



REFERENCE 5

Dawn of a New Age in Food Safety



BIOMUNE®
V A C C I N E S

FOOD SAFETY FOR POULTRY AND EGGS THROUGH SALMONELLA REDUCTION

INTRODUCTION

The safety of poultry meat and table eggs has become a topic of intense, serious discussion in the U.S.A. and around the world. Consequently in 1996, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) enacted regulations effective January 26, 1998, titled "Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems Final Rule" known as the "Mega Reg" for meat production including poultry. Additionally, the Food and Drug Administration (FDA) resumed responsibility for conducting traceback investigations involving table eggs as a suspected source of human food poisoning.

The Mega Reg established pathogen reduction standards for broilers including any salmonella found at the completion of processing.

The standard for salmonella contamination was based on a microbiological survey of processed poultry by FSIS to establish a baseline. Failure to meet this standard can result in a suspension of FSIS inspection services at the processing plant until compliance is achieved. Since uninspected poultry meat cannot be sold, suspension of inspection would have a significant economic impact on affected processors. While current regulations do not require USDA to inspect or test farms, "farm to table"

legislation has been discussed which would necessitate a scientifically based, on-farm salmonella reduction and control program.

It is evident from these new federal food safety regulations that there will be an increased emphasis on live production practices to comply. Salmonella control measures in broilers must occur prior to delivery of chickens to the processing plant since current in-plant procedures do not dependably reduce salmonella contamination if broilers arrive at the plant

with high rates of contamination. Irradiation of processed poultry has been approved by the USDA for pathogen reduction, however, irradiation has not been met with consumer acceptance.

Salmonella reduction and monitoring programs in the U.S.A. concerning shell eggs are substantially different from those covering meat-type chickens



product containing eggs is suspected in a human outbreak of food poisoning caused by *Salmonella enteritidis* or SE [proposed scientific nomenclature *Salmonella enterica* subspecies *enterica* serotype Enteritidis (McWhorter-Murlin and Hickman-Brenner, 1995)]. A traceback is conducted to identify the source flock of the SE pathogen. Differing from the Mega Reg governing meat-type chickens, SE but no other salmonella has been of concern in laying flock tracebacks. However, in a traceback there is zero tolerance for the isolation of SE from egg contents of the traceback flock. One SE positive finding from one pool of eggs is sufficient for the entire house (flock) to be considered SE positive. Consequently, all eggs produced by that flock are considered to be at risk of SE contamination and must be diverted to "breakers" (pasteurization plants) until there are four consecutive negative SE culture results in 1000 eggs tested at two week intervals.



This testing process usually takes a minimum of ten weeks, assuming that there are no additional SE positive findings. Depending on access to the breaker market, diversion of eggs originally intended for the shell egg market can represent a revenue loss of US\$0.05 to US\$0.30 per dozen or more. The economic impact can be devastating.

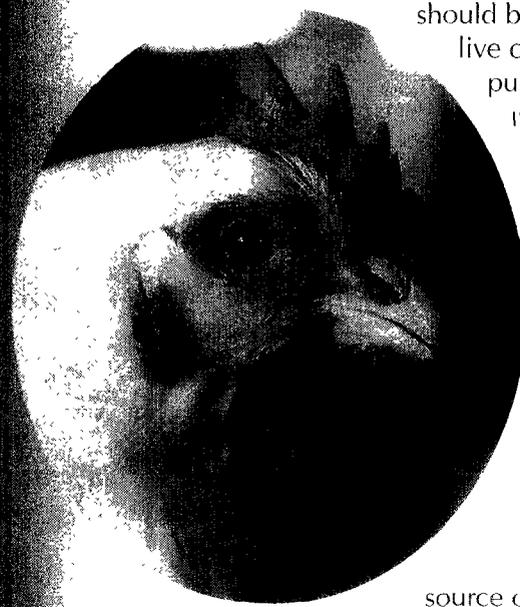
The traceback investigation by FDA includes bacteriological testing of eggs as well as the layer house environment. Because any phage type of SE is potentially as pathogenic to humans as another, FDA regulations do not require matching the SE phage type involved in the human outbreak to the phage type present on the farm before requiring diversion of eggs to pasteurization and mandating further testing. Once SE contaminates shell eggs diversion of those eggs to pasteurization is the only practical option. Therefore, salmonella control measures in laying chickens must occur prior to laying age.

Intensification of salmonella reduction practices at the live production level presents the greatest opportunity to reduce the risk of salmonella contamination of poultry meat and eggs. Such practices must be scientifically proven and should be cost effective if they are to become an integral part of live operations. A proven control measure is vaccination of pullets to provide salmonella-free chicks and table eggs in conjunction with rodent control, salmonella-free feed, biosecurity, cleaning and disinfection and insect control.

IMPORTANT ASPECTS OF SALMONELLA AND ITS CONTROL

Salmonella Contamination at Processing is Reflected by Salmonella at the Hatchery

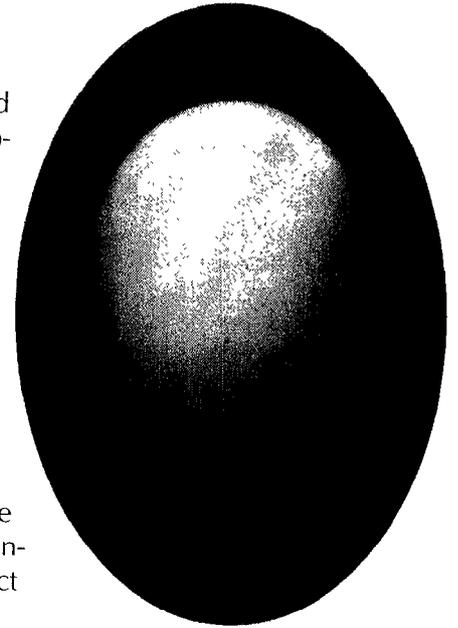
A recent study reported that the hatchery was the main source of salmonella found in processed broilers (Bailey et al., 1997). Further, the salmonella serotypes isolated in the hatchery were the same as those in breeders and the breeder house environment. It was



also shown by Bailey in field evaluations that there was a direct relationship between low prevalence of salmonella in the hatchery and a low level of salmonella in processed poultry.

