

Isabel Walls: Our next speaker is Dr. Sanchez-Plata, and Dr. Sanchez-Plata is an associate professor of Global Food Security at Texas Tech University, please go ahead.

Dr. Marcos Sanc...: Thank you very much for the invitation and thank you for letting us share some of the data we've been working with. So, my talk is about using bio mapping techniques for quantification of pathogens and indicator organisms and using that information to develop statistical process control parameters for food safety management. We have some data for pre- and post-harvest information that can complement some of the discussions we had earlier in this session. And what I would like to do first is to thank the industry for letting us work with them, into developing this industry data. So, this is all information, not from a lab, not from a laboratory, not from a challenge study, but rather data from plants collected throughout different weeks and months and analyzed in a way that would make sense of the data. So that's my first appreciation for this presentation.

What I'm going to try to cover here is understanding why we do *Salmonella* and *Campylobacter* quantification for developing baselines based on bio mapping, what parameters we're going to measure for process control, where, and when to take samples, how many samples and how often we do these studies, and then how do we use these data for the long term and how do we analyze the data?

We don't have to talk about the why much, because it has been this discussed here. The chicken and turkeys contribute to a quarter of the foodborne illness according to the attribution data from the interagency collaboration. So therefore, we need to make an impact to that contribution and that's what we're working for. Also, because plants and companies need to comply with the performance standards, therefore they work heavily on getting the performance standards and complying with them, and we help them into achieving that by either providing validation studies of some of the interventions on the processing schemes, for physical and chemical interventions, and also trying to develop management systems based on the data that they collect, either data for their own analysis internally or data that is used for regulatory issues. So, we work on that data and try to get actionable information from it.

The USDA FSIS came up with the recommendations on how to use bio mapping and they included developing maps at different locations during processing and collecting samples, not only of the indicator organisms but also of the pathogens. Then trying to co-relate the data of the indicator levels measured in log and scale with the pathogen prevalence that you can determine for the product and then find out if there is any type of connection when you have reductions in indicators, that they also co-relate with reductions in the prevalence of the pathogen. We're going to talk about this because it doesn't work that way.

The data is then supposed to be used to establish limits of process, where you can take the data, calculate an average, and then from that average, determine the variability and establish upper thresholds or lower threshold where you can measure the process and compare your performance on a monitoring basis, on a regular basis. And then take actions based on that information. And you are provided with data from a national baseline where they have calculated the median information on the different indicator organisms that can be measured and accounted for in poultry products. However, there's no data on the loads of the pathogens that you can find during processing.

This is the type of data bio mapping that we've been able to generate for the last six to seven years with different industry partners. This takes time because you have to collect samples, the different days, different weeks, and different stages during processing. But what you can see with these box plots is 50% of the data points on the boxes. The dark line in the middle is the median of the information and you see the logs of, in this case, aerobic plate counts, and how the process itself with the different stages of processing reduces the levels of microorganisms on the carcasses, all the way to the chilling steps. We can see the little triangles at the bottom represent different interventions applied throughout different stages of processing. So, this is the way we've been doing it with indicator organisms. We can determine the means and then try to come up with these statistical process control levels for indicator organisms.

We also use other types of indicators like *Enterobacteriaceae*, where you can determine that *Salmonella* is part of that indicator. So, the assumption is that if you're able to reduce *Enterobacteriaceae*, you are also able to reduce the levels of *Salmonella*, but with prevalence, we don't see that. So here you see that at the chilling steps at the end, the level of reduction of the process is very significant. You find several outliers, which are those circles, showing data points on some of those samples taken after the chiller. But most of the samples you can see are in that box. Therefore, they're showing you that the level of reduction is under 0.5 logs of *Enterobacteriaceae*.

We have been working with this in US facilities, but also in facilities in the Southern Hemisphere. They just happened to export the poultry products, not only to Europe, but also to the US. So, there's an interest like interesting dynamic there because on Monday, Wednesday, Fridays, they process for the European Union, and they cannot use chemicals because those are the restrictions from the regulators there. Whereas on Tuesday, Thursdays and Saturdays, they do work with chemicals because they export to the US. So that created for me, the perfect laboratory setting to compare the levels of indicator organisms and pathogens with and without the use of chemicals, which is one of the concerns that we always have. So based on that, we did several studies down in Chile, Brazil and Argentina, and then we tried to replicate those studies here in the US.

