 samples per flock) and serologically (60 samples pooled to 10 serum pools per flock) from age 12 weeks onward. There was no significant difference between SE-infected flocks in Groups A and C due to the small number of flocks in Group A. There was a significant ($P=0.02$) difference between SE-infected flocks in Groups B and C. **It was concluded that vaccination appears to contribute to the reduction of SE reinfection in broiler breeder flocks.** There is little reason to think these results would not also apply to commercial layer flocks.

A study (Ref. 13) from Singapore reported on the efficacy of vaccination of SPF leghorn chickens with a formalin-inactivated SE phage type 4 encapsulated in biodegradable microspheres. In one trial, 4-week-old chickens vaccinated intramuscularly with the SE microspheres developed anti-SE IgG antibody. These antibodies peaked at 4-5 weeks post vaccination and persisted at high levels at least 25 weeks post vaccination. They were also much higher and more persistent than the IgG response induced by ISA oil-emulsion SE bacterin. In another trial, 2-week-old chickens were vaccinated orally or intramuscularly with SE microspheres and challenged orally 6 weeks later with 10^9 CFU/dose of homologous SE. **Recovery of SE post challenge was significantly ($P<0.05$) lower from orally and from intramuscularly vaccinated, orally challenged birds than from nonvaccinated challenged chickens. Shedding during 3 days post challenge was significantly lower in the orally vaccinated group than the intramuscularly vaccinated group. In addition, shedding in both of those groups was significantly lower than in the nonvaccinated controls.** It was concluded that intramuscular vaccination elicited a higher serum antibody response than oral vaccination, but that oral vaccination elicited a significant intestinal mucosal IgA antibody response.

A review (Ref. 14) of Salmonella bacterins and subunit vaccines surveyed literature on bacterins, autogenous bacterins, outer-membrane protein sub-unit vaccines made with immunostimulating complexes or with microencapsulation, and killed vaccines made with highly fimbriated Salmonella strains. One experiment briefly described opened the possibility of improved bacterins, as vaccination of turkeys with a charged outer-membrane protein sub-unit vaccine adjuvanted with immunostimulating complexes led to significantly ($P<0.05$) higher antibody response than a whole cell bacterin. **The review concluded that killed vaccines and vaccines made from outer membrane proteins can significantly reduce fecal shedding of Salmonella from infected birds into the**

environment, can decrease lateral spread of Salmonella to susceptible birds, and that they should be used to supplement all other preventive and control measures.

Laboratory work was reported (Ref. 15) that compared an SE vaccine made of a sonicated extract of SE to one made of outer membrane proteins. **The vaccine made of outer membrane proteins was superior in terms of antibody response when given to birds about 8 weeks of age, and use of oil adjuvant also was superior to an aqueous suspension.**

A study (Ref. 16) was done of subunit vaccines consisting of 75.6 or 82.3 kDa outer membrane proteins administered subcutaneously to 9-week-old SPF chickens. The chickens were challenged with virulent *S. Enteritidis*, following which the numbers of challenge organisms recovered from small intestine and cecum of vaccinates was significantly lower than those recovered from nonvaccinated controls. **It was concluded that those proteins could be used as vaccines to reduce *S. Enteritidis* colonization of chickens.**

A study (Ref. 17) was designed to determine the genetic relationship between SE burden in specific organs in challenged chicks and systemic antibody levels to SE vaccine in broiler breeder chicks. Chicks from matched hatches from broiler breeder sires from a specific genetic line crossed with unrelated dams were vaccinated with an oil-emulsion SE bacterin (Biomune) and not challenged, or were not vaccinated, but challenged with SE. A negative correlation (-0.772) between antibody response to vaccination and number of SE in cecal contents was demonstrated. **This demonstrates that higher levels of serum antibody can be expected to reduce the SE burden in cecal contents,** and also that genetic selection for antibody responsiveness would also potentially reduce SE burden.

A Brazilian study (Ref. 18) examined the efficacy of vaccination with an SE bacterin (Poulvac SE, Fort Dodge Animal Health). Healthy, serologically negative commercial layers were vaccinated at 8 weeks and at 14 weeks of age with the bacterin, and were challenged orally at 24 weeks of age with 10^9 organisms per dose of a wild-type Brazilian strain of SE. SE isolation was performed in accordance with WHO methods at 1, 2, 3, 5, 10, and 15 weeks after challenge from liver, spleen cecum, ovary, and oviduct of vaccinates and controls using selective media and enrichment cultures. Bacteriological examination of eggs laid was performed weekly from 25 to 37 weeks of age. **There were significant differences ($P < 0.05$) compared with nonvaccinated controls in SE isolated from liver, spleen, ceca, and eggs.** Differences in ovaries and oviducts were not significant at that level. Vaccination reduced total organ isolations to fewer than 20% of those from controls. **SE contamination in eggs was reduced 89% by vaccination from 7.69% in controls to 0.85% in vaccinates. It was concluded that vaccination decreased bacterial shedding and egg contamination, and it was a successful tool in the control of SE in commercial table egg layer flocks.**

An unpublished study performed by a vaccine producer (Ref.19) compared four commercial bacterins for SE used in Brazil. Each of the bacterins significantly reduced ($P<0.05$) the number of SE isolations following challenge compared to nonvaccinated controls. **Each of the bacterins reduced SE isolation from eggs produced during the 13 weeks following challenge by 61% to 87% compared to nonvaccinated controls.** By 29 weeks following vaccination, ELISA serological results remained at or near 100% seroconversion for vaccinates using the two of the bacterins. Isolation of SE from eggs was inversely related to seroconversion levels.

An internal vaccine company study (Ref. 20) compared efficacy in commercial layer chickens from one-time vaccination with either of two SE bacterins (Poulvac SE or a commercial competitor bacterin) or three applications at 2, 4, and 14 weeks of age an attenuated live gene-deleted ST vaccine (Megan Vac1). At approximately 16 weeks of age, SE ELISA serological results were positive in 90% to 100% of layers with each of the vaccination programs. From then until 62 weeks of age, the SE ELISA serological results remained 80% to 100% positive with the two bacterins, but steadily declined with the live vaccine to less than 20% seroconversion by 62 weeks of age. Between 42 and 62 weeks of age, SE ELISA (IDEXX) measurements of anti-SE antibody in egg yolks from vaccinated hens varied between about 80% and 95% positive in bacterin vaccinates, while egg yolk ELISA results were between about 30% and 50% positive vaccinates given the live vaccine. **Following challenge with 1.32×10^7 organisms per dose of PT 13a SE, there was no significant difference ($P<0.05$) in recovery of SE from ceca, intestinal pools, or pools of internal organs between the groups given bacterins, but recovery was significantly lower from the bacterin groups than from the live vaccine group, which in turn was significantly lower than from SPF controls.**

