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FOOD SAFETY AND INSPECTION SERVICE

ADVANCES IN PRE-HARVEST REDUCTION OF *SALMONELLA* IN POULTRY

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MODERATOR:

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Food Safety and Inspection Service

C O N T E N T S

P R O C E E D I N G S

PROBIOTICS AND COMPETITIVE EXCLUSION

DR. BAUER: I want to welcome everybody back to the second day of Advances in Pre-Harvest Reduction of *Salmonella* in Poultry.

Our first session is Probiotics and Competitive Exclusion. For those of you that don't know me, my name's Nate Bauer. I'm a scientific liaison with the Office of Public Health Science.

Our next speaker, I actually team taught with -- actually he did all the teaching and we just hung around and watched him teach FSIS veterinarians. When Billy Hargis was at Texas A&M University, he would come over and talk about pre-harvest food safety issues to FSIS veterinarians at our training center there in -- collocated on the Texas A&M University campus; but we lost Billy Hargis to the University of Arkansas.

Anyway, Dr. Hargis received his Master's of Science of Poultry Science at the University of Georgia, his DVM and PhD at the University of Minnesota. He was a professor at the Department of Veterinary Pathobiology and Poultry Science at Texas A&M University. That was before joining the Center of Excellence in Poultry Science as a Professor and Director of the University of Arkansas Poultry Health Research Laboratory.

Dr. Hargis is a Diplomate of the American College of Poultry Veterinarians. He teaches in the undergraduate and graduate Poultry Science Program. He has an active research program in the area of poultry health and animal and food safety intervention with interests in poultry immunology and endocrinology.

His laboratory has been recognized by several awards and Dr. Hargis has advised or co-advised more than fifty masters and doctor of philosophy students and has published numerous manuscripts and book chapters relating to food safety and poultry health and poultry physiology.

Dr. Hargis is going to talk to us about gut maturation, prebiotics, probiotics and symbiotic interventions to reduce *Salmonella* in poultry. Dr. Hargis.

GUT MATURATION, PREBIOTICS, PROBIOTICS AND SYNBIOTICS - INTERVENTIONS TO REDUCE *SALMONELLA*

DR. HARGIS: Thank you, Dr. Bauer, and I appreciate the invitation to be here, it's an honor to be here. I bring you greetings from the University of Arkansas and the Poultry Science Center there, where I am very pleased to be.

I was going to stop Nate because he was cutting in to my time. This is the Poultry Health Research Lab there on the -- just off campus, they don't let people like me work on campus, you know. We've got a great group of people

that involves a number of scientists, a number of laboratories that are kind of working as a team. I'm presenting a lot of work that is certainly not mine. These guys deserve a lot of credit. I also think I ought to mention that a lot of our work really is an outgrowth from work that was USDA-ARS work in College Station a hundred years ago with Dr. David Nisbet, Dr. John DeLoach and the late Dr. John Corrier. Really fun times with those guys. Collaborations with Allen Byrd and David Caldwell are still intermittent and collaborations with and funding from numerous poultry companies and some collaborations with FSIS over the years, especially Dr. Robert Brewer.

Probiotics are defined as live microbial food supplements which benefit the host by improving intestinal microbial balance. There's a whole lot of things published on probiotics now and tremendous increase in research in this area just during the last five years or so. In addition to excluding pathogens such as *Salmonella*, there's evidence that certain probiotics can increase absorptive capacity, change the protein and energy metabolism, influence fiber digestion, changes in energy conversion, gut maturation and even immuno-stimulation.

There's a tendency to regard all microorganisms as harmful and nothing could be further from the truth. The number of non-pathogenic species far exceeds the number of

pathogenic species and many of the known bacteria are in fact useful, or even essential for the continued existence of life.

A lot of work now in the area of host-bacterial mutualism. Studies that are reviewing how the gut microflora has actually co-evolved with vertebrate animals. Now there are over at least 800 species of bacteria and probably a lot more than that if the truth be known and most of these are completely mysterious in the gut. We've got a long way to go before we understand the microbiome in the gut. It may harbor the genes that are located in these bacteria in the gut of animals, may harbor a hundred times more genes than the animal actually has and there are thoughts that it's fortunate that the vertebrate animals do not have to co-evolve all the functions that are encoded by the genes of the microflora. We're talking millions of years of evolution and animals could develop the means for supporting complex and dynamic consortia of micro-organisms and there's growing evidence that the animal can actually influence the microflora that colonizes the gut through the secretion of selected mucins and so forth in the gastrointestinal tract.

The gut microflora -- the size of the population is staggering. Up to a hundred trillion organisms in humans, perhaps five trillion in poultry. Bacteria living

in the gut achieve the highest cell densities recorded for any ecosystem on earth. This is ten times the bacterial microflora in the gut out number the somatic cells, the cells of the animal, by more than ten-fold. It's staggering. And it's important.

Early probiotic work really began in about 1972 with work by Nurmi and coworkers and basically what they did was showed that microflora from the gastrointestinal tract of adult healthy birds could actually prevent or reduce colonization by *Salmonella* in young chicks, prophylactic administration, if you will.

Since then, that's been supported by a great number of studies, undefined microflora cultures have been very useful. Increased productivity of poultry has been shown in a large number of refereed manuscripts over the years and, just as I mentioned, during the last five years there's been an explosion of research. Mostly NIH human/animal model type studies working with a group of bacteria known as lactic acid bacteria and the lactic acid bacteria include lactobacilli such as yogurt type cultures but many, many other lactobacilli that are adapted to ecosystems such as the oral cavity, the intestinal tract and so forth and different strains are able to colonize and do things in different parts of the animal. So when we say lactic acid bacteria, we're talking about organisms that are

closely related to lactobacilli.

The reports of benefits for enteric bacterial and viral diseases -- viral diseases in the human/animal model work, and also in children, reduced effects of mycotoxins, cancer intervention, increased absorption of macrominerals -- phenomenal things that are being described in the literature these days. Crazy examples, treating vaginal yeast infections in women, prevention of dental caries in people, allergies, autoimmune diseases, metabolic defects such as pancreatic insufficiency, really wide-ranging references talking about augmentation or modification of immune responses. And of course, a lot of studies that show increased productivity in poultry.

Now that sounds wonderful but we all know that there's been enormous numbers of failures associated with probiotics in poultry. Certainly the complex microflora or undefined cultures, things that are amplified from undefined cultures derived from healthy birds have been more efficacious in some studies in the past. They're presently not allowed in the United States because of the perceived risks. We could talk a long time about that.

Many of the products that are on the market world wide -- and we've surveyed a number of products from Asia, Latin America and the United States and find that a great number of the products that are commercially available

contain far fewer organisms than what the label would indicate and it does appear that live organisms are important for most of these probiotics to work. Many of the products that are on the market that do contain live organisms contain *Lactobacillus* and related species that are actually selected for yogurt fermentation, dairy product fermentation. Many of these are actually thermophilic organisms and really don't grow well at the body temperature of domestic animals. So it's unlikely that they're going to be very good as competitive exclusion or probiotic cultures.

We've had problems with antimicrobial or disinfectant interference with the number of effective products that have been on the market where -- trouble getting them to work because of these types of problems and some effective organisms are not compatible. That's something that I'll show you just a little bit of data that suggests that that's true and it's something that we were surprised by in our research.

A high dosage -- as you review the literature, it appears that a relatively high dosage of microorganisms is necessary for efficacy with many of the cultures that have been demonstrated in research laboratories to be effective. And, in general, it looks like around 10 to the 6 colony forming units, that's a million live organisms per milliliter or per gram of feed, are necessary to have the

effect. It's not at all clear whether it needs to be there constantly or whether intermittent administration is sufficient with different products that have had efficacy experimentally. Some products, you get some efficacy at 10 to the 5th or 100,000 organisms per milliliter or per gram of feed but in general, the point is you need a large number of live organisms to have the effect.

There's also a need for readministration after gastrointestinal disturbance such as unintentional feed restriction or therapeutic antibiotic use. And that comes back to cost and the ability to -- cost and -- not only in terms of product cost but in terms of labor and so forth associated with administration. What we did as a group about five years ago is we selected for facultative anaerobes. The reason for this was we made the presumption that strict anaerobes that truly cannot tolerate the presence of oxygen, are going to be expensive to propagate, package, and distribute under strict anaerobic conditions. And that they would be difficult to administer to poultry if we could not expose them to oxygen, so we started working with only facultative anaerobes.

Some products that have been efficacious in the past have contained very fragile organisms that were not very stable, even in a frozen state, so we looked at freeze tolerant organisms as a -- hopefully a selection for hardy

organisms. We looked at organisms which would grow in inexpensive medium, would be batch cultured, would compete with pathogens in vitro and then we finally selected for organisms that were compatible with each other. This represents a lactic acid bacterial colony that we selected and is typical of the others overlaid with *Salmonella* Enteritidis and they clearly produce products that retard growth of the *Salmonella* in vitro.

We -- Dr. Richard Ziprin back in the early to mid-'90s had some work that I'm not sure was ever actually published but it indicated that undefined cultures could sometimes -- not all undefined cultures but direct cultures that were briefly amplified in vitro from cecal content of adult healthy animals, these microflora could actually displace *salmonella* infections, so we know it's possible to have a therapeutically efficacious culture. Those cultures never bred true, shall we say, they were not propagatable. We lost the ability to exclude *Salmonella* from infected birds. But nevertheless it shows that -- it showed us that it was possible to achieve and so in our evaluation screening, we took it one step higher and we actually screened by inoculating chicks with *Salmonella* first and then treating at some point after, sometimes two hours, sometimes four days and so forth, to see if we could actually exclude or treat therapeutically birds that were

already infected.

The individual isolates that we selected were screened in groups of chicks that were infected with *Salmonella*. Many of the organisms that were effective in vitro were not effective in the live animals. Several combinations where we did see efficacy of single isolates, when we put those in combination, we lost the efficacy of the culture. They appeared to antagonize, so bugs that worked well individual didn't work well together -- really a frustrating situation. Eventually we found a group of organisms that did cooperate apparently and worked well together.

Let's go back, sorry. The culture that was developed contains eleven different isolates of lactic acid bacteria. Our laboratory designation was B11 and what we did was we infected the birds and then treated the birds with one of two doses as shown here and then looked at colonization at either 24 hours or 72 hours. And this is the percent *Salmonella* recovery from ceca pouches of the challenged birds. The control birds at 24 hours, very large positive population and considerably less in the treated groups regardless of dose; and a similar response, a little higher infection in the controls by 72 hours and a nice reduction in the treated birds. We saw those in a number of experiments and this was very consistent and reproducible in

our hands.

We looked at time course, just to ask the question of how fast is this acting and I'll just give you an example of one of those experiments. Looking just at the combined data here, control versus treated birds, I'll just move you over to 12 hours and we've -- we're 77 percent positive in the control group and 77 percent positive in the treated group but look what happens between 12 hours and 24 hours. The controls increase to about 96 percent in this particular experiment and the treated birds were 15 percent. Very rapid responses and I'll be honest with you; I'm not completely convinced that this is through conventional competitive exclusion mechanisms. We're working on that now.

We looked at spray application and with technology that was developed in the mid-'90s and the controls here were not treated or treated actually with water and these birds were sprayed with the B11 culture either 24 hours and 72 hours. We still see efficacy. We moved this to a field trial with commercial turkeys and this was kind of an ambitious experiment. We screened large numbers of turkey flocks to find turkey flocks that were absolutely red hot, I mean very, very infected as indicated by at least 6 out of 8 drag swabs positive for *Salmonella*. And the idea was is that if we really reduced or stopped shedding two weeks

before slaughter, the environment should go almost negative by the time the birds were transported to processing. And -- because we know that *Salmonella* half lives in the environment are such that that would be true. We identified a very large number of turkey flocks that were positive. We treated these for three consecutive days and then re-evaluated the environment right before slaughter, just immediately before live haul. And, in other words, about two weeks before live haul, the birds were treated and then immediately before live haul, we evaluated the environment again. Here you see the control group and we're about 85 percent positive and a non-meaningful reduction in *Salmonella* before live haul. We had a group that was treated with an organic acid preparation which appeared to be very effective as an antimicrobial compound in water and this had no meaningful effect. We treated another group of farms with the B11 culture and similarly we saw no meaningful reduction in the *Salmonella* recovery. But, when we pretreated with the organic acid mix and then treated with the B11 culture, we saw a marked and significant reduction in *Salmonella* in these flocks. We don't know why that is, but the assumption that we're making and the reason we did it was because the folks at Bayer sponsored some research showing that biofilms could capture beneficial bacteria in the drinking water. Biofilms that developed in

the drinking water lines and that shocking the biofilm with a sanitizer could increase the deliver of beneficial bacteria to birds back in the early '90s. That was the hypothesis we were working on and to this day we have not tested that hypothesis.

We did a similar trial with broilers and in this case we simply used written instructions to communicate with the live production people and provided the materials for treatment. We did the cultures for them after they took the drag swabs and block one -- and these also are not typical farms. These were highly selected. These were selected to be really high, highly contaminated farms. Block 1 we saw a tremendous reduction in *Salmonella* recovery. Block 2 nothing happened. Block 3 a reduction. Block 4 a reduction. So it's encouraging. Why? We don't know. Compliance may be a big part of this, we don't know.

We have a very recent commercial trial that we're just in the middle of trying to dissect and analyze the data where an entire complex was treated and *Salmonella* recovery in this complex from carcass rinses declined from about 45 percent at the beginning of the study to about 0 percent, actually 0, during two consecutive cycles. Now, whether or not it's necessary to -- whether there's an added effect of the second cycle treatment or not, we don't know. It may be part of a learning curve, getting everyone to participate

and do it correctly. We just don't know.

Drag swab recoveries decreased markedly through slaughter. There we went and visited one of the two farms where drag swabs did not decrease in recovery incidents and there was a non-compliance issue where the farmer thought it was a vaccine and was going to hurt his production and threw it away. Data from this trial are still being analyzed.

Production. One might suspect -- how am I doing on time? We're good. One might suspect that you would see a production benefit if you actually are excluding low level pathogens from the gut of birds. And production is important because unless you can achieve some production value, it's going to be hard to get this type of technology adopted in the U.S. market, I think. We looked at -- one of the first things we did was we looked at an idiopathic diarrhea feed passage problem that was pretty endemic in Arkansas at this -- at the time of the study. Very predictable problem that was occurring regularly and like many disease problems, it kind of went away over time, we don't know why.

But what we did was a little different. We decided to place pens right down the center of the commercial turkey house and expose them to everything we could possibly expose them to that's going on in the real world. Each pen had its own feed and water source. Birds

were wing tagged so that we knew the birds were staying in the pens and other birds weren't getting in. And we compared two cultures, the B11 culture and a simple culture that are components of the larger culture. Each of these treatments contained four replicate -- four replicate pens for each of these, the control, the probiotic -- simple probiotic B11 culture or a combination of amprolium and neomycin which was not our choice, it was what the company was actually doing. We did this precisely -- treated these groups or these pens precisely as the company was treating the block at large. And the results were fairly impressive.

The B11 culture was significantly better than the controls. Not different than antibiotic treatment and not different from the simple probiotic culture. This was repeated in four studies and this was recently published.

We looked at another field trial that hasn't been published yet where we looked at 118 commercial flocks, they were either treated or untreated and they were randomly assigned within service -- technical service person areas. So it represented the whole complex. And we generated data that looked like this. The treated birds are in the green and yellow here and this bar represents the mean of all the treated blocks versus the control. And you see data all over the place and that's really important when you're looking at performance because no product, no vaccine, no

medication provides ventilation and management, does not cure hemorrhoids or glaucoma.

(Laughter.)

DR. HARGIS: But when you recrunch data, we see a significant increase in body weight in the treated groups, that translate into an increase in average daily gain. A numerical drop in feed conversion rate but when translated to a cost per kilogram, that was highly significant and quite meaningful. So it makes sense that we might be doing that.

Broiler performance is fairly similar so far but it seems to be highly variable. Birds that are really good flocks don't seem to respond in terms of performance but at the same time, if you look at antibiotic growth promoting drugs, you often don't see performance value in really good flocks or under really good conditions so that may be the truth of the matter. Compliance seems to be a major difficulty for commercial applications and increased performance may increase the acceptability of doing something like this.

Prebiotics, I'm just going to mention real quickly, may selectively enhance beneficial bacterial populations in the gastrointestinal tract, that's what you're trying to do. Provide nutrients for the beneficial microflora, hopefully selective nutrients for beneficial

organisms. We've been able to see some good benefits with certain prebiotics. Lactose is one we worked a lot with and I'll just show you one trial that was done in commercial turkeys using the model that I showed you before. By 26 days of treatment, the two groups that received dietary lactose at .1 percent in the feed and the B11 culture were 173 grams heavier than the controls. We -- at the -- at 26 days we had to release these birds from the pens, they were light banded and released into the general population so no further treatment and that translated to somewhere close to a pound increase in body weight over the controls at the time of live haul, when we captured those birds and reweighed them. So, it's fairly exciting that we might be able to do something important. And again, very, very low levels of lactose here.

Ongoing work, we're looking at lactic acid bacterial isolates with increased efficacy or we're looking for them, I should say, and perhaps improved combinations. We know that's important. We're looking at prebiotics with multiple functions. The idea here is to provide more than a carbon source to provide the other nutrients that bacteria need. How much progress we can make, I don't know. And a lot of work in our laboratories is now focused on the mechanism of action, how this is actually occurring.

Thank you so much for your time and attention.

(Applause.)

DR. BAUER: Okay. Thank you very much, Dr. Hargis. We'll have the panelists -- we'll have the speakers from this session appear for a panel question and answer session when Dr. Morishita, Dr. Bailey and Dr. Byrd are through with their presentations. We do have Dr. Bennett on the front row keeping track of time and she'll flash a two minute warning to the speakers and then a no time left to the speakers.

Our next speaker is Dr. Teresa Morishita. She's a professor and extension poultry veterinarian at the Ohio State University. She received a bachelor's, and a master's in animal science from the University of Hawaii. Dr. Morishita subsequently received a DVM and Master's of Veterinary Preventive Medicine from the University of California at Davis. And then completed her residency in Avian Poultry Medicine at the University of California at Davis. And she is a Diplomate of the American College of Poultry Veterinarians. Dr. Morishita later received her PhD in comparative pathology from the University of California Davis and she has previously worked for ConAgra Butterball Turkey Company in Turlock, California and Eli Lilly & Company. And she has had her own private practice in California. And she has recently returned from Alaska on a bear project, is that correct? Okay.

Thank you very much. Dr. Morishita.

