Dr. Steven Ricke:

Well, thank you for having me here today to present some of the work that we've been doing here at university of Wisconsin on pre-harvest and post-harvest research strategies and control measures for Salmonella and poultry. I'm Steve Ricke, I'm the director of the Meat Science Animal Biologics Discovery program, or known as MSABD here in the department of Animal and Dairy Sciences at the university of Wisconsin. The MSABD program is a relatively new program, it's only been around for the last of years or so. And this is part of a new building, it was constructed over the past four or five years, a 67,000 square feet, 25,000 square feet of which is dedicated to meat and poultry processing, USD inspected plant and 8500 square feet that's dedicated to a food safety pilot plant and laboratory.

We focus on three areas, basically animal biologics, which is the discovery of unique animal proteins and peptides and antimicrobial compounds that can be used for biomedical purposes, as well as other uses meat processing poultry processing, Wisconsin's has a quite extensive meat processing industry and so we work a lot with that industry in terms of outreach workshops, discovery, research, etc. And finally, what I'm going to talk to you today a little bit about is our food safety efforts. And we do both research extension and outreach, almost to this research as part of our Land-Grant University Mission.

So our MSABD food safety and microbiology research program, as I mentioned, we have a bio safety level-2 containment pilot plant, as well as a joining lab that allows us to work with pretty much all pathogens. Some of the things that we study include meat microbial ecology. This relates both the shelf life, as well as pathogen, ecology, microbiome sequencing, and mapping. We are starting to employ microbiome mapping to some of the process steps that we're looking at in poultry processing, as well as other meat species equipment, sanitation evaluation. We're set up to bring in our test ourselves, spray cabinets and other types of sanitation equipment and sanitation compounds, etc. We do a lot of pathogen inoculated animal studies. That is the beauty of the having the processing plant. As we can start with pathogen, inoculated animal studies, live animals, and then bring them into the processing plant and follow the pathogens all the way through from live animal, all the way to the final meat product.

We examine feed additives quite a bit in our live animal side of things. There's an active industry in terms of prebiotics probiotics, bacterial flagella, as well essential roles, and a number of other feed additives that we've examined over the years to look at their abilities to control or limit salmonella establishment. In processing, obviously, we can add pathogens to the carcasses and follow their ecology as the carcass goes from initial processing steps to the final processing steps.

And as I mentioned already, we have the capabilities to do in longitudinal path studies, where we can start with pre-harvest infected animals either

deliberately or naturally infected and follow them all the way through post-harvest and be able to determine transmission routes and impact of where animals are infected in the gut versus other tissues and how that translates into what we see in the processing plant.

Now for our pre-harvest control strategies, we have a stepwise approach to how we do things, and this would be true of other pathogens as well. We've also looked at campylobacter, which obviously is another very important pathogen in poultry as well. Here we're going to focus on Salmonella for the purposes of this talk. And generally we start out with classic Salmonella challenge that is in vitro and in vivo studies, we have an in vitro SQL incubation by that we can use as an initial screen for doing high throughput testing, different dosages and different variations on particular feed additives, once we have that determined, we can take it on into in Vivo challenge studies and follow live animal response in the presence of that feed additive with and without the Salmonella challenge. While we're doing that, we collect samples and monitor the gut microbiome response, Salmonella is interacted with the gut microbiome as we started to find out in a variety of different ways.

And so it's important to follow those microbial communities and characterize them during the Salmonella infection phases. The information we get out of that I obviously relates to gut ecology identification, representative, gut microbes with feed additives what we're interested in is core microbiomes for particular feed additives. In other words, you want to know if a feed additive is actually being effective and if it's working and usually there's a signature or a core microbiome, that's representative of the presence of that feed feed actives, that's certainly true with feed prebiotics, but it's also true with other feed additives as well. And having those indicator organisms gives us a way to assess things in the field in terms of whether a particular product is working or not. Diversity, where we can identify differences in gut micro populations between feed additives and obviously control birds that aren't receiving the feed additive.

Again, this is a way for us to assess where there are impacts truly coming from the feed additive that is working against Salmonella establishment or limiting Salmonella. That's already established. One of the things that we haven't done as much as, but obviously the growers are quite concerned about this is bird performance. In other words, if we do a feed additive and we add it in there, how does that impact bird performance? Does it improve gut health? And do we see corresponding responses and bird performance? So we look at a variety of host gut responses, metabolomics, of course, where we can do a complete metabol act profile in that gut, transcriptomics and proteomics from the animal gut tissue standpoint, to see if we're impacting nutrient transport, immuno responses, etc. And so we can start to tie a complete picture together and get a very good idea of what a particular feed additive is doing.

