

or may not be true depending whether or not we have publication bias at work here.

Secondly, chances are that since we have conducted this review about a year and a half ago, there have been other studies. I have not revisited this issue since about November 2000.

And, thirdly, of course, there is always the chance that we missed a study somewhere, although we certainly failed to identify any additional studies, and the type of search we conducted was pretty extensive.

Another important issue is that these studies are limited to English literature studies. They may have been foreign language studies not translated into English that just missed the search.

(Slide)

What do we conclude?

And I have to say that each conclusion, you may see those are the conclusions of an outsider who is used to reviewing literature, but not necessarily reviewing literature on this subject.

It is generally the theme that seems to emerge from the reviewing of these studies that the published literature at least does not seem to reflect that there is a consistent association. Now there is no consistency between studies.

The reason a disagreement in salmonella challenge studies done in poultry, an important observation that almost all of the positive studies sort of seem to come from the same group, from the same lab. That is always a bit of a red flag, because you would prefer to see independent researchers repeating each other's result, replicating results.

Overall, I would decline to draw any firm conclusions one way or another based on the published literature. What probably needs to be done is a next step is expand this review beyond peer reviewed publications and look at unpublished literature, technical documentations, and so forth.

DR. LANGSTON: Just a reminder to the committee, I have been told that some people in the back of the hall are having trouble hearing us. So, please speak up into your microphone. Any questions for Dr. Goodman?

#### **Questions and Answers**

DR. KOICHEVAR: In any of the studies that you reviewed was data presented that linked increased pathogen load to increased incidence, or even increased risk of human disease? Was that addressed in any of the publications that you looked at?

DR. GOODMAN: No, this was beyond our scope.

This would be something that interests me personally much more than the scope of this project, but I think it is a different animal all together. This sounds like a great question. I do not have the answer at this point.

DR. KOCHEVAR: But with the search terms that you used, do you think you would have found that literature if it were there?

DR. GOODMAN: I have to say that 30 papers that are presented here are the ones that meet the inclusion criteria for our review. We reviewed hundreds of pages prior to narrowing down to these.

I do not recall seeing a study that I would say it seems like addressing an issue beyond the scope. I do not recall seeing a study.

DR. GLENN: Just to confirm all of these 29 studies, I believe, were sub-therapeutic use, correct? I mean, I can see that in the table.

DR. GOODMAN: Right.

DR. GLENN: Okay, just want to make sure.

DR. LANGSTON: Any more questions?

DR. ANDERSON: Just one question. In an attempt to try to get at the extent of publication bias, do you know who were the PIs on these studies? Did you do any sort of grouping of that? Were these academic scientists? They were from the federal government?

They were for industry?

DR. GOODMAN: This is just my recollection from reviewing the papers we have. This was not one of the data extraction criteria, but my impression is 50/50. And the more recent studies, we tried contacting researchers for the peer review and those were academics.

DR. WADDELL: You stated that all of these studies were sub-therapeutic, but the doses you just listed certainly are not consistent with what we would consider sub-therapeutic. What about the duration of treatment?

DR. GOODMAN: Yes, this information can be extracted from studies. With regards to whether or not the dosages were consistent with today's understanding of sub-therapeutic, I can see there may be a disagreement. But, nevertheless, what we are looking is the intent of using sub-therapeutic doses.

With regards to duration, I will have to go and go through actual papers. It has been awhile since I looked at them. It certainly can be extracted from there.

DR. WAGES: I am sorry. I am confused now. You are saying that all of the literature that you reviewed that is in this document are sub-therapeutic

uses?

DR. GOODMAN: Or, at least, that is the intent, the intent.

DR. WAGES: And how did you classify that? When you say that was the intent, who guided you on what levels were therapeutic or sub-therapeutic?

DR. GOODMAN: This was with my consulting with Dr. Matthew at the University of Tennessee, who actually is the author of one of the papers, 1999.

DR. WAGES: Because, clearly, some of these levels are therapeutic and not sub-therapeutic, not even close.

DR. GOODMAN: Well, this is something that certainly I could answer that question on a treatment in children, but not on food producing animals.

DR. GLENN: The route of administration for all studies was via the feed, correct?

DR. GOODMAN: Mixed with feed.

DR. GLENN: For greater than 14 days continuously, and they are high levels, some of them. Yes, I agree.

Isn't that our definition of sub-therapeutic, greater than 14 days? Didn't I see that somewhere today?

So, my point is that the studies appear to

have the route is through the feed, albeit, some of the levels are high, but it was a continuous thing, at least probably dosed over for over two weeks continuously via the feed. So I am just asking that.

DR. GOODMAN: You know, I would probably just defer that question to somebody in the audience.

DR. WAGES: For the committee's benefit, there is in the back of our book some of the studies that they did look at. You could probably get the answer there.

DR. KOCHEVAR: Do you happen to know on the -- and I am probably going to not pronounce it.