Unpublished data (Ref. 21) gathered by a vaccine company from the Pennsylvania Egg Quality Assurance Program (PEQAP) compared vaccinated flocks in the program to nonvaccinated flocks. Environmental testing in 1997 resulted in 2.1% of samples from 258 nonvaccinated flocks being positive compared to 0.0% of samples from 3 bacterin-vaccinated flocks. In 1998, 2.47% of samples from 295 nonvaccinated flocks were positive compared to 0.19% of samples from 21 bacterin-vaccinated flocks. Between January and September 1999, 1.89% of samples from 246 nonvaccinated flocks were positive compared to 0.25% of samples from 69 bacterin-vaccinated flocks. In egg testing of PEQAP flocks with an SE+ environment, in 1998, 10.8% of 295 flocks that were not vaccinated tested positive compared to no positive results from 1 bacterin-vaccinated flock; between January and September 1999, 5.7% of 246 flocks that were not vaccinated tested positive compared to no positive results from 2 bacterin-vaccinated flocks. Cumulative 1997 to 1999 results from the PEQAP flocks compared 799 nonvaccinated flocks containing 45.6 million birds to 93 bacterin-vaccinated flocks containing 8.2 million birds as follows:

- 1) **Among non-vaccinated flocks, 12.1% were environmentally positive, while 3.2% of bacterin-vaccinated flocks were environmentally positive, a 3.8-fold reduction;**

- 2) **From flocks that were environmentally positive, 21.4% of samples from nonvaccinated flocks were positive compared to 8.3% of samples from bacterin-vaccinated flocks, a 2.6-fold reduction;**
- 3) **The cumulative reduction in environmental contamination between nonvaccinated and bacterin-vaccinated flocks (3.8-fold x 2.6-fold = 9.9-fold), was 89%;**
- 4) **In other words, from nonvaccinated flocks, 2.2% of environmental samples were positive compared to 0.22% of samples from bacterin-vaccinated flocks;**
- 5) **Among nonvaccinated flocks, 8.1% had SE-positive eggs detected, compared to 0% SE-positive eggs detected from bacterin-vaccinated flocks.**

The technical bulletin (Ref. 22) of a vaccine company described mechanisms of how its bacterin helped control SE. The bacterin was said to work in two ways: 1) It aided in the prevention of organ infection by invasive strains of SE, including the ovary and oviduct, thus reducing endogenous egg contamination; and 2) It helped prevent intestinal infection, shedding, and contamination of the shell, thus reducing environmental contamination and exogenous egg contamination.

A later technical bulletin (Ref. 23) from the same company stressed that bacterin vaccination of hens resulted in high levels of protective maternal antibody that were secreted into eggs. Pullets were vaccinated at four and ten weeks of age with SE bacterin (Layermune, Biomune Co., Lenexa, KS), while other commingled pullets remained nonvaccinated. At 30 weeks of age, the vaccinates and nonvaccinates were separated so as to retain identification of eggs. **Progeny challenged orally at one day of age with 5×10^3 SE PT8 or 2.2×10^3 SE PT13a had fewer SE isolations one week later from liver, spleen, and ceca of chicks hatched from eggs of vaccinates than from the organs of chicks hatched from controls. Following PT8 challenge, maternal antibody positive chicks were 6.1% (2/33) positive while controls were 40% (8/20) positive. Following PT13a challenge, maternal antibody positive chicks were 23.5% (8/34) positive while controls were 60% (12/20) positive.**

Technical information (Ref. 24) from a vaccine producer detailed the benefits of using its bacterin demonstrated in company studies. **The bacterin, given to chickens in two doses 4 weeks apart and subsequently challenged orally with 3×10^8 CFU of SE, significantly ($P < 0.05$) reduced SE colonization of internal organs, including reproductive tract, at 12 weeks of age (91.6% protection of vaccinates compared to 40.0% protection in controls), 27 weeks of age (86.1% protection of vaccinates, 25.9% protection of controls), 55 weeks of age (100% protection of vaccinates, 71.4% protection of controls), and 64 weeks of age (100% protection of vaccinates, 78.6% protection of controls). Similarly, significant protection against intestinal colonization of the ceca was demonstrated at 27 weeks of age (80.6% protection of vaccinates, 29.6% protection of controls), 55 weeks of age (100% protection of vaccinates, 0% protection of controls), and 64 weeks of age (78.6% protection of vaccinates, 21.4% protection of controls). The bacterin significantly reduced SE contamination of vaccinates' egg shells for 14 days following challenge compared to controls. Maternal antibodies induced by the bacterin protected newly hatched**

chicks of vaccinates to a significant degree compared to chicks hatched from nonvaccinated controls **against oral challenge by SE PT4 (96.3% livability in chicks of vaccinates, 10% livability in controls), *S. typhimurium* (92.3% in vaccinates, 25% in controls), and *S. pullorum* (96.2% in vaccinates, 5% in controls).** Furthermore, the SE bacterin offered similar (approximately 80 to 90%) protection against organ colonization in young chicks against homologous SE phage type challenge or heterologous SE phage type challenge using 2 heterologous strains. Protection was further demonstrated against other group D Salmonellae (*S. gallinarum*) and against group B (*S. typhimurium*).

Other vaccine company technical information (Ref. 24) reported on reduction of SE in commercial layer flocks and their environment. During a 42-month period, 39 nonvaccinated flocks (3.3 million layers) were monitored for Salmonellae by testing pooled eggs, environmental samples from manure belts, and trapped mice. With an average of 6.4 tests per flock, 23 of 39 (59%) flocks tested positive for SE at least once, 8 of 39 (20.5%) of flocks were positive by egg or organ culture, and 58 of 248 (23.4%) of all samples were positive. **Following institution of vaccination with SE bacterin (Layermune SE and Layermune 3, Biomune Co.), 26 vaccinated flocks (2.2 million layers) were similarly monitored. No vaccinated flock was SE positive by egg or organ culture, compared to 20.5% before vaccination. Only 3 of 26 (11.5%) premises housing vaccinated chickens were SE positive by environmental or mouse samples, compared with 59% before vaccination. It was concluded that vaccination offered significant reduction in environmental contamination by SE and eliminated SE as evaluated by egg or organ cultures from commercial egg laying flocks.**