Environmental Influence on SE Egg Contamination

Findings of the SE Pilot Project established the influence of the environment and rodent population on the incidence of SE contaminated eggs in a large egg-producing region of the U.S.A. The SE Pilot Project's 1995 Progress Report identified a correlation between SE phage types isolated in rodents and the environment of laying flocks with those isolated from eggs of the laying hens. During the course of testing it was demonstrated that 50% of environmentally SE positive houses had SE positive eggs. Another conclusion of the Progress Report was a correlation between the number of positive environmental samples and the prevalence of contaminated eggs (Salmonella Enteritidis Pilot Project Progress Report, 1995).



Vaccination to Control SE

Successful results have been obtained by the scientific community through the use of vaccination with an SE bacterin as a salmonella control measure in chickens. Studies with SE bacterin demonstrated high serum antibodies in vaccinated hens, a reduction of SE colonization of the internal organs and a reduction of SE contaminated eggs (Gast et al., 1992). Additionally, studies with an SE bacterin demonstrated that fecal shedding was reduced in vaccinated hens challenged with SE (Gast et al., 1993). Stimulation of the chicken's immune system with vaccination has scientifically demonstrated to provide a useful tool to significantly reduce SE in laying chickens.



Electron micrograph of *Salmonella enteritidis* courtesy of E. Mallinson

Egg Antibodies Inhibit Growth of SE

Research conducted at the USDA-ARS Southeast Poultry Research Laboratory in Athens, Georgia, U.S.A. demonstrated that antibodies in table eggs laid by hens recently vaccinated with an SE bacterin had a dramatic effect in inhibiting growth of SE when SE was inoculated into contents of those eggs in the laboratory (Holt et al., 1996). These studies demonstrated that these egg contents, when artificially inoculated with SE, inhibited the growth of SE in a significantly higher percentage as compared to contents of eggs from unvaccinated control hens. This inhibitory effect was sustained after diluting egg contents from vaccinated hens 1:5 (v:v) with egg contents from control hens. This dilution was done to simulate the effect of blending practices

In addition, egg contents from vaccinated hens when detection of SE was possible showed a one million-fold reduction in the number of SE organisms as compared to the number of SE organisms recovered from inoculated contents of eggs from control birds. The author concluded that egg contents from vaccinated hens inhibited the *in vitro* growth of SE which may have important implications for the egg industry.

LAYERMUNE SE: A VALUABLE TOOL FOR SALMONELLA CONTROL

Layermune SE is the first and most thoroughly tested USDA approved *Salmonella enteritidis* bacterin for chickens. Data reported herein were collected by Biomune Co. and other independent researchers in controlled laboratory studies and through actual field use of *Layermune SE* in the U.S.A., Latin America, Europe and Asia. These data demonstrate the value of the killed salmonella vaccine (bacterin) in reducing salmonella in breeders, broilers and commercial laying hens. *Layermune SE* stimulates the chicken's immune system to reduce salmonella colonization of internal organs and the intestine. *Layermune SE* has been proven successful for millions of chickens around the world against vertical and horizontal transmission of salmonellae by providing the following benefits:

1. Aids in the prevention of salmonella colonization of chickens in an SE positive environment
2. Reduction of colonization of the internal organs to prevent ovarian transmission to the egg
3. Reduction of colonization of the intestinal tract to prevent fecal shed to
 - the hatching egg, thereby reducing salmonella positive chicks
 - the environment, reducing contamination
4. Maternal antibodies to progeny to prevent colonization during the most susceptible first two weeks of age
5. Cross protection against salmonella species of heterologous SE phage types, serotypes within the same serogroup and partial protection across serogroups

LABORATORY TRIALS DEMONSTRATE EFFICACY OF LAYERMUNE SE

REDUCTION OF SE COLONIZATION OF INTERNAL ORGANS AND INTESTINE

Immunogenicity studies were conducted in chickens vaccinated with *Layermune SE*. In all trials, two 0.5ml injections four weeks apart of *Layermune SE* were subcutaneously administered according to label directions. Unless otherwise indicated, chickens were orally challenged with a pathogenic SE obtained from egg contents at a dose of 3×10^8 CFU (300 million colony forming units), a challenge dose far greater than would be encountered in nature. The oral route of challenge was chosen to simulate a natural exposure. Non-vaccinated hens serving as controls and vaccinated hens were simultaneously challenged.

Cultures of internal organs (liver, spleen, ovary and oviduct) and the intestine were made to determine the number of hens positive for SE colonization in any tissue following challenge. Protection was defined by failure to reisolate SE.

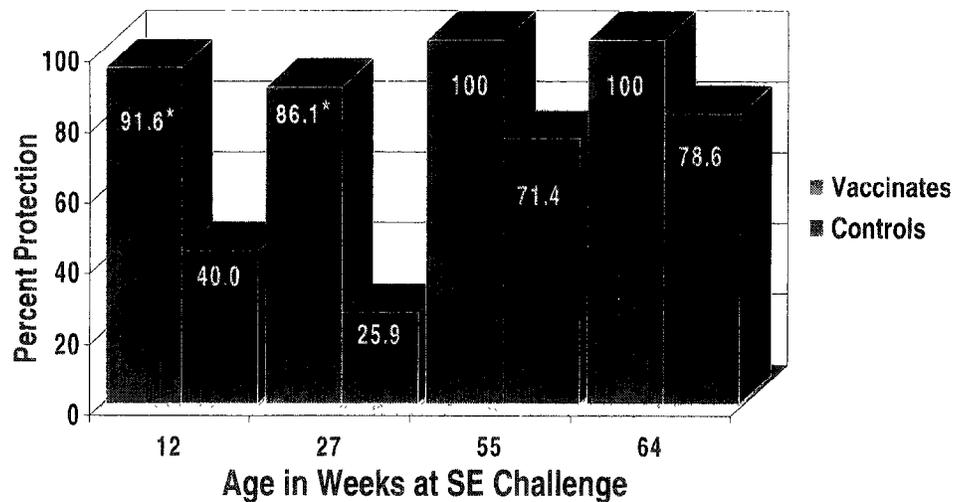


Fig. 1. Protection of internal organs following oral SE challenge with 3×10^8 CFU.
*Statistically significant difference between vaccinates and controls at $p < .05$.

