RESEARCH REPORT
PORK SAFETY

Title: Effects of egg yolk antibodies and antibiotic regimens on carriage and shedding of *Salmonella* Typhimurium - **NPB #03-120**

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Abstract: In two experiments, egg yolk antibodies with specific activity against salmonella were tested to determine if inclusion in feeds decreased carriage and shedding of salmonella in pigs. In Experiment 1, dried egg yolk containing anti-salmonella antibodies (EYA) was included in the diet of weaned pigs and tested against other diets containing either apramycin followed by carbadox, or oxytetracycline, or dried egg yolk without anti-salmonella antibodies, or spray dried porcine plasma, or no additives (control). One week following initiation of feed treatments, pigs were challenged with *Salmonella* Typhimurium. Fecal samples were obtained prior to initiation of treatments and at various intervals over 118 days for recovery of salmonella and non pathogenic *E. coli* to determine shedding patterns and for antibiotic resistance analysis. In Experiment 2, market-age hogs were fed diets containing EYA, or dried egg yolk without anti-salmonella antibodies, or no additives (control), or were subjected to ceftiofur injections. Two days following initiation of treatments, pigs were transported to another facility, challenged with salmonella, and mixed to simulate shipment and lairage prior to slaughter. Fecal samples were recovered prior to initiation of treatments and transport, and 24 and 48 hours following shipping for recovery of the challenge organism. In Experiment 1, antibiotic regimens decreased the percentage of pigs shedding salmonella, however the other diets, including that containing EYA, did not affect salmonella shedding in young pigs. Resistance to some test antibiotics was increased in *E. coli* from pigs consuming diets containing antimicrobial products, whereas other treatments did not appear to affect resistance patterns. In Experiment 2, EYA inclusion in feeds did not affect shedding of the challenge organism compared to the control diet. These studies indicate that in-feed use of dried egg yolk containing anti-salmonella antibodies is not effective in controlling salmonella shedding in either young or market-age pigs.

Introduction: Foodborne bacteria continue to cause concern among some consumers of pork products. Significant pathogens, such as *Salmonella enterica* Typhimurium, may reside in swine, producing few outward signs, and then spread rapidly among animals upon transport, in some cases contaminating processing facilities, equipment, and pork products. Thus strategies to reduce carriage and shedding of Salmonella by swine are currently being sought as part of pre-harvest food safety efforts. We have shown that nontherapeutic and therapeutic antibiotics can reduce shedding of salmonella in swine; however, concerns that such use may select for antibiotic resistant bacteria may eventually limit producer access to some antibiotics, and/or may cause additional concern among consumers of pork. Thus far, dietary strategies, including use of probiotics and prebiotics have not been consistently shown to provide protection against foodborne pathogens, and vaccination strategies appear to offer only limited effectiveness against colonization of pathogens in the gut, and offer little protection for the market-bound animal. Recently, however, investigations of egg yolk antibodies (EYA) have provided hope for a novel approach to limit colonization and/or proliferation of some pathogens in animals. To generate these products, laying hens are challenged with specific antigens derived from the offending organisms, and the hens subsequently produce significant amounts of antibodies against those antigens in their eggs. As such, inclusion of the EYA product in animal feeds might then provide protection against gut-borne pathogens.

We have in the past used disease challenge models in pigs to determine effects of production practices and various therapies on shedding of salmonella and on antibiotic resistance of those and other bacterial types. With this model, we were able to observe shedding patterns and effects of various treatments on the development of antibiotic resistance and the transfer of resistance. We used a similar model in this study to test the efficacy of EYA to reduce carriage and shedding of salmonella in pigs.

Objectives: Objectives for this study were: 1) to determine effects of in-feed supplementation of specific EYA and antibiotics on colonization and subsequent shedding of *Salmonella* Typhimurium in young swine and on antibiotic resistance patterns in fecal bacteria and 2) determine the effects of EYA inclusion in feed prior to shipping on shedding of *Salmonella* Typhimurium in market pigs.