Live Vaccines

A 1990 laboratory study (Ref. 25) tested the *S. gallinarum* 9R vaccine, given intramuscularly twice two weeks apart, and an experimental live SE *aroA* mutant vaccine, given twice two weeks apart both orally and intramuscularly, in 24-week-old hens obtained from a commercial layer flock. Two weeks following the final vaccination, the chickens were challenged orally with virulent SE PT4 at 10^8 organisms per dose. **Compared to nonvaccinated controls, the 9R vaccine significantly ($P < 0.01$) reduced isolations of challenge SE from liver, spleen, ovary, and laid eggs cultured individually, including both shell and contents.**

A 1990 laboratory study (Ref. 26) tested an experimental SE *aroA* mutant as a vaccine. SPF leghorn chicks were vaccinated orally with 10^9 CFU at 1 day of age, 10^7 CFU at 1 and 14 days of age, or 10^5 CFU at 1 and 7 days followed by 10^9 CFU at 14 and 21 days of age. **Following oral challenge with virulent SE PT4, all vaccinated groups had reduced fecal shedding of challenge organisms compared to nonvaccinated controls.** Chickens given four doses had significant ($P < 0.05$) reduction in challenge organisms in liver, spleen, and feces compared to controls following intravenous challenge. It was concluded that oral vaccination with the SE *aroA* mutant experimental vaccine provided significant protection from a chicken-virulent strain of SE.

A 1992 laboratory study (Ref. 27) of two SE PT4 *aroA* mutant live attenuated vaccines tested them by oral administration in SPF white leghorn chickens at either 10^9 CFU per dose at one day of age or 10^5 CFU per dose at 1, 7, 14, and 21 days of age. At one and at four days following intravenous challenge between 8 and 9 weeks of age with 10^8 CFU of virulent SE, there was significant ($P < 0.05$) reduction in SE recovery from spleens, livers, and feces of vaccinates with either vaccine than from controls. Following oral challenge with 10^9 CFU of virulent SE, **there was marked reduction in shedding of challenge strain in all vaccinated groups compared to controls.** It was concluded that live attenuated SE *aroA* vaccines were protective in chickens.

A 1993 report (Ref. 28) of experiments with live SE *aroA* vaccination of chickens used a model to simulate lateral spread of SE among chickens. In two trials, chickens were vaccinated at one day of age with 10^9 CFU of the SE *aroA* vaccine, and then housed at 3 weeks of age with nonvaccinated chickens that had been orally infected with 10^8 CFU of SE PT4 strain 109. **Over six weeks, none of the vaccinates shed SE PT4 strain 109 heavily, far fewer of them shed at all, and they shed for a shorter time than did nonvaccinated control chickens similarly exposed to infected chickens. It was also shown that systemic invasion by SE PT4 strain 109 was reduced compared to both the controls and the infected chickens, a suggestion that gut immunity was induced by the vaccine.** In another experiment, oral vaccination at 1 and 14 days of age produced solid cecal protection ($P < 0.0001$), and oral booster dosing at 16 and 18 weeks made cecal protection complete by further increasing the mucosal immune response, but did not significantly increase protection of livers, spleens, and ovaries. It was concluded that oral boosting before lay increased intestinal mucosal immunity, but that parenteral boosting might be necessary for protection of the ovary.

A series of laboratory studies reported in 1994 (Ref. 29) evaluated a live attenuated oral SE *aroA* vaccine created from a virulent SE PT4 isolate. Day-old chicks vaccinated orally with 10^5 or 10^9 CFU of the live attenuated vaccine then challenged intravenously with 10^8 CFU of virulent SE at 8 weeks of age gave protection to livers, spleens, and ceca from the challenge. Day-old chicks were orally vaccinated with 10^9 CFU of the live attenuated vaccine and one group of them were given an intramuscular booster at 16 weeks of age. One week after intravenous challenge at 23 weeks of age with 10^7 CFU of virulent SE, there was liver, spleen, and cecum protection in both groups of vaccinates compared to nonvaccinated controls. Spleen protection was greater in the group given the booster. Day-old chicks were vaccinated orally with 10^9 CFU of the live attenuated vaccine and challenged orally at 8 weeks of age with 10^9 CFU of virulent SE. Fewer vaccinates shed the challenge organism and they shed it for a shorter period than did nonvaccinated controls. Day-old chicks vaccinated orally with 10^9 CFU of the live attenuated vaccine and challenged the following day with 10^5 or 10^9 CFU of virulent SE had liver, spleen, and cecum protection from 1 day to 13 days following challenge with SE, but the same vaccination did not protect against challenge with *S. typhimurium*. **It was concluded that the oral live attenuated vaccine appeared to induce sufficient mucosal and systemic immune responses to reduce intestinal shedding, invasion from the gut, and colonization of internal organs from field challenge.**

A laboratory study (Ref. 30) of vaccination with an SE aroA mutant in day-old chicks followed by challenge one day later with SE PT4 was performed to investigate mechanisms of rapid onset of resistance to *Salmonella* intestinal colonization following inoculation of newly hatched chicks. **Vaccinates had a much lower number of challenge organisms in organs and cecal contents during the first days post challenge than controls. Immune cells infiltrated cecal lamina propria within 12 to 24 hours following vaccination, thus were in place by the time of challenge one day later, and those cells were theorized to inhibit subsequent colonization by a virulent *Salmonella* strain.**

A laboratory study (Ref. 31) of a live temperature-sensitive mutant of SE administered as an oral vaccine at 1, 2, 3, and 7 days of age at 10^9 CFU per dose, evaluated the response following challenge at 14 or 21 days of age with various *Salmonella* serotypes. Cecal colonization by challenge SE was significantly ($P<0.05$) reduced following challenge at 7 and 14 days, and organ colonization was significantly reduced following challenge at 14 days. **It was concluded that immunization of newly hatched chickens with attenuated live SE vaccine reduced *Salmonella* shedding, cecal colonization and internal organ invasion.**

Another laboratory study (Ref. 32) evaluated a live temperature-sensitive mutant of SE as a vaccine given orally, intraperitoneally, or by combination of routes at one day of age and again at 2 weeks of age. Challenge was performed orally at 4 weeks of age with 10^9 CFU of virulent SE, and protection was assessed by analyzing the ability of the challenge to colonize GI tract and spleen. Vaccination reduced the number of chickens shedding, and chickens vaccinated first orally had the fewest shedders. The numbers of challenge SE in cecal contents was significantly ($P<0.05$) reduced at all time points post challenge, and duration of colonization was reduced in chickens immunized first orally. Spleen colonization was reduced by vaccination, especially in birds vaccinated intraperitoneally first, then orally. **It was concluded that vaccination orally followed by oral or intraperitoneal boost with this live vaccine effectively reduced SE shedding and colonization of cecum and spleen.**