Results from these studies demonstrated that *Layermune SE* provided excellent protection against infection by SE of internal organs including the target organs of the reproductive tract (Figure 1). This protection was statistically significant at $p < .05$. The data also demonstrated an age resistance to challenge. It has been scientifically acknowledged that protection of internal organs, specifically the reproductive tract, reduces the risk of ovarian (vertical) transmission of salmonella to chicks or shell eggs.

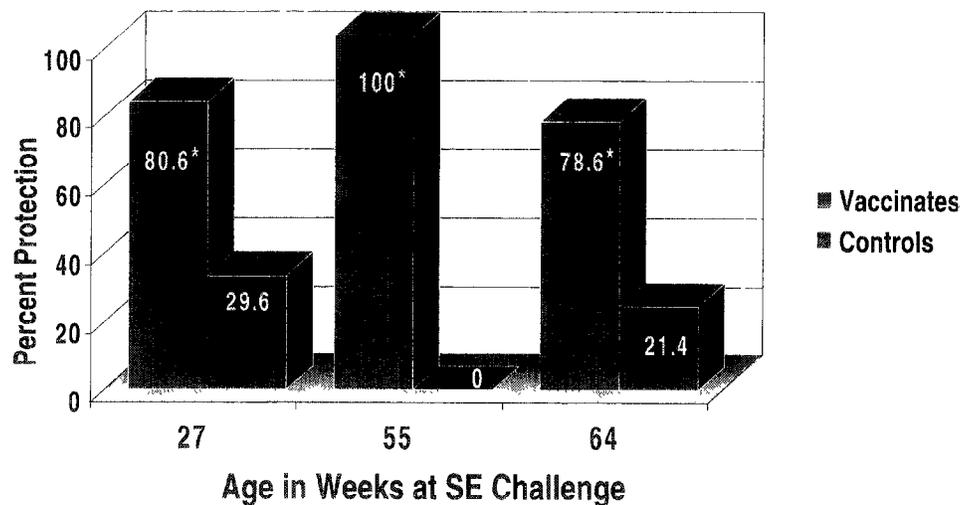


Fig. 2. Protection against intestinal colonization of the ceca following oral SE challenge with 3×10^8 CFU.
*Statistically significant difference between vaccinates and controls at $p < .05$.

Layermune SE provided excellent protection ($p < .05$) to the intestine against colonization by SE (Figure 2). Unlike internal organs, there was no age resistance to challenge evident in intestinal colonization.

In a study evaluating SE contamination of the egg shell surface, fecal shed of SE from vaccinated hens was significantly less compared to non-vaccinated hens for a

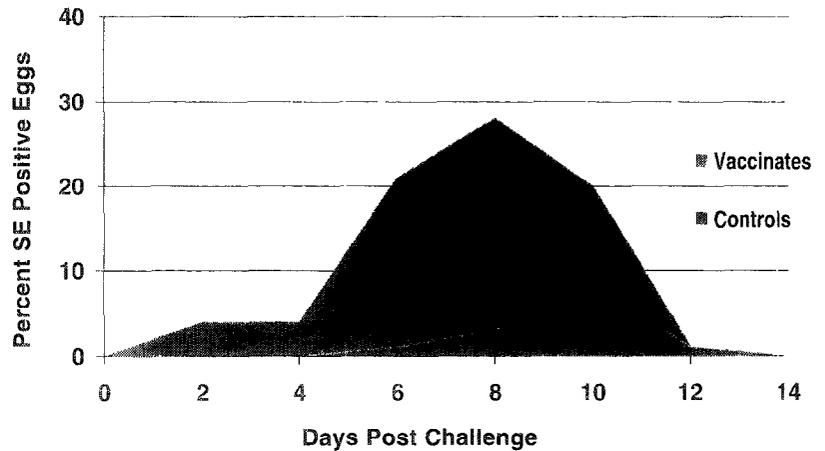


Fig. 3. SE isolation on egg shell surface 14 days following oral challenge with 3×10^8 CFU.

14-day period following oral challenge (Figure 3). This unique ability of *Layermune SE* to reduce salmonella colonization of the intestine reduces fecal shed and contamination of eggs, thereby reducing salmonella positive eggs and chicks as well as salmonella excretion into the environment.

MATERNAL ANTIBODY PROTECTION

Another defense mechanism provided by *Layermune SE* is maternal antibody protection conferred to progeny of vaccinated breeders. These maternal antibodies protect chicks during their most susceptible first two weeks of age against colonization by salmonellae.

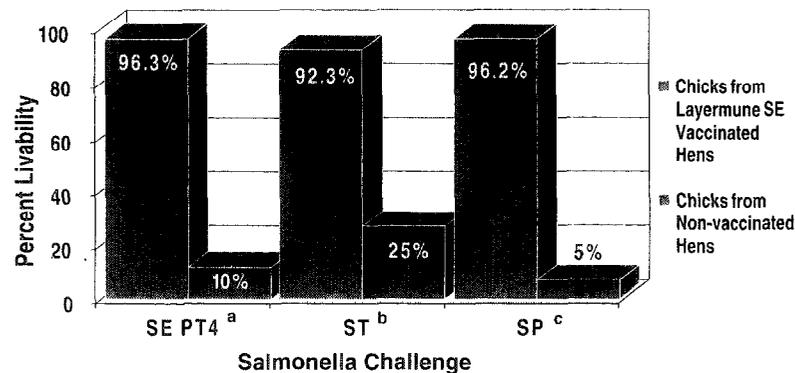


Fig. 4. Evaluation of maternal antibody protection in newly hatched chicks following salmonella oral challenges with 10^4 - 10^5 CFU.