MANAGEMENT PRACTICE IMPACT ON THE OUTCOME OF PROBIOTIC INTERVENTIONS IN BROILERS

DR. MORISHITA: Okay. Today I just wanted to share with you some of our preliminary studies that we did in California as well as in Ohio regarding management practices impact on the outcome of probiotic interventions in broilers. As you know, we are looking at *Salmonella*, the gram negative bacteria, primarily to reduce it for our producers in terms of for food safety in human illness. You learned about that yesterday.

Working as an extension poultry veterinarian, we could either target the pre-harvest or post-harvest areas and we decided to focus on the pre-harvest aspects. In looking at the pre-harvest food safety aspects, you want to take a look at the bird itself in poultry production. We took a look at the chickens, we know that it can spread horizontally and what kind of factors can impact colonization of the gut. We also had to look at the egg transmitted factors so going to the hatchery, doing air quality monitoring, especially when they're pipping to see the amount of *Salmonella* that could be harvested at the hatchery and that way we could probably gain some insights into how we can better manage or reduce *Salmonella* in our flocks.

For the chickens, again, looking at the environment and food, we know that we heat treat food so that shouldn't be a source of *Salmonella* but we have to look at the contamination factors around that, either from rodents or from the birds itself and how that food is managed, the height of the feeders and whether they can contaminate the feed.

We had to look at the water, again, Dr. Hargis mentioned about the impacts of water but looking at the water, checking each farm's well system to see if we've got *Salmonella* within there, looking at whether produced -- the farmers flush their lines and seeing if there's impacts and trying to culture the water well to look at potential sources for *Salmonella*.

We looked at the litter, how often they use -- reuse the litter, the amount of caking around feeders and waterers, looking at that as impacts for increasing *Salmonella* colonization in the gut.

And finally pests, how well is their pest control, be it fly control or rodent control.

So those are some of the aspects we had to look at each of the farms that we were studying, trying to see how each can best manage their farms to reduce *Salmonella*. In terms of *Salmonella* colonization for the digestive tract, as you learned in previous lectures, we look at the intestines

itself, we looked at the crop, but primarily we focus on the small intestine, the jejunum, and the cecum. So most of our studies have looked at there in terms of *Salmonella* colonization.

For intestinal microbiology, one of the most important things we have to do since we want to apply our laboratory research to field conditions, we would want to take a look at what's the microbial ecology of the chicken or we also did turkey poult intestines in the field. We know that if we've got good hatchery conditions, we can really clean up the *Salmonella* in the hatchery. So, we wanted to take a look at the birds in the field, what is their colonization rate of *Salmonella*. And on a lot of farms, what we could find is these birds would be clean at the hatchery when we took sub-samples of them to culture. Right when they come off the truck from the hatchery, we'd culture that, that would be free of *Salmonella*. But, if you follow on the farm, some birds can get in -- colonized with *Salmonella* as early as six hours after placement. So, we know that there must be some kind of area that they're picking up this *Salmonella* at.

And as we follow the chick's intestinal gut looking at the colonization rate, there's a variation. We see a lot of groups of organisms -- *Staphylococcus*, *Streptococcus*, you've got your *Salmonella* and there's a lot

of fluctuation from the day of placement up to about two and a half weeks of age but after two and a half weeks of age, most poultries we see a kind of stabilization of bacteria within the gut.

So, our main thing would be how do we stabilize intestinal flora within the gut. We know that at the day of placement here, we can have contamination of *Salmonella* as early as six hours. So, one of the things we thought we'd look at for our producers within the state is maybe probiotics and as Dr. Hargis mentioned, there's a lot of different types of probiotics. You could have the undefined products which have better success stories, or the defined. For our purposes, we wanted to focus on commercial products and focus on a defined product and see how that works, first in laboratory and then subsequently in field studies. And our main purpose again was trying to reduce the *Salmonella* presence within the gut. So, one of the studies that we did do was we looked at the avian-specific probiotic and *Salmonella* specific antibody in the colonization of *Salmonella* Typhimurium in broilers. For our particular study plan, again, the main goal was to reduce the prevalence of *Salmonella* within the intestinal system. We used our defined probiotic which primarily consisted of *Lactobacillus acidophilus* and *Streptococcus faecium*. And in this particular study, we also looked at *Salmonella*

Typhimurium antibodies.

Now what we did have is that we had four hundred birds in the study and we took a sub-sample of ten birds each every two to three days throughout the production cycle of 43 days. We kept it one extra day. Normally we would have it at 42 days that we'd send it to market but anyway we followed birds up to 43 days, cultured them, and in this particular study, when we applied the probiotic, remember I mentioned that we found *Salmonella* as early as six hours ahead of time, so we decided to administer the probiotic at the hatchery doing spray and for the first three days of life -- I mean, after they were placed. These birds were challenged with *Salmonella*, we decided to give them a high challenge dose of ten to the seventh bacteria of *Salmonella* and then we followed them again every two to three days culturing the intestines, looking at the presence for *Salmonella*.

And this particular study as we expected, you know, at day zero we're not going to culture anything because that was prior to administering the *Salmonella*. And as you can see, there's a slight increase in ten to the eight like there was an increase of growth in the organism but as we followed it down to day 43, you can notice the normal succession. Now the numbers highlighted in red, those -- we found that they were significantly different

here towards the end of the cycle and if we look at the numbers itself, like on day 43, we'll find 1.5 times 10 to the third versus 1.0 times ten to the fourth and you figure like that's a ten-fold difference, is it going to make that much difference or not. I mean, we're trying to reduce as much *Salmonella* as possible and that's a slight reduction. It seems to be significant. So we -- yet there is a positive result in the reduction but how is this going to be applied to field situations? Remember that we gave them an infectious challenge dose of ten to the seventh.

In previous studies, we've looked at the challenge doses of like 10,000 to 100,000 organisms but on this one we looked 10 million as a challenge dose. And that would be the worst case scenario possible and to see how this probiotics would work. So now, we said okay there's a little positive work in this in the laboratory conditions where everything is supposedly ideal. How's it going to work in field production? So in our particular field trials, what we did is we had twelve study flocks, twelve control and twelve treated flocks and again, most of these birds housed 18,000 birds per building. The treatment was the same; again we were getting probiotics at the hatchery through spray and then probiotics the first three days after placement. Of course, we couldn't do any infectious dose on the farm but these -- but these farms we had confirmed that

they had *Salmonella* through drag swabs earlier so we know that they were -- were *Salmonella* positive farms.

And our main goal was to compare these different farms in terms of prevalence. Again, we're looking for the *Salmonella* numbers in the intestinal tract and then if there was any changes in weight gain thereafter at slaughter.

For preliminary results, we did find a wide variation in farm results similar to Dr. Hargis, as he just mentioned. For the good managed farms, we didn't find any significant difference on the administration of probiotics but what we did notice is that on those poorly managed farms, we could find differences up to ten to the two log so that would be like a bird that had ten bacteria versus one that had a thousand. So, I mean -- so we did find that as a difference levels for the poorly managed farms and then when we compared weight, we found that those that were treated with the probiotics had about a .25 pound -- did better in terms of that.

In terms of the farm differences, we said like why is there such a difference in this, okay, they're poorly managed, is there a thing where a chick -- the chick sources and no it didn't seem to play a role in that, so the only thing that we could conclude was that there was some management differences in the farms and I'm going to talk a little bit more about some of the poorly doing farms.

In terms of the food, we noticed that the height of the feeders weren't brought up to the proper level, we had some birds in there you could observe some defecation in there too and that may serve as a constant source of potential *Salmonella*. The waterers, a lot of the farms that did have higher loads of *Salmonella* where the treatment didn't seem to work, they didn't flush their lines frequently, they didn't have a constant monitoring of their well system for the water. But I guess it was in the cleaning that we didn't find any main significant difference.

In terms of the litter, those poor doing farms, again I mentioned earlier, tended to have more wet areas around the waterers and as well as the feeders too and that wasn't properly managed.

The other thing would be the pests. Most of them had rodent control programs and they had said that their rodent control programs were fine but in some houses you could have seen some evidence of rodents.

In terms of other animals, we didn't find any difference in all the farms. None of them had like domestic cats around.

So the main thing that we looked at in terms of the management conditions for the poor farms, I would say, had to do more with the water and litter impacts.

In terms for our future studies, there probably is some application for the less ideal farms that are poorly managed. But I think we need to apply this for each company situation because if we also follow different farms over successive periods of time that was a different trial and there are variations in results as well too. If we started with a farm that had depopulated, totally cleaned out the litter, flushed lines at the beginning, that farm has no impact or the application of probiotics had no impacts on that -- the prevalence of *Salmonella* on those farms but as we got through successive flocks and the litter was not clean, then we start seeing some of those problems. And these were more we were following like poorly managed farms.

A lot for our producers, we always mention that probiotics is not the magic bullet, that they really do need to do good management practices and that's one of the things that we found out in the studies and again the management factors evidently played a larger role in this particular study in terms of the application for probiotics usage. And we still do need to do more additional studies needed on these applications.

But for these particular studies we'd like to thank the USDA-APHIS Vet Service for helping fund these as well as our Avian Disease Investigation Lab.

(Applause.)

DR. BAUER: Thanks, Dr. Morishita.

We introduced our next speaker yesterday, Dr. J. Stan Bailey. I did want to repeat a couple of things for those of you who weren't here yesterday. He's got a 21 year career here. I don't want to cut into his time. He -- okay. I'll go ahead and skip all that.

Stan's a poultry microbiologist here at ARS and I'll let him talk to you about competitive exclusion.

COMPETITIVE EXCLUSION

DR. BAILEY: Thank you, Nate, and good morning, everybody. I'm going to talk to you about competitive exclusion.

Competitive exclusion is a process that's been around forever and it's been understood in many different arenas for a long time. A lot of mouse model work back in the '50s and '60s and all kinds of different areas that you could work with competitive exclusion. The first work with controlling *Salmonella* with competitive exclusion in broiler chickens was done by Dr. Esko Nurmi and some of his students and co-workers first published in 1973.

There's a lot of reasons why competitive exclusion is and can be an effective process. Probably -- we don't really understand it totally but probably the most likely candidate for that is competition for receptor sites. Also, the -- it can produce bacterial volatile fatty acids which

can influence the make up, not just of the *Salmonella* there but also it will help select for other types of organisms in the gut.

Substrate competition is one of the theories as to why competitive exclusion work. Changes in redox potential also will favor certain organisms over others. And production of bacteriocins and other anti-*Salmonella* type compounds can also play a role.

So, competitive exclusion is not a magic process. It's a very natural, normal part of what goes on in a chicken or a human or any other animal. A couple of bullet statements that would summarize the competitive exclusion is that newly hatched chicks can be infected by a single cell of *Salmonella*. I've got a graph in a minute that Nelson had showed yesterday that will show you the difference in that progression over a short period of time. Older birds are far more resistant to colonization because of normal gut microflora. And the introduction of flora from an adult bird into newly hatched chicks speeds gut maturation so in a matter of a short period of time, minutes or hours, we can take a newly naïve gut of a chicken and make it as resistant as it would normally get to be on its own in a matter of days. And that's all competitive exclusion is. As I said, this is the slide that Nelson showed yesterday but this summarizes what we're talking about. A newly hatched chick

is easily colonized by even just a -- one or two or a handful of *Salmonella*. When that bird's a couple of days old, it's going to take many more, 100 to 1000 possibly and by the time it's three or four days old, it becomes more and more difficult.

And certainly that, in a way, one might sit there in the audience and say well, if it's so difficult to colonize older birds, why are we having all this problem. Well, two ways we can look at that. One, we get most of our problems or many of our problems early on when they're more susceptible and secondly, anything that happens to a bird later in life when it's stressed, when the gut flora is -- is messed up somehow from stress, subclinical disease, anything that would disrupt gut microflora makes that bird more susceptible at that time so it's a really complex world we're dealing with but it -- but competitive exclusion in and of itself is a very simple process.

In 1985, Nelson Cox, Norman Stern, Roy Blankenship and myself made the decision, as I mentioned yesterday, to start working on the live side. We evaluated all the techniques and technologies available and decided that competitive exclusion probably had the most promise of anything going and in doing that we also made the decision that undefined competitive exclusion -- and we'll talk more about that and you've heard that term referred to before,

had the best chance. It was the most effective of the processes. There were a lot of really brilliant people around the world who had tried to put defined cultures back together with some -- some success. Some you've seen reported today and many others have been reported in the literature. But the most successful competitive exclusion products were undefined. I think it's because there's so many different things in that undefined competitive exclusion. It's a multiple hurdle approach.

Salmonella has been around for millions of years and will probably be around millions of years after everybody in this room is gone. And as such, there's just so many complex ways that it can get into a chicken. It's a very complex environment, micro-environment, broad environment, and so we need as many hurdles as we can throw up and the undefined approach gave us that. There are regulatory issues and we'll talk about those but as a science, the undefined approach was clearly the most effective in the literature and so we went about seeing what we could do to improve on that.

So we developed a process for harvesting anaerobically the cultures and then propagating them anaerobically and then giving them back to the birds. And this patented process was then licensed to Continental Grain Company, later ContiGroup Companies (Wayne Farms, Limited),

and so the history of our product that we developed that I'll share with you here, was that in 1985, as I said, we first began working with competitive exclusion.

In 1988, we developed the mucosal competitive exclusion. That's what we call what we made because it was a mucosal scrapings and I'll give you some more details on that in a minute.

In '89 and '90, we did our first field trials in Puerto Rico. FSIS was doing some pilot work there and we worked very closely with them and we did our first field trial work and I'll report on that.

In '95, we -- the US and Worldwide patents for this process were issued. Also, in '95, as I said, Continental Grain licensed from USDA.

In 1999, the products were registered and approved for use in Brazil and Japan. A lot of large scale field trial work was done there. And I've been using this slide a long time so I don't know the answer to this last question. We'll talk about that a little bit at the end.

So how did we make it? We made our initial cultures by taking specific pathogen free adult broiler chickens, doing an anaerobic culture and scraping, we gently washed the luminal fluid from the inverted ceca and harvest the mucosal scrapings. The theory being that that environment, that micro-environment in -- where the

intestinal epithelium and the mucosa are would be containing the organisms that were of most critical concern that we wanted to get into the young chicks. And what we did was we assured the safety by showing what was not there. We assured that the culture we collected was free of all known avian pathogens, *Mycoplasma*, *Salmonella*, *Camplobacter*, *Listeria*, *E. coli O157* and the like. And then we also, in later studies, took this through birds we gave it to and followed up with all of those birds and we did this a number of times and the safety was actually early on signed off in our initial processes with FDA. They didn't really at that time have a particularly great concern about the safety. They signed off on the safety component. It was other issues later that was -- that were more of a problem.

So how did we apply MSC? We applied it by two processes. One, we sprayed in the hatch cabinet when the chicks were approximately 50 percent pipped out. Those of you who knew -- know a lot of the work we've done here know we recognized early on the role that spread of *Salmonella* in the hatchery can have, so we felt like it was critically important to get this culture to the birds as quickly as possible. So, we got it into the birds when they were approximately 50 percent pipped out but we wanted to follow up to make sure there was a good dosage so we also applied it in first drinking water for about approximately the first

four hours so we were hitting it twice. And we feel like in addition to the fact that it was an effective culture, that double treatment was very critical in the good effects we were getting.

So we went into field trial. First we went into Puerto Rico where we were working there and over the course of about a year and a half, we did a number of field trials and the composite of that was that on the farm, we had saw a reduction from 11 percent positive to 2 percent when we measured it in the controls versus the treated and then when we -- and something that's very unique to this process and this product is we have been able to consistently -- and it's the only product that I know of that has been able to consistently not only show effect on the farm but we took those birds to the processing plant and we followed them there and we had a reduction from 41 percent in the treated to 10 percent of the controls.

We repeated those trials in Georgia and we saw -- we were at a fairly low period then, I think a lot of us wish we could get back there right now, but we only saw 2 percent positives on the farm and that we reduced to zero and then when we took those to the processing plant, we saw a reduction from 9 to 4.5 percent. We worked with Dr. Eric Gonder who gave a talk yesterday with some turkey work up in North Carolina and we had really dramatic results through

the first six weeks while the poults were in the poult houses before they were moved and at -- up through six weeks we had seen a reduction in the treated to 3 percent from 40 percent in the controls. Unfortunately, with the stress of moving those birds, to the growout houses from the young poult houses, we lost that effectiveness and we, because of so many other things we're working on, have not really had a chance to do a whole lot of follow up work with that.

In our petition to FDA, we had to do three field trials in three separate locations. We did -- we used facilities in Georgia, Alabama and Arkansas. And in those three field trials we did a -- we composited all the data and again we saw significant at the .05 and .02 level on prechilled samples which were basically on-farm samples. We went from 23 out of a 180 birds positive to 12 out of 180 and after the post-chill, we went from 9 out of 180 to zero. Now, I'm not in any way claiming that if you use this type product, you're going to have zero *Salmonella*, but in these trials that was the results we got.

So what are the -- why CE? What are the advantages of using a competitive exclusion process? First it's fairly easily applied. This is not difficult. It doesn't take a rocket scientist as somebody said yesterday. It's low cost, at least this particular product we're dealing with. The anticipated cost was only in the

neighborhood of about a penny a bird. It's nonspecific protection. It's not a specific serotype of *Salmonella*. It was broad spread against all serotypes. There's a very rapid host response, as I mentioned earlier. What we saw -- the protection you got within a matter of just a few minutes or hours. And it's very -- seems to be very compatible with other issues. As I reported yesterday, it's very compatible with vaccination and pretty much anything else you'd be doing.

What are some of the potential limitations of competitive exclusion? Truly, for it to be the most effective, you need to start with *Salmonella* free chicks. If the chicks already have *Salmonella* then you don't have nearly the marked effect as you do -- as I have reported. You do need effective biosecurity particularly in the first 48 hours.

It does not prevent transmission by the egg so anything that's coming in ova, that will not show up as a -- you can't prevent that.

Protection can be weakened or lost due to bird stress. As I mentioned, even birds that have been on competitive exclusion, if they're older and they get hit with a significant stress and there's still *Salmonella* in the environment, then they're still going to be susceptible.

Some antimicrobial feed additives may adversely

affect protective microflora. But in the case of this culture, we tested against pretty much all of the common -- commonly used antimicrobials at the levels used and we didn't have any particular effect there.

Even under ideal conditions, protection is not absolute, rarely absolute.

So what is the status of this product, and I'm going to use this product and products like this in the discussion. In the US, the only approved competitive exclusion product was the defined product PREMPT and that company is no longer producing and selling the product. Worldwide, there are several undefined products including Broilact and Avi-Guard in addition to PREMPT.