A producer of a live attenuated vaccine (Megan Vac 1, Megan Health, Inc., St. Louis, MO) labeled for SE protection described how the live vaccine differed from SE bacterins (Ref. 33). Live vaccines cause the chicken to respond with secretory, humoral, and cellular immunity. Cellular immunity, which is best accomplished with a live vaccine, is the best form of immunity to protect against invasive SE strains. **The vaccine inhibited transfer of SE from hen to egg following massive challenge in pullets vaccinated at 2, 4, and 16 weeks of age.**

A laboratory study (Ref. 34) evaluated the impact of live and killed vaccines for SE on SE clearance in SPF leghorn chickens vaccinated at 2 weeks of age and again at 4 weeks of age with either a live attenuated *S. typhimurium* vaccine (Megan Vac 1, Megan Health, Inc., St. Louis, MO) or an SE bacterin (Poulvac SE, Fort Dodge Animal Health, Overland Park, KS). At 6 weeks of age, the chickens were challenged orally with 10^{10} CFU per bird of SE PT4 strain 338. At 7 weeks of age the birds were euthanized for evaluation of

immunological responses and SE clearance. SE shedding was reduced in chickens given live vaccine (most probable number = 20+/gram) compared to controls (most probable number = 210+/gram) or those given bacterin (most probable number > 1100/gram). The live vaccine potentiated cell-mediated immunity, but the killed vaccine suppressed it. In contrast, the killed vaccine induced the highest SE-specific antibody response. **It was concluded that SE infection in young chickens could be controlled by live attenuated *Salmonella* vaccination.**

A study (Ref. 35) reported on the efficacy of an avirulent live *S. typhimurium* (ST) vaccine at preventing colonization and invasion of layer hens by ST and SE. SPF leghorn chickens were vaccinated orally at 2 and at 4 weeks of age with 10^8 CFU/dose of the vaccine organism. Chickens were challenged orally with virulent wild type ST or SE at 3, 6, 9, and 12 months of age. There were 5 chickens in each challenge group. Eggs were collected following challenge at 6, 9, and 12 months of age. Challenged birds were euthanized 2 weeks following challenge and organ samples evaluated for Salmonella. The vaccine prevented colonization and invasion of spleen, liver, ileum, cecum, ovary, and oviduct by both ST and SE at each challenge time, with the exception of one magnum sample positive for SE at 6 months of age, but always was isolated from organs from groups of nonvaccinates. Salmonella never was isolated from eggs of vaccinates, but was frequently isolated from eggs of controls. **It was concluded that, although protection is not 100% effective, the live avirulent vaccine induced protection in layers against colonization and invasion of the intestinal tract, visceral organs, ovary and oviduct, and prevented egg and eggshell contamination by ST and SE for 11 months.**

A study (Ref. 36) reported a Dutch field trial in which 80 commercial layer flocks at increased risk of SE infection and placed on farms with a certified standardized biosecurity program were vaccinated subcutaneously at 6 weeks of age and again at 14 to 16 weeks of age with a live attenuated *S. gallinarum* 9R strain vaccine (Nobilis SG 9R, Intervet International, Boxmeer, The Netherlands). The vaccinated flocks were compared with 1854 nonvaccinated contemporary flocks monitored under the compulsory Dutch SE control program. The nonvaccinated control flocks were examined for SE at 72 weeks of age by serological monitoring. The vaccinated flocks were monitored at 4 weeks of age by routine bacteriology of pooled cecal droppings and also at 9 intervals by routine serology for SE. **Only one of the 80 vaccinated flocks was verified as SE-positive during the trial, while 214 (11.5%) of the control flocks became serologically positive, a significant difference ($P < 0.01$).** There was no evidence of spread of the vaccine organism into eggs. It was concluded that the vaccine contributed to the reduction of SE infections in commercial layer flocks.

In a study (Ref. 37) examining the case for SE vaccination, it was concluded that commercial egg flocks placed on farms with no history of SE should be protected with three doses of live attenuated *S. typhimurium* vaccine, and that such a basic program would provide a benefit to cost ratio exceeding 8:1, depending on risk of exposure, prevailing production costs, revenue for shell eggs and for added-value and further-processed products.

Live Vaccines Plus Bacterin

A review (Ref. 38) of the current state of research concerning SE was published in 1993. Regarding vaccination as a means of SE control, **the authors stated that the protective efficacy of live Salmonella vaccines was well established**, but that safety concerns prevented their use in some countries. **They reported that killed bacterins had proven to substantially reduce relevant parameters of Salmonella infection, including reducing fecal shed of Salmonella by orally challenged chickens.** They did not address the combined use of live and killed vaccines, which had not been studied at that time.

A study in Saudi Arabia (Ref. 39) evaluated the efficacy of the live 9R *S. gallinarum* vaccine given subcutaneously at 8 weeks of age in a combined program with SE bacterin given subcutaneously at 18 weeks and again at 22 weeks, compared to separate use of either vaccine alone (live vaccine at 8 weeks and again at 18 weeks, inactivated vaccine at 18 weeks and again at 22 weeks) in Dekalb Delta commercial layer chickens. The hens were challenged orally at 24 weeks of age with 10^5 CFU of SE PT4 and again at 27 weeks of age with 10^9 CFU of SE PT4 and again at 30 weeks intramuscularly with 10^6 CFU of SE PT4. Eggs were collected weekly for evaluation from each hen until the hens were killed at 33 weeks of age. Throughout the experiment, frequency of isolation of the challenge organism from eggshells and egg contents of nonvaccinated controls was higher than from eggs of any vaccinated group. Isolation of the challenge from cloacal swabs was significantly ($P < 0.05$) less in the groups given a combination of live and killed vaccine or killed vaccine only than the controls or the group given live vaccine only. After necropsy at 33 weeks of age, challenge was isolated from live and cecal tonsils of the group given live vaccine, from liver, spleen, and cecal tonsils of controls, but not from ovary or oviduct of either group, and the challenge was not isolated from any organ of the groups given live and killed vaccine or killed vaccine alone. The live vaccine produced no seroconversion, but birds in groups given killed vaccine all seroconverted. **It was concluded that the combined live and killed vaccination program, in association with improved hygiene, good biosecurity, and rodent control would provide an excellent comprehensive program for control of SE in layers.**

A paper (Ref. 40) examining the case for vaccination against salmonellae reviewed the characteristics of live and killed vaccines used against SE. The live attenuated *S. typhimurium* vaccine was avirulent and apathogenic, had very limited persistence in vaccinates, induced high homologous immunity against *S. typhimurium* and partial heterologous immunity against SE, reduced horizontal and vertical transmission and egg shell contamination by local and systemic immunity, and induced antibodies in the egg. The inactivated SE bacterin gives high homologous immunity to SE, induces systemic as well as local immunity in the intestine, reduces colonization of organs including ovary, reduces vertical transmission, reduces egg shell contamination, reduces shedding period in case of SE infection, induces high levels of SE antibody in the egg. The live vaccine provided some protection against SE, which then was strengthened through the use of the killed SE bacterin. **It was concluded that vaccination could provide a means of preventing spread of SE by keeping infection frequency and egg transmission at**

lower levels than was possible without vaccination, and that the reduced infection pressure should ease eradication of SE at a poultry production site.

