Isabel Walls: Our next speaker is Dr Michael Rothrock, and Dr Rothrock is a lead scientist and research microbiologist, with USDA's agricultural research service, at the US National Poultry Research Center. Please go ahead, Dr Rothrock.

Dr. Michael Rot...:   Yes, thank you very much for including me in this discussion. So if you can move to the next slide, please.

Dr. Michael Rot...:   All right. So yeah, you can go to the next slide. So we're going to be talking a little different now. So, for the past eight years, my lab has been focused on a alternative form of poultry production, known as pastured poultry production. So on the left here, we all know what the conventional poultry operations look like, but on the right hand slide there. So these are pasture raised, these are usually, relatively small. The flocks can go from extremely small, 25, 50 birds a flock, to about 1,500 birds a flock. So what they do basically here is, they raise them in these mobile houses. So every day they will, whichever level you're doing here, they will move that house to a new, fresh piece of pasture. So that's important to them when they define what pastured poultry is, it's they have to have access to fresh pasture, daily, but it's not free range, they don't get to go over the entire farm the entire time. They are contained, but they're constantly being moved onto fresh pasture.

And as they get bigger, they can range somewhat out of the house, but they're contained within mobile fencing. Next slide, please. So why focus on these pastured poultry producers? First off, there's a lot of interest in these kind of products being grown. If you look on the picture to the right there, this family farming model is how a lot of the globe does farming, while it's in my minority of the US poultry model. It's found throughout the world. So it's not a very unique system. It's just more unique in the US, itself. There is a shift in the marketplace, to go towards these alternative. There's lots of catchphrases, antibiotic free, all natural. I don't know what that means, because it means something different to everyone, free range products, but this is all something that these kind of farmers do.

So none of them are certified organic, but they all try to practice it as much as possible. And for me personally, to do the research, I was lucky enough to be able to get access to these farms a lot easier, than I was to the larger conventional farms. And in providing that access, they did provide some flexibility to help with some of the research goals along the way. They would change feed at times, for me and run different flocks side by side, depending on what I was interested in at the time. So it gave me a little more flexibility and to do some things. So next slide. Please.

So what we did from 2014 to 2017, we followed in the Southeast United States, 42 flocks from 11 different farms and we did a farm to fork approach. So a little picture diagram on the right there. We sampled multiple times during the grow-out on the pasture, we would sample the soil and the feces, of where they were at the time of sampling. Then we followed them to processing and we would do

ceca, take ceca samples, and also whole carcass rinses. And then, we would get the final product, whole carcass rinse as well.

So the difference between the two whole carcass rinses is that, the processing one is before any kind of storage, and the final product occurred after the storage and basically, what the final consumer, for whichever it was for that farm would get it. That's what we would look at as well. So from those samples, we would collect a lot of metadata. We just collect tons of metadata. We had questionnaires for the farmers to get an understanding of the management of the flocks. We would get physiochemical data from the soil and the feces, things like pH, moisture, nutrients, and elemental analysis.

Retrospectively, we got meteorological data from all the samples, on the sampling times based on the zip code, from where the farms were. And then, we have a bunch of biological data, both by traditional culturing methodologies and also by molecular methodologies. For the purposes of this talk, we'll be focusing on the cultural analysis, the cultural isolates that we got from the *Salmonella*, but we also looked at *Listeria*, *E. coli*, C*ampylobacter*, a lot of stuff. Next slide, please. So in terms of the *Salmonella*, so the table on the left hand of the side there, we broke all of the information down to look at the sample type effect, and then the farm effect. So the farms are just listed A through M in this case, to make them anonymous. So overall, for the pre-harvest samples, 12 to 15% we found were positive for *Salmonella*, and then post-harvest for the ceca, the final product.

It was about that same amount, but in the processing, it was a little bit higher, significantly higher. But it wasn't a huge difference in terms of sample type. But then, when we looked at farm level, there was a high variation between the farms, and some had no *Salmonella* at all, to lots of *Salmonella*. So you can see the right hand column is the prevalence and you can see the statistical differences there. So this is just overall numbers. The next thing we wanted to look at is, who are the main players, *Salmonella* players or the serotypes that you find and how do they relate to food safety targets. Next slide, please.

So when we did that, we found, which we've been hearing earlier today is that, Kentucky is by far the most prevalent, at almost 73% of all the serotypes was Kentucky. What we also did is, see which serotypes were in the CDC top 32, for human health importance. And only about 16% of all of the serotypes we isolated over the four years, were related to those kinds of serotypes. So there's a couple interesting things there, you see the second most prevalent one we found in Indiana. But interestingly, that came from two flocks, from one year on one farm.

