Megan®Egg – protection for commercial layers against *Salmonella enteritidis* infection for consumer protection

Karen E. Burns[A], Sandra M. Kelly-Aehle[B], Julie A. Lawrence[C]

[A] D.V.M., M.A.M, diplomate A.C.P.V, Technical Services Veterinarian, Lohmann Animal Health International, Inc., 1146 Airport Parkway, Gainesville, GA 30501
[B] Biologicals Development Director, AVANT Immunotherapeutics, Inc. dba Megan Health, Inc., 8620 Pennell Drive, St. Louis, MO 63114
[C]Research Scientist, AVANT Immunotherapeutics, Inc. dba Megan Health, Inc., 8620 Pennell Drive, St. Louis, MO 63114

In response to a rise in food safety concerns over *Salmonella enteritidis*, vaccination with inactivated bacterins started in the late 1980's in the United States. These bacterins offered reduction in *Salmonella* excretion in feces, reduced invasion by the challenge organisms and provided passive protection through antibodies being present in the egg (3,4,5,6,9). Science progressed to the development of live attenuated vaccines derived from *Salmonella typhimurium* (ST) that would offer cross protection to other *Salmonella* species, most notably *Salmonella enteritidis* (SE)(2,7). The first live vaccine of this type licensed in the United States was *Megan®Vac 1*– a double gene modified ST organism that was designed for protection of broilers through slaughter to reduce *Salmonella* contamination of carcasses (2). Another vaccine against SE infection has recently received USDA license. The researchers at Megan Health have developed *Megan®Egg*, a new vaccine for pullets. This product contains the same *Megan®Vac 1* organism, however, *Megan®Egg* is formulated for immunization of older birds.

Vaccine Characteristics: The vaccine is derived from a naturally occurring *Salmonella typhimurium* that has been genetically modified to impair two genes that are important for normal growth and metabolism. The modifications to the genes, *cya* and *crp*, result in an organism that is completely distinct from *Salmonella* species (2, 7). When cultures are examined using a rapid identification system, the results correlate with the species, *Hafnei alvei* (10). For this reason, along with the low shed rate of the vaccine strain, *Megan®Egg* will not interfere with environmental monitoring of a *Salmonella* reduction program.

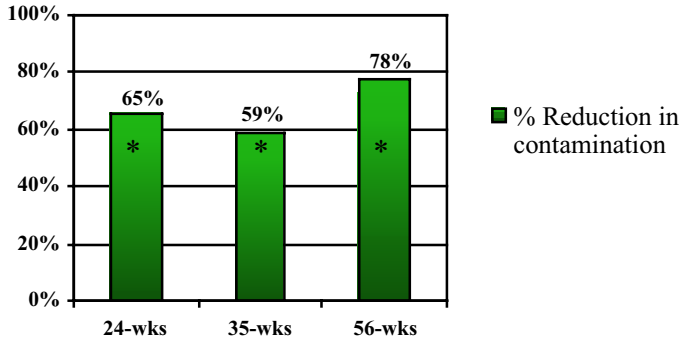
Label Indications: *Megan®Egg* is the first USDA licensed live *Salmonella typhimurium* vaccine to carry a label claim for protection of the ovaries and oviducts. This protection of the reproductive organs is significant against strains of *Salmonella enteritidis* proven to infect the ovary with consequent contamination of the egg. Additionally, *Megan®Egg* carries a label claim to reduce colonization of the intestinal tract and ceca by SE. As a result of the broad-based protection provided by the vaccine in the internal organs, ovaries, oviduct, intestinal tract and ceca, the risk of egg shell contamination is greatly reduced.

The label recommendation for age of application is 2, 4 and 16 weeks of age by coarse spray application. The vaccine has been used in field safety tests in commercial layer houses, with no adverse vaccine reaction observed.