In a recent study (Ref. 41), commercial layer pullets were administered live attenuated *S. typhimurium* vaccine twice within the first three weeks of life by coarse spray followed at 13 weeks of age by one dose of an SE bacterin (Poulvac SE). At 66 weeks of age feed was withheld to induce molting and a sample of birds was removed from the commercial flock and taken to research facilities. At 67 weeks of age, the chickens and SPF nonvaccinated controls were challenged orally with 2.17×10^6 organisms per dose of SE PT13a. Cultures taken seven days post-challenge gave significantly ($P < 0.05$) lower isolation results in the vaccinated chickens: reproductive tract, 3.7% isolations (1/27) in vaccinates compared to 40.7% (11/27) in controls; internal organ pools, 14.8% isolations (4/27) in vaccinates compared to 100% (27/27) in controls; intestine, 29.6% (8/27) isolations in vaccinates compared to 92.6% (25/27) in controls; and ceca, 7.4% (2/27) isolation in vaccinates compared to 81.5% (22/27) in controls. **It was concluded that the live plus killed vaccination program gave solid protection, reducing isolations by two-thirds or more, against a moderate level of SE PT13a challenge through the first production period (one full year beyond the final vaccination), and even beyond the point of feed restriction.**

In a study (Ref. 37) examining the case for SE vaccination, it was concluded that commercial egg flocks placed on farms with a history of SE infection should routinely vaccinate replacement pullets with two successive doses of live attenuated *S. typhimurium* (mutant or gene-deleted strain) vaccine by three weeks of age followed by one or by two doses of inactivated SE bacterin.

Vaccination in Combination with Molting

A review (Ref. 42) of the relationship between molting of chickens and increased SE shedding and greatly increased susceptibility of feed-deprived hens to infection by SE, examined at solutions to that problem. It was concluded that several, including antibiotic therapy, vaccination, and use of an altered molt diet, all dramatically decreased SE shedding during molt.

A study evaluated the effect of a live attenuated *S. typhimurium* vaccine (Megan Vac 1) given before molting on shedding of *S. Enteritidis* following challenge during molting (Ref. 43). Vaccinated and nonvaccinated SPF white leghorn chickens were housed in individual adjacent cages, 11 cages per row, in two trials. In trial 1, 22 of 44 70-week-old hens were vaccinated twice 2 weeks apart by spraying 1×10^8 organisms onto the beak and head of each bird. In trial 2, 33 of 66 81-week-old birds were spray vaccinated once with the same dosage. The chickens were molted 2 weeks following the last vaccination by light restriction and feed withdrawal. On day 4 of feed withdrawal, the center chicken in each row of both vaccinated and nonvaccinated chickens was challenged with 3×10^5 (trial 1) or 1.3×10^6 organisms of nalidixic-acid-resistant SE strain SE89-8312. Transmission to the nonchallenged hens was examined 3, 10, 17, and 24 days later. Vaccination reduced horizontal spread of SE in vaccinates compared to

nonvaccinates. Vaccinated hens shed significantly less SE on day 10 post challenge in trial 1 and on days 3, 10, 17, and 24 post challenge in trial 2. Vaccination also significantly reduced recovery of SE from ovaries in both trials and from livers/spleens and ceca in trial 2. **It was concluded that live attenuated vaccination is an important method of reducing SE problems in hens undergoing molt.**

A study evaluated the effect of SE bacterin given before molting on shedding of *S. Enteritidis* following challenge during molting (Ref. 44). Commercial single-comb white leghorn layer hens were removed from a commercial laying flock at 70 weeks of age. They were raised in individual layer cages in six groups of 10 birds each. Groups 2 and 5 were vaccinated with vaccine A (Layermune SE; Biomune, Lenexa, KS), and groups 3 and 6 were vaccinated with vaccine B (Inactivac SE4; Maine Biological Lab, Waterville, ME). Groups 1-3 were not molted, and groups 4-6 were molted 25 days following vaccination, according to the commercial company's procedure of light control and feed withdrawal until loss of body weight reached at least 30%. Chickens in all groups were heavily challenged on day 4 of feed withdrawal by oral gavage with a rifampicin-resistant SE HY-1 at 2.4×10^9 bacteria in 1 ml of heart infusion broth. Samples of cecal droppings were taken at intervals from day 1 to day 56 post inoculation and evaluated by enrichment culture and delayed secondary enrichment. Hens in the vaccinated, molted group shed about 2 logs less SE than hens in the nonvaccinated, molted group (shedding at 7 to 8 logs SE/g) during days 3-14 post inoculation, and thereafter SE shedding was similar in the 3 groups, at lower levels, near 3 logs on day 14, at control levels by day 21, and not detectable on days 49 or 56. Hens in the vaccinated, unmolted groups shed less SE than hens in the nonvaccinated, unmolted group, but differences were not significant except on one sample taken 6 days post inoculation. **It was concluded that vaccination with SE bacterin prior to molt could be effective in reducing SE problems in flocks where SE might be an issue.**

VACCINATION AND EGG ANTIBODIES AGAINST SE

The Agricultural Research Service (ARS) of USDA reported a study (Ref. 6) using an acetone-killed oil-emulsion bacterin made from 10^{10} cells/dose of phage type 13a SE, a common egg-related isolate, to vaccinate SPF leghorn hens. Hens housed in individual cages were vaccinated, every other one, at 23 weeks of age in one experiment and at 45 weeks of age in another. The bacterin was administered again 6 weeks later in both experiments. Three weeks following the second vaccination, vaccinates and nonvaccinated controls were challenged orally with approximately 10^9 cells of a highly invasive heterologous phage type 14b SE. Serum antibodies measured by a microagglutination test 2 weeks following initial vaccination reached 1:2389 in experiment 1 and 1:2198 in experiment 2. At 9 weeks post vaccination, antibody titers of vaccinates remained significantly higher than those of controls. **The incidence of SE contamination of egg-contents pools for all sampling intervals for the combined experiments was significantly ($P < 0.01$) lower among pools from vaccinated hens at 7.4% than among pools from control hens at 25.8%.** In the first experiment, significantly ($P < 0.01$) fewer hens laid one or more contaminated eggs during the first 10 days post challenge. In the second experiment, significantly ($P < 0.05$) fewer egg pools

from vaccinated hens than from control hens were contaminated during the second 10 days post challenge.