We sampled that same farm the year before and the year after this year, didn't see Indiana at all. It showed up one year and never came back and I didn't find it on any other farm. And then, the Infantis there, the third one, that only showed up on two farms and that showed up in the last year, in 2017. So it showed up there, right before you started to see the major outbreak. So just some

interesting things. So we were interested to see, again, did the sample type have an effect on the serotypes we find, or are they've more farm dependent? Next slide.

So what we found, this is for a sample type, and while there was some shifts, overall, there doesn't seem to be a huge effect of sample type on the types of serotypes that we recovered for the *Salmonella*. Next slide. But when you look over the farm again, it seems to be farm dependent, or farm specific, you can see just the colors alone. I know it's hard to read the actual serotypes, but just by the color patterns alone, you can see there's very different patterns. And it seems to be based much more on farm, than it is on sample type. Next slide, please. Since all of these farmers don't use antibiotics at all, I thought this was also an excellent way to get some baseline understanding of what antibiotic resistance is in *Salmonella*, without the use of any exogenous antibiotics.

So what we were able to find that, in the absence of any use, we still found about 27% of our isolates were multi-drug resistant, meaning to three or more, using the NARMS panel. And aside from the ceca, the MDR rates were relatively stable among sample type. But once again, it looks like the farm of origin, we found very significant differences between them. So you'll see, for instance, farm A, there was 75% of all drug resistance. Farm H was 57%, and then you'd have 0% on other farms. So it really seemed to be farm specific. And the next slide will show you this in more detail. So here, what we have is, these are some of the antibiotics, in the NARMS panels. We just selected some that seem to show the biggest difference. And on the bottom of the columns, you see the designation for the antibiotics.

But what we found was that, for two farms, you seem to have this pattern of the AMO, AMP, FOX TO and AXO, on antibiotics for resistances. That's the blue, it means resistance. Was present on those two farms, where we really didn't see it, almost at all, anywhere else. You would find the Streptomycin and the Tet resistance on other farms, but you wouldn't find this other combination. And the interesting thing about that was, farm A was at one location. And then, because it ran out of its lease, it had to move and it moved to a whole other state, which is what farm H was. So it was all the same infrastructure, all the same equipment, moved physically, to a different state. And there was a year gap in between, and you still saw this unique pattern show up in the *Salmonella* isolates and that.

So it was indicative of potential carriage and survival of this one gene on a plasmid, that could have just survived on the infrastructure within the stuff that was moved up to the new farm. Next slide, please. So now that we had all that, that's just understanding the *Salmonella*. We want to try to make it more applicable to the stakeholder or the farmer, to do something with the data other than, "Wow. That's interesting." So we partnered first, to do some predictive modeling with Dr Mishra's lab at UGA. So what we had is, we had all this management data and they collected a lot of the immunological data. And

so, we want to see if we could do some predictive modeling to see which of these variables

Dr. Michael Rot...:   were predictive of *Salmonella* prevalence. And we looked at both in the pre-harvest using feces and then in the post-harvest using the whole carcass rinse. So what we end up doing is, on the right hand side is random forest modeling, which basically we take all the data and it takes it on and it produces multiple tons and tons and tons of different models. And then it just finds all the models that give, they'll all give slightly different answers. And the ones that give, the most matches you find, they figure that's the most consistent model that you find over all your models and they build the, what they call an ensemble model to look at the data.

Next slide, please. So some of the findings are here. So when we looked at the meteorological factors, on the left hand slide, it just kind of ranked some of the most important meteorological variables that I found, with the max wind gust speed and the average temperature were the top two.

So then we looked at the data for those two, excuse me, those two variables. What we found is on the top there is the lower prevalence, our predicted prevalence when there was less gusty days. And as the gusts got increased, you saw a higher prevalence of *Salmonella*. And on the bottom, you can average temperature. When the temperatures were below 28 degrees, you found lower prevalence, but once they go above 30 degrees, then you saw the prevalence increase. And so these are just some variables and the breakdown of where the data shows that what may be affecting the prevalence, meteorologically, of *Salmonella* on these farms, and the right hand side just shows you how accurate our, this model is considered. So if you had an AUC, area under the curve, so if the line was a 1.0, it's perfect. From what I've understood from my colleagues, the 0.884 Is quite a very, it's a very good model, a very predictive and stringent model.