Vaccine efficacy in laying hens:
Vaccination protocol: Birds were administered *Megan®Egg* by coarse spray application at 2, 4, and 16 weeks of age. Nonvaccinated controls were administered distilled water by coarse spray.
Challenge: Birds were challenged with a very large dose of wild-type nalidixic acid-resistant *Salmonella enteritidis* organism, Phage Type 13a 8, 19 and 40 weeks after receiving the last booster vaccination.
Sample Collection: Eggs were collected daily for 5 days post challenge, then birds were euthanized and spleen, liver, kidney, ovaries, oviduct, duodenum, ileum, large intestine and cecal tissues with contents were collected for culture.

Results – Vaccine efficacy in laying hens:
Figure 1 shows the results of egg cultures post challenge from vaccinated and nonvaccinated birds at various ages. Protection provided by the vaccine is significant when statistical differences were found in the number of salmonella-free eggs from 24-, 35- and 56-week-old vaccinated hens compared to nonvaccinated hens. Long-term protection is evident 40 weeks after the last booster vaccination. A 65 to 78% reduction in the number of contaminated eggs cultured from vaccinated birds was observed during 3 periods during the lay cycle.

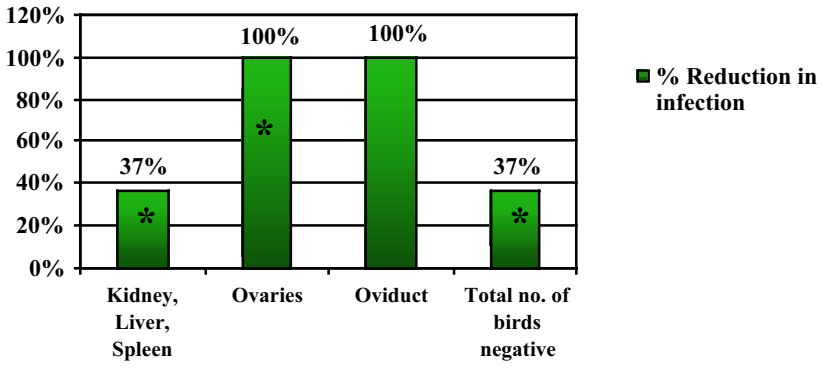
Figure 1. Reductions in *S. enteritidis* contamination of eggs of vaccinated hens compared to eggs of non vaccinated hens following wild-type SE challenge



*Significantly differs from nonvaccinated control group by Fisher's exact test P < 0.05.
% reduction of SE contamination rate = (%contaminated eggs of control group - % contaminated eggs of vaccinate group)/% contaminated eggs of control group x 100.

Vaccinated hens were significantly protected from SE infection of the kidney, liver and spleen than were nonvaccinated birds when both groups were challenged with wild-type SE at 56-weeks of age (Figure 2). In addition, significant protection provided by the vaccine is evident in the ovaries of vaccinated birds. Protection of the ovaries from SE infection is critically important in lowering or eliminating contaminated eggs.

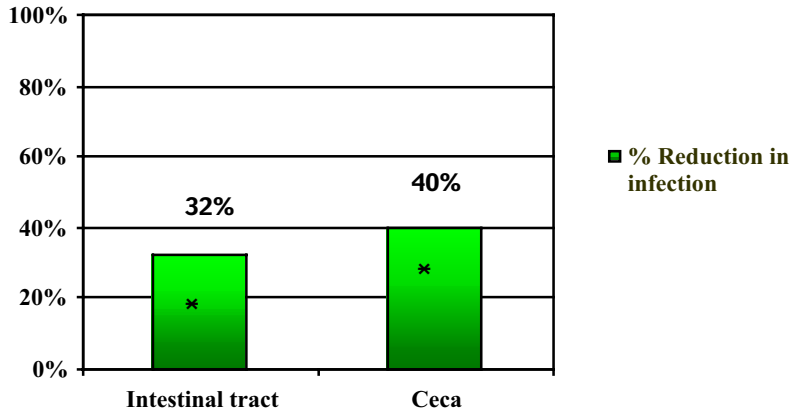
Figure 2. Reductions of SE infection in 56-week-old vaccinated and challenged hens



*Significantly differs from nonvaccinated control group by Fisher's exact test P < 0.05.
% reduction of SE infection rate = (%infected birds of control group - % infected birds of vaccinate group)/% infected birds of control group x 100.