An important 1996 study (Ref. 45) evaluated the effect of egg yolk antibody, which was known to closely reflect serum antibody status of the hen, in eggs laid by SE bacterin-vaccinated hens on the *in vitro* growth of SE in the egg contents. SPF leghorn hens housed individually in cages were vaccinated with an experimental acetone-killed, mineral oil-adjuvanted bacterin made with inactivated SE PT13a, while control chickens were vaccinated with mineral oil emulsion lacking the killed SE. In trial one, a single vaccination was given and eggs produced during weeks 2 and 3 post vaccination were evaluated, while in trial 2 a booster vaccination was given 5 weeks following initial vaccination, and eggs produced during 9 weeks beginning 2 weeks post vaccination were evaluated. Egg contents were evaluated for SE growth by pooling them, homogenizing them, inoculation with approximately 10 organisms of SE PT14b, culturing, and enumeration of organism numbers. Growth of SE was reduced in similarly inoculated egg contents from vaccinated hens at week 2 ($P < 0.001$) and week 3 ($P < 0.01$) post vaccination. The SE was detected in a high percentage of the pooled contents of eggs from control hens. Significantly fewer egg pools from SE-vaccinated hens contained detectable levels of SE at week 2 ($P < 0.005$), week 3 ($P < 0.001$), week 4 ($P < 0.005$), and week 5 ($P < 0.05$) post vaccination. The hens were revaccinated at week 5 and significantly fewer egg pools from vaccinated hens vs. controls were SE-positive at week 6 ($P < 0.001$), week 7 ($P < 0.005$), and week 9 ($P < 0.005$). The reduced percentage of SE-positive egg pools corresponded with elevated antibody titers. SE multiplied in egg contents from control hens to a mean density of 8×10^7 SE/ml, compared with less than 10^2 SE/ml in samples from vaccinated hens ($P < 0.0001$), and in a repeat experiment to more than 10^9 SE/ml in control eggs compared to less than 10^4 SE/ml in vaccinate eggs ($P < 0.0001$). The specificity of the anti-SE activity in the eggs was demonstrated by experiments inoculating *Proteus mirabilis* into egg contents in which growth was not significantly different between eggs of vaccinates and controls. Dilution of egg contents of bacterin-vaccinated hens 1:5 still resulted in significantly ($P < 0.005$) fewer culture-positive pools. When egg contents were inoculated with a 10-fold higher dose, eggs from vaccinated hens still supported significantly ($P < 0.05$) less growth in one trial, but differences were not significant in another trial. Addition of iron to the egg contents overcame the effects of egg antibody on suppressing SE growth. **It was concluded that in addition to the effect of SE bacterin to diminish the frequency of eggs containing SE, vaccination, by inducing antibody secretion into egg yolks could serve to block SE replication during incidences of egg mishandling, including pooling of egg contents, incomplete cooling of eggs, and allowing eggs to sit at ambient temperatures for long periods.** The antibody inhibition effect on growth of SE in egg contents during 24 hr. incubation at 37°C was 5 logs, sufficient to still inhibit SE growth in pooled egg contents from vaccinates and nonvaccinated sources. Production of eggs containing the inhibition lasted at least 8 to 10 weeks, but might be extended by use of other vaccines or other programs. The experiments performed were done with low numbers of SE, as typically seen in eggs from SE-infected flocks (10 to 20 organisms), but that inhibitory effect may be overwhelmed at higher challenges of more than 100 organisms per egg.

An SE vaccine producer reported (Ref. 46) the effect of its vaccine (Nobilis Salenvac T, Intervet) on inducing egg antibodies that reduced growth of SE in eggs produced by vaccinates. Eggs were obtained from age-matched commercial layer flocks (24-25 weeks old) where hens had been vaccinated with the Intervet vaccine or with a live attenuated SE vaccine, and control eggs were obtained from an SPF flock. Eggs from each flock were pooled, homogenized, and inoculated with SE at 10 to 100 cells per pool, then incubated at 37° C for 24 hours. **Vaccination with the Intervet vaccine reduced bacterial growth in egg contents by more than 99% compared to the nonvaccinated controls or the vaccinates with the live attenuated vaccine.** There was no significant difference in SE growth between the controls and the live attenuated vaccinates.

EUROPEAN EXPERIENCES WITH SE VACCINATION

A paper (Ref. 47) described the experience in the United Kingdom of controlling SE in broiler breeders using a combination of biosecurity and vaccination. SE, first noticed in 1985, became such a problem in the UK by 1989 that compulsory testing and slaughter legislation was passed. During the following five years, biosecurity measures such as cleaning and disinfection between flocks and rodent control were instituted and improved, and the incidence of SE declined. However, the cycle of hatchery contamination via eggs from infected flocks continued to create additional infected flocks. In 1994 an SE bacterin became available. Because early testing in commercial layers was promising, vaccination was instituted at 12 and 16 weeks of age. The vaccine helped in control of SE. Of the first 100 flocks vaccinated, 3 became infected, all 3 from farms on which the previous flock had been positive. The next cycle of vaccinated flocks on these premises remained negative. By 1996, no SE positive flocks remained. **It was concluded that vaccination helped control SE in the UK, when used with strict attention to biosecurity.**

In 2001 the UK Food Standards Agency's Advisory Committee on the Microbiological Safety of Food produced the Second Report on *Salmonella* in Eggs (Ref. 48). Chapter 7 of the report contains information on *Salmonella* vaccination in the UK. At the time of the report, only one vaccine, a killed, aluminum hydroxide adjuvanted bacterin (Salenvac, Hoechst Roussel Vet, Ltd.) was approved for use in the UK. In the field, it normally was given in two doses, one at 10-12 weeks and another at 14 to 16 weeks of age. Use of the vaccine was considered to be only a portion of a *Salmonella* control program, which also included proper hygiene and obtaining stock from noninfected sources. Vaccination first began in the UK in 1996, and by 1999 it was estimated that 85% of table egg layers were vaccinated under the British Egg Industry Council's Code of Practice for Lion Quality Eggs. The report stated that use of live *Salmonella* vaccines was not yet approved in the UK pending resolution of concerns about safety, specifically persistence of vaccine organisms in the environment, reversion to virulence, and human safety. At the time of this report, no independent studies had been performed to evaluate the effect of *Salmonella* vaccination in layers. The report cited studies by the VLA, which had found *Salmonella* vaccination highly effective in broiler breeder flocks. However, broiler breeder flocks were managed on an all-in, all-out basis, unlike most layer flocks, which were housed in multiple-age facilities or, sometimes, free-range,