Is it Huffton or Houghton Laboratories in the United Kingdom, the laboratory from which six studies showed significant shedding but within those studies were not able to be replicated?

I was just curious if you know whether that is a government lab, or an industry lab, or does anybody know that?

DR. GOODMAN: Yes, I need to go back.

DR. KOCHEVAR: That is okay. It is a minor point.

DR. GOODMAN: I know that the authors are the same. It is always Green and Smith in various combinations.

DR. WOOD: So it is not known though how many

of these studies might be related to 558.15 then, right?

DR. GOODMAN: I am sorry. There was some noise. I could not hear you.

DR. WOOD: It is not known how many of these studies might be related to fulfilling the requirements of 558.15?

DR. GOODMAN: No.

DR. WOOD: No. And would there be a different conclusion drawn -- I mean, you know, we go to the last slides, the last page of the report and look at the conclusions that basically say that overall there really is not much impact of antibiotic use on pathogen load.

But if you look at some of these reports individually you may come to a different conclusion, particularly, if you are looking at it in terms of a particular drug to that bug, you know, as it would be within NADA, as opposed to what this report does, which summarizes findings all together as one piece. And might there be different conclusions at that point?

DR. GOODMAN: You know, my impression is that there are very few drug bug combinations, if you will, that seem to have been repeated throughout -- more than once, or say more than twice.

For those that did exist, avoparcin and salmonella, there seems to be some disagreement. So my

impression is that there is no theme that really emerged from reviewing that literature.

DR. LANGSTON: I do not know why we are getting feedback on our mikes. You might just check if you cell phone or a pager or something like that that might be one explanation; otherwise, I have no idea why we are feeding back.

MS. SINDELAR: Sorry. Thank you, Dr. Goodman. With time constraints at hand for certain parties, we are going to change the schedule a little bit. And our next speaker will be Barbara Masters from USDA, and Karen Hulebak will be coming forward as well.

### **USDA Pathogen Reduction Measures**

**by Dr. Barbara Masters**

DR. MASTERS: Good afternoon. I appreciate the opportunity to be here. And Karen Hulebak will be joining me later in the presentation to provide some of the information. I certainly respect the committee and the challenge that you have at had.

So, basically, we were asked to provide some information to you about the agency's Food Safety and Inspection Service's final rule related to pathogen reduction in HACCP. It is a very brief overview of how we got there, and kind of each component to that regulation, and how it has been implemented by our

agency.

(Slide)

Basically, our pathogen reduction HACCP final rule does require that establishments develop and implement written sanitation standard operating procedures; that they develop and implement HACCP systems; that for all slaughter establishments that they conduct routine generic E.coli testing; and that we, as an agency, are conducting salmonella sampling with salmonella performance standards that we have expectations that be met for slaughter establishments and ground establishments.

(Slide)

Our pathogen reduction HACCP final rule was a long time in the making. After we proposed, we came back with a final regulation, and we had a fairly extended implementation process.

All establishments were required to meet the sanitation components of the reg., as well as the E.coli generic testing in '97; and then we staged implementation of the HACCP and salmonella criteria, performance standard requirements over three years.

We started with large establishments with greater than 500 plant employees in '98; went to the establishments with less than 500, but greater than 25

employees in '99; and then we implemented in all of those establishments that had less than 25 employees in 2000.

So, currently, all of our federally inspected establishments are meeting all the different components, the sanitation, generic E.coli testing, HACCP, and the salmonella performance standards are all in place in every size establishment.

(Slide)

What caused our agency to go with this new regulatory approach?

I think most of you were recall back in '93, the outbreak of E.coli O157:H7 on the west coast, certainly brought a lot of attention, put our agency at the forefront, and a lot of questions were raised about the effectiveness of our organoleptic system, and how effective that was in addressing the major cause of food-borne illness which, as we all know, is pathogens. So the agency went to work to address that concern and that criticism and we ended up with the pathogen reduction HACCP approach.

(Slide)

Having said that, in development of our final rule, it became imperative that our most important objective in putting this rule together was that we

would create a rule that would allow industry to build into their food production practices, as well as, us, as an agency, to build into our oversight in our regulations effective measures to reduce and pathogenic microorganisms of raw meat and poultry products.

So that was kind of the foundation of the guiding principal in putting together our final rule for pathogen reduction HACCP, is to ensure that the industry had a process and, we, through our oversight, had a process to ensure that we were in fact impacting on pathogen reduction rather than focusing on the organoleptic inspection that we had been doing.

I think as I walk through, I am going to walk through each of the separate components to the regulation. I think you will see how they work in concert together to reach this end goal.

(Slide)

The HACCP requirements, for those of you not familiar with HACCP, HACCP is hazard analysis critical control points. Basically, HACCP is implemented by the industry. The industry puts together and puts in place a system of process controls, and they are intended to prevent hazards to the food supply, and a tool for the industry to use to control, reduce, and prevent pathogens in meat and poultry.

