REFERENCE 9

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Comments wrote for the USAHA Salmonella Committee section on C&D plus the section on vaccinations that was chaired by Rich Dutton

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II. CLEANING AND DISINFECTION BETWEEN FLOCKS:

Between flock cleaning and disinfection (C&D) of houses that contained manure positive flocks has been a part of most state and industry SE reduction programs in the US. This between flock period of time while the house is vacated of birds gives the opportunity to reduce the number of SE bacteria in the house and also to reduce rodent populations. Egg layer production facilities are not easily decontaminated hence complete sterilization of the facility is not the goal, only reduction.

Historically, removing organic material using water has been used successfully to control viral diseases such as Marek's Disease. It has been determined that wet washing is **not** necessary for desired results of SE negative manure tested flocks the next cycle. In fact, there is evidence that the addition of water to the layer house environment may actually increase the numbers of bacteria. In addition, wet washing is costly, leads to greater equipment damage due to corrosion, waste water causes disposal issues in many cases, houses in cold climates are very difficult to wet wash as freezing may occur.

The Pennsylvania Egg Quality Assurance Program (PEQAP) has allowed between flock C&D without wet washing since early in 2003 (PEQAP Operations Annex section IV). Houses must qualify for this program however with a relatively low percent of positive manure samples (25% or less) and a low rodent index the last 3 months of lay (2 or less). The producer agrees to 1) vaccinate the incoming pullets using an approved program with bacterin, live vaccines, or both, 2) completely remove all organic material using physical means, 3) fumigate with formalin after dry cleaning, and 4) allow a minimum of a 10-day down period between flocks.

In determining if dry cleaning without wet washing was a feasible option, 7 houses in which wet washing C&D was performed in houses with manure positive layers, 100% of the next flocks were tested manure positive. These same SE contaminated houses were then allowed to use the Dry Clean Program (dry cleaned, fumigated with formalin, and placement of SE vaccinated pullets). Only 2 of these 7 flocks tested manure positive at either 30 or 45 weeks of age following the use of the Dry Clean Program. The use of vaccine is partly, if not largely, responsible for the success of this program but is one method of increasing vaccine use by allowing a trade-off for not having to use wet washing.

A major effort in reducing rodents during the between flock period is stressed heavily. At this time with a lack of feed and organic material, the rodents are much more easily baited plus harborage and entry sites can more easily be filled with rodent-proof materials.

The local/state SE Program authority, with the on-farm SE program coordinator, should coordinate the entire process of between flock C&D planning, execution, and evaluation of results.

III. USE OF VACCINES:

Killed vaccines for bacterial diseases have been used for many years. As an example, much success has come from the use of commercial vaccines for Pasteurella multocida in turkeys. In addition, Erysipelothrix bacterins for hogs and turkeys and Salmonella cholerasuis vaccines for hogs have been widely used. Salmonella vaccines have been used routinely in turkey breeder programs to prevent specific salmonella infections in poults.

With the advent of SE in layers it is only natural that killed vaccines should be attempted. The development of the live vaccines by recombinant gene research has been a tremendous benefit. One of the problems with killed vaccines is the loss in body weight due to the bacterial toxins. Two injections of a killed bacterial vaccine will result in a .3 to .5 lb loss in body weight before 16 weeks of age. The advantage of the live recombinants is the oral or spray application and no injection. Since the vaccine can be used in young birds the immune system is prepared for the killed vaccine or another stimulation by the live vaccine booster. Another live vaccine developed for the European and South American conditions has been the 9R Salmonella gallinarum vaccine used in breeder conditions where challenged with Salmonella gallinarum have been very severe. This vaccine which is also a group D vaccine has been very successful as a live vaccine without the spread of the original organism. USAHA has in the past opposed the use of 9R vaccine due to concerns about safety as the US has eradicated Salmonella gallinarum.

Technical proof of efficacy

There are many researchers who have studied the use of vaccines in layers. Dr Richard Gast, for instance, is quoted in a recent personal communication.

"I have attached the Salmonella vaccine section from my last Diseases of Poultry chapter (with a list of cited references). I thought this might provide a useful summary of published research results on the topic." (References are found at the end of this section).