^a*S. enteritidis* phage type 4.

^b*S. typhimurium*.

^c*S. pullorum*.

Source: Ohta, H., 1998.

4 (SE PT4), *Salmonella typhimurium* (ST) or *Salmonella pullorum* (SP). Maternal antibodies conferred protection to 96.3% of chicks from vaccinated hens while only 10% of chicks from non-vaccinated hens survived challenge with SE PT4. Using ST as the challenge organism, 92.3% of chicks from vaccinated hens survived compared to 25% of chicks from non-vaccinated hens. *Layermune SE* vaccination provided protective maternal antibodies against SP challenge to 96.2% of chicks from vaccinated hens; only 5% of chicks from non-vaccinated hens survived SP challenge (Figure 4). In non-challenged progeny there was no significant difference in mortality in chicks from vaccinated or non-vaccinated breeder hens (Ohta, H., 1998).

Oral Challenge

In a study conducted in Asia that evaluated livability, three groups of newly hatched chicks (25 chicks per group) from *Layermune SE* vaccinated hens were orally challenged with 10^4 – 10^5 (10,000 – 100,000) CFU of SE phage type

Intramuscular Challenge

Significant differences were observed in a study conducted in Europe when 20 chicks per group were challenged by the intramuscular route with SE PT4 known to cause high mortality in chicks and associated with the most serious cases of human food poisoning (Vielitz, E., 1994). In this trial chicks from both *Layermune SE* vaccinated and non-vaccinated hens were intramuscularly challenged at 3 days of age or 15 days of age. Resistance to challenge was measured by livability of chicks. Livability of chicks from vaccinated hens challenged at either 3 or 15 days of age was 100%, compared to 25% and 10%, respectively, of chicks from non-vaccinated hens (Figure 5).

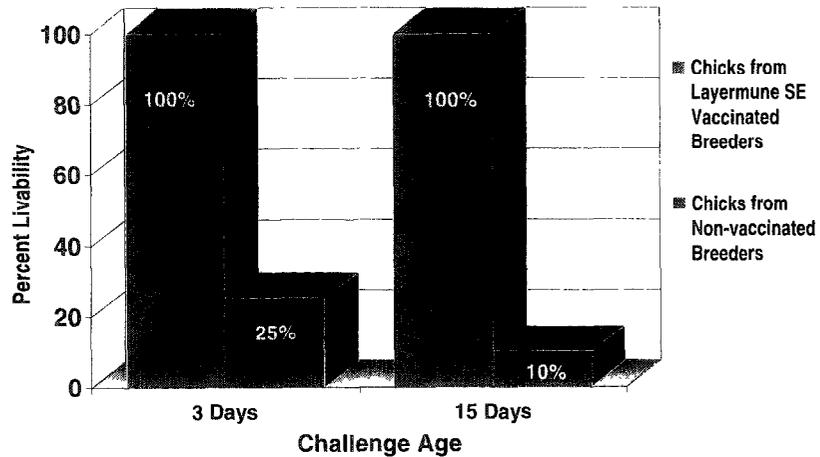


Fig. 5. Maternal antibody protection following intramuscular SE PT4 challenge of chicks. Source: Vielitz, E., 1994.

CROSS PROTECTION BY LAYERMUNE SE

Protection Against Different Phage Types

Layermune SE was shown to be equally effective against SE challenges with heterologous phage types (not contained in the bacterin) when compared to a homologous phage type challenge. Studies conducted by vaccination and subsequent oral challenge of 35-40 young chickens per group demonstrated equal protection of the internal organs against oral SE challenge with 1×10^8 (100 million) CFU of heterologous phage types compared to the homologous phage type challenge results (Figure 6). Although phage type is an epidemiological concern, these studies

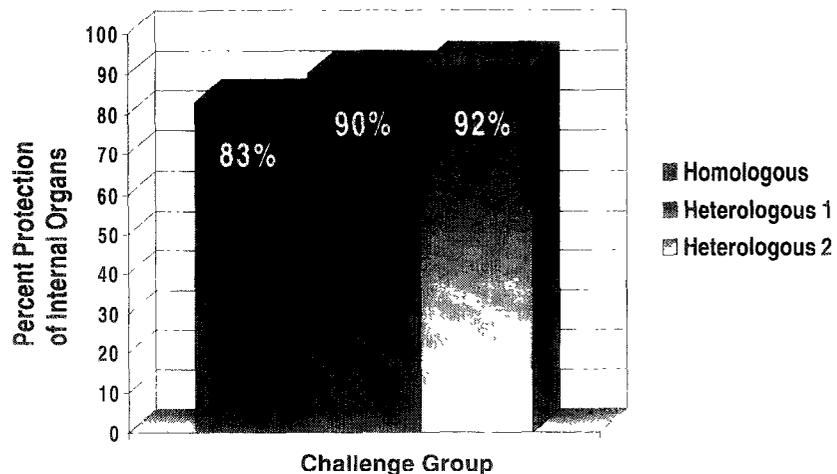


Fig. 6. Demonstration of effectiveness of *Layermune SE* using homologous or heterologous phage type oral challenges with 1×10^8 CFU.

demonstrated that *Layermune SE* provided immunity to the chicken irrespective of the phage type of the challenge organism.