Materials and Methods: Experiment 1 consisted of two replicate trials using a total of 144 weaned pigs, verified to be salmonella free and with no history of antibiotic use. Pigs were moved to a research facility near the campus of the University of Tennessee, Knoxville and randomly divided into 6 groups, with groups being housed in identical SEW nursery rooms with separate environmental and waste removal systems. Strict biosecurity was maintained between rooms to limit transfer of organisms between treatment groups. Pigs were randomly assigned to feed treatments including: 1) control (no antibiotic, no EYA) meeting NRC requirements; 2) a diet containing apramycin at 150 g/ton, for 14 days, followed by carbadox at 25g/ton until pigs reached approximately 75 lb.; 3) a diet containing oxytetracycline at 100 g/ton of feed until pigs reached approximately 75 lb, 4) a diet containing anti-salmonella EYA at a level of 5 lb/ton of feed for 14 days, 5) a diet containing a commercial dried egg yolk, lacking specific antibodies to salmonella, and 6) a diet containing spray dried plasma protein at a 5% level in the diet for 14 days. Diets were formulated to contain similar energy, lysine, methionine, and total protein. EYA targeting *Salmonella* Typhimurium antigens, which included fimbrial (F), outer membrane protein (OMP), and lipopolysaccharide (LPS) antigens, were generated at the University of Manitoba, using procedures already established at that site. Briefly, EYA were generated by initially inoculating chicken hens

with isolated and purified Salmonella antigens. Hens were subjected to a second, booster injection with those same antigens three weeks later, and following confirmation of sufficient antibody production, eggs were collected and the yolks extracted and dried for use in the study.

On day 7 following initiation of feed treatments, all pigs were challenged intranasally with approximately 10^6 colony forming units of *Salmonella* Typhimurium derived from a confirmed case of swine salmonellosis and identifiable via a nalidixic acid resistance marker. Fecal samples were collected prior to application of feed treatments, just prior to salmonella challenge, 24 hours postchallenge and at various time points over the following 118 days. Fecal samples were cultured for isolation of *Salmonella* and non-pathogenic *E. coli* using standard enrichment and microbiological recovery techniques, to determine shedding (*Salmonella*) and antibiotic resistance patterns (*Salmonella* and *E. coli)*. From each sample, a maximum of 4 *Salmonella* Typhimurium, and 4 *E. coli* colonies were randomly selected and each isolate was tested for resistance to apramycin, oxytetracycline, and carbadox, using a broth dilution minimum inhibitory concentration (MIC) procedure. Prevalence of the challenge organism was determined at each sampling period to characterize treatment effects on colonization and shedding of *Salmonella*. Health status and performance of pigs were monitored via fecal scoring, body temperature, incidence of disease, weight gains, and feed efficiency at predetermined intervals during the study. Data were analyzed using Proc Mixed procedures of SAS.

Experiment 2 consisted of two replicate trials, each using a total of 32 market-age pigs, confirmed to be free of salmonella. Pigs were divided into 4 treatment groups which included: 1) control group fed a typical finisher diet meeting NRC recommendations, 2) a group fed a similar diet but with the inclusion at a level of 5 lb/ton of feed of a dried egg yolk product containing anti-Salmonella EYA, 3) a group fed a similar diet but containing a commercial dried egg yolk without anti-salmonella antibodies, at a level of 5 lb/ton of feed, and 4) injection of ceftiofur sodium at 2.27 mg/lb of body weight on each of two days prior to shipping.

Forty eight hours following initiation of treatments, pigs were challenged with the same *Salmonella* strain as in Experiment 1, transported to finishing facility located approximately 1.5 hours from the initial site, randomly mixed and divided into two pens of 16 pigs each, with groups having direct fence line contact, to simulate transport, mixing and holding at a slaughter facility. Fecal samples were obtained from each pig prior to initiation of treatments, prior to challenge and transport, and 8, 24, and 48 hours post transport, for the recovery of the challenge organism. Salmonella data were analyzed as in Experiment 1 to determine efficacy of each treatment to reduce shedding

Results: In Experiment 1, antibiotic treatments resulted in a lower percentage of pig shedding salmonella postchallenge (Figure 1), compared to the control, spray dried porcine plasma, dried egg yolk, and EYA diets. All test groups showed a decrease in shedding over the length of the study. Treatments did not appear to affect response of pigs to the salmonella challenge, with most pigs demonstrating an increase in rectal temperature following the challenge, followed by a return to normal body temperature within a few days.

