Isabel Walls: So sorry. I didn't realize I was muted. We now have two more speakers. The first is Dr. Jeff Buhr. And Dr. Buhr is an animal physiologist in the US National Poultry Research Center at USDA's Agricultural Research Service. Please go ahead, Dr. Buhr.

Dr. Jeff Buhr: Next slide, please. I'm going to talk about the challenges and hopefully explain some of the concerns we had about animal production. When we're talking about *Salmonella* and broilers first thing we need to remember is it's asymptomatic, like almost commensal. We can't tell which chickens or feed have it. They show no difference in production.

Jeff Buhr: We're generally looking at less than 1% of the intestinal bacteria. The birds tend to be intermittent shedders in the feces. And they're only around for three to nine weeks of age. I'm sorry. Yeah, nine weeks of age, until they're processed. Next slide please. If we look at recommended, like we talked about vaccinating breeders, it works well in a low challenge, to the point where commercial, primary breeders actually have insurance that they're chick's eggs are salmonella free.

We need to talk about litter management, because we're going to reuse litter when we're doing broilers and there are many treatments are added there. We talk about feed. Most broiler feed is pelleted up to 170 degrees. So *Salmonella* transmission in the feed ingredients is not usually a problem, but there is transmission in the house, among the feeder pans, and at the end of a flock, any residual feed go somewhere else. There are many water treatments we can add. Biosecurity is a big, important thing and it varies tremendously between farms. It has to do with the animals, the humans and insects. Next slide please.

Here's the study, actually done at PDRC, where they vaccinated the breeders and they looked at the broiler ceca, and as you can see here, if we look at Kentucky, the vaccinated birds were 58, non-vaccinated 86. But if we look over to Enteritidis on the right, 98% of the birds, the broilers were still positive in the ceca. So vaccinated didn't seem to work when they challenged the broilers from those breeders, a day of age with 10^6. So this was a challenge trial. Next slide, please. This slide shows the relative reduction, and we're talking less than half a log from 18 to 24 days of age of these broilers. Next slide, please.

There's another trial they did here. They vaccinated the pullets five times. In this study, they had four pullet flocks, which became four broiler breeder flocks. From each of those, they raised four flocks of broilers. We did get a significant reduction in *Salmonella* from litter samples. These are all litter samples here, and we went to the processing plant. Although the levels increased, there about a 10% reduction. This was a natural contamination of what was on the farms. Next slide please. So overall with breeders, it doesn't work very well, if we have high challenge. Challenge the birds orally a day of age challenge, all the birds. If we have low challenge, we can get some reduction and that's quantifiable on the broilers, in the processing plant, after feeder draw. Next slide, please.

One thing we talk about broilers, we need to talk about litter. Many times people have thought, well, new litter would be the answer, where really, we need to reuse that litter, because it gets in, the bacteria, for the mature gut flora. And we also need it now, for the Coccivac programs to work. There's going to be killing of the litter, windrowing or composting in the broiler house, between flocks. The main treatment in litter is going to be to control ammonia. We need to reduce ammonia during the first two weeks of brooding. It also reduces mortality and minimizes foot and hock burns on the chicks. Next slide, please. Here's the study out of Australia, and what we see here is, the use of new versus used litter. New is in the purple and the pink. You can see those on the left side of the slide, the brooding time period.

If we go to where the dash line is, that's when they release them to the rest of the house. Most growers do partial house brooding to save on energy. And also, it works better with the Coccivac program, to have them in half of the house. However, when we look to the right, we see that the new litter had just as high or higher recoveries, and all the peaks are from the new litter, rather than the used litter, rest of the house. So what they're doing is, in most houses, it's 100% used litter. And this study, on the brooder side, it was brand new litter, and it was the same for the other farm, farm B. We look at the next slide. This one's going to do serovars. And we see, even on new litter, we pick up some serovars on the top half of the graph. We picked up very few during brooding, in the used litter. But the peaks you see in the top half of the graph, we see many more serovars than we do. So new litter versus used litter, that doesn't appear to be the answer. Next slide, please.

Here's a study out of Brazil where, this company did seven flocks in a row, where they recycled the litter. And you can see, these are litter samples from the flock that gradually decreased the number of positive samples from a high, the first flock 43, in the end, only 11. So we're getting a good reduction in *Salmonella*, in the litter with recycling. Next slide, please.