Obviously, it is not exclusive to meat and poultry. HACCP is used in a lot of the industry. But, based on our regulations, this was the intent of HACCP and the meat and poultry establishments.

(Slide)

The specific regulatory requirements we have in place, basically, the industry is expected to make a flowchart of their process, and look at every step within their process, and do a hazard analysis of each of those steps in their process to determine which hazards might be introduced at each step in their process.

While we, as an agency, only have regulatory authority at these slaughter and processing establishments, we do expect the establishments to consider hazards that might be brought into their establishments such as on live animals, as well as hazards that might be introduced when it leaves the establishment such as in the transportation process, et cetera.

So they are considering each of those hazards as they do their hazard analysis. They are also considering the intended use or consumers of their product. And, in that process, they are going to determine which food safety hazards are inherent in

their process.

(Slide)

Once they do that, they then identify those critical control points are those points in their process that are critical to ensure these hazards are under control. They are required to establish critical limits. The critical limit should be that limit that is essential to be met to ensure that the food is safe.

They may have quality limits beyond their critical limits, but the critical limit in a HACCP system by regulation should be that limit that must be met to ensure the food is safe.

So, if I need to cook my food product to 160 degrees to get an effective kill of salmonella in my hot dogs, for example, and I do not reach 160 degrees, then I might have a product that has a food safety concern.

I, as a company, may choose to cook it to 168 degrees for quality purposes, but my critical limit in my HACCP program will be 160 degrees or focusing on the safety requirements of my system.

The agency's regulation requires the industry to have monitoring procedures and frequencies. These should be adequate to demonstrate that the process remains in control. They have to list their corrective

actions to be followed in response to any time they do not meet the critical limit.

Our HACCP requirements and our sanitation requirements are the two places in our regulations that we require, through regulation, specific corrective actions, and these corrective actions include preventive measures.

The corrective actions in a HACCP system oftentimes go beyond what is happening at the in-plant level. I talked about the hazard analysis may take the industry back to the farm level. An example might be in a slaughter establishment that as an identified pathogen associated with fecal contamination is a hazard.

If they are dealing with continuous contamination of carcasses with feces, their corrective actions, their preventive measures, often take them back to the farm level to address feed withdrawals, and those situations on the farm that can effect animals coming into the plant, and how much fecal contamination they are getting at the plant.

(Slide)

As part of their HACCP system, they are required to keep records. They must have verification procedures and frequencies associated with that verification. Verification is oftentimes testing of

some sort, to verify that their system is in fact effective in producing a safe product.

They have to sign and date their HACCP program. And we did put in a requirement that these HACCP programs be developed by someone that has completed a course of instruction in the application of HACCP for meat and poultry.

We did not require any specific certification, or any specific course to be completed. We did lay out the guides that we expected that course of instruction to follow to be considered a HACCP trained individual.

(Slide)

The next requirement that I am going to walk through briefly is our sanitation standard operating procedures. Basically, the four components to our SSOPs are that the plants implement them; that they maintain the effectiveness of their program.

Again, the specific corrective action requirements are spelled out in our regulations, and there is also recordkeeping requirements with out sanitation standard operating procedures.

(Slide)

Basically, we are looking at a establishments to conduct both pre-operational, as well as operational sanitation procedures. Those are the steps that they

take to keep their plant clean for the production of food products. And they are also required to monitor those procedures on a daily basis.

(Slide)

They are expected to routinely evaluate the effectiveness of their SSOP. I talked about how you would see some of these working in concert.

An example might be listeria monocytogenes, where if they are finding listeria in ready-to-eat product, that may be an indication to them that their sanitation standard operating procedures are not effective for controlling listeria in the environment, and it might be something they are doing related to their sanitation that is causing them to have the listeria showing up in their products through their HACCP system.

If their HACCP system has, in fact, been validated, and is effective to destroy the listeria, then they are probably dealing with a concern in their environment. And that would cause them to also have to reevaluate their sanitation standard operating procedures. And, again, they should revise those if they determine them to not be effective.

(Slide)

For corrective actions related to SSOPs, if

they find that they have direct production contamination, they have to dispose of the product, make a proper disposition of it. They need to restore the sanitary conditions. And, again, to highlight, they need to prevent the recurrence.

(Slide)

A third component of our pathogen reduction HACCP rule is our E.coli performance criteria. I want to specify performance criteria, because a performance criteria are not specific standards that we are enforcing.

They are criteria that were based on a national baseline data. And they are criteria that the industry is expected to test for, and test against to use to determine whether or not their sanitary dressing procedure and processes are working.

They are responsible to take the samples. They conduct the E.coli, the generic E.coli sampling. It is effective in all of our slaughter establishments. So they are expected to take them. And our inspection personnel verify that the testing is being done, and that they are taking the results of that testing to go back and look at their sanitary dressing and modify their dressing procedures as appropriate.