Resistance of recovered Salmonella to antibiotics tested in this study was not affected by treatments (data not shown). Resistance to apramycin was higher in *E. coli* recovered from pigs receiving apramycin in their diet, and resistance to tetracylines was higher in *E. coli* recovered from pigs on antibiotic and dried egg yolk diets, compared to the control and EYA diets (Figures 2 through 4). Resistance to carbadox was not affected by treatments.

In Experiment 2, shedding of Salmonella was not different between treatments (Figure 5). Most pigs shed salmonella within eight hours of challenge, with a significant decrease in the percentage of pigs shedding salmonella at 24 hours post transport, followed by a subsequent rise in the percentage of pigs shedding at 48 hour post transport. This pattern was noted across all treatment groups.

Discussion: Our results indicate that in-feed inclusion of dried egg yolk containing specific antibodies is not effective in reducing shedding of *Salmonella enterica* Typhimurium in weaned pigs, whereas inclusion of antibiotics, including apramycin followed by carbadox or oxytetracycline appeared to reduce shedding. These results are in contrast to those noted for egg yolk antibodies with specific activity against enterotoxigenic *E. coli* in pigs and mice. We suspect that the invasive nature of salmonella in pigs may result in a systemic infection, by which organisms bypass the gut, thus avoiding feed based treatments, and move through vascular and/or lymphatic routes directly into the colon, thus contaminating feces just prior to excretion. Studies have indicated that market hogs can become rapidly infected (within a few hours) by salmonella upon transport and lairage at slaughter facilities and other investigations demonstrated that a rapid movement of the organism occurred within the animal, resulting in deposition in various tissues and organs, including the upper and lower GI tract and lymphatic tissues, within a few hours following challenge.

As was noted in several investigations by our group and others, use of nontherapeutic levels of antibiotics in diets resulted in an increased resistance to some of the test antibiotics (apramycin and tetracycline) by *E. coli*. We have consistently shown a rapid increase in apramycin resistance, when that product is used, and a subsequent decrease in resistance to that drug following its withdrawal. As apramycin is primarily used in younger swine, this transient rise in resistance would likely have little impact on swine of market age or resulting pork products. Also in agreement with our previous studies, we noted little change in resistance of salmonella due to feed treatments, including treatments that contained antibiotics.

From this study, we conclude that use of egg yolk products containing antibodies specific for salmonella antigens is not an effective strategy for reducing colonization and shedding of salmonellae in weaned pigs. Antibiotic products continue to present a more effective means to reduce the incidence of specific pathogens, including foodborne organisms; however, use of those products can result in increased antibiotic resistance, primarily noted in nonpathogenic, naturally occurring *E. coli*.

Lay Interpretation: Salmonella may reside in pigs during all stages of growth and may later contaminate pork products, thus presenting a foodborne hazard for humans. Often, salmonella are passed among hogs during transport to and holding in slaughter facilities, thus increasing the potential for contamination of pork products. This research tested an egg yolk product that contained anti-salmonella antibodies as a feed additive to determine if such products can reduce salmonella in pigs. Experiments were conducted to determine the effectiveness of the egg yolk product in young pigs and in older hogs bound for market. The results of these studies indicate that egg yolk antibodies are not an effective means of reducing salmonella in pigs, whereas diets containing some antibiotic products can reduce salmonella in younger pigs. However, as antibiotic use can lead to drug-resistant bacteria, the search for alternative feed additives or management schemes that reduce foodborne bacteria should remain a priority for the industry.