conditions that would complicate *Salmonella* control. Nevertheless, preliminary evidence from the VLA indicated that vaccination greatly reduced the level of environmental contamination. That, in turn, would induce an environmental competitive exclusion effect as normal environmental flora replaced and competed with environmental *Salmonella*, leading to noninfected houses. It also was concluded that the effects of vaccination could be cumulative, as lower shedding levels made disinfection more effective and thus reduced carryover of *Salmonella* to subsequent flocks. The report concluded that, **“Commercial studies carried out at VLA have demonstrated a substantial reduction in shedding of *S. enteritidis* in faeces of vaccinated birds. This is likely to reduce shell contamination and internal contamination due to breaches in the integrity of the shell.** These studies also indicated a probable, but unconfirmed, effect on internal contamination of eggs as a result of reduced systemic infection.” Another conclusion of the report was that the decline in human salmonellosis was “probably due to a reduction in the prevalence of *Salmonella*-contaminated eggs,” and that **“vaccination has had a significant effect on the prevalence of egg contamination and human infection.”** The report recommended that the government Food Standards Agency explore with the poultry industry “the means by which the wider use of vaccination can be promoted.”

In launching the report, the UK Food Standards Agency issued a press release (Ref. 49) in which the Committee’s chairman, Professor Douglas Georgala, said, “We believe we are seeing a real success story here. **There has been a sustained drop in human *Salmonella* cases since 1997. We believe that this reflects a corresponding fall in the levels of *Salmonella* in eggs. There are reasons for believing that these improvements flow from the widespread vaccination of egg laying flocks against *Salmonella enteritidis*, combined with improved flock hygiene measures.**” The chairman of the British Egg Industry Council, Andrew Parker, responded (Ref. 50) to the report that confirmed, “the success of the British Lion salmonella vaccination programme,” saying of the voluntary industry program “More than 80,000 Lion Quality eggs were independently tested last year and all were free of salmonella.” Mr. Parker went on to say that the industry had voluntarily spent £12 million over the past 3 years, but now that three-fourths of UK eggs were covered by the successful Lion scheme that the industry association called on the government to insist that all eggs sold in the UK be produced according to those high standards.

The British Egg Industry Council on November 1, 1998, instituted the Lion Quality Code of Practice (Ref. 51), with a requirement for vaccination of member flocks. The following describes it. It is a national program for shell egg producers that included a number of stringent new food safety procedures incorporating the latest research findings to ensure the highest standards of food safety in the world. These include compulsory vaccination against *Salmonella Enteritidis* of all pullets destined for Lion egg-producing flocks, improved traceability of eggs and a "best-before" date stamped on the shell and pack which shows that they are fresher than required by law, and on-farm and packing station hygiene controls. The Lion Quality mark on eggshells and egg boxes means that the eggs have been produced to the highest standards of food safety in the world. **In 2001 a Government committee (the Advisory Committee on the Microbiological**

Safety of Food) produced a report highlighting the effectiveness of poultry vaccination in reducing human salmonella cases by half. This has since been reinforced by the Food Standards Agency, which has confirmed the success of the UK egg industry in overcoming salmonella in eggs. UK egg producers are now calling on the Government to ensure that all eggs sold in the UK are produced to the same high safety standards as those stamped with the British Lion mark. More than 28,000 UK-produced eggs were tested by the FSA and no salmonella was found inside any of them. Only nine eggs had salmonella on the shell; these would not normally pose a health risk if the eggs were handled correctly. This contrasts with the most recent Health Protection Agency tests on imported Spanish eggs, of which nearly seven per cent tested positive for salmonella. Spanish eggs have also been linked this year with a food poisoning outbreak at a cafe in central London, with one-third of the Spanish eggs used by the café testing positive for salmonella. More than 80% of UK eggs are currently produced under the industry's voluntary Lion Code of Practice, a comprehensive program incorporating the highest standards of food safety. British Lion egg producers now believe that two of the Lion Code's key elements — salmonella vaccination and a 'best before' date stamped on every egg — should be imposed on all eggs sold in the UK. The Lion Quality mark, which is a registered trademark identifiable by the Lion Quality mark on the egg shell and box, can only be used by subscribers to the BEIC on eggs which have been produced in accordance with UK and EU law and the Lion Quality Code of Practice. Lion Quality eggs currently account for more than 80% of the total UK egg market.

An unpublished slide presentation (Ref. 52) produced by a Scottish veterinary group servicing the table egg industry indicates that vaccination "clearly has an important place in Salmonella control." That group preferred the use of the live SE vaccine licensed for use in the UK in 2001 because it could give protection from one day of age and was safe for both poultry and operators.

A study (Ref. 53) of an iron-restricted SE PT4 aluminum hydroxide-adjuvanted bacterin (Salenvac, Intervet) used to control food-borne SE as a component in the UK egg quality assurance program, compared two programs for use of the vaccine in ISA commercial layers: IM administration at one day of age and again at 4 weeks of age (V2) or at 1 day and 4 weeks with a third dose at 18 weeks of age (V3). Vaccinates and nonvaccinated controls were challenged intravenously with 5×10^7 to 7.5×10^7 CFU/dose of SE PT4 strain 109 (group V2 at 8, 17, 23, 30, and 59 weeks of age; group V3 at 23, 30, and 59 weeks of age) and then observed for 21 days during which eggs were collected daily and cultured in batches of 1 to 8 eggs. Eggs and shells were cultured separately. Postmortem tissue samples were taken for culture from chickens 21 days following challenge. Reduction of shedding of the challenge SE was significantly ($P < 0.05$) less in group V2 compared to controls at all times, but was significantly less than controls in group V3 only at 30 and 59 weeks, but not at 23 weeks; overall, reduction in shedding from the V3 group compared to controls was statistically equivocal ($P = 0.11$). The number of tissues culture positive for the SE challenge was always higher for nonvaccinated controls than for vaccinates: V2, 60 of 480 (12.5%) samples were positive compared to 136 of 480 (28%) control samples positive; V3, 14 of 240 (5.8%) of samples were positive compared to 91 of 240 (37.9%) of control samples positive. Reductions in tissue colonization were

statistically significant in 5 of 8 test groups. In total, 1249 eggs were collected from all groups of challenged birds. For V2 bird egg batches, 16 of 216 (7.4%) shells and 18 of 216 (8.3%) egg contents were positive for SE, while for V3 bird egg batches, 12 of 223 (5.4%) shells and 8 of 223 (3.6%) egg contents were SE positive, and for control bird egg batches, 42 of 252 (16.7%) shells and 51 of 252 (20.2%) egg contents were SE positive. Over all age groups, both vaccination regimes led to significant ($P < 0.001$) reduction in both egg content and shell contamination. **It was concluded that the data pointed unequivocally to protection of the egg in vaccinated birds, and that the bacterin afforded protection against SE challenge by the various measures of assessment used in the study.**