So what are the issues? Why are we having so much trouble getting approval for either this product or any other undefined product? Because I truly believe that undefined competitive exclusion has the potential to be a tool in our toolbox for fighting *Salmonella*. And it's a very complex process and we're going to need all the tools we can get. No one thing is going to solve all the problems. The regulatory issues, our concern is one of -- is competitive exclusion a drug? That decision was made early on and shifted over to FDA-CVM because it's a very complex logic that I don't really follow, never did. It's not a drug. And so I -- that is an issue that maybe needs

to be revisited. Within that process that we're going through, though, as I said earlier, safety was not the issue. Safety was approved. It was a question of, could we, in an undefined product, show consistency of product from day to day and batch to batch over a long period of time. We feel like we were able to do that.

There's an issue that came into the process late in the game was the potential for antibiotic resistance transfer. So what we had to do was prove what wasn't there but there are some issues with antibiotic resistance transfer that I'll talk about on the next slide. And there is always the potential with 80 to 85 percent of the U.S. market using in ova Marek's vaccine treatment by Embrex and then using the antibiotics in that that you would always have to on -- watch out to make sure whatever antibiotic was used in your Embrex machine would not adversely effect any potential competitive exclusion product.

But from the industries point of view, they don't really care about all that. I mean, they want to make sure a product's safe, but all the industry cares about, does it work? Will the product improve performance and I didn't have time to talk about it today but in a lot of those field trials we did in Brazil and Japan and other countries, we showed some fairly significant improvements in production. And how much does it cost? Those are the issues that the

industry cares about. And if the product doesn't work, it won't make it. If it works, it will.

As I said, there's some risk benefit issues around antimicrobial resistance and I thought I'd highlight those because they became a big part of the discussion. There's a potential for direct transfer of resistance from bacteria in the cultures. There's also potential for genetic transfer between bacteria in the CE mixtures. That's the down side -- I'm almost through.

CE has been shown to significantly -- now these are the good things though. Those are the potential down sides and they are things we have to recognize. But on the alternative side, as we pull antimicrobials in the marketplace now, we're seeing a tremendous increase in necrotic enteritis. This type of product has a high probability of being able to reduce those problems, thereby leading to reduced antimicrobial usage. And it's likely, because of that -- and just performance issues, it's likely that CE will allow significantly less antimicrobials to be used. So what's your trade off? A remote possibility of genetic transfer versus significantly less antimicrobials used. So it's a risk benefit that we always have to look at.

So in summary, I would say that the combination of eliminating *Salmonellae* from breeder flocks, hatcheries and

layer flocks followed by the treatment of new hatchlings with competitive exclusion cultures before exposure to environmental salmonellae provides a realistic opportunity to produce poultry products with significantly reduced salmonellae.

And that's all I've got. I'm over my time. Thank you.

(Applause.)

DR. BAUER: Thank you, Stan.

Our next speaker is Dr. J. Allen Byrd. He's project leader for pre-harvest food safety research at the Food and Feed Safety Research Unit, ARS, College Station, Texas.

Dr. Byrd received his B.S. in Animal Science, Master's in Nutrition, PhD in Poultry Science and Doctor of Veterinary Medicine at Texas A&M University in College Station, Texas. He's been a scientist at ARS in College Station, Texas since 1997 and he was a post doc there in '96.

He's here to talk to us about development of cost effective means for the prevention and control of *Salmonella* -- *Salmonellosis* in poultry.

DEVELOPMENT OF COST-EFFECTIVE MEANS FOR THE PREVENTION AND CONTROL OF *SALMONELLOSIS* IN POULTRY

DR. BYRD: Thank you very much. I appreciate -- I

take it as an honor to be able to speak to you today but what my talk is actually about, I'm going to continue on the competitive exclusion talk and talk about how we developed a defined competitive exclusion culture in our laboratory and Dr. Bailey just mentioned the product was PREMPT was the only FDA approved product and it has since then been, not taken off the market but the company went bankrupt that was producing it.

And I always like to put up a slide like this, to again to let you see what we're up against and something's that's eight foot tall is hard to go against when you've got little cultures to work up against. But, we always forget what we're going up against, and it helps to visualize this in a slide.

What happens in a newly hatched chick, and some of the slides I'm going to be repeating what you've seen previously. During the first few days of life, we know that the ceca is usually the principal site of colonization for *Salmonella*. And normally when a chick hatches they essentially have a sterile gastrointestinal tract. And in the olden days when we had the hens still sitting on the eggs they would provide the culture, the beneficial bacteria of these birds. Since that time, of course, we got modernized in technology, we've taken the eggs away from the hens.

Now, they have to get their bacteria some other place. The first place, as Dr. Bailey has mentioned as well, is in the hatchery. So, if you have pathogenic bacteria in there, that's a place where they grab hold and it's hard to get rid of once they have already established or colonized the birds.

Secondly, we have the broiler house, there's always potential to those newly hatched chicks when they first get to those broiler house to be exposed to pathogenic bacteria. Again, it takes about four hours for the -- four to 12 hours before the gut starts to mature and essentially four days before it becomes somewhat totally mature for this bird to be producing. Of course we have ubiquitous presence of *Salmonella*. The hatchery on egg shells, on belts, of course man may bring it in on their clothing. In some cases you have workers who will have it on their hands and then handle the birds. We also have rodents and wild birds that may enter the place. And the birds can either pick it up through an oral route or they actually pick it up through a cloacal route as well.

So what we like to do is take the newly hatched chick, put it in a sterile environment and throw it into a chicken house with 18,000 of his closest friends and relatives and allow them to grow under these conditions in the sterile environment where they can't be in contact with

pathogens. And then, grow them out to 42 days of age or however long your company happens to grow them. But we also, that by putting them in sterile environment we also affect production. We've heard other competitive exclusion cultures, probiotics that would increase production. So, the bacteria that's present inside the gastrointestinal tract are there in symbiotic relationship where we have beneficial for both parties.

So what do these bacteria do? What are the benefits of a healthy microflora? Well, they exclude pathogens within the G.I. tract. They compete with *Salmonella* for like I said the binding sites and nutrients and they create a hostile environment for these pathogens.

Now, essentially when we can get a gastrointestinal -- we get beneficial bacteria within the gastrointestinal tract that acts as a barrier to protect it from the outside world. And basically, what affects this barrier that goes on. Essentially any stress that causes a bird not to eat. The environment, he gets too hot, he gets too cold, it affects how often these birds are going to consume feed. And when they're consuming the feed, again they're not consuming it just for the birds themselves they're feeding the bacteria that are present there.

Delayed access to feed and water. Again, remove the feed, those bacteria are basically starved and you'll

get different populations develop. And this allows pathogenic bacteria to move in. Water, if you don't have water usually you get the slowing of the feed going through the system. Or if you get changes in feed consumption, again these beneficial bacteria in there are depending on that feed. You change the feed source and they go through a change in their environment, because such bacteria -- certain bacteria are depending on that source and then when you change that feed source it causes competition between the bacteria.

Vaccination, we know that vaccination is just a controlled disease. Usually a little lower level but any time that you have anything that influences an animal it tends to make it at some point feel bad or they may not eat as well as it should until they respond back. And of course disease itself, if the bird's not eating then their immune system may go down and the bacteria, the beneficial bacteria tend to drop off.

And of course the last thing is antibiotics. Antibiotics are made to kill bacteria. They're produced by bacteria to kill other bacteria. And any time we put that into the mix, we are also affecting beneficial bacteria as well as pathogenic bacteria.

It's a hard slide to see but Dr. Bailey as well talked about Nurmi's concept, where Dr. Nurmi took adult

microflora and gave it to chicks, newly hatched chicks and basically matured the gut a little faster as into natural conditions.

So what I did is -- again here I'm going to talk about how we developed a competitive exclusion culture and the culture that we developed was a defined culture. Now, what are the benefits of a defined culture versus undefined cultures? In a true defined culture all the organisms are identified. You don't have any questions about what goes on, what bacteria may be present or not present. For the most cases many of the -- undefined cultures are just as safe and -- but if you know all the components in there you're truly indeed safe.

We know there's no avian or human pathogens in there, and quality control and reproducibility, we know can evaluate what -- or what bacteria are in the culture through different means and we are able to reproduce it and it was required by FDA for commercial licensing and regulations.

Now the first thing that comes up, one of the last slides Dr. Bailey mentioned was resistance. And whenever you're dealing with any type culture system, any type bacteria, that means the single strain of bacteria cultures that we're giving to them all the way to the undefined or define cultures. The bacteria have some type of resistance in there. They either have innate resistance which we have,

basic bacteria or natural resistance to antibiotics.

Innate, they do not have structural or metabolic drug targets and they're not easily transferred from one bacteria to another. But they will increase in the presence of populations, the populations will increase in the presence of antibiotics.

Now the thing that we're most worried about in dealing with bacteria is the acquired resistance and this occurs either by a mutation in a target gene within that bacteria or they may be able to acquire mobile DNA through plasmid, transposons integrons or phage DNA and this could occur at any level. And it could even occur in a controlled defined culture if there's contamination within the production process. But under strict quality control procedures this should be -- should be avoided.

How we started off looking at this thing, one of the scientists in our laboratory was Dr. David Nisbet and he came from the University of Georgia here and he was a microbiologist. And he utilized a continuous flow system, and basically what it does, it models the large intestine or it models the rumen of a cow. And he thought that we could use this technology to produce an exclusion culture. And what it is, is basically we just have a -- a vat here, a vessel and in that vessel is where we put our beneficial bacteria. And we can put a control substrate media that the

birds or the bacteria can grow on. And then, we can collect the product that's here. And that's essentially what a continuous flow system is, you continually add media. It goes in here and provides a feed -- food to the bacteria and then we collect the products that are being produced here. Within the system we can control temperature in the redox, the carbon source which is our media, the nitrogen source, we can keep it anaerobic or aerobic. We can change the pH if we need to. The turn over rate which in the -- our culture we utilize basically the turn over for the ceca of the bird. So essentially once a day we would turn over the culture. And many other things that can be manually operated.

Here is just an actual picture of one of our chemostats and it's hard to see but right here is our vessel, it's a liter vessel, and then our control media and then down here we collect the product. We have different gases that we want to add to a system and then the computer to monitor pH and the turn over rate within the system.

And the rationale between it again, it models the intestine cecal ecosystem, we can maintain many of the products. And it would be well to do a defined culture within the system. Our first culture we evaluated we called CF1 and what we did was take a culture and adapt it to a lactose diet essentially. Lactose, as Dr. Hargis, showed

earlier is a compound that when given to birds, tends to reduce *Salmonella* on its own. So we thought that we could provide a lactose and a competitive exclusion culture to the birds, we were able to more dramatically control *Salmonella*. And what we have here, essentially we provide lactose and we saw basically a 2 log reduction in *Salmonella* just providing that; a culture by itself again a 2 log reduction in *Salmonella* which is about 100 organisms. And then, when you combine this -- this culture with lactose we saw -- we reduced that down to 3.5 log reduction of *Salmonella*.

The original culture consisted of 11 total organisms and eight gram positive, three gram negative and it was a facultative and obligate anaerobe. The problem we were having is that the lactose become a little too expensive and we thought it would be best if we went to a different approach, because as many of you know, we're here to make money and the poultry companies are no exception. And they need to be economical as far as controlling their costs. And when you get -- competitive exclusion cultures get too expensive, then it's not economical for them to use them.

So, our next approach was a product we call CF3 which became PREMPT and it was originally worked with by David Nisbet, the late Dr. Don Corrier and Billy Hargis helped us and of course John DeLoach was the research leader

at the time. And we went through and identified 15 different facultative anaerobes in this culture; as you can see *Enterococcus*, *Lactococcus*, *E. coli*, *Citrobacter*, *Pseudomonas* were some of the ones present. And also obligate anaerobes. *Proprionobacterium* and *Lactobacillus* were some of the cultures, some of the bacteria present consisted of 29 bacteria in this entire culture. And this is what it looked like as an experimental thing, basically it looks like cecal soup inside of a bottle. And we -- they were shipped, it was frozen and it was shipped to the different laboratories for research. And we would provide it to the birds. First our gold standard of course is just giving it directly to the birds orally. Dr. Corrier illustrated here, followed with a -- we talked about Puerto Rico trial with the mucosal starter culture. This was also done in Puerto Rico as well, where they just sprayed it on with a hand sprayer. And eventually when it got to production, we used a sprayer which came right after the vaccines sprays in the culture.

We had -- this is just to illustrate a culture -- a chick ceca that was exposed to -- was not exposed to PREMPT and you can see the crypts, the openings here where there's hardly any bacteria. And then, we got the presence of PREMPT we were able to see that it indeed filled some of the binding sites the crypt sites on this study.

We looked at litter on our initial field trial that we have here and we had controls for litter at day 21, went from 603 organisms down to nine with our treated control. On day 43 from five to two organisms.

The actual birds themselves, we had seeders, birds that were challenged with *Salmonella* and birds with contacts that were not challenged. In our birds that were challenged in this study we got -- we went from 11 percent down to zero and contacts from 6.7 down to zero.

The product was again, was 29 anaerobic bacterial isolates, pathogen free, it was spray conventional application which is one of the reasons that it's probably not on the market today, is that there needed to be a different approach in the application. It had five years commercial testing and it was the only product FDA approved in the United States.

Some of the bacteria that was effective against, just the general *Salmonella* species. We also saw that it was effective for *Salmonella* Typhimurium DT104, *gallinarum*, and Enteritidis and *E. coli* O157*:H7*. It had some effect against *Clostridium*; and although a small effect, it had some effect against *Campylobacter* as well.

And a final word, I know that we keep saying this is that whenever we start a food safety control, program you can't just give it one time or one thing to affect it in a

sense of competitive exclusion, we usually give it early on. But if you have something like a feed change or lack of delivery of food, we would tend to get, you lose the effect of some of these cultures. And again, it starts prior to hatching, the breeder operation and finishes when we get the chicken to the consumer.

Thank you.

DR. BAUER: Could we have the panel, the speakers up here for a short panel question and answer session. And do we have our microphones for the audience?

Questions for our panel. Go ahead, Norm.

DR. STERN: Dr. Morishita --

DR. BAUER: Norman, they want you to identify yourself.

DR. STERN: Norman Stern, Agricultural Research.

DR. BAUER: Make sure you have a green light on there, Norm, okay.

DR. STERN: -- you had indicated that several of your chicks at six hours have become positive for *Salmonella*, yet the hatchery was supposedly negative. You think these were all environmental *Salmonella*?

DR. MORISHITA: Well you know, when you get a whole bunch of chicks we can't like culture each one. There could have been one that was cultured at the hatchery. So potentially it could have come in there, you know, we

suspect.

DR. STERN: But it in fact, do you think, make a difference in our control?

DR. MORISHITA: I think we can, but for -- I guess for experimental studies that's the best that we can do. We can't really culture every single chick. So we just have to take a sub-sample and we find that negative, you know, it's -- it's kind of hard to say, you know.

DR. STERN: In terms of treatment, does it make a difference if it's from the hatchery or it's from the environment?

DR. MORISHITA: In terms of treatment, if I find -- like I cultured like a hatchery and I find they have *Salmonella*, then I think that you should apply the product at the hatchery, that would be most important.

DR. BAUER: Other questions, we have a microphone on this side also. It's on, can you turn that microphone up. Okay, try it now.

QUESTIONER: Anyone can answer this question. The question I have is, especially when you receive, (inaudible) birds that were positive once or negative (inaudible) that period. Where is the (inaudible); the question that I have is how do these cultures work when you have (inaudible)?

DR. BAUER: Who's the question for?

DR. BAILEY: It was for anybody. I'll start

first. We don't know fully and it's probably not always the same place where the *Salmonella*'s hiding. It could be intra-cellular, it could be internal organs. And it also can be in the environment, because when birds are disrupted and they're exposed to it even in the environment they can pick it up and -- and it can spread very rapidly. But at the same time we are keenly aware that it could be in any of the internal organs and kind of in -- in a benign state so to speak, just hanging out waiting for an opportunity to spread.

So we don't fully know, and I don't think it's always the same. I think that's part of the problem with *Salmonella*. We try to put it in little boxes and make absolute statements and I don't think that works very well with *Salmonella* a lot of times, I think it's a very complex situation.

DR. BAUER: Did any one want to address the unabsorbed yolk, *Salmonella* being found in the unabsorbed yolk, that Nelson Cox talked about.

DR. BAILEY: We can all talk about that in a minute. I think that Billy was going to respond.

DR. BAUER: Okay.

DR. HARGIS: I don't want to talk about that, but I'll talk about this.

DR. BAUER: Okay.

DR. HARGIS: Paula Cray here in this building did something really neat with pigs that -- it's mind boggling really. She actually ligated and severed the esophagus of pigs -- and I'm putting it in very simple terms. But squirted *Salmonella* in the month of these pigs, and 15, 30 minutes later, something like that, could find that *Salmonella* in the lower gastrointestinal tract of these pigs. So there is an extra enteric circulation of *Salmonella* and probably it exists in poultry as well. The organism can be dormant intra-cellularly. I think a true cure rate is probably going to be difficult to achieve. We've got something like 10 percent of horses carry *Salmonella* and something like 10 percent of humans carry *Salmonella*. So, I think it's going to be a tough one to get away from. But fortunately we don't have to. I mean, think what we're trying to do is it's a number game. Trying to reduce the numbers that are being shed, reduce the percentage of carcasses or meat products that are contaminated and reduce the load that's on those carcasses. I think that's the goal.

Somewhere way above zero will be an acceptable level of *Salmonella* and it will drop off the radar screen. Clearly there is an acceptable risk; where it is, we don't know. It's a social issue, not a scientific issue. But we know cars kill people and we know electricity kills people

and so forth. There's an acceptable risk at some point. Perhaps not where it is right now.

DR. BAILEY: Nate, to your question about unabsorbed yolk, Nelson Cox and I and some of our co-workers have be doing work looking at unabsorbed yolk in all ages of broilers both for *Salmonella* and *Campy* and other things. But clearly we are able to demonstrate that that unabsorbed yolk which is in a higher percentage of birds than one would think even as they get older, is carrying a fairly high percentage of the time or -- in certain situations, not always, of the various pathogens. So, that's an area. But that's just one of many. There's all kinds of things looking at -- at bursal involvement, B-cells and macrophage and things. There's a lot of ways *Salmonella* is moving around, as I said earlier. It's just a very complex situation, it's not real simple.

DR. BAUER: Other questions, right over here.

MR. HOLDER: Tom Holder with Allen's Hatchery, somebody on the panel might not be able to answer this, but somebody in the audience might be, if some of the guys are still here from yesterday. I think we get the feeling from industry that we know what's coming. It's train heading down the tunnel and we're in the headlights, so we've got to do something about *Salmonella*. But here we are, from what we've heard this morning and from personal experience, we

know that these products will help us reduce *Salmonella*. But yet we don't have it available to us. How can we get this available to us, and did I hear somebody yesterday that it's moved from FDA back over to APHIS now with --

DR. BAILEY: No, no.