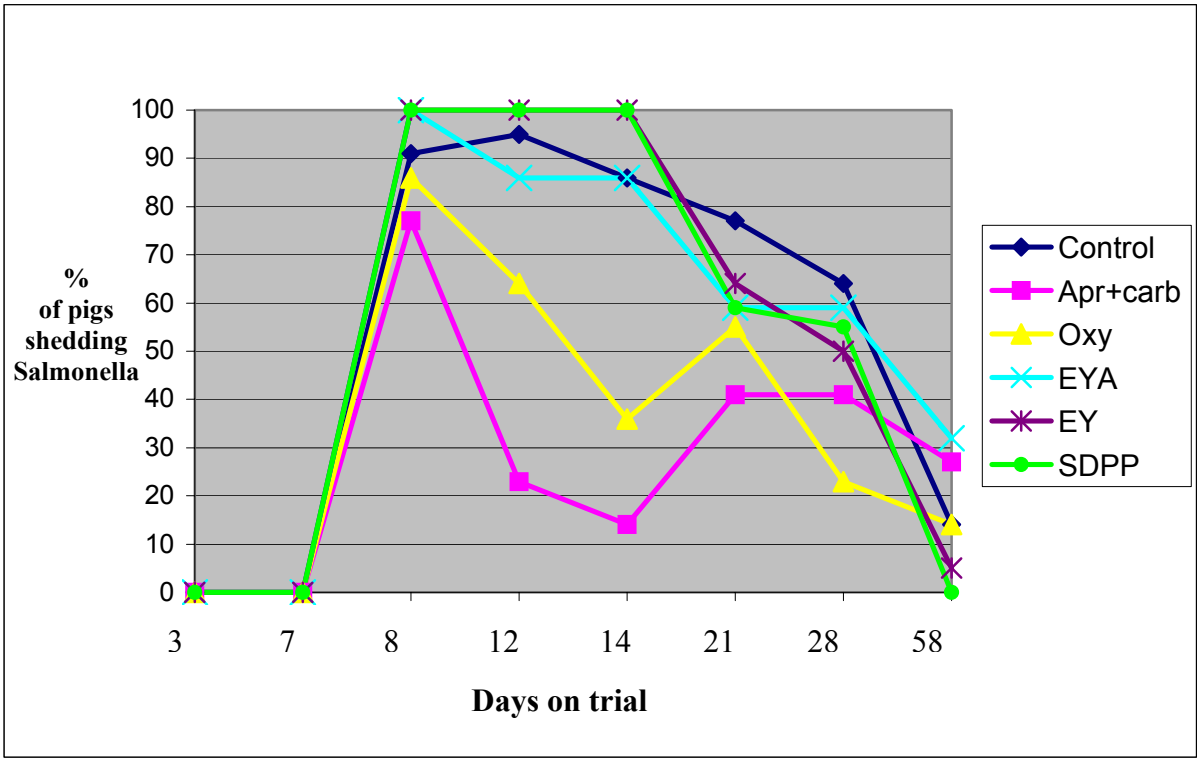

Figure 1. Effects of treatments on percentage of weaned pigs shedding salmonella across sampling days in Experiment 1. Pigs were challenged with salmonella on day 7. Control = phase diet without antibiotics or egg yolk products, Apr+carb= apramycin followed by carbadox, Oxy = oxytetracycline, EYA = egg yolk containing anti-salmonella antibody, EY = egg yolk without anti-salmonella antibody, SDPP= spray dried porcine plasma.