So, rather than enforcing the standards or the

numbers, they are not standard in criteria, we look at what the industry is doing as they watch what is happening with their generic E.coli numbers.

(Slide)

Basically, they should assess these bacterial counts. And they are basically comparing those counts against what was done in the national baseline. And they look at these results and compare it to what is going on with sanitary dressing, and take whatever actions are necessary to lower those counts. So that is in all slaughter establishments.

So I have now walked through the HACCP sanitary E.coli. Karen is going to walk you through the fourth component which is the salmonella performance standards, and then both of us will be happy to entertain any questions that you have. Thank you.

**by Dr. Karen Hulebak**

DR. HULEBAK: Thank you. I would like to -- I am Karen Hulebak. I would like to thank the committee myself, along with Barb, for the opportunity to talk to you about pathogen reduction and performance standards.

My remarks are intended to give you a quick glimpse of how salmonella performance standards have helped FSIS achieve the goal of protecting the public's health by significantly reducing the prevalence of food-

borne pathogens in meat and poultry products.

I am going to start with some background information on performance standards, give you an update on where we are with the standards, some reasonably current data, and talk to you about where we plan to be going in the future.

As Barb mentioned earlier, FSIS issued its groundbreaking pathogen reduction HACCP systems rule in 1996. This rule established salmonella performance standards for all meat and poultry, slaughter plants, and for plants that produced raw ground product.

FSIS chose salmonella as the target organism for pathogen reduction in performance standards for several different reasons:

First, salmonella was the most common cause of food-borne illness associated with meat and poultry products; second, it occurs at frequencies that permit changes in its occurrence to be detected and monitored; third, testing methodologies are available to easily recover salmonella from a variety of meat and poultry products; and, finally, interventions aimed at reducing salmonella are likely to have the effect, a beneficial effect, in reducing contamination by other enteric pathogens as well.

(Slide)

A different salmonella performance standard was established for each meat and poultry species slaughter, and for each raw product category based on nationwide prevalence studies.

For example, the standard for steers and heifers is different from the one for cows and bulls; and the standard for ground beef is different from the standard for ground chicken.

FSIS measures plant performance against the standard by collecting a series of samples to comprise a single sample set. Within each sample set, a maximum number of positive samples are allowed, and still meet the performance standard -- and the plant still meets the performance standard.

The sample set demonstrates whether plants are or are not consistently achieving an acceptable level of performance with respect to its HACCP controls.

(Slide)

Next, to just reiterate, the salmonella performance standards measure, are set for slaughter, and raw ground product plants. They measure process, HACCP process effectiveness in limiting contamination with this pathogen. And, finally, I should note that they are not used to determine product disposition.

(Slide)

As I said, the salmonella performance standards serve as a yardstick for FSIS to measure the effectiveness of industry HACCP control. They provide industry with objective, measurable standards that can be used to calibrate their HACCP systems.

And, most importantly, these performance standards help FSIS achieve our goal of protecting the public's health by significantly reducing the prevalence of food-borne pathogens in meat and poultry products.

(Slide)

So far, we are pretty pleased with what the salmonella performance standards have been able to achieve in concert with the other provisions of the HACCP rule. The latest complete progress report, April 2001, shows that the prevalence of salmonella in raw meat and poultry has decreased significantly since the implementation of HACCP in '98.

These are the first aggregate data I am going to show you on all sizes of meat and poultry plants. And let me quickly review them for you; broilers, average salmonella prevalence of about 10 percent under HACCP, as compared to 20 percent before HACCP; market hogs, 7 percent under HACCP, compared to 8.7 baseline; cows and bulls, 2.1 percent under HACCP, compared to 2.7 baseline.

(Slide)

Steers and heifers, .3 percent now, compared to 1 percent prior to HACCP; ground beef averages 3.7 under HACCP, compared to 7.5 percent pre-HACCP; ground chicken, 14.4 percent, compared to 44.6 percent baseline; and ground turkey, 29.7 percent under HACCP, compared to 49.9 percent baseline pre-HACCP.

(Slide)

In addition to these data showing declines in the prevalence of salmonella on various raw meat and poultry products, the Centers for Disease Control and Prevention has reported reductions in the incidence of food-borne illness.

FSIS believes the performance standards working in concert with HACCP are one of the factors contributing to these food-borne illness reductions. While all of this is good news, we know that there is more room for improvement.

This is why two activities are currently underway to review salmonella performance standards, what they have accomplished, and how they might be improved.

The National Academy of Sciences and the USDA National Advisory Committee on Microbiological Criteria for Foods are both studying the issue of microbiological

performance standards.

Performance standards will remain an important part of HACCP for FSIS. Exactly what those standards should be, and how they should be applied, are questions that the agency seeks answers from the scientific community.

(Slide)

And, next, just to summarize and bring salmonella together with E.coli performance criteria, we see the standards, the salmonella standards, and the E.coli criteria as complementing one another.