MR. HOLDER: That was wishful thinking.

DR. BAILEY: No, that was wishful thinking.

MR. HOLDER: Can we make that more than wishful thinking and get it of dead center through some political clout here if we're going to be under the gun. We need some help?

DR. BAUER: Did somebody on the panel want to address that?

DR. BYRD: No.

(Laughter.)

DR. HARGIS: Tom, that was a snide remark and a statement, not a question. But it -- it is a major issue. The issues with autogenous vaccine requirements to be able to provide recurring isolates to the need to go to incredible extremes to guarantee safety of -- of perfectly harmless cultures.

DR. BAUER: Another question.

MR. BOLDEN: Yes, thank you, Steve Bolden with Pilgrim's Pride.

DR. MASTERS: Can I quickly address --

MR. BOLDEN: Excuse me.

DR. MASTERS: Dr. Raymond unfortunately had to leave, but I at least want to share with you his vision to work from the Office of Food Safety very closely with Dr. Lester Crawford, at the FDA. And he's at least had this issue brought to his attention. And while I can't guarantee that he can have FDA make approval from CVM, I can at least assure you that he's heard this. That's why we're having this meeting and he can at least share across departments the concern that we have to make sure that these kinds of issues are brought from one agency to another agency and the concern that we have these kind of products available and the knowledge that he has to make improvements in the industry and in public health and in food safety. And so these issues have been brought to his attention. And he spent the day before the meeting with the Centers for Disease Control and with the industry and heard these issues the first day, he heard them yesterday, and so I can assure you that one of his goals and I think he mentioned this in his opening remarks, is to work across departments and to have collaboration.

And so I can assure you that's he's at least heard these issues and that he'll be bringing these to Dr. Crawford's attention. So at least he's sharing his goals for public health and food safety. So, at least we'll be

bring these to their attention. So, I'll at least address them from that perspective.

DR. BAUER: Thanks, Dr. Masters. We have another question back here.

MR. BOLDEN: Steve Bolden, with Pilgrim's Pride. I hope that those things that are being brought to his attention, match -- I hope product availability matches regulation, okay. I think that would be great.

To spend a penny a bird within our organization would be $15.6 million a year. Keep that in mind, I know we got product development companies within this audience and we need to get that cost down to about 1/10 of cent per bird. It's got to usable in a spray cabinet or in ova or it has to survive a feed ration. It cannot be administered at the farm level in the water, that's a pipe dream, with a large integrator.

So as we move forward on these products they've got to be administered en masse, and it's got to be economical. And I give you the target of 1/10 of a cent per bird to work with. Just a suggestion. Thank you.

DR. BAILEY: I -- we recognize I think those of you in the audience and most of the industry people here know that I've worked, -- we've worked, it's not just me -- my colleagues and I have worked with pretty much all of the industry for years and years. And we are -- we pride

ourselves on our practicality. I hear much of what you're saying particularly about application methods. Allen referred to that earlier and that's something we know we need to work on to be sure.

There's a couple of factors that I'd like to point out from a slightly different perspective. Yes, that is a lot of money, if you look at that over the course of a company as large as your's over the course of a year. But if we look at public health and if this is related to public health -- and that's a different debate that we can have. But if we look at public health, then the amount of money you're talking about is minuscule considering what the public health return would be. It also depends on where you're sitting, and I'll just philosophize just a second. I'll take my prerogative as almost being old enough to retire, as Nelson said yesterday. And they probably couldn't fire me before I got to that age. But it depends on where you're sitting whether you think that's a lot of money. If your regulations change next week or next month or next year based on public health or what the regulatory agencies say then your perspective on what you'll be willing to pay for something may be entirely different.

So, I hear you and I understand what you're saying and I think we all are wanting to work to provide as effective tools as we can as cheap of cost as we can. But

it just depends on where you're sitting and what the environment is on any given day what you will actually be able to afford to do. And that's my personal philosophy, that's not an ARS position, that's just me speaking.

DR. BAUER: Other questions? One right back there.

DR. HARGIS: May I make another comment?

DR. BAUER: Oh, sure.

DR. HARGIS: You know, times are changing in terms of the number of tools that are available. And you know when we lose some of our better growth promoting antibiotic tools, when we lose some of our therapeutic drug tools as is happening, the inability to re-treat through the drinking water might be revisited, Steve, especially if we are able to do -- use this type of approach for controlling other pathogens that really do make a difference in terms of production cost and production efficiency.

So, I think the evidence is really out there. Stan, talked about it with mucosal starter culture, with turkey operation moving from the brooder to the growout houses. Time of stress, interruption of feed, feed change and so forth. A serious problem with maintaining homeostasis of the enteric microflora. If it's going to work you're probably going to have to re-treat those birds after a point like that. And if you can't or won't, then

it's not going to be a good approach. But a single inoculation and trying to maintain that through a broiler flock with significant feed changes, it's going to be tough. Without ever re-treating those birds.

But again, cost/benefit ratio. Not just in terms of public health, but I think the evidence is clear that it's possible to get returns on the investment that more than pay or offset the cost of a competitive exclusion/pro-biotic approach.

DR. BAUER: Question back there?

MR. CERVAISTES: Hector Cervaistes with Phibro Animal Health. More than a question, it's a comment, because I may not be here for the final Q&A.

I see Dr. Masters is also leaving, maybe she can carry the message to Dr. Crawford.

I've seen the program and I see everything about competitive exclusion. We're talking about -- she was talking about going back and looking at all the past research and the tools that we have to go -- to decrease *Salmonella* shedding in poultry. And I can't help but to notice that antibiotics are blatantly absent. And I know it's an unpopular stand to take, but we still have antibiotic feed additives that are approved by FDA to be used today and research by my esteemed colleague Dr. Nelson Cox and others at this group have shown that it will reduce

Salmonella shedding in turkeys, as recently as a couple of years ago published in the Journal of Live Poultry Research. That's one observation.

The other is that we see the difficulty in getting these defined cultures approved by FDA with a legal claim for *Salmonella* control. It would be nice to add performance enhancement if that's the case.

And finally, I do want to say that in my opinion there is such a thing as a magic cure and it's actually the simplest and cheapest intervention available, it's called handle, store and cook your food properly.

Thank you.

DR. HARGIS: Amen.

QUESTIONER: Just a couple of questions. Any one of you can handle this. We've heard from for five, six, seven years about *Lactobacillus*. Like Steve Bolden mentioned it's not a very easy product to apply. We've seen products come and go that are based on *Lactobacillus*. The literature also tells us about other products, other microorganisms that do the same kind of thing such as *Bacillus* that are much easier in the application, added to the feed and such. Why people or not talking about other organisms which are a lot easier to apply that also has other efficacy controlling *E. coli*, *Clostridium*. That's one.

The second issue I have is some of the work like

Margie Lee and people like that are doing indicate that *Lactobacillus* really serves its function the first two to three weeks of gut maturity. After that, you have other organisms taking over. Are anybody looking into those kind of aspects?

DR. HARGIS: We've looked -- Dan, I think as you know we've looked at a number of *Bacillus* isolates and we would like to have *Bacillus* that could be stable for either environmental application, drinking water application, or feed application. The problem that we've had is we haven't been able to find one that or any one single isolate that has a significant impact on *Salmonella*. We're still looking.

Another problem with -- the feed applications sounds good and there's a -- a couple of *Bacillus* strains that have been researched and shown performance advantages and I think you're exactly right. That they may influence other disease causing organisms. The problem with those studies that have been published is the number of organisms that are required. The number of spores or colony forming units per gram of feed. You're up to 10^5, 10^6 to get any effect at all. And in some of those studies 10^7 is where you start to see effects. Now you start talking a million to 10 million organisms per gram of feed,you think a penny a bird is expensive, you're talking really enormous numbers of

spores. And those products have been commercialized, there's one in Japan that was commercialized and very nice research showing that it worked very well. But when it was commercialized it was 4 logs lower for feed application than what was effective in the research studies.

DR. BAUER: We have time for one more question.

Dr. STEWART-BROWN: You know, I thought a couple of years ago that maybe competitive exclusion was just about ready to work. It was -- and it was kind of a product where bird microflora, the fluctuation from day old to processing had evened out to the point where there was now not so much stressful -- so many stressful time periods in the growout that competitive exclusion could hold. Perhaps even from day old, but at least from a couple of applications, and we'd get that to work a lot better as the research indicated that it might.

And you know, Teresa mentioned these problem farms and some of the pieces where -- where you have these big and -- and Billy, you mentioned about when you move turkeys. Migration practices in the chicken house can be pretty violent I think to the microflora mix. And if you have a real cold winter and fuel prices are as they are, I don't think migration is going all that well in those kinds of winters.

In other words you open up the house to let them

have more of the house but they -- they're not going there. And it's just hard to get migration like you needed to for the microflora to hold the competitive exclusion approach. And I think we're even worse than that these days because we've taken some of the crutches out of the process with the antibiotic practices. And I understand all that, I want it all to be good. I want to do all the right things.

Having said that, when you take some of that microflora management tool away and then you add a real cold winter and a real poor migration process, really, really hard in my estimation to get that competitive exclusion approach to hold up. And I -- I think all of you, I know all of you understand that because we talk about it all the time. And I just want -- I think competitive exclusion research looks -- is hard to get it to the chicken house, especially when you're pulling other stuff away that makes it even more fluctuation. And I welcome any criticism or comment about those feelings because I want it to work.

DR. BAILEY: For the monitor, that was Bruce Stewart-Brown, from Perdue.

Bruce, I agree with you. I think almost every body who spoken here in the last two days and certainly I did tried to emphasize that each of these is a tool in the tool box. They're not absolutes and -- and I have never in 15, almost 20 years now, working with competitive exclusion

advocated that it was -- it was a magic bullet that was going to answer all of your problems.

What I do believe is that it can be along with vaccination and biosecurity and a lot of other things -- they can contribute in small ways to reducing the overall load. And I think ultimately that you and I and many others in this room have discussed it is a load issue. The things that are being done in the processing plant, this is not a post-harvest meeting, but the things that are being done in the processing plant are actually good. And if you keep the load at a manageable level coming into the plant, then those effective treatments can -- or those treatments that we're doing in the industry in the processing plant have a chance to keep you where you need to be. But, if you -- even effective systems can be overwhelmed if the numbers are too large. So, that's why each of these tool where they are only incremental can be additive in effect and help keep those levels down. Or at least that's kind of how I see it.

DR. HARGIS: Bruce and Steve also, I hear you loud and clear. I mean I understand completely what you're talking about. You got any ideas, talk to me. I think we're going to have to -- I think that to get maximal effect of this approach is going to require intermittent application. Perhaps not multiple times or many times, but more than once in a broiler flock and probably several times

in a turkey flock. Intermittent application is certainly more appealing economically than continuous application, if the numbers that have been published so far hold out in terms of achieving efficacy. So, I -- I don't know, the solution.

DR. GONDER: Just one more brief comment if I could.

DR. BAUER: Eric Gonder, go ahead.

DR. GONDER: I can deal with the continuous administration, but if we don't get the product and we can't get the product, the administration method becomes moot.

(Laughter.)

DR. GONDER: And I think it's time to very seriously return to the issue of why these products cannot be cleared. Why we're being held to one schedule and the regulatory establishment for the approval of these appears to be held to no schedule at all? I don't know if that needs a Congressional fix, that seems somewhat extreme. But we really don't seem to be making much progress here.

DR. BAUER: And on that happy note, let's give our panel another round of applause.

(Applause.)

DR. BAUER: And let's take a break til 10:35.

(A short recess was taken.)

INTERVENTIONS AT POULTRY GROWOUT

DR. GOLDMAN: All right, thank you, we are ready for the last session of the day. The birds are moving closer to the slaughter house. We're going to have a series of presentations on interventions at growout. We actually have four presentations in this session and then, the fifth presentation is not part of the session strictly speaking but it will be the last presentation and that will be a presentation by Dr. Bailey on his experience and knowledge about *Salmonella* control in Scandinavia.

But to begin this session on interventions at poultry growout we will start with a presentation by Dr. Eric Line, on litter management.

Dr. Line received his PhD in food science and technology from the University of Georgia in 1993 and has worked as a research food technologist at USDA ARS for the past 12 years and he's just from the fifth floor upstairs here.

His primary research interests are in finding on-farm interventions for foodborne pathogens such as *Salmonella* and *Campylobacter* in poultry. And in improving microbiological detection and enumeration techniques.

Please welcome Dr. Line.

(Applause.)

LITTER MANAGEMENT TO REDUCE *SALMONELLA*

DR. LINE: Good morning. Thank you so much for

the opportunity to speak with you today. I feel like I'm speaking to the home team crowd here, since I had to come from all the way on the fifth floor to be here.

Any talk about litter management to reduce *Salmonella* means we must first ask the question of what is litter? Poultry litter is poultry manure mixed with absorbent bedding materials, we call that litter of course. The constituent properties are going to vary widely depending upon how the chicken are fed because about on average 20 percent of the chicken feed will wind up in the manure. Depends of course on the chickens' age and their size and of course on the age and the type of absorbent bedding material that makes up the litter.

The composition and content of the litter varies by region. Typically we'll see things used such as wood shavings or rice hulls, peanut hulls, straw, all of these can and have served as absorbent bedding material in chicken litter. And it kind of depends on what is most readily available in the region of the country in which you are raising the poultry and cost, of course, comes into play here.

Litter management practice vary widely. Not only within the United States and around the world, but even between integrators. It's typically several that several flocks are likely to be raised on the same litter. The

total clean out of a house may only occur once a year, sometime less often than that. Depends on the integrator. It may not be a bad thing as I'll point to in just a moment. Cake removal, which means taking the hard crusted upper layers of litter and tilling them to reincorporate them into the overall mass of litter is a common practice between flocks as is top dressing. Which means taking a little bit of fresh litter and scattering it across the surface of the spent litter between flocks.

Litter typically will build up in the houses until it's 15 to 20 centimeters deep on average and dirt or clay floors are common in the United States, whereas concrete floors are more prevalent in the European Union and specifically in some of the Scandinavian countries and I think that may be one of the difference that makes comparison of intervention methods between some of the Scandinavian efforts and our efforts different. You can imagine it's much more difficult to thoroughly clean out and sanitize a dirt floor as opposed to a concrete floor.

Well, as I mentioned a moment ago it might not be bad that we tend to grow birds -- several flocks of birds on the same litter, and this is because there's a demonstrated bacteriocidal effect of used poultry litter. Back in 1967, Tucker reported that the persistence of *Salmonella pullorum* and *Salmonella gallinarum* varied from three weeks in old

litter to 11 weeks in new litter. And in general moisture ammonia and pH increase with a period of litter use; the longer the litter is used, the higher the moisture ammonia and pH levels tend to be. And the *Salmonella*-cidal activity was theorized to be a result of a water activity that was unfavorable to cell viability and a high pH from the ammonia in the litter.

In a study done in Australia more recently, which looked at about 20 flocks through growout, it was noted that *Salmonella* was much less frequently isolated from flocks that were reared on old litter than on new litter. So, this bacteriocidal effect of used poultry litter is a very real phenomenon.

As you can imagine there are numerous challenges associated with controlling pathogens in the poultry house environment. This is not a sterile surgical suite in a hospital. This is dirt floors, grimy conditions, dust, high humidity. The temperature is just perfect for outgrowth of a number of bacterial species and including some bacterial pathogens. The humidity levels will vary widely and can get into a range that will support growth of pathogens. There's plenty of nutrients around for bacteria to grow on, from leftover feed that's scattered in the litter et cetera. And the pH of the litter typically without a -- without a litter amendment is going to be just slightly higher than neutral.

And typically not high enough to inhabit many pathogens growth.

Cross contamination occurs rapidly within a house like this. This is a typical, I think this is a turkey house, but a typical broiler house with 20,000, 25,000 birds you can imagine, because of the natural coprophagic activities of birds -- that means that they are going to peck at each others droppings in the litter, it's just a natural things that birds do. In fact it's been estimated that as much as 10 percent of a bird's diet may be made up of what it picks up off the floor out of the litter. So, you can imagine the fecal-oral passage happens very rapidly and bird-to-bird transfer organisms can happen very rapidly under these sorts of conditions.

There are many different vectors of contamination. I'll speak a little bit more about that in a moment. Poultry growers face two problems relating to *Salmonella* in their houses. The first one would be resident *Salmonella* which may persist in houses from flock to flock even after cleaning. And this is especially a problem in houses that are difficult to -- to thoroughly sanitize and thoroughly disinfect.

Problem two is even if you are successful in thoroughly decontaminating the house, *Salmonella* contamination may be reintroduced into those clean houses

from a wide variety of outside sources. We heard many of these sources mentioned earlier yesterday and today. Vertical transmission of *Salmonella* Enteritidis and Typhimurium especially from parent flock to day of hatch chicks has often been reported.

And of course horizontal transmission vectors do occur also for *Salmonella* and these are probably more important here in the U.S. because of our decrease levels of biosecurity in our houses as compared to say some of the other European or Scandinavian countries. But we've mentioned all of these earlier -- feed, water, insects, rodents, wild birds, domestic animals, human contact,-- a lot of different ways *Salmonella* can get into that house. Eriksson reported in 2001 that *Salmonella* contamination loci are not equally distributed in poultry houses. There are going to be hot spots.

Greater *Salmonella* populations in litter samples were found when there was a water activity greater than .9 and a moisture content of greater than 35 percent. And at reduced water activity and moisture content levels the numbers of viable *Salmonella* were found to be lower.

Hayes, et al in 2000 found that *Salmonella* are unequally distributed in commercial poultry houses. They found a low water activity environment of less than .84 likely represents a physical barrier to the establishment of

Salmonella contamination. And he went on to theorize and to propose that a water activity below .84 and a moisture content between 20 and 25 percent would actually serve as a method of pathogen control, specifically for *Salmonella* and perhaps for some of the other foodborne pathogens of concern.

An interesting correlation was made by Mallinson et al in '98, between the litter surface humidity and *Salmonella* contamination. And this study was done looking at the results from a total of 67 broiler flocks. Here you see the average litter equilibrium relative humidities, this is the same thing as water activity times 100 basically. Divided into a series of ranges from 78 to 83, >83 to 87, >87 to 90, >90 to 95. So increasing water activity of the litter. And then you looked at the litter surface drag swab results. Generally looking at six swabs per flock. And you see that the number -- that the percentage of *Salmonella* positive swabs increased greatly as the water activity of the litter in those houses with the *Salmonella* contaminated birds increased. There was also a very noticeable increase in the percentage of flocks with at least one *Salmonella* positive swab. As you went from 78 percent humidity and only 17 percent of the flocks positive to 86 percent of the flocks being positive in a water activity range of .9 to .95.