Here's a turkey project, which it's divided into two parts. We have turkeys at three weeks of age, and turkey at 19 weeks of age on the right. One thing in turkeys, they have a separate brooder house, and then they're moved to a grow-out house, mainly because 19-20 week old turkeys would destroy the equipment for the younger birds. These flocks are unrelated and they ranked them in the prevalence in the counts of *Salmonella*. And we can see, it goes from 2.8 to 5.3, the black diamonds show the moisture content. So if you look at three weeks of age, it looks like moisture content is an important factor. However, at 19 weeks of age, *Salmonella* is relatively lower. Moisture content is higher in all of them. So this is not the answer. Next slide, please.

When you're treating litter, we want to minimize the volatilization of ammonia during a brooding period. We also decrease beetles, which can carry *Salmonella*, decreased moisture. We're also decreasing *Salmonella, Campylobacter* and perfringens, which interacts with coccidiosis and get necrotic enteritis, and poor

performance and death. Next slide, please. They're typically going to windrow or composted. There's a gap between 14-21 days between flocks, and want to get that temperature up to about 130 degrees. They'll turn it twice. It's beneficial to remove the caked, wet or broke up lump litter, for optimal performance. Next slide, please.

On the top one, with the John Deere tractor, you can see they're pulverizing the litter and then forming a windrow. On the right, they're doing with just a tractor, and you can see you'll see the chunks in there. One thing to notice, is the feeder pans and waters are all hoisted up the roofs. In between flocks, these are just blown out with a leaf blower. Next slide, please. If we look on the left here, we can see what benefit from de-caking 1, 2, 3 windrow. Block mortality for this company was 6.2% de-caking, only 4.9 after one, 4.1 after two, 3.2. So we have improved livability. And then, we noticed that we were able to eliminate darkling beetles. And if we look down there, that's supposed to be 103, the box is on top of it, per 1000 birds. The 20,000 birds in a house, that's $2,000 per house. Next slide, please.

Here's a study they did at Auburn, they made artificially contaminated litter with about 10 logs. They either placed it on top of the compost, which would be on top of the windrow, uncomposted, or buried in the middle. And if we look in the middle, the *Campylobacter*, *Campylobacter* didn't survive in that environment, either way. *Salmonella*, who was on top, we got about 1.9 logs, a reduction from 10 logs. It was composted. We couldn't find it. The problem is over here with clostridium perfringens, even though we're at 1.4 log on top of the compost pile, still had 8/10 of the log. So this is the one that's hard to get rid of in composting. Next slide, please.

There are many acidifiers that are added to litter, to convert ammonium to ammonia, and keep it in there, and they're active. Mainly concerned during brooding, the rest of the time they can control ammonia by ventilation. Next slide, please. This is what a house would look like when it's first put in there with brand new, fresh litter. We see the feeder pans, and then there's paper put down so the chicks can spill the litter out there to get them started. This house has got four... I'm sorry, three nipple drinker lines and three feeder lines. Next slide, please.

This is what brooding would look like on used litter. You can see that, and you can tell it's a brooding picture because between the five water line sets, they've got two feeders that had to be filled by hand, which would not be practical in the commercial. So this is probably, one of these 60,000 bird houses. Next slide, please. When we're sampling litters, there's lots of different ways. We talked about litter samples. We talked about socks. We'll talk about drag swabs, the shoe covers. And we came up with a intermittently stepped on, drag swab. And I'll explain that in a minute. Next slide, please.

The top left would be a litter grab, usually doing 25 grams of litter. It can be a composite and we have the drag swab, the sock boot cover, and the shoe cover, all three of these need to be wet. They need to be wet so they pick up stuff in the litter. You can't just wait on the moisture in the litter. Next slide, please. You can see in the study, look at experiment two here, this regular drag swab, we got 44%. We increased that to 69% by stepping on it. So that added pressure of you stepping on it, we got the recovery 25% higher. Next slide, please.

This is our typical setup. When we do research, we have six adjacent pins here, and plastic wire between them. Next slide, please. And what we have, is a challenge pen on the end and a dayson pen in the middle pen. The top row, we have DS for drag swabs. Bottom row, intermittently stepped on drag swabs. If we have a high challenge in the end pens, there's no difference between them. What happens in the middle pens there, the one on the left, the regular drag swab missed the *Salmonella* four times, the one on the right missed it three. So in low levels, drag swabs aren't going to pick it up, but in high levels, there's no difference. Next slide, please.

So basically, we're increasing recovery, but we're getting fewer false negatives, and that's the biggest problem we have with sampling on the farm, is false negatives. Next slide, please. There are many different ways to sample birds. This one I'll talk about later, coacal swabs, feces, drag swabs. We have to look at composite are a lot better when the salmonella levels low. Ceca individual. Ceca an individual would be at necropsy. Next slide, please. Here's an example where we did a *Salmonella* vaccination. This vaccination worked, everything's negative. Next slide, please. However, in this one, we had positive ceca, positive sleeve, good correlation. Only two coacal swabs positive. Next slide, please.