E.coli testing is a good indicator of fecal contamination. But it is not directly correlated with salmonella contamination which is affected by a number of other factors as well including the condition of incoming animals.

The salmonella standards will continue to be used by FSIS as an indication of which plants are not currently meeting the standards, and which plants therefore need to be making -- need to be taking further steps to reduce pathogens that can cause food-borne illness.

I wanted to leave you with one final note as well. You have some very big questions in front of you, and answering them is going to take some hard work. And

I wanted to note for you that the agency has, as you may or may not know, developed a risk assessment of farm to table process risk assessment for E.coli 0157:H7.

When I say farm to table, I mean to say, that the risk assessment models, the entire farm to table process of the production of ground beef from slaughter to consumption, and looks at the effect, potential effect, of various interventions on the load of pathogens in a final product, and estimates the likelihood of illness that might occur from consumption of that product which actually speaks to I think a question one of your members asked earlier this afternoon.

Indeed, the model is anchored in epidemiologic data for o157:H7. Now while the model has not been constructed exactly to answer the kinds of questions you may wish -- you might theoretically wish to ask of it, we believe that it could be used, at least theoretically, to ask more quantitative questions about impact of incoming pathogen load on likelihood of human disease -- incoming of incoming pathogen load in an individual animal, or a lot of animals on output or pathogen load in a final product.

Thank you very much. Barb and I both thank you. I will conclude my remarks, and we are happy to

answer any questions you might have.

### **Questions and Answers**

DR. KOCHEVAR: Can I assume from what you just said that there is not data available yet from that system?

DR. HULEBAK: That is right. The model has not been used ever to ask that specific question, but it is a public document. It is not complete, not that any risk assessment every is, but it is available to the public. It is out, and, in fact, currently undergoing a rigorous peer review by a committee of the National Academy of Science; ipso facto, it is a public document.

DR. KOCHEVAR: So that sounds like a very comprehensive process that is going to go on there. Short of that, is there any evidence from FSIS where USDA conducted studies that, if animals come in with a higher pathogen load, just to the slaughter plant, discounting for now conditions on the farm completely, there is no data at this point that shows that those animals create a higher risk for contaminated in-product from that plant?

I mean, that is the kind of the thing that will come out from these other studies, but it is just not there right now.

DR. HULEBAK: Right. That, again, is the kind

of question we have not asked of that model.

DR. KOCHEVAR: Okay, thank you.

DR. LANGSTON: I would like to compliment what you have accomplished with HACCP. I think it is wonderful. I thought the comment on the temperature on hot dogs was very interesting, but there are still, of course, big issues.

For example, E.coli 0157 in hamburger; listeriosis in cheese, sausage; salmonella in eggs, all of which could be addressed by gamma irradiation which is an improved technique. Yet, I have yet to see any information in the press or elsewhere explaining to the public that it does not make the food radioactive.

It actually prolongs the shelf life. And if used to eliminate the pathogen rather than sterilize the food, per se, in essence, a pasteurization, it does not effect the taste, and presumably not the nutritional value.

Two questions: (1) Have you looked at, included irradiation in your risk assessment, and what that would do for those pathogens? And (2) If what I have said is correct, why has there not been a consumer education program?

DR. HULEBAK: I will take the easy one first. And that is I am not sure that we have looked at

irradiation as an intervention in the process risk assessment for E.coli 0157. It certainly could be done.

Second, as the regulatory agency that approves such interventions for use on raw products, which we have done, we feel that -- and I think the rules are quite clear, that we cannot be in the business of promoting one intervention over another intervention.

We believe that it is the job of other agencies such as the Centers for Disease Control to make that kind of a public education message very clear. And I believe the CDC has said that in more than one venue that it believes that kind of message needs to be gotten over clearly to the public.

You can understand. There is sort of a conflict of interest for us as a regulatory agency approving interventions, one range of interventions. We cannot mark it one over another. But CDC, which does not have that conflict of interest in the way that we do, has said that it is interested in making those kinds of points.

DR. LANGSTON: So I realize milk is a little bit different, because it is handled at a state level. But you do not require pasteurization of milk?

DR. HULEBAK: We are not in the milk business at all.

DR. LANGSTON: So that is a state requirement, I see.

DR. HULEBAK: Well, it is also FDA's billy wig.

DR. WOOD: A question was asked earlier about the data coming in the front door, in terms of pathogen load, and going out the back door of the processing plant, I think is the question of the day.

I mean, is there any data available at all that we could look at related to that question that you were talking about that was in the risk assessment on E.coli, in terms of the pathogen load walking in the front door of the establishment on the hide of the animal?

And is there any data available on that, that is available?

DR. HULEBAK: There are voluminous data that were used as inputs into that risk assessment, and I am sure they could made available to you.

DR. WOOD: How about the number of farms where those farms have been seen are identified by establishments as a critical control point regarding the pathogen load on the hide, and where steps then were made or taken by that establishment to work with those farms with regard to HACCP or whatever? Is there data

on that?