Protection Against Salmonella Gallinarum, Another Serogroup D Salmonella

Table 1. Salmonella Gallinarum Challenge Results of Pullets Vaccinated with Layermune SE at 10 and 14 Weeks of Age

Group	SG Positive (%)		Mortality (%)
	Liver	Ceca	
Vaccinated	8.3	0	0
Non-vaccinated	100	17	34

Orally challenged at 16 weeks of age

Organ culture at 7 days post challenge

Source. Instituto de Investigaciones Veterinarias - Ministerio de Agricultura y Cría, 1997.

Salmonella gallinarum (SG), a serogroup D salmonella as is SE, remains a significant disease problem in poultry in some parts of the world. To evaluate the effectiveness of *Layermune SE* against SG challenge, an independent study was conducted in Venezuela by the Instituto de Investigaciones Veterinarias –

Ministerio de Agricultura y Cría. Chickens vaccinated with *Layermune SE* at 10 and 14 weeks of age and non-vaccinated chickens were orally challenged with a field strain of SG at 16 weeks of age. Parameters for evaluation were mortality and SG reisolation from the liver and ceca. Vaccinates had no mortality and minimal reisolations from internal organs with SG being reisolated from the liver of 8.3% of the chickens and no reisolation from the ceca. However, the non-vaccinated chickens had 34% mortality and were 100% and 17% positive for SG reisolation from the liver and ceca, respectively (Table 1).

Protection Against Salmonella Typhimurium, a Serogroup B Salmonella

Benefits provided by *Layermune SE* are not limited to serogroup D protection. A study utilizing *Salmonella typhimurium* (ST) as the challenge organism demonstrated that *Layermune SE* provided partial cross protection. Unlike SE, a serogroup D salmonella, ST is classified in serogroup B. A significant cause of food poisoning outbreaks in humans, ST can contaminate poultry food products, particularly poultry meat. Cross protection trials followed the same design as previous immunogenicity

studies with pullets in a layer operation vaccinated at 10 and 14 weeks of age with *Layermune SE* and orally challenged at 55 weeks of age with ST at a dose of 3×10^8 (300 million) CFU. Unvaccinated hens served as controls and were challenged along with the vaccinates. Salmonella recovered from either the liver, spleen, ovary, oviduct or intestine

Table 2. Cross Protection Results of Layermune SE Group D Bacterin Following Salmonella Group B Challenge

Treatment	Number of Hens Protected Organs and Intestine
2 Doses of Layermune SE at Pullet Age	12/16 (75%)
Non-vaccinated Control Flock	5/12 (41.7%)

S. typhimurium challenge at 55 weeks of age with 3×10^8 CFU

denoted a non-protected hen. Protection for the vaccinated group against a heterologous serogroup B challenge was 75% versus 41.7% for the challenged control group and was statistically significant at $p \leq 0.1$ demonstrating partial cross protec-

RESULTS OBTAINED FROM FIELD USE OF SALMONELLA BACTERINS

Layermune SE and *Layermune 3*, a combination killed vaccine containing *Layermune SE* and inactivated Newcastle and infectious bronchitis viruses, have been closely evaluated under various field conditions across the United States and around the world. Their success is unmatched, providing the single most effective control measure for salmonella.

Salmonella Reduction in Broilers Through Breeder Vaccination

Case 1: Salmonella Reduction in Broilers at Processing Age

The use of broiler breeder vaccination to impact the incidence of salmonella in broiler chickens at processing age was evaluated by a broiler integrator in the U.S.A. Since this integrator had broiler contamination determined to be salmonella groups B and C, but not group D, *Layermune SE* was evaluated for cross protection against heterologous serogroups.

The field study consisted of vaccinating all pullets at 10 and 18 weeks of age in one complex for one year. A separate complex with a comparable rate of salmonella contamination in broiler flocks at processing served as the non-vaccinated control. Six large-bird (six pound) broiler farms were designated as "study flocks." Three farms received broiler chicks from the vaccinated breeders, and three farms received chicks from non-vaccinated breeders. Each of the six study farms received new litter in preparation of placement of the first test flock only. Thereafter, broilers were placed on used litter. Broilers on each farm were evaluated for salmonella contamination in three consecutive chick placements during the year by culturing cecae from 30 birds from each flock at the processing plant. A broiler was considered negative if no detectable salmonella was cultured from the ceca. Only 7.1% of broilers from vacci-

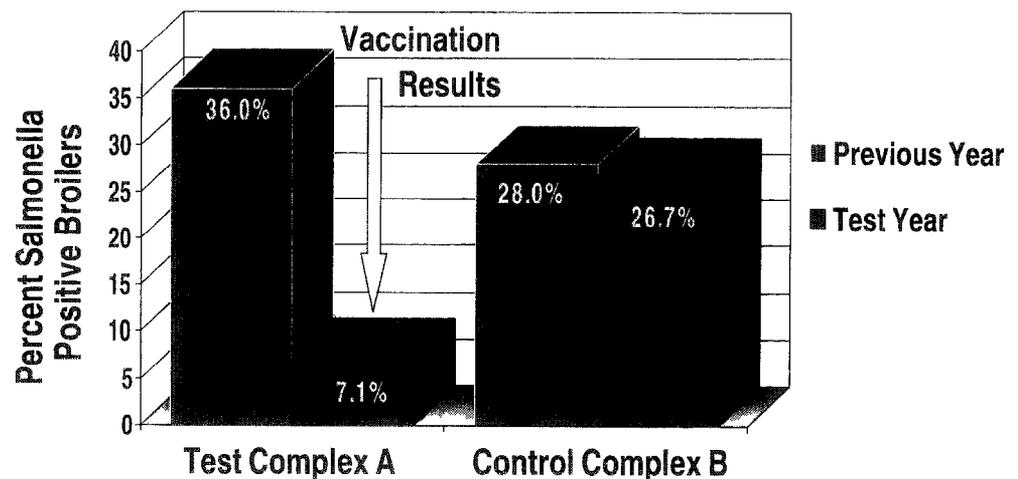


Fig. 7. Use of *Layermune SE* in breeders to reduce the number of salmonella positive broilers at processing age. All breeders in complex A were vaccinated in the test year. Complex B served as the non-vaccinated control.

nated breeders on the test farms were salmonella positive compared to the previous year's baseline of 36% positive broilers. Intestinal salmonella contamination in broilers from non-vaccinated breeders was 26.7% comparable to 28% from the previous year (Figure 7). Breeder vaccination significantly reduced the number of broilers with intestinal contamination by effectively reducing salmonella shed from breeder hen to egg and, therefore, from hatchery to broiler.