And if it's effective, which of these variables are contributing to that effectiveness. And we think mechanistically that's quite important as we go

forward to start to sort out which of these feed additives are going to likely be the most consistent and the most effective. So some of the things that we've seen in the past few years is and some of these have been surprises. For example, as I mentioned earlier, Salmonella and the gut microbiome are interactive and that's not a complete surprise. We know that Salmonella can induce inflammation and other host responses, which can impact the gut microbiota as well. One of the things that we were looked a lot and developed some Salmonella vaccines. One of the things that we picked up on as we were developing those vaccines is the presence of a vaccine strain does impact the gut microbiota.

In other words, there's a detectable difference in gut microbiota when Salmonella vaccines are present. What really surprised us was that there was detectable gut microbiome variation, even with closely related genetic vaccine variants. In other words, the parent strain was the same with just minor genetic modifications in the vaccine variant. And we still could see detectable gut microbiome difference. In other words, those variants impacted the gut microbiota somewhat differently between each other to the point where there were detectable compositional differences.

What we've concluded out of that is that when we think about vaccine strategies, we need to think of it in terms of a three way interaction, obviously the Salmonella and the host, but the Salmonella, the host, and the gut microbiota, all three probably contributing to the overall host response to that vaccine strain. And the host is ability to limit Salmonella establishment.

And so going forward, I think it's going to be important to include all of these metrics when we go to evaluate vaccines. Probiotic mechanisms, discovery, we've done a lot of Probiotic work over the years. And what we found out is as prebiotics come from more complex sources. We see a much more complex gut microbial response. Classic probiotics typically was always believed to select foreign enrich for certain beneficial organisms, such as lactobacillus or bifidobacterium. What we're finding out now is, it doesn't stop there, that there are a lot more other microbes in the gut that also respond to the presence of not just these probiotics, but certainly the much more complex ones as well. What we've also started to discover is there are other sources of prebiotics beyond what I would call the traditional sources.

For example, serial grain brands have proven to be illicit prebiotic type effects when they're fed to birds or put into our in vitro system and can limit Salmonella growth in those types of environments. What's interesting is that even within a single cereal grain, such as rice, for example, different colon bio have different prebiotic properties. And so going forward, we really think that Salmonella is going to be a very effective screen for identifying which of these serial grain brands are the best probiotics to use in a actual practical setting. Salmonella, pathogenesis, in poultry. We've done what we call Salmonella barcoding, where we can put a strip of DNA on Salmonella genome, in a place

that doesn't affect any kind of physiological activity or doesn't affect its infectivity, but is detectable by a PCR or sequencing.

We can easily identify different variations of that barcode to identify different isolate to Salmonella. We've used that to identify transmission routes. One of the things that we were interested in barcoding helped us to elucidate this to some extent as we've always assumed that birds are primarily orally infected by Salmonella. In other words, they consume something contaminated with Salmonella and then it gets into the gut and then we have an infected bird. What we found was is aerosol transmission routes where the bird literally breathes in Salmonella is also an important route too, that we should not preclude. And therefore things like house management and those sorts of things probably are bigger factors than we realize in terms of monitoring Salmonella, as well as being able to control Salmonella infection in a flock. The other thing that came out of barcoading was, we could put different barcodes within a single-serovar population.

In other words, having different strains with different barcodes. And there was heterogeneity in that population. In other words, some went to different routes in terms of infection, survival rates in the the gut, etc. And so whenever we consider a particular serovar whether Typhimurium or Enteriditis as to whatever, there probably is some heterogeneity within that population of an inocular or colony on a plate or whatever. And so that probably contributes to the variation of what we see sometimes with the responses in terms of infectious dose, etc. Now for we'll switch gears and talk a little bit about poultry processing. Again, what we look at is we're using the microbiome in conjunction with Salmonella loads. Here, we're looking at processing microbiome again, our 16 S gene targeted sequencing of the entire microbial community. We have used this in a variety of ways, somewhat unique applications that we just came across as we were working through how to use this type of technology.