The numbers of SE challenge organisms recovered from intestinal samples from vaccinated and nonvaccinated hens were compared. Figure 3 shows significant reduction of SE infection in vaccinated birds. The number of SE organisms recovered from the intestinal tract and ceca from vaccinated birds were significantly less than those recovered from the nonvaccinated birds.

Figure 3. Reduction of SE infection of the intestinal tract and ceca of 56-week-old vaccinated and challenged hens



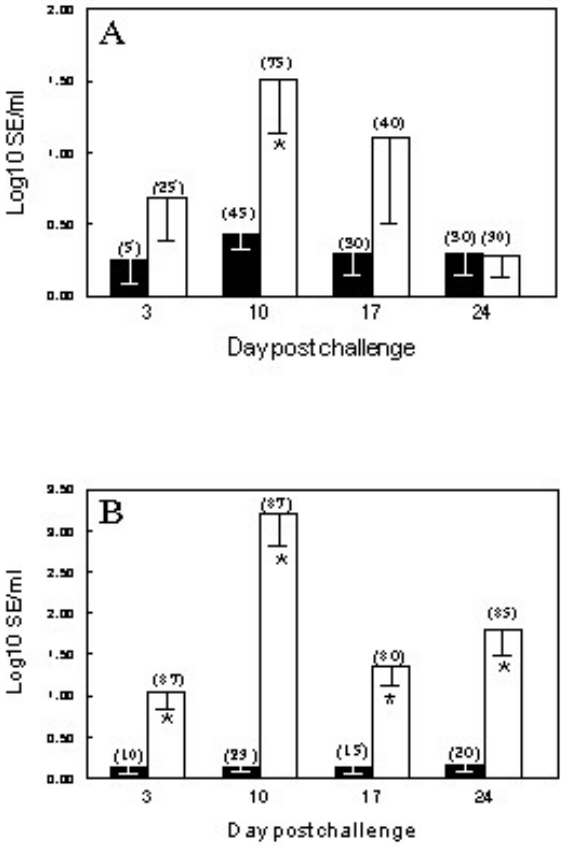
*Significantly differs from nonvaccinated control group by Welch's two-tailed t test $P < 0.05$.
% reduction of SE infection rate = (%infected birds of control group - % infected birds of vaccinate group)/% infected birds of control group x 100.

Vaccine efficacy in molted hens:
In addition to the data above describing protection provided by the vaccine, studies have been conducted to show the benefit of vaccinating molted hens. The results of two trials summarized below demonstrate that immunization of hens with the live *S. typhimurium* vaccine helps reduce SE infection in birds undergoing molt. This work is described in Holt, et al (8).

Two trials were completed using 70- and 81- week old hens. In the first trial, an increased dosage of *Megan®Vac 1*, equivalent to one dose of *Megan®Egg*, was administered by coarse spray at 4 and 2 weeks prior to molt. In the second trial, birds were vaccinated only once 2 weeks prior to molt. The hens were molted 2 weeks after the last vaccination in both trials by a modification of procedure previously described (1). On the 4th day after feed withdrawal, the center bird in each cage row of 11 hens was orally challenged with either 3×10^5 or 1.3×10^6, respectively, of wild type *Salmonella enteritidis* for both trials. Intestinal shedding of the challenge organism was measured through intestinal secretions collected on 3-, 10-, 17- and 24-days post-challenge. At 11 days post-challenge, ten birds from both trial 1 and trial 2 were euthanized and the spleen, a section of liver, and one cecum were cultured for the wild-type SE challenge organism.