Case 2: Reduction of SE Positive Chicks at Hatch

In Germany, vaccination of breeding and laying chickens against *Salmonella enteritidis* and *typhimurium* (SE and ST) is mandatory as explained in the April 11, 1994 *Regulation on Protection Against Certain Salmonella Infections in the Chicken*. *Layermune SE*, also known as *Talovac 109 SE*, has played a vital role in Germany's successful salmonella control program for breeders and layers. A field study in Germany evaluated two vaccination regimes to compare SE shed to broiler chicks in an integrated poultry operation. In one vaccination program 137,000 breeders on five farms were vaccinated via drinking water a total of eight times with a live ST vaccine commercially available in Germany. In the other program 177,600 breeders on six farms were vaccinated via water five times with the same live ST vaccine and twice with *Layermune SE*. Results were evaluated by monitoring meconium cultures from weekly hatches of broilers through 34 weeks of production. Samples consisted of pools of meconium from 250 chicks per flock. One hundred seventy three pools were tested from the live vaccine program, and 219 pools were tested from the live vaccine plus *Layermune SE* program. The incidence of SE positive meconium pools from chicks was 42.2% for the live ST vaccine program versus 5% for the program that included *Layermune SE*. The difference was statistically significant at $p < .05$ (Table 3). This study demonstrated that the most efficacious vaccination program to reduce SE must include *Layermune SE*.

Table 3. Results of SE Monitoring of Weekly Hatches of Broiler Chicks Through 34 Weeks of Production

Vaccine Program	Number of Meconium Pools Tested	Number SE Positive
① <i>S. typhimurium</i> live vaccine ^a (8 times)	173	73 (42.2%)
② <i>S. typhimurium</i> live vaccine (5 times) + <i>Layermune SE</i> (2 times)	219	11 (5.0%) ^b

^aZoosaloral vaccine commercially available in Germany

^bStatistically significant difference at $p < .05$

Source: Vielitz, E., 1994

Case 3: Use of a Flock-Specific Salmonella Group D Bacterin

A broiler breeder company in the U.S.A. eliminated a group D salmonella from its operation with the use of a Biomune custom (autogenous) salmonella bacterin when other salmonella control measures had failed. Although SE and other invasive group D salmonellae such as *Salmonella gallinarum* and *S. pullorum* were not prevalent, chicks serologically positive at hatch to group D salmonella presented export marketing problems for this producer.

The salmonella vaccination program consisted of vaccinating breeder stock pullets on known positive farms at 12 and 18 weeks of age. The incidence of salmonella was monitored by monthly environmental cultures of all vaccinated breeder flocks and meconium cultures of every hatch comprising more than 1500 cultures per month. Salmonella monitoring results were compared with the previous year's prevalence on known positive farms. Reduction of group D salmonella in chicks was evident the first year after vaccination began as young pullets replaced older,

non-vaccinated hens. Elimination of the group D salmonella from the breeder chicks at hatch occurred shortly after all breeders had been vaccinated twice and has continued for more than three years (Figure 8).

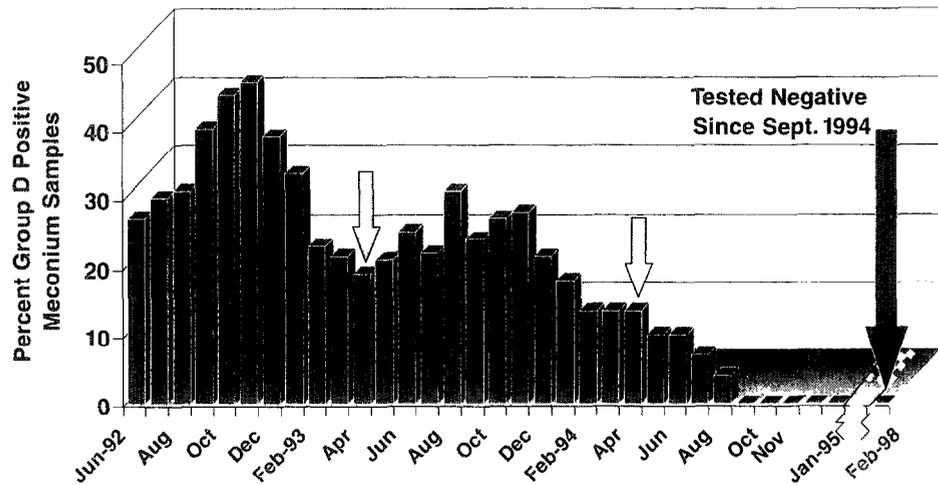


Fig. 8. Monitoring results of salmonella group D in chicks with use of a salmonella bacterin in broiler breeders. Vaccination of pullets at 12 and 18 weeks of age began in April, 1993. By April, 1994 all breeders had been vaccinated 2 times.

Salmonella Reduction in Commercial Layers and Eggs Through Vaccination

Case 1: Reduction of SE in Layer Flocks and the Environment

Extensive use of *Layermune SE* and more recently *Layermune 3* in the U.S.A. by a large commercial egg producer over a three-year period resulted in total elimination of SE as evaluated by egg and chicken organ cultures and a significant reduction of environmentally SE positive premises. These results demonstrated the ability of *Layermune SE* to control SE in layer flocks.