And, if so, is it a large number of farms? Are the number of farms increasing? And is there any assessment or assessment being made on what kind of impact those HACCP plans have had on the pathogen load at the front door of the processing plant?

DR. HULEBAK: That is a more fine grain question that I do not believe has quite made it into the risk assessment type work.

Barb, are you familiar with any studies that we have done, carried out, or aware of?

DR. MASTERS: We, as an agency, would not have that data. I can tell you anecdotally that we have had some, particularly, poultry establishments that have failed to meet the salmonella performance standards; that after they have failed their third set, they have to reply to us with corrective actions.

Their corrective actions included measures taken on the farm, and they did take those measures. And they did pass a fourth set of salmonella testing; and that I can think of several example where that has happened.

But we would not have the specific information, as far as to how many farms they worked with, the specific interventions, et cetera. The

industry might be able to make that available to you, the National Chicken Council, National Turkey Federation. So, anecdotally, yes, but no specific data.

DR. KOCHEVAR: So their interventions might have included other things besides the on the farm stuff?

DR. MASTERS: That is correct, yes.

DR. KOCHEVAR: So it would hard to sort that.

DR. MASTERS: And they did include other things, correct.

DR. GLENN: I have a question. I would also like to compliment FSIS on these improvements that have been seen, and in their relationship to CDC's report.

Since 1998, when we first implemented HACCP, and we were looking at the salmonella reservoir, as we are trying to look at today, you indicated that broilers had been sampled, and that there is this prevalence. Tell me exactly where you sample a broiler, and a market hog, and a cow, and a bull, steers, and heifers.

DR. MASTERS: Going from memory, I certainly can get that information to you, but the broilers undergo a whole bird rinse. So the entire carcass is put in a bag and rinsed with fluid.

Turkeys, we started out with a whole bird rinse and it became an ergonomic concern, so we switched

to swabbing. And the turkeys are swabbed, and my memory is not with me on the swabbing, but they are swabbed in those locations we believed to be at the highest risk for fecal contamination. There are specific areas that are swabbed.

Swine carcasses and cattle carcasses, it is the flank area, the brisket area, and the sternum area. So we can get that information to you, if that is helpful, but they are specified and considered to be those areas most at risk for fecal contamination.

DR. GLENN: Okay, great. And that is what I wanted to know because in our definition of pathogen load, we are looking at feces. And I wondered if you were out in the lot sampling feces. I wanted to make sure that was not true.

DR. MASTERS: No, actually, the animals, the carcasses that are selected have been in the chiller for 24 hours, and typically are chilled carcasses when they are selected.

DR. GLENN: Chilled, okay. And has there been any coordination as this policy is developed with FDA, CVM, as we, across the government, emphasize a farm to table approach?

Has there ever been any liaisoning to say, you know, if load is an issue then we might want to do

something closer to the fecal source at the slaughter plant?

DR. HULEBAK: That is why we are here.

DR. MASTERS: As Karen says, that is why we are here. There has been a lot of communication between ourselves, FDA, APHIS, specific to the level you are talking, I do not know about that detail. But, yes, it has been an ongoing, unified effort to try to make that effect.

DR. GLENN: Okay. And do you expect further improvements of this magnitude as we go on down the road, and we are farther out from the implementation of HACCP regarding the salmonella reservoir? I am just curious.

DR. HULEBAK: It really is kind of a crystal ball. I mean, clearly, there is a great deal of interest in performance standard as a concept in making performance standards even stronger, tools more deeply and firmly rooted in science, what that is going to mean, in terms of their structure, their focus.

What pathogens we are talking about, you know, that is anyone's guess. There are two very good groups of highly qualified scientists involved, and that is more than one hand, you know.

DR. LANGSTON: Thank you very much.

DR. MASTERS: Thank you.

DR. HULEBAK: Thank you.

DR. SUNDLOF: While Aleta is switching the slides, we are back in the normal order now. And I would like to introduce Dr. Scott McEwen.

Scott is a professor of food safety and epidemiology at the Ontario Veterinary College, University of Guelph. His research efforts is on determinance of infection with antimicrobial resistant bacteria and Shiga toxin-producing E.coli in food animal populations.

Scott has been involved for quite awhile in the whole issue of antimicrobial resistance. He served on the WHO panels that have been dealing with this issue. He was at our Threshold Workshop. And he is currently a chairman, the chairman of the Canadian effort to look at the issue of antimicrobial resistance in food producing animals in Canada.

So, Scott.

### **Epidemiological Evidence of Pathogen Load Effects**

**by Dr. Scott McEwen**

DR. MCEWEN: Thanks very much, Steve, for very kind words and introduction, and greetings to everybody from the Great White North. It is a pleasure to be here as always, take part in this important public health

issue.

(Slide)

I am mindful of the time -- and I will try to be quick on areas that we have already discussed.