So perhaps we can borrow the multiple barrier or hurdle approach that is common to food safety to look at some of the good litter management practices that we can see in poultry production. The first hurdle that we might consider would be maintaining adequate ventilation in the house. The second hurdle might be properly maintaining water devices in the house to prevent leakage. And third might be to utilize appropriate litter treatments. I'll talk a bit more about each of these.

It's been well established that a continuous uniform air flow will lead to drier litter. Which creates very unfavorable environmental conditions for the growth of enteric bacteria and this of course leads to a lower *Salmonella* contamination on the birds in the house and then on the carcasses in the plant.

Our second hurdle, good water control practices, working to prevent leakage from the watering system, working to prevent caked, built up litter that's wet and containing a lot of moisture under these drinkers is important. You're creating fewer hot spots in the house that way that is favorable for the growth of enteric bacteria. And this could also lead to lower *Salmonella* contamination of carcasses.

At an interesting aside I was speaking to Trisha Marsh-Johnson, just a few moments ago during the break and

she was telling me about a new trend in broiler management where they use these radiant tube brooders now which heat the litter close to the surface until you get a high temperature increase very close to the surface of the litter. And that temperature increase causes the relative humidity to rise very close to that litter and in that litter. So, you may have relative humidity approaching 100 percent right underneath these brooders. So, that's something else that could potentially create a hot spot favorable for growth enteric bacteria.

Our third hurdle was potential litter treatments to reduce populations of *Salmonella* and other pathogens. These litter treatments primarily are acidic in nature. You see a long list of acids here. There's one hydrated lime, which is basic. I'll just touch on a few of these. There are many properties of these acidifying litter treatments that theoretically should be useful in reducing *Salmonella* populations. And the first one, and probably most importantly is the reduced pH. All of these acidic litter treatments are basically a granular acid in some sort of carrier usually, that is applied directly to the litter surface. So, you can imagine if you're spreading acid on to something you're going to get a very rapid drop in pH. And that's what does happen with most of these products. If you can achieve a pH below 5.0, we know that's unfavorable for

the growth of *Salmonella*. That would be the goal.

The reduced pH is also going to reduce ammonia volatilization from the litter. That means less ammonia present for the birds to be exposed to. We know ammonia in excessive levels is very stressful to chickens and other poultry. Reducing this stress on the bird may help reduce pathogen colonization of those birds. Reducing the pH also has been demonstrated to reduce insect populations in the litter. And this is another important factor. Insects we know can serve as a -- as a vector for *Salmonella* contamination in houses. Vector has been documented in studies showing where darkling beetles had pick up a particular serotype of *Salmonella* from a flock. That flock was then killed and the house was thoroughly decontaminated, cleaned out as best that they could. Sent in a new flock of birds and that flock developed the same strain of *Salmonella* as the darkling beetles had previously. And it was a very clear demonstration that the beetles had been the reservoir of *Salmonella* for this -- this next flock. It doesn't happen all the time but it is a potential that we need to be aware of. And many of these treatments also reduce the water activity. They're hydroscopic in nature, they will draw out water from the litter as they're hydrolyzed. So there's some reduction in water activity.

And another good thing about the acidified litter

treatment is that many of them may be used during growout, so practical application is possible. We always have to have a mind set that if we're working on a particular intervention, if it's not practical at the end of the day, if it's not something the farmer can feasibly do in the course of a day, it will never get adopted no matter how good it works. Many of these acidic litter treatments can be applied before the birds go into the house. Some of them do have to be reapplied, which is a bit problematic for the farmer, but not impossible. So, there's still some things to be worked out here I think in terms of making the best application.

We studied through these litter management years ago. We looked at the effect of sodium bisulfate which is also known as PLT and we looked at aluminum bi -- aluminum sulfate which is commonly known as alum. And when we introduced these compounds into litter we saw a very rapid decrease in pH, as we would expect. But the amounts that we included we lost that pH effect over time. By the time that we were back up here to about four weeks of age we were getting back up close to the same level of pH as the control. So we were beginning to lose any effect of the lowered pH. And when we grew birds on this acidified litter we actually, we were successful in showing some reduction in colonization by *Campylobacter*. But *Salmonella* was a

different story. We -- we really didn't see much of anything in terms of reduction in *Salmonella* in these treated birds and actually we saw some increases in *Salmonella* as compared to control, which was interesting.

I think that was better explained by a study that was recently published by Susan Watkins out of Arkansas where she evaluated a couple of different litter treatments and looked at their effect on *Salmonella* in poultry litter. She looked at Poultry Guard, which is a sulfuric acid product. And she looked at the PLT, which of course is the sodium bisulfate. And looked at it at different levels incorporated into the litter. And when a 100 pounds of product was utilized of the Poultry Guard, you see there was about a one log decrease in *Salmonella* in the litter as compared to control. The same thing for PLT, when 100 pounds per thousand square feet was utilized, there was about a log and a half decrease from the control to the treated.

What was interesting, when they used lesser amounts, look up here to the 25 pound level, control 2.7 treated 3.43. You actually had an increase in *Salmonella* in the litter. And that may be what we were seeing in our -- in our trials with actual birds. The lower inclusion rate may just not impact the pH enough to impact the *Salmonella* it may be reducing the populations of less acid-tolerant

bacteria that are present in the litter. And therefore creating less competitors for the *Salmonella* to then outgrow and to bloom in the litter. So that -- I guess a caveat of this sort of study is if you're going to utilize it, make sure you utilize them at the recommended levels and not less then the recommended levels or you could cause yourself problems that you were trying to solve.

It's been published that citric acid, tartaric acid, salicylic acid have all inhibited the growth of *E. coli*, *Salmonella*, *Proteus*, *Pseudomonas* in inoculated poultry litter. So these things work as well. I don't know about any commercial applications available for these products as of yet. There may be and I just -- I just may not be well informed.

One I wanted to touch on here was one -- the kind of switching sides from acid treatment to a basic treatment. Look at the effect of lime on *Salmonella* Enteritidis survival in vitro. Hydrated lime historically has been used as a sanitizing agent, used to control a variety of bacterial pathogens and parasites. An in vitro study was conducted using *Salmonella* Enteritidis inoculated litter, which was treated with 0, 5, 10, or 20 percent lime and the results demonstrated a marked pH increase. It jumped all the way to pH 12 even with only the 5 percent lime included. So the study was successful in reducing *Salmonella* recovery

in three out of three trials at the 10 percent lime inclusion level. And in two out of three trials at the 5 percent level. I've not seen any data yet from these authors on any in vivo studies that have been done with lime. I expect they will probably do some, but I haven't seen that published yet.

One caveat here may be that the lime actually increased ammonia volatilization. So, there's some potential to run into some problems there.

Fumigants are generally ineffective for *Salmonella* reduction in litter. Most fumigants would be useful only for litter disinfection between flocks or actually after the litter has left the house. We had hopes for chlorine dioxide because there's a new product available, chemistry available for generating chlorine dioxide from a dry chemistry base. You don't have to have the big chlorine dioxide generators that were common to poultry processing plants. And that product has been shown to be useful in decontaminating buildings that are contaminated with *Bacillus anthracis* spores, for instance. We tested it in shoe boxes full of chicken litter that was inoculated with *Salmonella* and we could get no effect. We had -- similar results have been shown for formaldehyde, methyl bromide, glutaraldehyde all of these things are relatively ineffective in actually decontaminating the organisms while

they are in that litter environment. And as Eric Gonder informed us yesterday, you can't simply disinfect manure.

Another publication shows that gaseous ammonia can effect some effect on pathogens in litter, a 2 log decrease in *Salmonella* was observed in 24 hours. If the litter was dried first and then gassed with ammonia, a 3 log reduction was realized. If the exposure time was increased to 72 hours, you got an 8 log reduction. That all sounds very interesting in -- in a very artificial test system. This was done in actually petri dishes. But a practical field application was not addressed and I'm not sure how you would practically apply this in the field. That would be a difficult technological challenge, I would think.

So, we know that multiple pathogen vectors require multifaceted interventions. We've talked about many of these throughout the course of the last two days. There's no silver bullet as it's been mentioned many times also. I did want to point out some of the potentials here for specifically bacteriocin, looking forward to Norman Stern's talk in a few minutes. He'll be talking about our progress in this area. While we've not really considered bacteriocin as a litter treatment, let's face it, anything that reduces -- reduces the level of pathogens in the bird is going to be reflected in the load of pathogens carried in the litter. The same thing for competitive exclusion. If the birds are

shedding less, then you're going to have a reduce primarily *Salmonella* load in the litter.

We had a visitor here earlier this week, [J. A] Wagenaar from the Netherlands who did a simple calculation to show the -- the tremendous number of fresh *Campylobacter* that are deposited in a broiler house on a daily basis. If we follow his lead, we could do a similar calculation for *Salmonella*. If you have a typical 25,000 bird house, let's say conservatively 30 percent of the house is positive, that would be 7500 birds times 25 grams of litter, excuse me, 25 grams of fecal matter per day per bird times let's say -- and it's variable, but let's say 10^4 is an average for the number of *Salmonella* being excreted per gram of fecal matter. Multiply all together you get close two billion fresh *Salmonella* being multiplied and deposited it on to the litter every day by poultry. So that just emphasizes the importance of litter treatment. And the management of *Salmonella* in these sorts of operation.

I think the number of *Campylobacter* was like 6 times 10^{14} because there's a higher carriage rate there. But the *Salmonella* is -- the *Campylobacter* is more fragile and would be dying off. *Salmonella* is very hearty it's going to be surviving and staying around for a long time in that litter.

So, anything we can do to reduce the primary load

in the litter is going to help us. Not only do we need to think about the litter in the house and the treatment in the house, we've got to think about what's going to happen when the litter leaves the house. We know there's more than seven billion, probably eight, close to nine billion broilers produced now annually in the U.S., which leads to more than 15 billion kilograms of poultry manure and litter produced annually. This is enough litter produced annually to cover a two lane highway 1619 miles long to a depth of three feet. Now, why the National Agricultural Statistic Service chose to send this theoretical litter highway from New Orleans to Fargo, North Dakota via Chicago I'll leave that for you to figure out. But the utilization may be problematic for manures, but we have to remember poultry manure is a valuable resource. There have been calculations done I think by the Alabama Cooperative Extension Service maybe about ten years ago, suggested that the nutrient value in -- in poultry litter was something in the realm $25 to $37 dollars per ton. So, it does have some value. And it's up to us to determine the safest ways to utilize that litter and to prevent spread of pathogens in that litter to the environment.

So accomplishing our research goals will help us to protect and improve the safety of our food supply and as always food safety starts with food production.

Thank you for your time and attention.

(Applause.)

DR. GOLDMAN: Thank you, Dr. Line.

The next presentation will be by Dr. Allen Byrd, who was introduced to you earlier this morning in one of his presentations. He is an ARS researcher at the Food and Feed Safety Research Unit in College Station, Texas. And he will speak on the Impact of Feed Withdrawal on *Salmonella* Prevalence; Use of Organic Acids and Sodium Chlorate to Reduce *Salmonella* Prior to Transport.

Dr. Byrd.

IMPACT OF FEED WITHDRAWAL ON *SALMONELLA* PREVALENCE; ORGANIC ACIDS AND SODIUM CHLORATE TO REDUCE *SALMONELLA* PRIOR TO TRANSPORTATION TO SLAUGHTER

DR. BYRD: Thank you. Again, it's a pleasure to talk with you this morning. This is some research that we began when Dr. Billy Hargis was at Texas A&M University and continued on to the our ARS laboratory and I guess it spilled over into Dr. Hargis' laboratory since he's moved to Arkansas.

What is feed withdrawal? We remove feed prior to going to processing to minimize the GI and gut contents and reduce the visible contamination. This evacuates the crop and reduces the pressure on the GI tract to help minimize the rupture as it goes through the processing plant. And

the main thing that many companies had that minimized the loss of that feed, that feed that goes into the GI tract is not absorbed, that's just lost money. So if you reduce that money you're also saving a little money on the whole production of the bird.

Well, there's several factors that influence the withdrawal as you go through a system. The actual time off of feed. If you go to some of these growers that we work with there's supposed to do an eight to 12 hour feed withdrawal and you go talk to them and -- you come in to do studies ant that type of thing, you'll see the birds been off feed 14 to 16 hours. And you ask them why? And they're like well, I had to go to church and I didn't want to leave it beforehand. Or they'll have some -- some reason why they do these things. So, the time off of feed actually affects the fragility of the gut and actually the bacteria that's present within the GI tract.

The nutrition, some of the feed that are being produced, if you have a higher fiber content it increases the gut flow as opposed to other feed sources.

The size and the sex of the bird. Larger birds tend to eat less frequently. They usually have large meals like every six hours as opposed to a smaller bird that eats more frequently. So, if you happen to hit that bird with feed withdrawal when it's about to feed and you give it

another eight hour feed withdrawal, you've got 14 hours on that bird itself. So depending on the size of bird that affects how the feed withdrawal performs in your processing plant.

And the flock health, again, if the bird's not healthy it may be eating less frequently than a bird that's a healthy bird, so increasing the flow of the contents throughout the GI tract.

And of course you have factor interaction between all of these different factors.

We know that food deprivation alters the intestinal micro-environment, these beneficial bacteria, which decreases the normal resistance in every species of mammals and avian species. And this promotes the growth of microbial pathogens. We also in in vitro experiments have suggested that in the micro-environment of the host may modulate virulence, factors which regulate *Salmonella* invasion. Juliet Durant with Dr. Corrier, four or five -- three or four years ago, found out that birds in a molting situation undergo basically a two to three fold increase in the virulence of *Salmonella* during feed withdrawal. So, essentially take the feed away, you take away the food for these beneficial bacteria and these bird -- the bacteria -- the pathogenic bacteria are more virulent so they can invade more easily in these birds and that causes more stress.

Some of the factors that influence or what goes on within the gut contents with the feed withdrawal period is that proventriculus-ventriculus junction, jejunum, and ileum have been shown to reduced in size. So it supposedly decreases the chance of cutting into these organs as it's going through the processing plants.

Now when we usually focus on in the processing plant, it used to be thought that the ceca was the main source of contamination in the processing plant. It has -- the highest number of *Salmonella* are found in the ceca, -- in the ceca. And our laboratories focused on the -- I call it the upper gastrointestinal tract -- the crop, the proventriculus and gizzard. And so, we basically said the upper gastrointestinal tract and lower gastrointestinal tract going from the gizzard up and the small intestine back into the lower gastrointestinal tract.

Dr. Hargis did a study I believe it was in Puerto Rico where they were observing that as chickens go through the processing plant, as the carcasses move through there they found out that the crop ruptured more frequently or leaked more frequently than the ceca. There's about an 84 fold increase when the crop went through the processing plant. And basically we think of the crop as just a big balloon that has two ends on it. And if you grab one end things are coming out the other end and if you grab the

other end things go out this way. So whenever we work with these things in the processing plant we've got to consider the potential of contamination by crop positive.

So we did a study, went out to the field and evaluated feed withdrawal period to see if it increased or decreased or maintained *Salmonella* in these birds. Essentially, after -- oops. Let's go back. At about five plus hours of feed withdrawal, we started seeing an increase in the number of *Salmonella* in the crops of birds. And overall in the study found a five fold increase in *Salmonella* in these crops of these birds, suggesting that there is a potential for contaminating the carcass when it's going through the processing plant.

As when you compare it to the ceca of these same birds we saw that essentially that it maintained the same, running around 5.8 percent to 7.9 percent, suggesting that, again, no changes are seen in essentially in the ceca and -- but we saw differences in the crop.

We know that some of the work done here in Athens, Georgia, they also saw increases during the transportation phase, increase in the *Salmonella* and I believe *Campylobacter* as well when they're being transported to the plant. And so another avenue that we could have increasing levels of *Salmonella* entering the plant.

So, the question would always arise, still is the

crop really that big of a beneficial thing. So we wanted to demonstrate there was a potential for crop contamination of the carcass as it's going to the plant. So, we went through and wanted to visually show workers within plants and to demonstrate the potential for this contamination. So what we did, we developed a fluorescent marker and it consists of an agar and some cotton -- cornmeal and a fluorescein dye. And this is right here is a bird that we gave this ten mls of this fluorescein dye marker inside the crop right here. And if you look closely you can see there's feathers found here. What it is, we gave this 30 minutes before it went to the processing plant to follow through the processing plant til it got to pre chill system. And I wanted to illustrate the birds indeed have been pecking on litters or been picking things up, because they're basically programmed to do a few things -- eat, sleep, defecate and the most important thing is grow. And if you take that feed away from them they're looking for something else to eat. And birds are notorious they look at -- they peck at contrast.

So, if you have that dark litter that Eric was just talking about on the floor and a white feather there they gobble those things up. You have the feed trays where we have this yellow litter or just the feed pans itself and mice happen to run through there and they leave these pellets there convenient for them to pick up. That could

have up to million organisms just in those little pellets. They gobble those up like candy. So, during this feed withdrawal period there's the potential of this contamination occurring.

This is a different bird and what we have here is the thoracic inlet, right here the thoracic inlet. The breast would be up here and here's the neck of a bird that was gone through the processing plant or gone through processing. Same -- a different bird with the lights on and off, this is just a black light. And you can see that a crop ruptured here, here's the neck, the wing here and then the breast here, and the same bird with contamination here.

And then a bird basically where the proventriculus-ventriculus ruptured as it was being pulled out through the pack man. And you can see this contamination going on there. Since I'm showing these things and just visually showing where potential contamination could occur, this is not saying that's all *Salmonella* going to be there. It's just saying that the potential is there.

Now, this is what we saw on most of the birds. We've seen a single pinpoint of light. The single pinpoint of lights right here, but, you know, some people say that's not that big of a deal. But when you're doing testing inside these processing plant and you get a positive test,

it can only be one little point and still give you a positive test. And if you get enough of those you've flunked your testing procedure.

Now, what did we actually find? First let me back up and say that this fluorescein dye acts a lot like the bacteria. It doesn't usually stick real well to the stainless steel equipment in the plant. It does stick to the skin of the bird and it can be washed off -- it's hard to wash it off the bird. And what we found just when we get to the rehang station, it can either be manually or automated, around 64 percent of the carcass was contaminated with this dye. And as we went through the processing plant through the pack man and the post packing it moved up to 90 percent with our highest level at the post-crop removal. And if you've ever been in a processing plant and looked at the cropper, the cropper is essentially a piston that has hooks on it and again, if that crops happens to be still in there it grinds up in there. Grabs ahold of that crop and again it has a balloon with two ends and you squeeze it things are coming out one end. So we get the contamination and then we go through the final wash and the numbers have dropped back down to 62 percent. Again this can be just a pinpoint on the bird. It doesn't necessarily look like it's all contamination of the whole bird. It could be a pinpoint and it was just illustrating the potential that

contamination could occur.