For a 42-month period preceding *Layermune SE* vaccination, SE culture results on 39 unvaccinated layer flocks comprising 3.3 million layers established the prevalence of SE. A part of the salmonella monitoring program in this company consisted of testing pooled eggs,

environmental samples of manure belts, egg belts and mice captured in commercial traps. Organ samples from 2 cases of peritonitis were also tested. An average of 6.4 tests per flock were conducted. During this 42-month period 23 of 39 (59%) non-vaccinated flocks or houses tested positive for SE at least once. Eight of these 39 (20.5%) flocks were SE

positive by egg or organ culture. Of 248 samples tested, 58 (23.4%) were SE positive in either the environment, eggs, mice or chickens. There were 16 SE isolations from

Table 4. Comparison of SE Isolation Before and After Layermune SE Vaccination in a Commercial Layer Operation

Vaccination Status	Number of Flocks	Source and Number of SE Positive Samples/Number of Samples Tested				Total
		Environment	Mice	Egg Pools	Peritonitis	
No	39	37/168	5/23	14/55	2/2 ^a	58/248
Yes	26	2/64 ^b	1/13	0/7 ^b	N.T. ^c	3/84 ^b

^aTwo field cases.

^bStatistically significant difference at $p < .05$ between vaccinated and non-vaccinated flocks.

^cNot tested.

Layermune SE vaccination became an integral part of the health program in November, 1994, and to date has been evaluated in 26 layer flocks comprising 2.2 million layers. During this period no vaccinated flock has tested SE positive in egg pools or organ cultures compared to 20.5% positive flocks prior to vaccination. Further, only three of 26 (11.5%) premises housing vaccinated chickens have been SE positive as determined by 2 environmental and one mouse culture compared to 59% positive flocks or houses prior to vaccination (Figure 9). These results demonstrate that *Layermune SE* vaccinated chickens are protected against SE infection in SE positive environments.

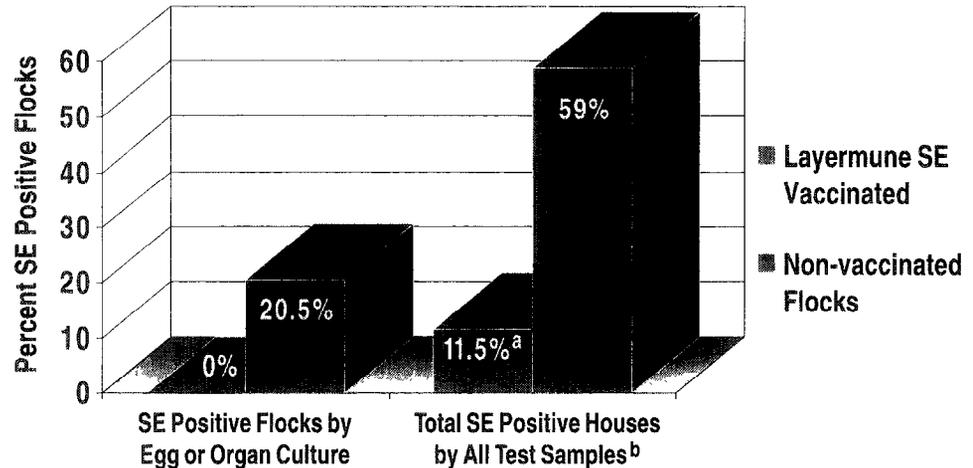


Fig. 9. Reduction of SE positive commercial layer flocks by using *Layermune SE*. Study included monitoring of 26 vaccinated flocks and 39 non-vaccinated layer flocks.

^a3/26 SE positive samples were 1 mouse and 2 environmental samples.

^bConsisted of environmental, egg, mice and organ cultures.

After vaccination, only three of 84 (3.6%) samples have been SE positive, consisting of one mouse and 2 environmental samples compared to 58 of 248 (23.4%) SE positive tests prior to vaccination as detected by environmental, mice, egg and chicken organ cultures. (Table 4). Since this layer operation utilizes SE monitoring and vaccination as part of its egg quality assurance program, the use of vaccination has resulted in a lower incidence of SE and the subsequent need for intensive testing, reducing in half the number of SE monitoring samples per flock from 6.4 to 3.2.

A summary of the egg producer's field use of *Layermune SE* provided in Table 5 shows elimination of SE in the flocks as evaluated by egg and organ cultures, plus a dramatic 80.5%

reduction of SE positive premises as determined by environmental testing. Use of *Layermune SE* vaccination has resulted in fewer SE positive samples thus reducing the need for extensive testing. *Layermune SE* vaccination of SE positive flocks frequently changed the environmental status to SE negative within two to three weeks following vaccination. This change to an environmentally negative SE status can be explained by the immediate effect of *Layermune SE* to reduce

Table 5. Results of *Layermune SE* Vaccination in a Commercial Layer Operation

Reduction in Number of Flocks with SE Positive Eggs or Internal Organs from 20.5% to 0%

Reduction in Number of SE Positive Flocks or Houses from 59% to 11.5% (80.5% Reduction)

Vaccinated Layers were Protected Against SE Infection in Houses with SE Positive Environments

Case 2: Vaccination Changed a Layer Operation from SE Positive to SE Negative

An 82,000 layer operation in the northeastern U.S.A. introduced *Layermune 3* vaccination at 14 weeks of age in response to an SE positive replacement flock. This flock was SE positive at day of age as determined by culture of meconium on chick pads and also SE positive at twelve weeks of age as determined by manure and chicken organ cultures in accordance to the state's voluntary egg quality assurance program. In addition, the prior year's flock had tested SE positive late in the production cycle which resulted in eggs being diverted to a breaker plant in the last twelve weeks of production with a consequent economic loss. The vaccinated flock was placed in the same house as the prior year's SE positive flock. Breed and management practices remained unchanged in this operation. Testing of the environment and bird mortality for the presence of SE was conducted monthly according to a protocol established by a university diagnostic laboratory.