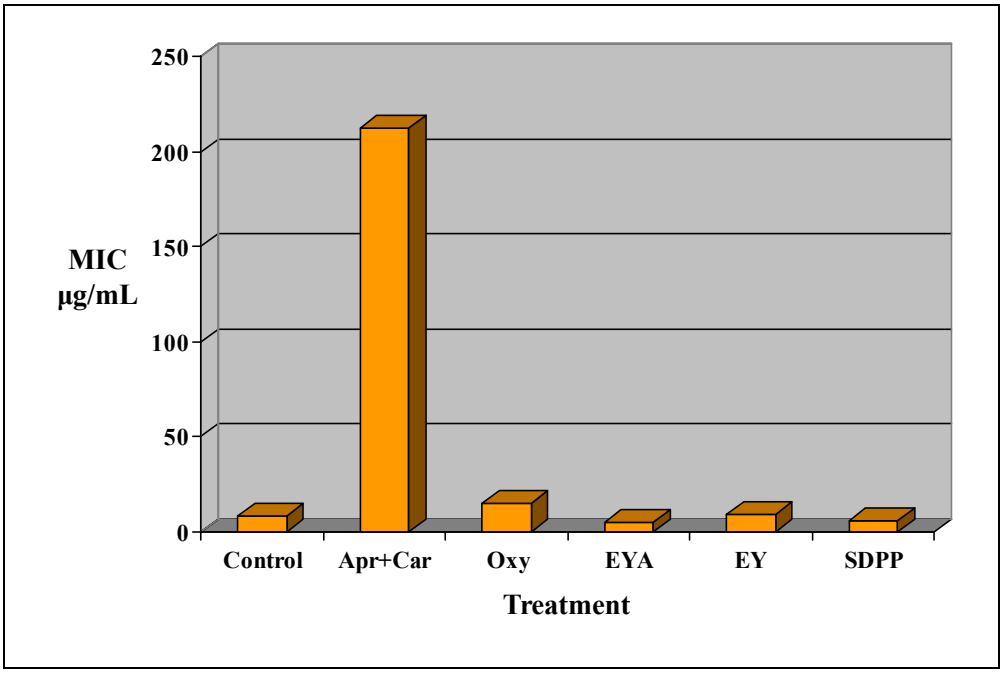

Figure 2. Sensitivity to apramycin by *E. coli* recovered from weaned pigs 14 days following initiation of feed treatments in Experiment 1. Data are means of minimum inhibitory concentrations (in micrograms per milligram) of apramycin. Control = phase diet without antibiotics or egg yolk products, Apr+carb= apramycin followed by carbadox, Oxy = oxytetracycline, EYA = egg yolk containing anti-salmonella antibody, EY = egg yolk without anti-salmonella antibody, SDPP= spray dried porcine plasma.

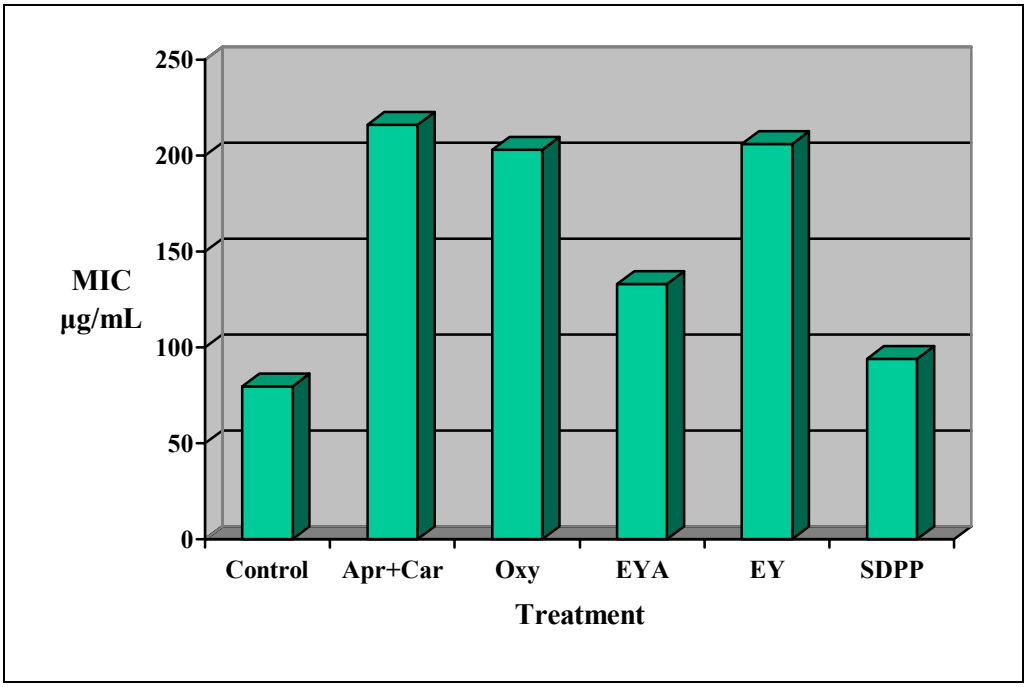

Figure 3. Sensitivity to Oxytetracycline by *E. coli* recovered from weaned pigs 14 days following initiation of feed treatments in Experiment 1. Data are means of minimum inhibitory concentrations (in micrograms per milligram) of oxytetracycline. Control = phase diet without antibiotics or egg yolk products, Apr+carb= apramycin followed by carbadox, Oxy = oxytetracycline, EYA = egg yolk containing anti-salmonella antibody, EY = egg yolk without anti-salmonella antibody, SDPP= spray dried porcine plasma.