Certainly, conventional plating has done a lot for bio mapping and processing to get an idea of what overall bacterial loads are typically, aerobic plate count plates, et cetera. What we've done is overlay that with microbiome mapping, where we can look at the same sites that we normally take for plate counts, and we can actually do microbiome sequencing of those same steps, and then compare the two in terms of microbial composition. We've also introduced the concept of pathogen quantitation while we're doing that. In other words, we can look at the total microbial ecology, and then look at the presence or absence, or even more importantly, the quantity of salmonella or campylobacter or some other pathogen within that microbial community. Certainly, identification of individual taxonomy taxa bacteria to find what I would call ideal representative indicator microbes. In other words, we've always based our indicator methodologies off of what we've seen in the literature and used in other ecosystems.

With microbiome sequencing, we can actually use the carcass microbio that we identify and figure out which ones that those are most likely to parallel what a

pathogen such as salmonella is doing, and then use that population and develop detection assays around that particular population of organisms, whether it be pseudomonas or some other organism that continues to parallel and behave similarly to salmonella. We can also identify signature microbial populations for interventions as well. For example, particular acids or whatever, we'll usually select for a particular population that will be evidence of the presence of that antimicrobial. We've also used it for screening of culture, both nonselective and selective plating and validation. What we find is that non-selective plating is maybe more selective than we realize, and that selective plating or selective plates may actually be less selective than we've realized. We've certainly seen this with certain pathogens, such as campylobacter. That's been pretty evident.

So some of the results and accomplishments we've had so far within our research on the processing side is we've developed a salmonella rapid quantitation most probable number qPCR method, which has a high degree of sensitivity. We've used a non-selective media combination with qPCR with the idea that we could recover viable, but injured cells that would normally maybe not grow on a selective media or a selective enrichment, and we can speed it up because salmonella grows so much faster on this non-selective fairly rich media and then qPCR can distinguish salmonella against the background microbiota that's coming up in the non-selective media. So we've been able to shorten the quantitation time considerably, and then still use the precision of an MPN here on a targeted plate to where we can reduce media volume. With microbiome sequencing and mapping, we've been able to detect a shift from fecal to spoilage microbiome as we go through processing. In other words, there are certain steps where one population starts to fade away and the other population becomes much more prominent.

As I mentioned already, candidate indicator microbes. Intervention signature microbial populations and profiles. I think that's going to be important for validation studies on effectiveness of antimicrobials going forward, especially if we can line that up with good indicator organisms of pathogens so that when pathogen levels are low or infrequent, we can still get a pretty good idea of how effective our intervention methods are. Shelf-life modeling is something we've gotten into more recently. Idea is that knowing what that population is of the raw product coming out, the raw poultry product, and how long and how fresh is it going to stay and when do we start seeing a shift over to more of a spoilage that's detectable? Then of course, selective plate ecology. We think there's quite a bit of information to be gathered off of sequencing of some of those colonies off of those plates. So I think our future focus, at least in our program for salmonella research in poultry is going to be along the lines of continuing to use this barcoded technology for longitudinal studies.

In other words, being able to infect animals with different barcoded serovars and follow them all the way through to processing to identify transmission routes, tissue tropism, et cetera, in terms of where salmonella might be going in certain tissues in the bird and if that presents another problem in terms of controlling salmonella. Identifying salmonella virulence markers. We've done

some early work along those lines of being able to show that's certain virulence genes correspond to organ invasion in laying hens. Some of this may very well be true in broiler as well, such things as feed withdrawal and other things can actually impact salmonella virulence and make it more invasive. I think there are things to be explored and developed there, especially with the microbiome approach to being able to look to see if the microbe ecology of the gut also changes during those times. Pre-harvest strategy additives and house management combinations. In other words, I think feed additives help, but I think house management has to be considered as well.

In processing microbiome mapping in conjunction with salmonella quantitation, I think is going to be important because I think they are somewhat interconnected. I think the ability to be able to follow both through processing will be really important. Salmonella and processing ecology, processing plant ecology. We focus mostly on the birds in the processing lines, but I also think parts of that processing plant, the environment, sampling of that and seeing how much that contributes to both the general microbiota on carcass as well as particular pathogens, such as salmonella will really be important to determine as we go forward. Salmonella serovar and strain differences. We, like everyone else, is probably focused on just a few serovars for these mechanisms. I do think we need to look at the broad range of serovars because I would suspect that not all serovars behave the same way and that some are going to be more or less virulent, more or less influential on the gut microbiome, behave differently in the processing plant, et cetera. I think we'll need to determine those things. With that, I thank you and will be happy to entertain any questions.