Here, we had positive birds, no coacal swabs positive. Next slide, please. And here, we can see in a cage layer situation where they had 50 cages, had 12 birds per cage. If they sampled the feces, was a composite sample, they got 46 out of 50 cages positive. If they did coacal swabs, three per cage, only six out of 150 were positive. Eggs, we got more positives on the shell because they're rolling around the cage. It's a composite sample. Fortunately, inside eggs are all negative. Next slide, please. So using samples other than coacal swabs, feces, spleen litter, they're all pretty good samples. Composite is what you have to do, rather than individual, if you have small, low numbers. Next slide, please. Competitive exclusion has been recommended for broilers. It was suggested in the past, but this is what we're getting with that mature litter and reusing it. There are many feed and water pre and probiotics, bacteriophages, and bacteriocins, probably fit best in the processing plant on the final product. There are many antimicrobial compounds that are added to feed, and they so, shall benefit. Next slide, please.

Probiotics are defined as live cultures. Prebiotics is everything else. And I'll give an example of some organic acids in the next slide. Next slide, please. Here's 66 examples of prebiotics that have been fed to broilers. Next slide, please. The

way organic acid works is, they're lipid soluble so they can diffuse into the cell. They can dissociate if the pH is 3.4 . I'm sorry, three to four, or less. This disrupts cell function and death. Gram negative bacteria can metabolized longer chain, fatty acid. So it has to be the medium and short chain. Next slide, please. Here's the diagram, what I said, but thing to remember, we need to get that pH to 3.4 so that we're pumping in the acid faster than they can pump it out, with this ATPase, the hydrogen ion. Next slide, please.

Here, we did some work, also from PDRC. We're looking down here, where the red arrows are. We're looking at relative, low number of bacteria, *E. coli* and *Campylobacter*. Next slide, please. In the jejunum, less than 3% are gram negative, ceca, less than 8%. Next slide, please. Here's an example where, they did acetic, lactic, and formic acid in water 0.5%. You can see, they had a significant reduction in *Salmonella* positive in the crop. No effect on the ceca. Next slide, please. Here's an example where they added lactic acid in the water, and they looked at positive crops, but not a significant difference. However, after a feed withdrawal for 10 hours, they got a significant difference. It increased when they looked at the carcass rinse, but there still was a significant difference. So this was a positive effect. Next slide, please.

This is a slide with *Campylobacter*. I know this is *Salmonella* meaning, but in the early studies, they show that feed withdrawal, I mean full fed, they had a significant reduction in the quantity of *Campylobacter*, and it held up during feed withdrawal. This was when it was only provided for three days. Next slide, please. Oh, this one's a little smaller. It didn't hold up with seven days. Next slide, please. We did a study, where we looked at formic acid and lactic acid in the feed. Our typical challenge would be three positive chicks, in a 33, take them out at a week. So this low challenge. Next slide, please.

We looked at litter. We looked at ceca, and we did whole carcass rinse, after processing, and whole carcass enrichment. Next slide, please. Okay. There's our whole carcass rinse. Next slide, please. 30 mls of buffered peptone water. The remaining 400 windrow hole carcass rinse. This theoretically, can pick up eight cells. Next slide, please. We struck the *Salmonella* on plates, looked at zones. Next slide. This shows what the challenged pens, gradual decrease at six weeks. Next slide. This was adjacent pen, not nearly as high a challenge. Next slide. Here's what we see, what happens at three weeks. We look at ceca, pretty high that's the time to sample them. Decreases at six weeks, comes up with feed withdrawal, higher, whole carcass rinse, higher whole carcass enrichment. Next slide.

Here's what happens in adjacent pen. Were low challenge. Highest at three weeks, nothing increasing feed withdrawal, but the control, whole carcass in rinse, nothing, whole carcass enrichment, nothing. The problem here is, the treatments in a low challenge were not different from the control. So it's hard to convince them to do something. Next slide. In immersion chilling, is the main critical control point, in any processing plant. Next slide, please. Here's the

digestive tract of a chicken. It's not really possible to get these oral interventions to affect the digestive tract. Next slide, please. Here's the pH in the gizzard, it's already down to 2.5. Next slide, please. This is taking longer than I thought with the changing. We can push it down a little, but we're not going to affect the lower digestive tract. So next slide. So we have minimal effects in the crop and the duodenum, and not in the jejunum and ileum. So I can stop there if my time's up. Yep, that was it. Time's up.