Briefly, I would like to go over some of the plausible mechanisms of a pathogen load effect from different types of antimicrobial use in food animals, what I think is relevant information from non-food animal observational studies, some general characteristics of the epidemiological studies that reviewed for this presentation, a brief summary of the evidence from food animal studies that I obtained from literature.

This is basically a pub med search, plus some information I had in my own files as a teacher and researcher in this area. But I am sure a keen student came up with some extra studies that I missed.

(Slide)

Some of this has been touched on already. I think the first possible effect of antibiotic treatment, whether it is sub-therapeutic or otherwise, on animals could be an increased susceptibility of animals to infection, essentially, by reducing the infectious dose.

Two possible ways this could work: If there is treatment before exposure with the pathogen, that is,

a previously uninfected animal, it could increase the susceptibility of the infection by suppressing normal flora, thereby diminishing the animal's colonization resistance.

The second mechanism treatment, during exposure to a resistant pathogen facilitates infection by the selective effect of resistance.

(Slide)

A second possible mechanism of treatment is increased duration of shedding or concentration in feces. We have talked about that, possibly due to related mechanisms to greater degree of colonization, perhaps, or attachment sites either intra- or extra-intestinal infection in lymph nodes or elsewhere, and possibly with disruption or normal flora.

And a third major effect antibiotic treatment could have I think on pathogen load is to diminish it by decreasing the prevalence or duration of shedding due to treatment.

(Slide)

Just, in general, we have talked a lot about experimental studies today, that is where you randomly allocate treatments to animals in groups typically. I am referring to epidemiological studies. And in today's context those are observational in nature, both from

respect to exposure to the pathogens of interest, and to exposure to drug treatment.

So these both occur as they would on the farm. That is one of the major benefits of conducting such studies. The other major benefit is that multiple causal factors or risk factors can be investigated, and there is many of these that Jeff touched on very well in his presentation relating to the agent host and the environment.

And one other thing I would mention is that another important aspect of animal husbandry in today's world is it is very hierarchical in nature. And epidemiological studies that are designed properly can take into effect things like group effects, herd effects, hatchery effects, and that sort of thing.

We always have to take great care in conducting these studies to try and design them appropriately and analyze them carefully to avoid the many biases which these studies are subject to.

Some of these have been mentioned; things like, selection bias, misclassification bias, confounding, and other things. Epidemiologists spend a great deal of time trying to prevent or to explain these sorts of biases.

(Slide)

And I should mention that not all of these studies are the same type, and different designs have various strengths and limitations with respect to causal inferences.

(Slide)

A few points about some related studies in other species that I think are relevant. There has been several case control studies in humans, in particular, showing prior antimicrobial treatment as a risk factor for clinical salmonellosis, and recently a paper showing its effect for campylobacteriosis.

We have had similar observational studies conducted in horses, hospitalized horses and dogs with nosocomial outbreaks of salmonella associated with prior treatment with antimicrobials.

There is some evidence for causal role of drug treatment enterocolitis of horses and rabbits. I should emphasize that these observations involve clinical disease and not subclinical infection.

(Slide)

The first example is from the human realm. It involved a case control study that was described about 10 or 12 years ago, an outbreak due to an antimicrobial sensitive strain of salmonella havana.

In this study group, prior antimicrobial

therapy was a risk factor for clinical disease. And the odds ratio is 4.3 indicating a fairly strong association with prior antibiotic use.

(Slide)

From horses, a study conducted in California, I believe, case control study of salmonella St. Paul infection in hospitalized horses. Horses receiving parenteral antimicrobials prior to the first culture for salmonella were at 10.9 times greater risk of infection than horses not being treated.

(Slide)

Before going on to some of the food animal studies, I think it is important to talk about both the effect measures and outcome measures that were used in these studies. It is quite a range.

Most of them measured farm level culture status, that is, the prevalence of infection at the group level, a qualitative, yes or no. Some assessed individual animal status, or the proportion of the herd that were shedding, so a more quantitative outcome.

Duration of shedding/infection, concentration of pathogen feces were not measured explicitly, although, to some extent, these weigh into a prevalence measurement because prevalence is a function of incidence, the number of new cases that occur over time

and the duration of infection in the population.

One important point I think is that most outcomes were relative unrefined. Multiple serotypes were -- usually no attempt to differentiate serotypes or other important agent or host characteristics.

(Slide)

Similarly, most studies had somewhat aggregated exposures, measures, or risk factors. Most measured treatment at the herd level, which is often appropriate if we are talking about sort of group treatment effects, were difficult for individual treatment.

Growth promotion, yes or no, in some cases, specific drugs were mentioned, others not. And, sometimes, they quantified therapeutic. Was therapeutic drugs administered to this flock or herd? Yes or no.

If you looked at individual animal treatment effects, usually not drug-specific, none measured the duration of treatment, and most treatment variables were unrefined, as I have mentioned.