The degree of protection afforded by Salmonella vaccines (including cross-protection by vaccines of serotypes heterologous to the challenge strain) has generally been good, although rarely complete. Such protection rarely approaches 100% efficacy and can usually be overcome by high challenge doses. However, even partial protection can be very useful toward achieving the long-term goal of reducing SE incidence in successive laying flocks in a house or farm. Data from the European programs that use vaccines certainly demonstrate that vaccination can contribute to SE control in a very positive manner. In my opinion, one of the real weaknesses of the "test and divert" emphasis of the FDA proposal is that it only address the public health risk of currently infected flocks, and has minimal value for preventing recurrence of infection in subsequent flocks on the same site. Vaccination is a powerful tool that should be utilized (in addition to cleaning and disinfection) for this purpose. Identifying infected flocks might

protect consumers against an immediate disease threat, but true long-term protection of public health will require additional efforts to break the cycle of continuous re-infection of flocks from environmental reservoirs.

Vaccination with either killed or live preparations has been found to reduce the susceptibility of poultry to paratyphoid *Salmonella* infection. Live *Salmonella* vaccines have often been associated with a stronger or longer-lasting protective response in poultry, perhaps either because of adverse effects on relevant protective antigens during the preparation of killed vaccines or because live vaccines present relevant antigens to the host immune system more persistently (23). Killed vaccines may also fail to fully elicit the cell-mediated portion of the protective response (343). Nevertheless, both killed and live vaccines have been associated with significant protection against salmonellae, although neither type of vaccine has consistently provided an impenetrable barrier against infection. Moreover, feed or water deprivation and environmental stresses such as heat may compromise the effectiveness of vaccines (346). Like competitive exclusion, vaccination is most effectively used as a component in a comprehensive program of risk reduction practices.

Interest in the use of killed vaccines (bacterins) in poultry has been renewed in recent years by escalating concerns about *S. enteritidis.* Subcutaneous administration of adjuvanted oil-emulsion bacterins to laying hens has been reported to reduce significantly the incidence of fecal shedding and the numbers of *S. enteritidis* shed in the feces, the frequency of isolation of *S. enteritidis* from various internal tissues, and the incidence of production of eggs with contaminated contents following subsequent oral challenge (173, 174). Chickens vaccinated with bacterins have been reported to exhibit reductions in mortality, lesions, clinical signs, and organ invasion for up to 12 wk post vaccination when challenged with *S. enteritidis* by intravenous or intramuscular routes (441, 442). Field studies have associated bacterin administration with a reduced incidence of *S. enteritidis* infection in Dutch broiler breeder flocks (145), but could not document any significant effect of vaccination on the isolation of *S. enteritidis* from the environments of Pennsylvania laying flocks (122). Subunit vaccines, composed of *Salmonella* outer-membrane proteins administered with adjuvants or incorporated into lipid-conjugated immunostimulating complexes, have been efficacious against *S. enteritidis* in chickens (332) and against *S. enteritidis* or *S. heidelberg* in turkeys (62, 63). Another potential benefit from vaccination was evident in a study that reported reduced multiplication of *S. enteritidis* in eggs from bacterin-treated hens compared to eggs from control hens (238).

Live attenuated vaccines need to persist in tissues long enough to induce a protective immune response, but should be avirulent and eventually cleared from vaccinated birds. PT *Salmonella* vaccine strains attenuated by several different approaches have been tested for their protective efficacy in poultry. Oral or intramuscular administration of various *aroA* mutants of *S. enteritidis* (auxotrophs that do not grow well in vivo because of their inability to synthesize particular aromatic compounds) reduced organ invasion after intravenous or aerosol challenge and reduced fecal shedding, horizontal transmission, and egg contamination after oral challenge (27, 76, 77, 78). This protection has been found to persist for up to 23 wk after administration of the vaccine strain (79). An orally administered *cya crp S. typhimurium* strain (a double mutant with deletions of both adenylate cyclase and the cyclic AMP receptor protein) provided very strong protection against intestinal colonization and organ invasion by *S.*

typhimurium (204). A temperature-sensitive mutant (59) and a strain attenuated by repeated passage in chicken heterophils (282) have also been shown to protect chickens against *S. enteritidis* infection. Hassan and Curtiss (205) reported that vaccination of hens with an avirulent *S. typhimurium* strain reduced intestinal colonization of their progeny when challenged with virulent wild-type strains. Evidence for cross-protection by live vaccine strains against other epidemiologically important *Salmonella* serotypes has been inconsistent. An avirulent *S. typhimurium* vaccine reduced colonization, organ invasion, and egg contamination by *S. enteritidis* (206), but *aroA- S. enteritidis* strains did not cross-protect very effectively against *S. typhimurium* challenge (78). Several safety concerns about live *Salmonella* vaccines have been raised recently, based on evidence that some vaccine strains may be genetically unstable (19) and can be detected for longer than anticipated in vaccinated hens if sufficiently sensitive culturing methods are used (428).