We developed a standard procedure for designing a baseline, which is about doing at least five different days to consider different flocks and day effect. We can modify their interventions scheme for the plant, with and without chemicals or with low levels of chemicals. We always take samples in the morning and the afternoon to consider shift effects. And we also look at different locations throughout the processing, depending on the stages of the process where interventions are applied. At least 10 samples per location for a total of at least 50 data sets, for each point to be analyzed as a minimum sample size. And then we look for quantification and the prevalence of pathogens like *Campylobacter* and *Salmonella* and also the indicator organisms, total aerobic counts and *Enterobacteriaceae*.

This is some data with indicator organisms that what you see here. On the Y axis is the total count of aerobic plate counts and on the X axis, all the stages where we took samples. The blue boxes there represent samples collected during days where we cut down the level of chemicals in the process. We call it the naked process, because we have a minimum amount of chemical there and we can see really what's going on with those samples. With the red, you have the regular process of these facilities, where they have a series of chemical interventions applied and the reductions that they can achieve. So, what you can see here is that the dispersion of the data without the chemicals tends to be larger. It makes sense because the chemicals will homogenize the levels of organisms on the samples, but then you can see that some of the interventions may not be doing as big of a job as we think sometimes if we don't measure it. So, we've been able to do with these studies is to optimize the process, cut down interventions that don't make much reduction and then concentrate on the strategic places where you get significant reductions to cut down the levels of chemicals and also the levels of organisms in the samples. This is with aerobic plate counts.

The next chart will be with the *Enterobacteriaceae* organisms in the samples. You can see some stages you may even see a distortion of the information because again they're collected at different days, different days of processing, but once you get a significant number of samples, you can determine a process variability and establish statistical process control parameters. We are just about to publish this paper and it's already submitted, waiting for some reviews from the journal, and the idea is that we will provide these comparisons between low levels of chemicals to the conventional levels of interventions and measuring the organisms, not only indicators, but also pathogens.

When the pathogen perspective comes into play, we tried to do a statistical process control with prevalence data. What you see here is prevalence on the Y axis throughout two years of data sets from a particular facility, this is chicken wings, taking samples. What the size of the circle tells you the number of samples we collected on a particular time period. And we established, with the prevalence percentage, an upper threshold, what you see there around 28, 29% as the upper threshold of the facility. So, this light system with green, yellow,

and red was to determine days or flocks that were more problematic, and then days where we had everything good, to learn from the process.

The green circles that you see there show you days where the control was significant at the operation, and maybe coming from farms with better levels of *Salmonella*. The red circles above the line gave us homework to do, which was going back to the farms where those flocks came from and trying to find out why those numbers were higher than usual when we took samples of the wings at the end of the process. So, we did this with prevalence data, but we were always looking for a way to account for the pathogens in the system. So, we got some improvements in this facility for over a three-year period, and they've been able to cut down, as you see on the right, they tend to be more on the green side of the process and they just get some outliers from time to time when you got a problematic flock.

So, the question is, if we do just detection and you get a low count bird, you're still going to get an enrichment and you'll be able to determine that it was positive for *Salmonella*, but we don't know how much positive it was. Whereas if you had another bird that is loaded with *Salmonella*, you're still going to get a detection and the answer going to be the same. So how high was that concentration? So that's the question. We've been able and lucky to partner with major developers of methodologies for quantification based on PCR and we'll explain that.

If you look at it from a picture of the process, you can see this process where you have 18 birds, out of those, four are positive, and the range of contamination is one cell versus a million cells on another one. So, if a person buys the chicken on the left side and cooks it is going to be certainly eliminated, the risk of getting foodborne illness, because the numbers to reduce are significantly lower. And even if you do cross contamination in the kitchen, you're still going to have very low levels of the organism being transferred. Whereas if you're the lucky winner of chicken number three in the picture, then you may be able to get significant numbers of *Salmonella* transfer or under cooking, and then getting sick. We cannot just come up with a prevalence number to say the risk is the same. The presence of the hazard doesn't necessarily mean the risk is the same, so we needed to account for that difference.