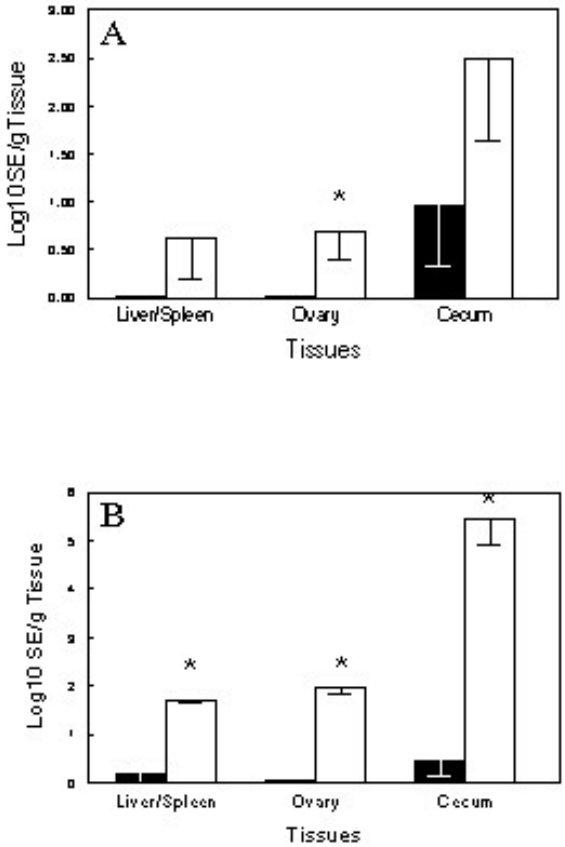
Figure 4 shows a comparison of SE shed by vaccinated and nonvaccinated molted exposed hens for both trials 1 and 2. In trial 1 where two doses of vaccine and the lower SE challenge level were used, the shedding rate is dramatically lower in vaccinated hens compared to nonvaccinated hens. The positive samples from the vaccinated groups were only detected after enrichment. The nonvaccinated birds showed a higher level of reisolation with four of 22 birds containing greater than 10^3 organisms/mL. The same results were observed again in the second trial where only one dose of vaccine was needed to provide significant protection to molted SE-exposed hens.

Figure 4. Comparison of SE shed by vaccinated and nonvaccinated molted exposed hens in trial 1 (A) and trial 2(B). Asterisk indicates value is significantly different from vaccinated hens (P<0.05).



A comparison of SE recovery from internal organs revealed significantly higher levels of SE in nonvaccinated exposed hens compared with vaccinated hens in both trials (Figure 5).

Figure 5. Recovery of wild-type SE from internal organs of vaccinated and nonvaccinated molted exposed hens in trial 1 (A) and trial 2 (B). Asterisk indicates value is significantly different from vaccinated hens (P<0.05).



In trial 1, significantly higher levels of the challenge organism were detected in ovaries of nonvaccinated hens, while numerically greater levels were seen in ceca and liver/spleen. In trial 2, protection against SE transmission due to vaccination was dramatic. Significantly higher levels of SE were recovered from nonvaccinated hens compared with vaccinated hens.

The aerosol route of delivering the vaccine to hens was effective and has the advantage of mass immunizing large numbers of birds without handling the birds. The SE transmission challenge model used in these trials accurately depicted real-world conditions for hens undergoing molt. Use of this live attenuated *S. typhimurium* vaccine prior to inducing molt in commercial layers, significantly reduced shedding and infection by *S. enteritidis* following challenge in these trials.

There are several benefits to using a live vaccine in a commercial setting. No adverse reactions are caused by coarse spray application of the vaccine. The ease of mixing and applying the vaccine by coarse spray reduces injuries due to bone breakage by handling. Hands-free vaccine administration means less labor and man-hours to vaccinate. The vaccine is easily stored at 45°F. Preventive measures should include vaccination to prevent and reduce infection. Including *Megan®Egg* in a *Salmonella* control program is a cost-effective approach and a powerful tool to effectively minimize the risk of infection to poultry flocks.

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