A 2003 review (Ref. 54) of SE in the UK surveyed the history of the SE epidemic and the nature of the SE organism. A dramatic decline in human Salmonella infections in the UK since 1997 followed the introduction of measures to control SE in laying hens and in meat chickens, including the Lion code for egg producers which led to more than 80% of British eggs being produced by vaccinated hens. The most widely used vaccine was a bacterin, but as of 2003 a live vaccine also was in use. The decline in SE infections was concomitant with the introduction of vaccination, however it is likely that other factors such as improved flock biosecurity measures also played a part.

A 2001 UK study (Ref. 55) examined environmental sampling for SE from cage-layer, barn-egg, and free-range egg layer flocks. **One of the conclusions of the study was that the prevalence of *Salmonella* was low in most of the vaccinated flocks sampled.** While vaccination made it more difficult to detect infected flocks, vaccination also appeared to be effective in reducing the rate of egg contamination.

A UK government-funded study (Ref. 56 and see correction to the paper at Ref. 57) evaluated, by sampling feces and environmental samples, the effect of introducing vaccinated commercial layers onto farms that previously housed flocks infected with SE. The farms included free-range flocks, barn-egg flocks, and caged-layer flocks. Comparing 13 cage layer flocks before vaccination with 20 vaccinated flocks (and 2 flocks treated by competitive exclusion), the incidence of SE in bulked fecal samples was reduced from 40.5% to 5.9% in vaccinated flocks ($P < 0.001$), and in vaccinated flocks SE was isolated from 16.1% of environmental samples compared with 29.5% of such samples from nonvaccinated flocks. In barn-egg flocks, SE was found in 58% of bulked feces samples and 71.4% of environmental samples from nonvaccinated flocks, but from 7.1% and 6.3% respectively in vaccinated flocks ($P = 0.12$). In nonvaccinated free-range flocks feces samples were 63.8% positive and environmental samples were 46.6% positive, compared with 0.7% ($P < 0.001$) and 0% ($P < 0.001$) in vaccinated flocks. **It was concluded that vaccination was to be recommended for all commercial laying flocks to reduce fecal shedding of SE, and that elimination of SE from large multistage laying farms without vaccination was not likely to be possible.** Good cleaning and disinfection were also required. In most cases of poor vaccine performance, there were severe rodent control problems and a poor standard of cleaning and disinfection.

A UK study (Ref. 58) evaluated eggs collected monthly from 12 commercial cage-layer flocks on four farms where SE was present in vaccinated flocks despite vaccination with an SE bacterin (Salenvac, Intervet) at 4 and 18 weeks of age. The eggs were cultured in batches of six. Overall, for the 12 flocks, 24 of 13652 (0.18% single egg equivalent, 0.11 to 0.26 CI⁹⁵) shell batches were SE positive, while 6 of 13640 (0.04% single egg equivalent, 0.02 to 0.10 CI⁹⁵) egg content batches were SE positive. Three other batches contained SE in both egg content and shells. Therefore, total SE contamination of eggs, both contents and shells, was 33 batches of 13682 eggs (0.24% single egg equivalent, 0.17 to 0.34 CI⁹⁵). SE was found in 67 of 699 (9.6%) of vaccinated spent hens and 64 of 562 (11.4%) of bulked fresh fecal samples from the laying houses. By contrast, in eggs from three nonvaccinated flocks tested before the present study, 21 shell batches of 2101 eggs (1.0% single egg equivalent, 0.63 to 1.56 CI⁹⁵) were SE positive and 6 batches of egg contents of 2051 eggs (0.29% single egg equivalent, 0.11 to 0.64 CI⁹⁵) were SE positive. **It was concluded that vaccination had a beneficial effect on egg contamination** but that there is still some contamination risk associated with the presence of SE in infected vaccinated flocks, and that combination of vaccination with good husbandry was essential.

An October, 2004 report (Ref. 59) from a United Kingdom multi-agency national outbreak control team reported a near doubling between 2000 and 2004 in the number of reported laboratory-confirmed infections caused by non-phage type (PT) 4 SE, mostly due to PT1 and PT14b. This contrasts with the continuing rapid decline in human disease cases caused by PT4 SE, the most common SE phage type in the UK and the one responsible for the egg-borne Salmonellosis epidemic of the late 1980s. **The report attributed the decline in PT4 infections to, in part, the SE vaccination of broiler breeder flocks since 1994 and of commercial laying flocks since 1996 in the UK.** The report continued that the increase in non-PT4 cases appeared to be due to the use, mainly by commercial establishments, of eggs imported from Spain, based on descriptive, statistical, and microbiological evidence. Between October 2002 and September 2004, the Health Protection Agency sampled over 11,000 raw shell eggs from 79 premises thought to be linked to Salmonellosis outbreaks. *Salmonella* spp. were isolated from 5.6% of Spanish eggs used in catering premises, compared with 1.1% of non-Lion Quality UK eggs sampled. Meanwhile, no UK Lion Quality eggs sampled were found to contain *Salmonella*. The Lion mark on British eggs certifies that the egg laying flock has been vaccinated against SE. A recent FSA [UK Food Standards Agency] survey of British produced eggs showed no traces of salmonella within any of the eggs (Ref. 61), and it also showed a significant fall in shell contamination with only 9 shells testing positive out of a total of 28,518 eggs.

Government regulations in Germany mandate vaccination against *Salmonella* in any egg production flock larger than 250 animals (Ref. 40). Vaccination may be accomplished using SE vaccine or *S. typhimurium* vaccine. Grandparent stock is not vaccinated, but broiler and layer breeders must be vaccinated and table egg layers are vaccinated using both live and inactivated vaccines. The most recent German zoonosis report (Ref. 60) contains an English language summary of the *Salmonella* situation in laying flocks [p. 27], which states, "In contrast to the previous year, a decrease in *Salmonella*

contamination of layer flocks could again be established continuing the trend since 1996. The development which has been seen after 1995 is regarded as a success of the provisions on the immunization of layer breeding flocks on the basis of the Regulations on Salmonella in Chickens of 1994, as last amended in 2001. In 2002, this successful trend obviously continued. The contamination of layer flocks was even somewhat lower than that found in 2000.”

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