So, what we wanted to do again was to illustrate that the bird indeed are grazing on the litter. Dr. Corrier and I myself went into this processing plant, excuse me, a chicken house and we evaluated how often these chickens pecked on the litter. So, yes, we were official USDA pecker counters.

(Laughter.)

DR. BYRD: So we go through these things and basically find out that during the feed withdrawal period, during the eight hour feed withdrawal period that the birds would peck on the litter four-fold higher -- four times more frequently then they did prior to feed withdrawal, picking up those litter contents which could potentially be contaminated with pathogens.

So, what goes on in that crop when they do pick that stuff up? We know that the crop pH increases and the thought process behind that is that *Lactobacillus* numbers tends to drop because you're taking the feed away from these bacteria as well. And because they're falling off we also see the *Lactobacillus* decreases, there causing the increase in the pH of the crop. And then the potential of a contamination with the feces or pathogens.

So an early study or a study that was done actually here by Dr. Arthur Hinton, said, well what can we

do just to replace the energy to these beneficial bacteria? And basically he found that indeed we had a glucose supplement at 7.5 percent, we could reduce the pH from a 6.5 to a 6. And the actual log of *Salmonella* from like a 2.75 to zero. Now, I'm not suggesting that you put a sugar system into your water system. But it's just demonstrating that if we give nutrients to the beneficial bacteria, it could help oppose these pathogenic bacteria.

The next phase that we evaluated, I'm being trained more or less an endocrinologist. And what do endocrinologist do? They basically take out an endocrine organ grind it up put it back in and see if you see any effects. So, we want to see the same thing we added acid back into the water system. Mainly we looked -- wanted to focus on lactic acid. The acetic acid or vinegar, growers have been doing that for a long time, many times they don't know why they do it. They just put it in their system and it helps the birds do better and helps the chlorine work better is what they tell us.

And in our formic acid, and the reason we chose formic acid is that we use to have chick studies and in Texas and I guess most of the south, you have fire ants that go every where. And those chicks would just gobble up the fire ants and we couldn't challenge these birds with *Salmonella* because it was killing them. So we decided to

look at formic acid, indeed we found reduction from 53 percent down to 31 percent in our lactic acid. Really no difference in the ceca. The one drawback that we had using these acids is that usually we have a reduction in the water consumption, usually around 15 percent reduction in water consumption, which played a role in of course the yield in the plants itself.

So, we wanted to go out and evaluate in the field to see if there was indeed an effect with our lactic acid. And what we found is that prior to feed withdrawal we saw around 12 percent kind of maintained there until we went to the processing plant. We jumped up about two fold from post-feed withdrawal. But in treatment we saw a reduction from post-feed withdrawal to 16 percent down to 3.4. And in our pre-chilled carcass for instance, reduction about half, from 31 down to 15 percent. So indeed we saw an effect and there are some poultry companies that would swear by using this lactic acid procedure and other companies I think have different type water type things, hard water or soft water which may affect the effectiveness of the lactic acid. And as well as they use different levels for longer time periods or shorter time periods.

Some of the other studies that we looked at, we looked at some of the inorganic compounds, we looked a lactic acid versus sodium bisulfate. And found out indeed

we saw a reduction from our controls to sodium bisulfate similar to what we saw in lactic acid and our levels of our -- the actual log numbers from the crop went from 2.02 to 1.04.

Also, propionic acid has been evaluated in our laboratory and basically we saw similar reductions. Where -- here's a case where we actually saw consumption per hour reduced almost in half by using lactic acid, which we didn't see that with propionic acid. And the *Salmonella* reduced from 17 [of 20] to 9 [of 20] -- to 9 essentially, small numbers from 165 organisms down to 16 or 28.

Nowadays many, many companies out there have different acid products, essentially they're all working similar in that they're reducing pH. Some will last longer, some will have calcium components such that it causes the crop to contract and empty better and reduce the levels. But essentially they work the same way, reducing the pH on these levels and I only talked about the ones that I personally have worked with and not even going to elaborate to others.

The next area we're moving into is a product that we've been working with that -- it's called a chlorate. We call it experimental chlorate product, and how it works is that the family of bacteria -- Enterobacteriaceae possess some bacteria mainly *E. coli* and *Salmonella* and *Wolinella*.

And they have an enzyme that's called nitrate reductase, which basically allows it to survive anaerobic conditions. And interesting if we put chlorate into the system it allows it to -- it takes chlorate and produces a cytotoxic chlorite and only those bacteria that possess those enzymes are affected. So it doesn't affect the overall population of anaerobic bacteria. And if some of you like cartoons, basically we have a chlorate, intracellularly, with nitrate produces nitrite or chlorate can produce chlorite and again it dies as it goes through the system.

The animals will drink chlorate solutions very easily and some places there's a slight increase in the consumption of chlorate. And it can be broken down from chlorate to chlorite and chlorite into the systems. It's been used as an oxidizing agent in lozenges and gargles in Europe, a diuretic and cardiac stimulant. Veterinaries use it as an oxidizing agent and antiseptic. It's been approved for use in the UK for toothpaste and some medicines. In the United States most of the paper you have has been dyed white with this chlorate product. And it's also been used as a defoliating agent, for like cotton harvesting.

And being that I come from a laboratory where we develop a competitive exclusion culture, since this doesn't effect the overall population of total anaerobes, we wanted to see how this actually worked against birds that were

previously exposed to *Salmonella*. As previously been mentioned, these competitive exclusion cultures usually don't work when they've been previously exposed to *Salmonella*. So we thought we could come in and provide this competitive exclusion product with our experimental chlorate product and see how it responds.

And what we have here are seeders, birds that have been previously given *Salmonella* and we found that our controls was 100 percent and just our CE product alone again does not work when the birds have been previously exposed to around 87.5. But again, with the experimental chlorate product it went to 41 percent compared to 100 and combined with the other, it's 37.5. No real differences there. However, when we look at contacts, birds that were in the pens with these challenged birds, we saw that the numbers dramatically changed where our controls were 85 percent and we went down to 15 percent just with the ECP; with our competitive exclusion, a 33 percent reduction. Or down to 33 and combined together, we said 2.5 incidence in the number of birds affected by *Salmonella*.

So, we wanted to look at what time frame do we look at in these birds and another area we wanted to look at is right before the birds go to processing plant. During the feed withdrawal period or a couple of days before the feed withdrawal, we would provide our sodium chlorate or

experimental chlorate product to the birds and look at the crop contents. And basically from our controls at 37 percent we reduced it down to 1.7 after two days of being feed this product. And this is with an eight hour feed withdrawal.

Some of the things we found with this product was that ECP in day of hatch broiler chicks reduced *Salmonella* from 32,000 organisms down to two. In the pre-harvest we found that crops were reduced from 36.7 down to 2 percent. In the ceca from 53 to 31 percent and we actually saw *E. coli* reduced 1 log from 6 to 5.

We also -- because the lactic acids was sensitive to different pH or changes in the water, we also looked at it in the presence of pH of .4 and -- 4 or 9, and really there was no significant differences in our normal distilled water control compared to our controls.

We also added it in as a feed supplement and some companies, as y'all talked about earlier, it's hard to add things to into the water system. We wanted to see if we could put into a the feeding system into the feed -- the process -- the feed mill. And basically our controls went from 93 percent down to 23 percent when fed for four days. And this basically a seven day trial, looking at these birds.

In a different one we looked at the different

concentrations on seven days and we saw the numbers go from 60 percent down to 15 percent in our water versus 38 percent in our controls, in five percent of the feed, during a seven day trial going through a eight hour feed withdrawal.

And we also want to demonstrate this is the work done by Dr. Randy Moore, who is now here in Athens and he basically found out it worked just as well in turkeys. Not dramatic differences but we saw from 65 percent down to 5 percent at 26 hours on a log of 1.8 down to .17.

Now, this was done in pigs, but again we wanted to illustrate that it did not affect the overall anaerobic populations when exposed a chlorite with our chlorite being our yellow bars. And indeed we had some slight increases, although not significant, but in their rumen, the ceca and the rectum -- excuse me that must have been cattle.

And then resistance, always the resistance question comes up when you're talking about something to replace antibiotics. And indeed if you expose this bacteria, in this case we looked at *E. coli* O157:H7 exposed it to chlorate and adapted it, we indeed would find that these bacteria would become resistant, in a pure culture, to our chlorate. And in the case here, a similar thing is that we took these pure cultures and then took the influence away and we found out after eight passes we'd lose the resistance to the chlorate. But if you maintained in that 10

milliliter chlorate course it's maintains in this test tube.

We took this same material in a batch culture and took sterilized fecal fluid and basically put these bacteria that have become resistant to the chlorate into the system and found out it indeed maintained resistance. But you put this same bacteria into fecal fluid fresh from a cow and you indeed see that they cannot compete with the other bacteria then and these bacteria tend to die off. And here in this case 25 hours.

And the other question is does it change the bacteria profile or the antibiotic profile in the bacteria itself. And here's a case we looked at two different *E. coli* O157:H7 and saw that in two different strains, there was really no significant differences between any of these groups. We did see a slight increase from the tetracycline from a 1 to a 2 micrograms per ml and the spectromycin in the second one went from eight to 16. And again, once you put these bacteria into a mixed culture population, they tend to -- cannot survive as well and they tend to die off.

So our conclusions about this chlorate, it does decrease *E. coli* here, both the wild type and the O157:H7. It reduces *Salmonella* we recently -- not recently but in the last year or so, we found that it's also effective against controlling or reducing *Clostridium* species and have been giving birds that have had necrotic enteritis given this

product and it tends to reduce the lesion scores in these birds and stop the stunting that you normally see associated with a necrotic enteritis. And it does not decrease the potential beneficial anaerobes.

Again, it's just been said over and over again pathogens they're essentially in all phases. *Salmonella* is in all phases, all the way from grandparent flocks all the way down to the -- all the to the consumer. And once it enters the flock, it's hard to get rid of it. Even with this, as you see this chlorate product tends to work fairly well, but it doesn't necessarily eradicate anything. There's no magic bullet and it must be implemented early on in the process during the -- control programs must be implemented early on in the production process.

And this is what we said earlier and I appreciate your time. Thank you.

(Applause.)

DR. GOLDMAN: Thanks again, Dr. Byrd.

Next we'll hear from Dr. Norman Stern, who's a research microbiologist here at the Poultry Microbiological Safety Research Unit here at USDA here in Athens. He conducts a research program of poultry production food safety primarily to control of *Campylobacter*. And he'll explain to us how this is a model that can be used for *Salmonella*.

Dr. Stern has more than 25 years of research experience and his findings are documented in six patents and more then 300 scientific publications. Dr. Stern has received numerous research grants from sources outside of ARS. He's nationally recognized and internationally recognized for his work in the area of pathogen control in *Campylobacter* sampling methodology.

He was elected as chair of the Food Microbiology Division in the Institute of Food Technologists, is also a Fellow in the American Academy of Microbiology, a consultant to the World Health Organization on control of *Campylobacter* and has participated teaching at various university level courses.

Please welcome, Dr. Stern.

BACTERIOCINS AS INTERVENTIONS TO REDUCE PATHOGENS

DR. STERN: Good morning, I want to begin by acknowledging the audience and really appreciating that an esteemed group of you would take time out to consider new options that might be available to address the *Salmonella* problem. It has been paramount to make some forward progress in this area and so we decided to take a slightly different approach in our attempts to control *Salmonella* and for that matter *Campylobacter*.

I wanted to show this gentleman named Esko Nurmi, because I consider myself a friend of Esko and he is indeed

the progenitor of many of the ideas that have been presented today.

I felt particularly good -- Dr. Line and I had the opportunity to go from Russia on our way to a *Campylobacter* meeting in Denmark, we made a special point of visiting Dr. Nurmi and taking him out to for dinner. And I really respect this guy. When I took him aside later that evening and showed him some of the data that we had gathered on the application of bacteriocins and in my case I was most interested in *Campylobacters*. But when I reviewed the data I then had, he acknowledged that we really have come up with a very significant observation and probably had done a considerable amount of defining the mechanism of competitive exclusion. And so that's maybe -- that was gratifying.

Next, I do wish to acknowledge my colleagues at the State Research Center for Applied Microbiology. This is a former bioweapons group in the Soviet -- in the former Soviet Union. But these people are amazing microbiologists and each of them have a considerable expertise. And I don't need to go through each of their expertise. But they are certainly part of a team that's been a great joy for me to work together with. And I think we've had some useful progress.

This is Dr. Svetoch, he directs probably about 50 senior scientists, I don't know really how many support

staff go with him. But what Dr. Svetoch tells his people goes. It's a remarkable difference in procedure as compared to what many of us experience. So I envy Dr. Svetoch and he's truly another gentleman with a great deal of class and his group are very hard working.

Okay, we've all looked at competitive exclusion now, and I kind of wanted to understand it a little bit better. As most of you know, we looked at CE for *Campylobacter* and that's the reason I have anything to say is that I've been working with *Campy* for years and CE doesn't work for *Campylobacter*. Or if it works, it's pretty minimal. So we took the approach to look at variety and we were very wide open and definitely considered every aspect going from bacteriophage through competitive exclusion. We just hadn't found the right one.

So, very briefly I want to just show you the protocol that we used. These 25,000 isolates are from chickens, so we can be worried about bacteria from chickens, but we're probably up to 35,000 now. And we screen each individual isolate from a variety of *Penibacillus*, *Bacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus* and yes, we found inhibition of *Campylobacter* from a number of these genera.

So what we did was to take these isolates and we -- we took the isolates and looked for zones of inhibition, a fairly traditional approach, no amazing microbiology

happening there. And maybe .5 percent of these had interesting zones of inhibition that warranted further study. And as I said we had a number of different gnera that were useful.

What we did was to take the most promising cultures, grow them in broth and did some fairly routine biochemistry, although I must acknowledge that Dr. Eruslanov probably is one of the world great biochemist and I've seen a few. He is amazing. And so, we precipitated out the cell-free fermentation and we got our crude antimicrobial preparation. We dialyzed against that to get rid of the salt, so it was back in solution and we went through a series of molecular sieving and protein charge purification and chromatography to derive our bacteriocins and indeed in this particular work, we micro-encapsulated the bacteriocins in PVP, a substance that many of us took this morning with our pills, and incorporated that material into our chicken feed.

So, this doesn't look remarkable, we have a zone of inhibition and indeed this is a *Campylobacter* lawn and this is a plug coming from our -- our potential antagonist and we see zones of inhibition and that's all this was. And we selected against *Campylobacter* and there were -- retrospectively some of this is useful to select against *Campylobacter*, but I'm here to talk about *Salmonella*

control. Yet we selected our antagonist against *Campylobacter* but we still found them to be quite effective against *Salmonella*.

I should make mention of the perhaps two dozen strains that were most effective against *Campylobacter*, when we put those live antagonists into chickens repeatedly we had no benefit again, either prophylactically or therapeutically against *Campylobacter*, so I don't think CE works for chickens.

To kind of let everybody in the audience understand what kind of tests we did. We've been measuring our crude antimicrobial preparation -- I'm going to have a hard time. Do we have any other? Okay it's back on for a little while. We had -- there were four *Campylobacters*, we had it looks like seven bacteriocin producers and we had differences in the activity units per milliliter, and that's shown here. We made one to two dilutions as is commonly done for MICs and here you see a number four for this particular combination of *Campylobacter* and bacteriocin, at one to 128 we had inhibition. Well, that was on a per 10 microliter drop so, therefore you multiply by 100 and you're at 1280. So, I'll be referring to this as we go along.

So taking our bacteriocins, our crude antimicrobial prep, we needed to -- we more or less decided that we're not going to be able to do anything to abet the

presence of *Campylobacter* to the going into the final couple days of production. Consequently we took a very different approach and what we wanted to do was to demonstrate, we could effectively kill *Campylobacter* at the end of the production. So we needed to know how to make more of the bacteriocin and we did that by doing our isoelectric focusing of the crude antimicrobial preparation. We did polyacrylamide gels to determine the molecular weight and we did the spot test to demonstrate efficacy. Then we went down to larger scale purification and we went through a series of chromatographic procedures to pull our bacteriocin out in pure format.

On the basis side here, what we learned for -- OR-7 it turns out was a *Lactobacillus salivarius* which was effective against *Campylobacter*. And this particular one, what we did was run our polyacrylamide gels, we took the gel and renatured it and put it at the bottom of a petri plate and poured *Campylobacter* inoculated over it and you see our molecular standards. You see the crude antimicrobial prep with a zone of clearance around approximately a six kilodalton protein when we absorbed the CAP against the *Campylobacter* we lost this particular protein and when we purified these particular polypeptide we -- we again saw the zone of inhibition surrounding that protein.

This is an example of our isoelectric focusing.

We had a very different isoelectric -- we had an isoelectric point at 9.0 for the purified protein, and again no zone of inhibition against the crude microbial prep at other molecular weights.

A different bacteriocin, this was our *Penibacillus* and indeed this particular bacteriocin is -- was published and the results are in -- in the literature. Same procedure, and again, you see the zones of clearance surrounding the particular bacteriocin here weighing 3.5 kd. Same bacteriocin, only isoelectric focusing. So we had characterized these, different bacteriocin.

We were still needing to prove to ourself that we had a bacteriocin and so to do that, we demonstrated that when we subjected the bacteriocin to proteases here, beta chymotrypsin, proteinase-K or papain, we see no activity -- no residual activity against *Campylobacter* for these -- they were -- the activity was ablated by these -- this degradation of the proteins when we subjected the bacteriocin to lysozyme lipase and here notably it was stable at 90 degrees C for 15 minutes. And we have other bacteriocins that are considerably more stable. This one I believe -- yes, is a -- was a bacteriocin to -- from a *Lactobacillus* and although I would call this heat stable, it's still was less stable than some of our other bacteriocins.

All right, pH activity of again OR-7, you can see that it is highly stable over a range of pHs and over a range of temperature treatments and time as illustrated. It was only when we got out to a pH of ten that we lost activity. So, again, I'm demonstrating that these bacteriocins are quite stable.

All right, one of the criticisms levied against competitive exclusion is that our CE was not defined and even those, -- okay, I won't go there.