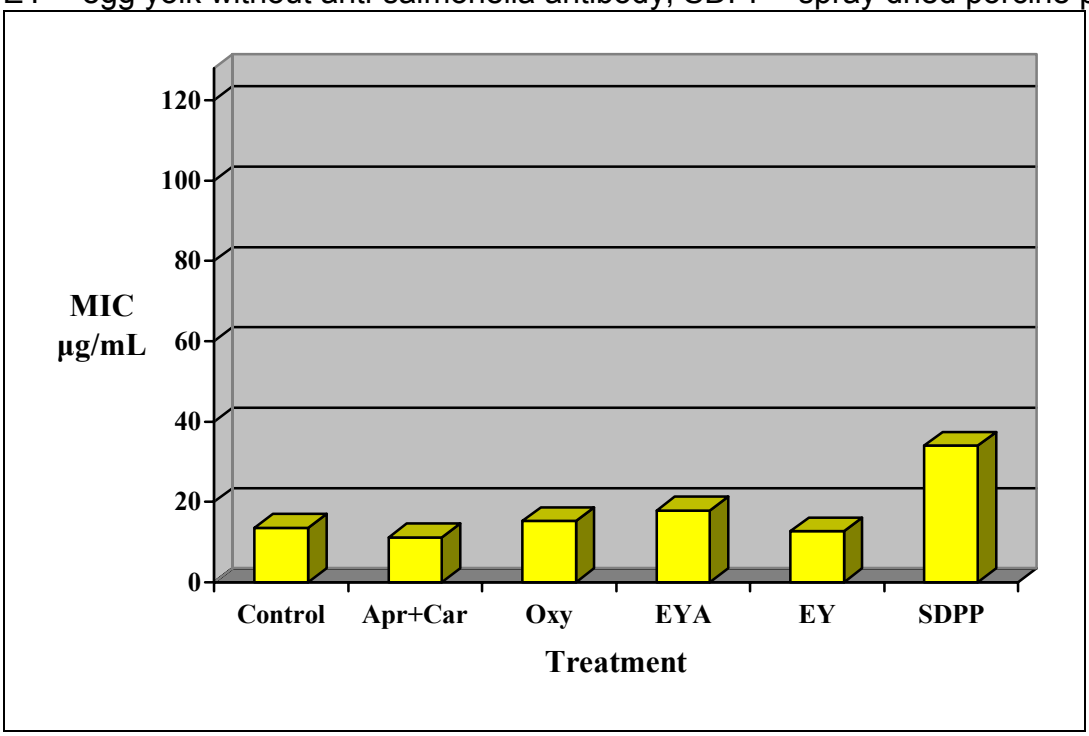

Figure 4. Sensitivity to carbadox by *E. coli* recovered from weaned pigs 14 days following initiation of feed treatments in Experiment 1. Data are means of minimum inhibitory concentrations (in micrograms per milligram) of carbadox. Control = phase diet without antibiotics or egg yolk products, Apr+carb= apramycin followed by

carbadox, Oxy = oxytetracycline, EYA = egg yolk containing anti-salmonella antibody, EY = egg yolk without anti-salmonella antibody, SDPP= spray dried porcine plasma.

Figure 5. Effects of treatments on percentage of market pigs shedding salmonella across sampling days in Experiment 2. Pigs were challenged with salmonella and transported 48 hours following initiation of treatments. Control = finisher diet without antibiotics or egg yolk products, Egg yolk= dried egg yolk without anti-salmonella antibodies, EYA = egg yolk containing anti-salmonella antibody, Ceftiofur = IM injection of ceftiofur sodium on two days prior to challenge and transport