The risk assessments have determined, and from outbreak data, that the probability of illness on the Y axis versus the log dose of the organism here in the X axis for *Salmonella* show that if you need at least four logs of the organism in the raw chicken to cause about 50% of making people sick under current cooking practices. If you improve the practices, then you reduce that risk. But if you cut down the numbers of *Salmonella* and sample, you can cut it down to 20%, and then if you cut it even further, less than 10% of chances of getting sick, again without including the cooking step in the process.

The quantification methodologies, there are several methods, and we are currently working with quantitative PCR, allowing us to estimate the levels of pathogens in the sample.

This is some data showing prevalence data on the right side, you see the prevalence as a percentage, you see the reductions on prevalence on a facility with about 80% incoming positives, and then with a whole bird at the end, with 27%. And when you go into the parts room, you can see that before and after chemical interventions, you can reduce the prevalence, but you still think that this facility will be high compared to the performance standards.

But if you look at the same chart, when you have counts of *Salmonella* on the Y axis on the left and then prevalence on the right axis, you can see that even though the prevalence may be still high at 27% of the samples being positive, the loads of *Salmonella* on the birds that were positive are significantly reduced by the process itself and that reduction stays even in the cut up room where we think that cross contamination is increasing because of the contact between the birds and the samples. So, this is showing you the importance of quantification, because you can see the difference between the prevalence results, and you can also measure the interventions and effectiveness and the interventions when you apply them in the operation to cut down the numbers of *Salmonella*.

So, what do we do with that data? We need to analyze it, and the statistics tend to be complicated because when you have low counts, you have to apply non-parametric statistics and that's a tricky situation. I'm just going to mention that here.

This one is another facility where we do look at the low and the high chemical interventions. You can see the red line is the prevalence of the high chemical, and the blue is the prevalence of the low chemical. When you see that data, it has both the prevalence on the right side and the quantification on the left side.

So again, it's allowing this facility to measure the reductions of the process by physical and chemical interventions, and then to optimize those processes with and without the chemicals themselves, and especially cutting down those unnecessary stages where chemicals don't do much on a reduction from the statistical perspective in the process.

We can do this with several different facilities, this is comparing plants. So, you can see the prevalence on the right side and the reduction on the lines that you see, the percentage prevalence reduction, and then the quantification of the samples at different stages, comparing one plant to another one. This is allowing us to find out from one plant what practices make better interventions compared to other ones, and then test those interventions in another facility to see if you can get an overall improvement of the process. What I want to highlight here is that even with two facilities with different performance, the levels of contamination are always below 0.5 logs on the samples that you get at

post chilling, even in the cut-up room and the operations in the room. If you remember the risk assessment, the risk of getting illness from this will be minimized.

So, how do you use this data? If you disassemble the data by date, then you can start collecting information about the performance of different flocks. The blue line here is with chemicals and the red line is without chemicals. We're able to identify flocks where we have higher numbers than our statistical process control numbers and we do homework to go back to the farm and try to see if they come from farms that we have classified as high risk. And how do we classify with high risk? With bootie swabs. They were talking about the boot swabs earlier.

We have data showing that you can actually rank the farms based on the levels of the pathogen in the boot swabs and determine the flocks that come from the high level of contamination and the low level of contamination. You will go to the flocks with high levels and see if you've identified the reasons why things are wrong. We have done some biosecurity surveys, and we kind of collate the biosecurity score with the levels of *Salmonella*. So, farms with higher biosecurity get lower levels of *Salmonella* and farms with the low biosecurity rankings get tend to have higher levels of *Salmonella*. It's not a clean difference between them, there's a lot of noise, there's a lot of confounding factors, but we see the trend when we look at data from the plant and what type of farm coming from the origin is.

So that's definitely a promising because we consider that live receiving and bootie swabs could be used as a pre-harvest monitoring system to determine the type of processing in the facility. For example, we can use high chemical schemes for high level farms and then conventional chemicals or optimized chemical schemes for the farms with the regular levels of production. I'm running out of time, so I'm just going to leave it there. But there's a hope where these pre-harvest monitory systems can help you determine and improve your food safety performance. Thank you.