I would say that we have defined our bacteriocins. These are the amino acid sequences as indicated and we don't memorize that, yet we can see that this is a consensus sequence for the class 2A bacteriocins and there are disulfide bridges that are part of the class 2A. But it's the tail end that perhaps gives the differences in bacteriocin activities. But each of these are clearly defined now, and we can produce batch after batch with the particular bacteriocin.

We were very interested in the toxicity of the bacteriocin. When we used the crude antimicrobial preparation against both viral and Hep 2 cells we saw toxicity. When we worked with the purified peptide, there was no toxicity manifested against this bacteriocin and others that we have looked at.

Now we get to the stuff this group may care about,

yes I'm going to get the *Salmonella* -- this is still *Campylobacter* and this particular strain is in the culture collection in Peoria. What we did was to take four different *Campylobacters* gathered around the country and some of out -- am I out of time? No. And basically, we decided that we were never going to get *Campylobacter* therapeutically, prophylactically out of the chicken because *Campylobacter* has a way of coming over and over again. So we accepted that the birds were going to be colonizing. We challenged the birds with enormous loads, ten to the eighth of each of these strains. Ten chicks per strain. The chicks that -- the control group never were treated with anything and then, the treatment groups were treated for three days, seven to nine, and I believe in this case it was 125 milligrams per kilo. And so, this was repeated for each of the four strains. What we saw was a remarkable reduction in *Campylobacter* from $10^{7.2}$ per gram of ceca material to not detectible, 7.1 to .7, 7.8 down to 1.3 and 10 to the 6.6 to un-detectible. And yes, our control group never having seen *Campylobacter* did not produce *Campylobacter*. Different bacteriocins from our *Penibacillus*; same pattern, same project. Ten to the 7 -- same experiment, 10 to the 7 to .4, 7.1 to .3, 7.8 to 0.3, 6.6 to 1.2. I know these are only ten day old birds. We have considerable data for 42 day old birds that reflect the same type of data and we repeatedly

get the same kind of outcome.

All right, I have to talk about *Salmonella* in this particular presentation and I can say that we have considerable data against a wide number of other pathogens, so I don't believe that I have time to -- well I know I won't talk about that today.

We used a antibiotic resistant marked *Salmonella* Enteritidis, we challenge enormous levels. We probably gave the birds ten to the nine orally, and lo and behold our controls were indeed colonized ten to the 9.5. The liver was colonized, the spleen were colonized as expected. When we gave the birds ten milligrams of this particular bacteriocin, we could not find similarly challenged *Salmonella* in the cecum, in the liver, or the spleen. When we used five mgs per bird we were down to 14 birds we did not find SE in the cecum, 11 in the liver and 11 in the spleen and yes four of them did manifest some level of *Salmonella* Enteritidis in the -- in the tissue study. And the effect was approximately the same way when we treated each bird with 2.5 milligrams.

There now we're in broilers and the same sort of set up; yes we looked for *Campylobacter* and SE that was provided. *Campy*, we can always find a *Campy* positive bird. So our positives were in at 10^7 and at 10^7 for the cecal load. In the liver it was 10^7 in this experiment. But when

we treated the group with 3.7 mgs of this bacteriocin we eliminated the *Campylobacter*, eliminated the SE from the GI tract and we almost eliminated the SE in the liver. When we were down to -- when we were at 7.5 mgs per bird, further improvement in the liver.

We have looked at full market age birds and indeed we have again seen these same sort of six and seven log reductions for other *Salmonella*. This is not just peculiar to the *Salmonella* that we used for these studies.

So briefly and thinking about this, competitive exclusion had been ascribed to a variety of mechanisms and we've heard them already today. Substrate competition, colonization, site competition, volatile fatty acid, rapid rates proliferation and people threw in bacteriocin. I'm well convinced that each of us that have a gut in this room is having competitive exclusion happening right now and that the bacteria in your intestinal tract are producing bacteriocins and are the center of the gut ecology questions, and turns out bacteria kill one another so they can survive on their own.

Bacteriocins are defined as short chain protein -- susceptible to proteolysis. So we don't expect too much to be excreted from the treated host. Nevertheless, there's likely to be residual excreted still. We somehow -- we know that proteolysis will destroy these and there's a lot of

proteolytic activity going on in the gut. However, all that needs to be done is the bacteriocin intact has to see the target organism and the target organism is then inactivated.

All right, we need to be clear on distinguishing bacteriocins from clinical antibiotics. This really needs to be appreciated so that we don't have to go down the same road that I traveled down 15 years ago with FDA and I think there's a notable difference between bacteriocin. And we could talk about that if you care to.

The bacteriocins attack the host cell surfaces and cause the bacteria to leak out their cytoplasm, it's fairly straight forward. They're putting a hole in the target bacteria.

In FDA -- what's the number? No time, I'll finish this. Okay, if it's good enough for people it's good enough for chickens. Bacteriocins are consistently effective.

A couple of take homes. It is lethal to the target organism, effective against the mucosal surface targets here. Effective against antibiotic resistant bacteria. Leaves limited residues and creates no resistant target bacteria.

And I do want to acknowledge the ARS for their contributions to this work and to my salary and to giving me the opportunity to work here for a few years. I appreciate the Office of International Research in ARS, U.S. State

Department and my colleagues at the State Research Center for Applied Microbiology.

And yes, we do intend to go to a field trial in Russia. So with that I will close and thank you.

(Applause.)

DR. GOLDMAN: Thank you very much, Dr. Stern.

The last presentation on interventions at growout will be given on Bacteriophage Reduction by Dr. Stuart Price.

He's an associate professor in the Department of Pathobiology, College of Veterinary Medicine over at Auburn University. And prior to arriving at Auburn he earned a BS in microbiology from Oklahoma State University and was awarded a pre-doctoral fellowship at the University of Oklahoma Health Sciences Center, where in 1984 he received his PhD in microbiology and immunology. He received post-doctoral training in microbial pathogenesis at VPI from 1984 to '86 and at the University of Kentucky Medical Center from 1986 to 1990.

His lab at Auburn University focuses on pathogens that cause food borne disease including *Salmonella* and *E. coli* O157 and his ongoing efforts include developing pre-harvest food safety interventions using lytic bacteriophages to reduce pathogens in both poultry and cattle.

Please welcome Dr. Price.

(Applause.)

BACTERIOPHAGE REDUCTION OF *SALMONELLA* FROM INFECTED CHICKENS

DR. PRICE: First I'd like to thank Nate Bauer for inviting me to present today. It's good to be here. I also thank my collaborators Shelly McKee and Haroldo Toro at the university. Shelly is a food safety poultry scientist and Haroldo is an avian disease veterinarian, we work very well together. Finally, like to thank U.S. Poultry and Egg Association for supporting parts of this work.

Our goal in this project has been to combine some traditional intervention methods, including vaccination and CE treatment with bacteriophage to further reduce shedding of *Salmonella* in poultry. Bacteriophages are viruses that use bacteria as their host. Lytic phages replicate in the host bacterium and are released in an environment following lysis of the host cell. Bacteriophages occur naturally wherever population of bacteria exist. And of course maybe you heard of phage typing, it is used to distinguish closely related bacterial species and strains from one another. We routinely have isolated phages that infect *Salmonella* from samples coming in from our clinical laboratories from our teaching hospital. And also our diagnostic laboratory from many poultry samples.

This electro chromatograph is a picture of one of

our bacteriophages we call S9. These phages have a fairly normal looking appearance compared for instance to human bacteriophages with *E. coli*. They have CAPs containing a head and a tail. They also, have whiskers which can't be seen in this photograph. Phage treatment, phage therapy is nothing new. There are others working in this area both in pre-harvest food safety and also in using phages to perhaps remove or at least replace some antibiotic therapy in humans suffering from sepsis. Phage therapy actually dates back to the pre-antibiotic era where much work was done in Russia and Eastern bloc countries.

Bacteriophages can be grown to very high titer in either liquid or broth culture using their host, in this case *Salmonella*, for that growth. Phages form clear plaques on lawns of their host. As you can see this here with these circles growing on lawns. We can thus in the laboratory isolate these phage from samples and purify them by plaque purification. And also use plaques for titering samples of stocks of bacteriophage.

We can grow these, again, in very high titer and store them and they store for a long periods of time without loss of viability. This photograph is a composite of three petri plates in which we were growing a Typhimurium lawn and have pure cultures of three different phage used this study, S2A, S9 and S11. Note that each of these phages produces a

distinct lytic or clear plaque on these lawns.

Now, we chose Typhimurium as our model organism here for a couple of reasons. For one, as was talked about previously, Typhimurium is certainly isolated frequently from broiler chickens as shown in this FSIS table from a couple of years ago. And also it is one of the several serovars isolated from humans and spreads from animal to humans, at least we think it does.

We chose from a library of 36 bacteriophages, five that appeared to be distinct from one another and their plaque morphology and in their lysis patterns on seven serovars of *Salmonella*. Shown here we have S2A, 4, 9, 11, and 13 grown on one of seven serovars of *Salmonella* and each of these five phage had a different lysis pattern in terms of pluses being ... plaques and minus being not ... plaques on these individual strains. We feel like at least from plaque morphology and from growth on these strains that these five phage are different from each other. And that's important. We have worked towards developing what we call a phage cocktail versus using individual phage for treatment, in that bacteria can rapidly develop resistance to individual phage. But using multiple phages in a cocktail decreases that possibility dramatically.

Now, before we can actually do any kind of testing of bacteriophage in chickens shedding *Salmonella*, we had to

determine a couple of things. Although we don't know yet how we're going to end up delivering the phage to chickens, we decided to start with drinking water, and so we had to first of all determine if these phages that we have actually survive in drinking water. Now, also we had to make sure that these phages actually would transit through the chicken.

For our water survival, we used 10^6 phages and took readings at times zero and at time 48 in three different kinds of water. We used tap water, which at Auburn contains one part per million chlorine, deionized water and deionized water containing skim milk. Then we incubated these samples 26 degrees at room temperature and again at two days we compared the titer in these waters versus a T0 reading.

We found that all five strains survived well in the deionized water shown in the first two bars of this graph where we have the phage. This is on the X axis and the log 10 PFUs per mil on the Y axis. We also had no loss of viability of phage in water, this deionized water that contained milk, this would be the purple columns across. However, when we put the phage into -- into regular tap water, again this contains one part per million chlorine, three of our phages showed dramatic decrease in two days. Phage S4, S9, S13 showed tremendous multi-log decreases in

viability, most likely due to the fact that they may be chlorine sensitive.

So obviously one of our parameters for delivering phages to chickens experimentally in this work was that we have to use deionized water. In future work, of course we need to go back and try to find phage that are resistant to chlorine as are S2A and S11.

We then tested each of the five bacteriophage in groups of seven chickens. And inoculated each chicken with 10^7 PFUs of individual phage and then at day eight we gave *S.* Typhimurium to these chickens -- again this would be the host for these bacteriophage. And then sampled fecal samples for the presence of phage out to day 11 when the chicks were euthanized.

Four of the five bacteriophage did transit through chickens. S2A actually was shed beginning the first day after phage was administered, administered on day five and day six we began to start seeing phage. The other three were not shedding until late in the experiment. One of them actually the day of euthanasia. S13, phage S13, was not shed at least in this experiment in its seven chickens and therefore in the experiments that I'm going to be showing you, this phage may not have actually played any role in any of the results. In optimizing the cocktail then, we wanted to use phage that are resistant to chlorine and that do get

through chickens very well.

In our first experiment we inoculated chickens with our phage, and we had three different experimental groups and a control group. The X axis of these graphs will have the actual group listing, while the Y axis will be measuring CFUs per ml of homogenized ceca. So we're actually counting the number of Typhimurium in the ceca of chickens and each of these groups has 12 chickens.

In the first experiment we had one group that received the cocktail phage to the seven of all five phage. In the second group we mixed the phage with a CE product called Protexin made by Probiotics from England. The third group received antibody only. This antibody was hyper-immune chick antibody made against this strain of Typhimurium. And the positive control group of 12 chickens received just the ST challenge.

We noted that comparing these groups the means of the 12 chickens in each groups total ST ceca counts to the positive control, all three different groups did show a decrease that was significant compared to positive control. And that the group that received both phage and the CE product showed a decrease significant even compared to the other two treatment groups, the phage alone and the antibody alone.

This surprised us somewhat. So we decided to

expand the experiment and examine some of these same groups again, but also add to that a group where we mix phage and antibody and also looked at CE product alone. And those results are shown here. But once again, we're looking at total cecal counts of Typhimurium with chickens, we had one positive control where we put ST only in them. The other five groups each received a treatment, again we have a phage only group, antibody only group, and a new phage plus antibody group and new CE group only and then we repeated the CE plus phage work.

And once again we found that all treatment groups showed a significant difference in cecal numbers of ST counts comparing again, 12 chickens per group with the positive control. And as in the previous experiment the group that received bacteriophage and CE product showed a difference from the other four treatment groups.

Now, our phage were delivered to the chickens on -- between day eight and 12 through the water and again the water that we used here was deionized water. The CE product was given to the chickens on days 1, 2, 3. Antibody were given intramuscularly on day eight and then all chickens in all groups were given ST orally on day nine.

To summarize, bacteriophages do seem to show some promise in augmenting some traditional intervention methods in reducing *Salmonella* in poultry.

Our future work needs to be focused in two areas. We need to optimize the bacteriophage cocktail that we're using. We need to find phage that are resistant to chlorine and also phage that do transit the chick intestine completely. And we need to move this work into the growout and look at what's going on in the field and see if we see similar results.

As mentioned yesterday, sometimes food safety experiments work one time and not another. And of course for this to be promising at all as augmenting any type of pre-harvest reduction, we need this to work each time.

I'd like to acknowledge the workers at Auburn that helped with this work, I mentioned Haroldo Toro and Shelly McKee; Fred Noerr is our state diagnostic veterinarian, heads up our state lab there, provides many samples, they have a large number of poultry samples that come in weekly to that laboratory. Laura Bauermeister and Milla Kaltenbroct which are associates in the laboratories and technicians include James Krohling, Shara Murray and Michelle Purdue.

Thank you for your attention.

(Applause.)

DR. GOLDMAN: Thank you, Dr. Price.

We're now at the last presentation of the meeting. You've heard previously from Dr. Bailey, so I won't

reintroduce him. He's going to make a presentation on *Salmonella* Control in Scandinavian Production Systems Compared to Production Systems in the U.S.

Dr. Bailey.

SALMONELLA CONTROL IN SCANDINAVIAN PRODUCTION SYSTEMS COMPARED TO PRODUCTION SYSTEMS IN THE U.S.

DR. BAILEY: Thank your, sir.

We've heard several references the last couple of days to what is going on in Scandinavia and I had opportunity to develop a project with Dr. Tonya Roberts from the Economic Research Service, like a year or so ago. And we spent a couple of weeks in Sweden and Denmark where we toured a lot of facilities and met with a lot of people. So I want to share with you -- we've heard a lot of rumors about what's going on, but to tell you what's really happening there.

To start with, I need to frame things though. We need think -- we need put things in scale when we're going to have this discussion. U.S. -- as we heard before, the industry grows about 8.5 billion broilers a year. That's 80+ million broiler breeders. That's about a 100,000 broiler houses more or less. And that's important because if you think about retrofit or do anything differently it's not an insignificant cost.

And other facts, in the mid to late '90s the

pathogen reduction HACCP baseline studies showed approximately 20 or slightly more than 20 percent *Salmonella*. In recent years that's been running somewhere in the neighborhood of 11 to 13 percent. Clearly we know that the data for the last six months is up considerably over that.

But why have we seen those reductions from the baseline or had we been seeing them? I will maintain that primarily we're seeing those because of elevated levels of chlorine in the chill tank; secondary antimicrobial treatments in the inside/outside bird washers. There's a lot of different things being used in the industry. And the improved *Salmonella* status of breeder stock and hatchery sanitation. Some of the work we talked about earlier in this meeting. I might throw in as an aside right now that I've heard a lot of good thoughts and patting on the back that all these other pathogens that we're seeing reduction in, including in human population, including *Campylobacter*. Whereas our interventions that I'm talking about for *Salmonella* right here aren't totally effective for *Salmonella*. I think this is the only reason I'm seeing the reduction in *Campylobacter*, because we're doing nothing else significantly different in the U.S. poultry industry to reduce *Campy* levels. So it is these antimicrobial treatments that we're working on for *Salmonella* that's led

to those reduction in Campy, at least in my opinion.

So significant improvements will likely require on-farm interventions. You saw this slide from Fred Angulo and I never thought I'd be using the same slide as Fred Angulo day to day. With that said, just a couple of points, when on-farm interventions were implemented, this light purple, in the broiler industry, you did see this consistent reduction in *Salmonella* in Sweden. Similar for their pork industry when they implemented new on-farm control strategies and *Salmonella* Enteritidis in table eggs. So Sweden and Denmark have instituted effective on farm-programs.

However, the size and maturity of the U.S. industry would be very expensive and will make implementations of these exact programs difficult if not impossible. In fact we grow more chickens in Athens, Georgia than the country of Sweden and we grow more chickens in Athens and Gainesville, Georgia than Sweden and Denmark combined.

So what are their programs? They're fairly simple, and I've got a set of a couple of slides for each country I'll run though. They're really fairly simple programs, they don't involve any in-plant interventions at all. It's 100 percent on farm. The Swedish program requires no *Salmonella* in the breeder flocks and if they

have any breeder flock positives they totally eradicate. When the program was initiated back in the late '80s, early '90s, the government paid for this program. It's since switched over to an insurance funded program where everybody pays into the insurance program based on the number of birds they produce. But they haven't had to eradicate any breeder stock in the last three years. So, they've essentially got that under control.

Eliminating all *Salmonella* from feed, have an active surveillance program to monitor *Salmonella* status at all times. They have an active government input in the process and this is a very critical point for Sweden -- no *Salmonella* positive chickens are allowed to be sold.

So, the model then, as I said, is the breeder flocks have to be *Salmonella* negative, they test all, they kill the positive, they dispose, indemnify the farmers initially by the government, now by an insurance program. They clean and sanitize after each flock and they have very effective rodent control programs.

For feed, they have to be *Salmonella* free-- they test all and they dispose the positive or they reheat treat it and retest it. For broilers, they test all, kill any positive, dispose, indemnify, clean and sanitize after each flock. And I think it's a fairly interesting and important point that no *Salmonella* label claims are allowed, because

all fresh chicken sold in Sweden is considered by statute to be *Salmonella* negative, therefore they don't have any claims.

So, the principles behind this is it's pretty simple, straight forward. If you don't allow it in, you don't have it in your breeder stock, you don't have it in your environment, you have good biosecurity, then you can't have any *Salmonella*. Then you test the program and if you have any, then you kill the birds. So what's the status of what's going on now? All fresh processed broilers that are sold are *Salmonella* negative.