Following monitoring at 22-weeks of age which resulted in one dead hen positive for SE, the monthly environment and organ culturing results were SE negative

Table 6. Performance of an SE Vaccinated Flock to Previous Year's Non-vaccinated Flock

Year of Production	Number of Birds/Flock	SE Vaccination	SE Status	Livability	Eggs-per-Hen-Housed
1996	82,000	None	Positive	93.33%	233
1997	82,000	<i>Layermune 3</i> ^a	Negative ^b	94.14%	237
			Difference	+0.81%	+4

^aContaining *Layermune SE* plus inactivated Newcastle disease and bronchitis viruses.
^bFlock and environment tested negative after 22 weeks of age.

and thereafter remained SE negative throughout the production cycle. Livability to 60 weeks of age in the non-vaccinated flock of the prior year was 93.33% compared to 94.14% livability in the current year's vaccinated flock. Eggs per hen housed to 60 weeks of age averaged 233 in the non-vaccinated flock versus 237 in the vaccinated flock (Table 6). *Layermune*

3 vaccination resulted in an SE negative status for the flock and environment while providing equal or better flock performance in egg production and livability.

Case 3: Layer Vaccination Prevented SE Contamination of Liquid Egg Product

Field use of *Layermune SE* by a large commercial egg producer and processor in the Far East resulted in the failure to detect SE in liquid egg product. This endemic SE PT4 layer operation vaccinated 3 pullet flocks with *Layermune SE* at 9 and 13 weeks of age. Three comparable pullet flocks were selected as unvaccinated controls. Samples of 500 ml per ton of liquid egg product were tested for SE when flocks were 36 weeks of age. Egg contents from all three vaccinated flocks had negative cultures while two of the three control flocks tested positive for SE PT4 with 427 to 1600 SE organisms per 100 ml of liquid egg (Table 7). This study demonstrated the ability of *Layermune SE* to prevent colonization of the ovary and subsequent deposition of SE in the egg

Table 7. Evaluation of SE Contamination in Liquid Egg Product Following Vaccination of Commercial Layers With *Layermune SE*

Flock	Vaccination Status	SE Culture Results of Liquid Egg
A	<i>Layermune SE</i>	Negative
B	<i>Layermune SE</i>	Negative
C	<i>Layermune SE</i>	Negative
D	None	427 SE organisms per 100 ml
E	None	1600 SE organisms per 100 ml
F	none	negative

Case 4: *Layermune SE* Vaccination Controlled Mortality Caused by *Salmonella Gallinarum*

The ability of *Layermune SE* to cross protect against another salmonella serotype within serogroup D was demonstrated by field use in Latin America (Norton, E.C.G. and F. Lozano, 1997). A company located in an area of high bird density with endemic *Salmonella gallinarum* (SG) used *Layermune SE* in one house on a nine-house farm with 112,000 multiple age birds and high mortality due to SG. Replacement pullets were vaccinated at 10 and 14 weeks of age. Cumulative mortality for an 11-week period was only 1.8% in the vaccinated house compared to an average mortality of 8.1% (ranging from 4.2 to 12.5%) in the non-vaccinated houses (Figure 10). The producer credited *Layermune SE* vaccination with saving his operation from the devastating clinical disease and mortality caused by SG.

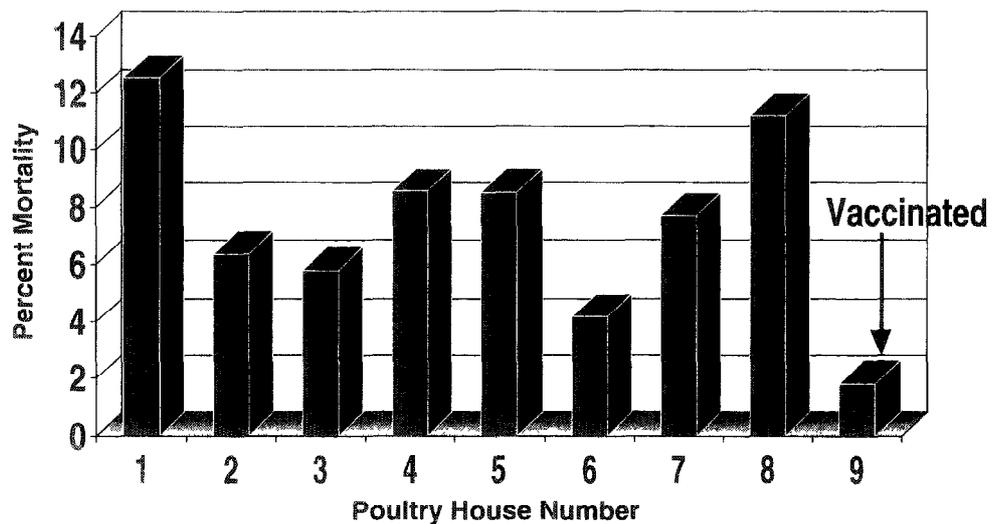


Fig. 10. Comparison of 11 week cumulative mortality between a *Layermune SE* vaccinated flock and 8 non-vaccinated flocks on a *Salmonella gallinarum* endemic farm. Source: Norton, E.C.G. and F. Lozano, 1997.

SUMMARY

Biomune's *Layermune SE* and *Layermune 3* have achieved an extraordinary record of success in controlling salmonella infections in breeders, broilers and layers. This record has been substantiated by poultry meat producers and table egg producers across the U.S.A. and around the world. These control measures have helped reduce the risk of salmonella contamination of poultry and egg products to the consumer. Vaccination to increase resistance to salmonella colonization has proven an excellent option for flocks to reduce shed of salmonella from the breeder to the chick and from the layer to the egg. While complete eradication of salmonella by any one control measure may be an unrealistic goal, significantly increasing the immune status of the flock will effectively halt spread of infection through the reproductive system into the egg and through the intestine onto the egg shell and into the environment. *Layermune SE* utilizes the immune system to prevent the establishment of salmonella colonization in breeders, broilers and layers to provide the most proven and effective salmonella control measure that has been thoroughly tested in the laboratory and in the field.

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