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Dr. Nagaraja in a personal communication sums his years of research and field studies in the following statements. "I am a strong proponent of vaccines for salmonella. At the present time we have the emergence of antibiotic resistant strains of Salmonella ex. DT104, of Salmonella typhimurium, SE PT4, and penta-resistant strains of other salmonella. Poultry industry is constantly under the threat of losing the availability of antibiotics. Available tools are decreasing. We have to look for alternate ways which are successful in reducing the risk of spreading salmonella. Vaccines are one of the effective tools we have today both in human and animal industry.

If salmonella are cycling in a breeder flock the best way to stop cycling of salmonella in that flock is through vaccination. I have demonstrated this in many experimental trials both in turkeys and chickens. I understand the advantages of both killed and live vaccines. Both have their own inherent advantages and disadvantages. Nevertheless the use of vaccines will play a significant role in reducing salmonella in poultry. Birds vaccinated will be less susceptible for infection with Salmonella and shed less into the environment."

In addition to the above comments USDA approved vaccine efficacy studies by several commercial vaccines companies are being sent under separate cover. These studies represent many years of research, which have resulted in the granting of many licenses to manufacture and distribute under a USDA approval based on efficacy and safety in the US.

Experiences in use of vaccine in the field

Dr. Mike Opitz after many years of work as a field research person has made the following statement in recent personal communication, 2004.

"Inactivated vaccines have been a very effective means of reducing environmental contamination when used in combination with rodent control, sanitation and housing of SE clean replacements. The use of vaccines is one of the most prudent (effective, practical) SE risk measures to be taken for multiple in-line layer complexes. There is overwhelming information available from abroad and the US on its effectiveness and limitations. Vaccines have been consistently very successful in reducing detectable environmental contamination in our experience. The verdict on live vaccines is still out. I agree with the FDA proposal that vaccinations should not be required, but left to local veterinary/company decision makers."

Dr. Rich Dutton has shown that the control of SE cannot be done without vaccine use in conjunction with rodent control, dry cleaning, disinfection, and biosecurity measures. Killed and live Salmonella vaccines have been used to:

1. Eliminate an SE break in a large complex by testing, removing a flock, dry cleaning a house, and vaccinating the new flock. The 30 plus house complex is now negative and has been for several years.
2. Vaccinate all flocks going into a complex resulting in a conversion of many houses from positive to negative.
3. Revaccinate positive molted flocks with low incidence SE, which converted to negative and maintained their negative status until the end of lay.
4. Spot vaccinate resulting in a lowering of incidence to negative.
5. Vaccinate pullets during grow, move into a positive layer house in a complex and never have a positive SE isolation after 2 more flocks in the same house.
6. Vaccinate a positive pullet flock in a pullet complex and with cleaning, isolation and disinfection not have another occurrence in other houses or in the same house the next grow period or on the complex again.

The Pennsylvania PEQAP program has already reported the results of several years of vaccination. The data associated with that program was reported in the Maryland FDA SE open meetings.

International programs and benefits

The international community has had many experiences with Salmonella enteritidis PT4. Only in the past few years has England been able to reduce their problems by requiring mandatory vaccination of birds where eggs are being sent to the market for consumer use.

In Germany where just a few years ago the incidence of Salmonella was approaching epidemic proportions until a vaccine was required. Currently, as reported by the Euro- Surveillance Monthly data from 15 countries in Europe report the incidence of PT4 has declined from 61.8% in 1998 to 32.1% in 2003.

To be included under separate cover will be efficacy data concerning the use of vaccines by commercial companies in Europe.

Summary

The evidence for the efficacy of the Salmonella vaccines although not perfect is very good under the most challenging of research and field circumstances. When either live or killed vaccines are used with appropriate programming, and associated with bio-security measures, testing, and rodent control the result will be a reduction of SE. An SE program is always a work in progress and not a course to elimination.

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