The EU has allowed this as a restrictive trade barrier. And it's the only restrictive trade barrier in the poultry in the -- in the EU. That means that you cannot import from even other EU countries that don't prove *Salmonella* negative status fresh product. Now, that doesn't mean you can't import chicken. That's a very limited definition of fresh product. If it's marinated or manipulated in some other ways, they're imported. And I can tell you that I was there and I was on a lot of these farms. The industry's under intense economic pressure to remain competitive. And to a certain extent it's because of these extreme measures they're taking for their *Salmonella* control.

The Danish program is quite similar to the Sweden

program, although it's not the same. They do have control in the breeder flocks, control in the feed, active surveillance but they don't have an eradication program for broilers. *Salmonella* positive chickens are allowed to be sold. So the Denmark model is all the same 'til you get down to the broilers. It's the same as it was in Sweden, but in the broilers, they do test and if they test positive they reschedule for either Thursday or Friday of the week or at the end of a processing day. So, because of that, they do have *Salmonella* label claims. They can either be labeled *Salmonella* negative -- I mean *Salmonella* negative, they don't -- I don't they label them *Salmonella* positive. But they can label them *Salmonella* negative.

So the Denmark status is that there's less than 2 percent of broilers on the market that carry *Salmonella* now. The control responsibilities again, the government had some input but it was turned over the industry in 2002 and they're working on an insurance program similar to what they have in Sweden.

So, the basic principles are similar to the Sweden model. The difference being that Denmark only kills breeders and indemnifies for them. If it's broilers, they test them, they reschedule and sell them at a different time. So, what can we take out of this as a potential for a U.S. model. Certainly, I'm in no way advocating this

similar program. It's just in my opinion would not work with our system as large as it is. But I think that we can learn some lessons from it. We need to work to have principles that are -- that we can take something from them. And breeders, I would advocate that we would work to have as reduced as possible *Salmonella*. And we would -- I would advocate getting there with increase biosecurity and use of vaccination and competitive exclusion.

In the feed, we can make *Salmonella* free feed, that's not a problem. What's the problem is keeping it *Salmonella* free after it's pelleted. And there are things we can do in -- and that could cost a little money. But that is something that could be done.

In the broilers, we want have reduced as much as we can, and again, the same things we talked about before -- using chicks that are as *Salmonella* free as possible. And we do that by what we do in the broiler breeders. I would advocate using competitive exclusion and some of the other types of things that you talked about today.

So, it's a cumulative effect of trying to get to some of the same principles that we're driving what was going on as an effective control program in the much smaller industries in Sweden and Denmark.

Again, we're -- not to beat a dead horse, but control in breeder flocks and in the broilers and I've

already talked about how. So we want to work to achieve the similar results that we see in Sweden and Denmark, but we have to do it in a cost effective and practical manner for an industry that's the size of ours. And rather than eradicate breeders and our broilers I would propose using other intervention treatments to achieve similar results. And those again are vaccination, competitive exclusion and increased biosecurity and the like.

So, what's the current U.S. status? It's highly unlikely that the government is going to assume any of the cost in the development of any *Salmonella* reduction program. And unless federal regulations are changed, the industry will not likely adopt changes that are cost prohibitive or put them in a competitive (sic) advantage. So, changes are going to have to take place. They're going to have to be done sort of universally across the board, so one company is not singled out.

And I did want to take the last 30 seconds of my talk to tell you about a program that I'm involved in now. It's called Collaboration Animal Health and Food Safety Epidemiology. And it's a program that's been initiated with the pork industry, very active input in the pork industry. We're looking at some pilot programs in the beef industry, the dairy industry and eventually into the poultry industry. It's a collaboration between APHIS, FSIS and ARS, where we

look at health issues and we look at issues of pathogens and we look at antimicrobial resistance, and other things. I just wanted to make you aware of that program and to take this opportunity -- I've worked with several of the companies this year in this room who have some issues and problems. And I'll lay this on the line for you from my 32 years experience. Almost always data is going to help in almost every way. It occasionally can give you a blip that's a heartburn. But the more data you have, the more you understand your process, the more you understand what's happening in terms of pathogen control, antimicrobial resistance, all of those things; we're all better off as a society and you're better off as a company because you know how to address things to solve problems with data.

So, that's all I've got and I thank you very much.

(Applause.)

DR. GOLDMAN: Thanks, Dr. Bailey.

If we could quickly get the presenters from this last session to the stage we'll have time for maybe a couple of questions and then I have a very brief summary and then we'll get everybody out of here close to on time.

Any questions or comments from the audience before this session?

DR. MARSH-JOHNSON: Hi, I'm Trisha Marsh-Johnson with Jones-Hamilton, for the moderator. I have a comment

and then a question for Dr. Line and for Dr. Byrd.

One of the management techniques that Dr. Line mentioned was adding ammonia to the house at higher levels. That really can be done and we do see growers doing that today. But they do it by manipulating the natural ammonia that's in the litter. So if a grower would shut the house up completely at bird movement you can get ammonia levels well above 300 parts per million and they maintain that for several days. So, I think that you can achieve that, you know, pretty much at no cost.

Question for Dr. Line. The data that you presented on the study with the sodium bisulfate and aluminum sulfate, when you published that study, the carcass data did indicate that there was a decrease in carcass positive for *Salmonella* at least with the sodium bisulfate treatments. But your comments seemed to contradict that and I was curious if you had done additional work that didn't support the trend of your published work?

DR. LINE: No, I went back and reviewed that published work and maybe we're talking about two different studies. But the results for *Campylobacter* did show reductions. The results for *Salmonella* weren't as promising. And we actually did see some of those slight increases, because we were using lower levels at the five pounds per 100 square feet was our level. Which would

equate to 50 pounds per 1000 square feet if you equate that to the Susan Watkins work. And at those levels, she also showed slight increases in *Salmonella* in the house, in the litter. So, I think our only difficulty in those experiments was just that we were not using enough product. These studies were done at a time when we were still learning how much of the product was going to be necessary to be incorporated into the litter to achieve significant results. And we were trying to make it as cost effective as possible, so we followed the advice of the companies that were involved as to how much product they felt might be economically feasible to incorporate into the litter for this sort of study. And in these early phases at least it turned out that perhaps it was wasn't enough.

DR. MARSH-JOHNSON: I just wanted to comment on Susan Watkins' study, actually at 50 pounds per 1000 there was no change from control. It was only an increase when they used sub-therapeutic levels.

DR. LINE: That's right.

DR. MARSH-JOHNSON: The other question I had was for Dr. Byrd. I think the chlorate product is probably one of the most promising things, you know, that we've seen data from. Where are you as far as getting FDA approval for that product?

DR. BYRD: We're in the process of -- they just

did -- the National Cattlemen's Beef Association -- provided funds to do residue trials in cattle. And we did the trials with that and found out there was no high levels with the residue. We're still waiting to do that in the chicken aspect. And we're looking at chlorate as a feed additive. And I think they just got the go ahead to try some of the chlorate in beef cattle studies in the field. But we're still waiting on the go ahead for chicken field studies.

And right now, in the laboratory we're looking, making sure that the pelleting and mash issues is not affecting the product itself. And still looking at both the water form and the feed form. So right now, it's still in FDA's hands.

DR. EWING: Hi, Marty Ewing, Sanderson Farms. This is for Dr. Bailey. I was just wondering could you describe the type of samples and when the samples are taken in the Swedish and Danish programs? And do they ever verify their *Salmonella* free status by either carcass rinses after the chiller or product testing itself?

DR. BAILEY: The -- most of -- the samples for broiler flocks are taken a week before they're sent to the processing plant. They're done primarily by using sort of their equivalent to a drag swab, it's -- it's a foot -- it's like a -- a large, it's about the size of a wrist band that slips around the ball of the foot and they walk through the

house with it. They do that also at -- I forget the number of days, but at -- it's several times during the breeder production. They do verify occasionally. I don't know the percentage but they do, there is verification with the live bird from -- there is a process but I don't remember the number of samples.

I was very skeptical about the methodology; as many people in the industry, we've heard many things. But when I was there I was actually convinced that generally speaking they do. It is for all species, there's been talk -- a lot of their programs are set up around Enteritidis and Typhimurium, but they do test for all species. So, I think the methodology is pretty solid. So, I tend to believe the figures more or less are as they're being reported.

DR. RICE: John Rice, with Sanderson. Stan, has the reduced instances of *Salmonella* in the broilers in Scandinavia that led to a reduced instance of *Salmonella* food borne illnesses in humans?

DR. BAILEY: That's a good question, John. They -- they have a very intensive reporting system and they do attribution a great deal. And the answer to your question, as I know it, is I'm not sure that their overall *Salmonella* levels are down. Their attribution to certain species are down. But they still have a lot a "travel related *Salmonella*" and the like. So that I'm not sure the answer

ultimately to that question. I think the levels have remained fairly steady. It's just that the attribution to say poultry or to pork or whatever, from that domestically consumed -- produced and consumed -- has gone down. I'm not sure that they've seen a huge drop off in the human side, because they relate most of what they have now to travel. So, I understand your question but I -- I'm not sure that the overall level is down that much. It's just that they attribute that to certain species.

MS. MOSINYI: Boitumelo Joy Mosinyi from Kansas State University.

So, Dr. Bailey, what you are saying is that Sweden and Danish program are effective on a small scale?

DR. BAILEY: I'm not sure I understood the question.

MS. MOSINYI: You talked about the Sweden model of *Salmonella* free and the Danish program, and you say that the in the U.S., you don't really agree with both programs that they have, because it wouldn't be cost effective? And since the poultry industry is large, it would not be kind of feasible, is this essentially what you're saying? Did I get you right that these programs will then be feasible on a small scale?

DR. BAILEY: I don't have any doubt in my mind that many of us in this room can grow chickens with at least

very low levels of *Salmonella* by doing very similar programs to what they're doing in Sweden and Denmark or the same program. The question is how can you translate that thought process of very, very stringent requirements where you have zero *Salmonella* in your breeder stock and in the case of Sweden -- in Denmark they even laugh and say that the Swedish program is not feasible. So, even if we drop back to the slightly less stringent Danish program, you still have to control 100 percent your *Salmonella* in your breeder stock or else you're going to have to eradicate. And at the size and scale of our industry I just think that would be very difficult to implement a program like that.

I do believe that if we are very stringent in using all of these weapons that we've talked about and -- and very stringent in -- with a commitment from the top of each company down, so that everybody understands the role and importance, that we can achieve somewhat similar results with fairly low levels of *Salmonella*. I would never say that I think we could get down to zero or even one percent or anything. But I think we could get to considerably lower.

I mean there are also secondary issues. As we talk about human illnesses going down in all these other pathogens most of those are directly attributable to certain specific things. With the *Salmonella* issue, there's a

separate issue than what we're dealing with here. If we reduce the level of *Salmonella* by 50 of 75 percent, are we going to see a -- a similar drop in human illness? We don't know, because we don't understand attribution of *Salmonella* good enough across all species and all sources.

So even if we do get that reduction in poultry we may -- we have to be prepared that we may not see that dramatic reduction like we saw with *E. coli* O157 in beef. Because there it's only coming from -- primarily coming from one thing. With the *Salmonella* it's -- we think it's coming from many sources.

DR. GOLDMAN: We have time for one last question.

QUESTIONER: This question is for Norm. Norm, have you've done any cost analysis of bacteriocin product or scale up -- large scale production of it yet of different form?

DR. STERN: Thank you, Jean. When -- we're still at the laboratory scale, but we project if we're going to make kilogram quantities that we can likely get this done with our current technology that we have in hand. It's something on the order of a penny a bird. But you know, that still remains to be demonstrated.

DR. GOLDMAN: We have time for one more one last question.

Dr. SCUPHAM: Alexa Scupham from ARS at ADC.

Norm, you said in your talk that you don't believe competitive exclusion will work for *Campylobacter*. I realize that we're not here to talk about Campy, but that implies a unique positive functional niche for *Campylobacter* in the intestinal community. Do you have an idea of what that niche or that function might be and how we might turn it against itself?

DR. STERN: Well, that part of that was really interesting. *Campylobacter* has been -- it's been published that *Campy* lives within the crypts of Lieberkuhn within the intestinal tract and really you don't see a lot of other bacteria occupying that deep crypt and *Campylobacter* is unique with that crypt. However, when we solubilize the bacteriocin, it is able as it's soluble to get and reach the *Campylobacter* within that unique crypt. In as far as *Campy* versus Campy, the answer is yes, and we have done studies I think -- I know we've published on that study. And yes, I think there are bacteriocins being elaborated every where.

DR. SCUPHAM: Have you tried expressing any of your bacteriocins in a less virulent Campy?

DR. STERN: Yeah, I think that was the topic of the paper in *Applied Environmental* that we published. And in that one I wouldn't attribute it solely to the bacteriocin, because we didn't study it. However, we could demonstrate that we would have a specific *Campylobacter*

actually displace the primary colonizer, so indeed that would take a different type of study for us to address whether it was bacteriocin. But I might bet on it.

DR. GOLDMAN: Okay, thank you, very much. Let's give one last appreciation to our panel.

(Applause.)

DR. GOLDMAN: I will be very brief.

What I thought I'd do by way of a summary would be to share a few bullet points with what I call recurring themes. I think you'll all nod your heads when you hear some of this.

First, food safety research is difficult. And we heard it in two ways. One, results in the lab don't always replicate in the -- on the farm or in practice and that similarly there is often a lack of correlation between in vitro finding and in vivo findings.

One thing I'm very impressed with is that there has been a lot of research done over the years, much of which demonstrates that *Salmonella* can be reduced in birds prior to slaughter.

Another recurring theme, probably the most popular recurring theme, is that research is ahead and in some causes way ahead of the government's readiness to approve interventions. I think we heard that loud and clear with respect to many of the interventions, including some of the

most recent interventions we heard about in this last panel. I think it is worth noting that approval of any biologic drug vaccine or any kind of intervention that requires regulatory review is always based on two things, efficacy and safety. We heard a lot about the efficacy here. We heard at least today a mention about concerns of -- about safety. And of course, safety has to do with human safety, where as efficacy has to with efficacy in the birds. So, something to bear in mind as all of the researchers pursue their efforts to get approval for their various interventions.

There were several presenters who mentioned cooking as a solution. And I think I would -- I would at least say that cooking is part of the solution. Someone today added handling and storage of finished products as part of the solution. I think that is a better way to characterize the consumer intervention, if you will. I do think it is worth noting that unlike problems we've seen with *E. coli* and *Salmonella* that result from ground beef in which undercooked ground beef or even raw ground beef has been associated with illness, we don't tend to see as much in poultry related illnesses regarding consumption of uncooked poultry. So, I think it is really an issue very much of handling and cross contamination.

We also heard that simple things do work --

biosecurity measures, environmental interventions, some of the litter management activities that we heard about this morning. We also heard clearly that the kitchen sink approach is probably not the best approach. That we should consider individual interventions and that even a single effective intervention will reduce the microbial load of *Salmonella* that results in *Salmonella* in these birds. And that can -- that effect can be amplified through the production process. And we can see some great advantage just with single interventions.

We also heard I think a good review of the National Poultry Improvement Program. And it providing at least a model for consideration in which industry played a great role in developing interventions for avian disease serotypes of *Salmonella*. And then more recently SE as a food safety issue.

And I think we also heard repeatedly that competitive exclusion products can be useful when added to various other interventions. So, CE has been combined as you heard with various things including vaccination, experimental chlorate and bacteriophages.

So, those are some of the, what I would recall recurring themes here. I think the attendance at this conference or at least the attendance that we had a little bit earlier, demonstrated that both the research community,

the regulatory community and the industry appreciates the reasons why we're here today.

And I think the opening remarks we had from Dr. Masters and Dr. Raymond yesterday simply punctuated the clear need for FSIS at least to exert its public health role in helping to address salmonellosis. I think it is worth my pointing out that FSIS is both a regulatory and a public health agency. And some of you may not know, we heard some references to the healthy people 2010 goals. Actually FDA and FSIS are the two lead agencies for this country's attaining all of its food safety goals. So, it's not CDC, it's not public health departments in the states, it's these two federal agencies which are responsible for this country trying to attain its food safety goals for healthy people 2010.

And I think I -- to me salmonellosis is first and foremost a public health problem, which is why FSIS is here, why we have hosted this meeting, and it is one nevertheless that as we've heard in the last two days, despite the best efforts of researchers in the industry and researchers in academia and researchers within the federal government, it has seemed to elude a good control to this point.

FSIS has been developing over about the last six or nine months a comprehensive *Salmonella* strategy. This meeting marks kind of the first official effort in that

direction. But it will also include as Dr. Masters alluded yesterday, perhaps some additional public meetings. We will be beginning a risk assessment for *Salmonella* in raw products. We will also begin baseline studies. You know, we have a current baseline that's just beginning for beef trim, looking at *E. coli* 157, but we'll also broaden those baseline studies after the first of the calendar year. So those will be part of our comprehensive *Salmonella* strategy.

Finally, I think some tangible products for those who asked earlier. We will have a transcript of this meeting available on our website when -- as soon as we are able to get that up. And as Dr. Thaler mentioned yesterday, we hope to be able to develop some production guidelines, compliance guidelines for the industry based on what we've heard in the last couple of days.

And I think maybe a less measurable outcome would be the collaborations that have occurred in the last two days, the chance for industry representatives to hear directly from researchers and to identify for themselves, some research gaps and make connection with researchers who may be able to help them with those efforts.

So, I think I will end those comments, but most importantly I need to end by saying, thank you to a number of people. First and foremost, Drs. Masters and Raymond, our leaders in FSIS, who've given us this very clear charge.

Dr. Nate Bauer that you see down in front who has been here, there and everywhere, was really principally involved in putting this meeting together and most of the researchers and others who presented heard directly from him. Also, on that staff Drs. Alice Thaler and Bhabani Dey, both help moderating the sessions. Ellyn Blumberg from FSIS Office of Public Affairs and Outreach. Dr. Patty Bennett, who was also here in the front holding up the signs telling us that we're out of time is from our Office of Policy. And then our host here in Athens, my folks, meaning from OPHS, Dr. Pat McCaskey, Dr. Lynda Kelley, Dr. Phyllis Sparling, Susan Brantley and Debbie Perry.

Very importantly, I want to thank the Area Director Darrell Cole and the Center Director Woody Barton and their contractor Four Seasons I think initially were concerned about the crowd, but I think managed us very well.

And finally, thanks to all of you in the research community, in the industry, and those of us in the regulatory world for our combined efforts and wish you safe travels home.

Thank you.

(The meeting was concluded at 1:10 p.m.)