Next slide, please. When we looked at management factors, the top half here shows the pre-harvest samples, so the fecal samples. So some, the relative importance, the years farming and the constituents of the pasture feed seemed to be the two most important variables. So when you looked further into it, if you are less than seven years farming, there was a lower rate prevalence. When you got above seven years, farming, the prevalence increased. That, excuse me, could be due to biosecurity on the farm and how well they manage to clean and do sanitation on the farm. So the more it builds up over time, the higher chance you have of *Salmonella*. And the second one there, it looked like you had higher prevalence when corn was the major constituent of the pasture feed, as compared to when wheat and oats were the major constituents of the pasture feed.

The second part looks at the whole carcass rinse post-harvest samples, and we found that the flock age and the brood feed were the two most important. And you can see that again, corn seemed to be, the prevalence seems to be higher

when corn is part of the highest component of the feed versus the lows without corn. And then you can see there, the flock age seems to be two peaks with a dip in the middle of age there. I'm not sure why, but just that's what the data showed. Next slide, please.

So we started doing those kind of models, and then we ended up having a NACA agreement with Mississippi State University. And that's Dr. Nanduri and Ramkumar there. So to do some more intense modeling of this kind of data. So what we did, basically, what we just presented, was using a single model and the random forest modeling. The problem with that is that sometimes based on the model, you'll get different, important values. Like any kind of analysis you do, if you do multiple different techniques, you're going to get slightly different answers. So what they decided to do is look at the data where you take a more of an ensemble approach.

So basically for that is what it does is it looks at five different, completely unique ways to build the models, Random Forest, XGBoost, and then three of those five were deep learning models. You can list them there. So really to get into the data. And then the idea is that you're going to do these five different models and the results you get from the five models, the ones that match between the models, or the ones that match between most of the models, is the more consistent or more confident model are answer to your thing. And then we can build some recommendations for farmers based on this data. Next slide please.

So here we have the input variables that we used and the ones I've already showed you. What we added to those input variables was the physiochemical analysis of the feces in the soil. That was performed just to look to see what environmental impact variables could have on *Salmonella* prevalence. Next slide please.

So here we have the different for the feces and the soil and the different models is in the second column. And basically the ones with the boxes around them show that when you rank them the most important to the top five, I think they went to the top 20 most important variables for that given model, if you had here, this was, magnesium, for example, with feces, for four out of the five models, it was in the top five. So that's an important one. So you can see which are the most important ones for each sample type. Next slide please.

So what we could do with that data is we could give some recommendations to reduce the prevalence. So in the feces, these are things that the farmers could potentially influence. So, the level of potassium, if you want to reduce it, you want to have greater than 5,000 ppm potassium. I mean, feces is hard, but you can basically implement more potassium in the feed, using what kind of feeds you use. No, don't use GMO free feed. And then even in the soil here, you can see, because this is a pasture system and they're on the soil all the time and they're pecking from the soil, they can pick up the stuff from there. And it can just show you, that shouldn't be ppm for birds, but it shows you recommendations that we're able to give to the things that they can test for,

and maybe modulate on their own farms to reduce prevalence. Next slide, please.

One of the interesting things we found was flock size was a confounding variable. So what we ended up doing was breaking down, doing models separate for what we considered professional pastured farmers, where they did greater than three flocks a season, versus ones that are more hobby or small scale ones that did fewer than three flocks a season. And then we were able to do, because there is a vast majority of different types of these types of farms, we can make recommendations based on flock size.

Next slide. So here's just another list, you can see here. I won't go over them, but we can make different recommendations, based on the size of your farm of things that you can test for and potentially amend or change in your management to try to reduce *Salmonella* on their farms and in their practice, to ultimately produce a safer food. Next slide, please, and the last two slides.

Well, so future directions, we want to integrate, we have microbiome data from all of this, so we can look also at the biological variables, not just in addition to the meteorological and the ones we showed previously. For example, we can look at the relationship between *Salmonella* prevalence in that data set to, for example, pre or pro-biotic taxa and how these relationships change based on the management variables we looked at. We would also like to further explore the link between the pre-harvest and processing variables on the final product prevalence using these deep machine learning methods.

And to expand this beyond just pasture poultry, this highlights potential for these kind of approaches in conventional systems, especially as they adopt to systems where boilers are gaining more access to the outside. And more data points for this is, and for the metadata you have makes these models better. And I know FSIS has lots of access because these models are built on, I think my data set had about 2100 samples and about 250 columns of data for each sample. So I mean, it was a lot, but I mean you can always add, the more you add the better it gets. And it really highlights the potential to provide this actionable data and recommendations to stakeholders to enhance the poultry food safety. So the last slide is just acknowledgement. So the people who helped me along the way.