(Slide)

This slide is a summary of the results of these studies presented in a qualitative nature, a format. The number in brackets represents a number of studies over the last 20 years or so, which looked at

pathogen load effects in that species of animal, and species of bacterium.

The NS means it was not significant. The drug did not significantly associate with pathogen shedding; minus sign indicated a protective effect, or a negative association; and positive, the opposite.

In poultry, three studies looked at salmonella epidemiologically; two, there was no significant difference between treated and untreated animals. In one study, there was a protective effect. In swine, one study showed a positive effect of treatment; two showed no significant difference. In cattle, similarly, one showed a protective effect; two, no significant difference.

With respect to E.coli 0157:H7 in cattle, four studies were in the literature. One study showed a protective effect; one a sort of positive effect, which I will talk about in a few minutes; and two, not significant. In campylobacter, two studies in poultry, one protective, one not significant; and in cattle, one not significant.

(Slide)

I will go through a few studies done in animals to illustrate some of the principles and findings. The first was a study of E.coli 0157 in dairy

herds in the Pacific Northwest, United States. In this study, they undertook regular monthly fecal culture of cattle and classified farms as to positive or negative.

The investigators reported a tentative association of E.coli 0157 prevalence, and the feeding of ionophores in heifer rations. This was an unconditional association, and the authors did not undertake any multivariate studies on this particular outcome.

(Slide)

Second example, a large study conducted here in the United States. Again, cross-sectional study of U.S. feedlots. I believe it was a cattle on feed evaluation study conducted by USDA. And they were undertaking to identify risk factors for E.coli 0157 shedding.

Sixty-three of the hundred feedlots examined had at least one positive sample, and these investigators found no significant association between positive fecal culture samples and ionophore use, or with feeding of antimicrobials in these feedlots.

(Slide)

The third example is, in this case, a pig study. This was undertaken in the Netherlands, and involved 353 pig farms. And the outcome in this case

was serological or seropositive status of blood samples of herds taken at slaughter.

In this instance, the use tylosin as an antimicrobial growth promoter in finishing feed was significantly associated with a higher salmonella sero prevalence on farms.

(Slide)

Fourth study, very large, well-conducted, comprehensive study undertaken in Denmark was a cross-sectional study of about 4,000 broiler flocks, 12.6 percent of which were positive on fecal culture to salmonella typhimurium.

In this study, the use of unspecified antimicrobials was associated with reduced risk of salmonella infection, specifically in flocks that came from salmonella negative parent flocks; association did not hold in flocks coming from positive parent flocks.

In this study, growth promoters was not significantly associated with salmonella infection.

(Slide)

In summary, I have only shown you a few of the studies involved that is really representative I think. We have only had a modest number of epidemiological studies which have looked at this issue of pathogen load, approximately 15, by my count; none assessed

carcass contamination or other public health effects.

Most evaluated salmonella. Fewer looked at Shiga toxin-producing E.coli and campylobacter. And most studies, I think, importantly, sought to evaluate a broad range of potential risk factors, in essence, screening studies. None were specifically designed to assess pathogen load.

(Slide)

I think one sort of caveat that took place on this is, given the exploratory nature of these studies, and the comparatively unrefined nature of both the exposure and outcome variables, important associations may have gone undetected. And I think any future epidemiological studies in this area should be specifically designed to address this topic.

Another point, it may seem obvious, is that such studies are inherently post-approval because you cannot undertake observational studies until the drugs are on the market.

(Slide)

So, in conclusion, most studies found no evidence of a pathogen load effect; some found evidence of a protective effect; and some found a positive effect of drug use on pathogen load.

Overall, I believe that current

epidemiological evidence suggests that undesirable pathogen load effects of antimicrobials used in Europe and North America, if they indeed exist, are probably minor. Thanks very much.

DR. LANGSTON: Questions?

**Questions and Answers**

DR. LANGSTON: I think on your slides, 7 and 8, where you give examples, one a human study, and another in horses, where antimicrobial treatment increased the odds ratio in developing salmonellosis, do you have any idea whether those salmonella were susceptible to the antibiotics that were used in either the humans or horses?

DR. MCEWEN: Well, in the human study, this strain was reported to be antimicrobial-sensitive. So, I gather that means universally-sensitive to antimicrobials tested. I do not know what range of drugs they tested. And I cannot recall from the horse study whether or not they tested the resistance or the susceptibility status of that strain.

DR. KOCHEVAR: As a follow up to that, in any of the other studies was there any consideration given of incidents of drug resistant strains in any of the questions that were asked epidemiologically?

DR. MCEWEN: Yes, I mean, I do not have any

general conclusions. I explicitly left out studies that sought to evaluate effects on resistance, and tried to select those which either ignored resistance or did not measure it.

I mean, it is difficult I think to separate completely the issue of resistance from pathogen load because the mechanisms do overlap. So it is a bit artificial to do that. But, in the case of this particular exercise, that is what I did.

DR. WAGES: Scott, does Canada have anything in place in their drug approval process that requires pathogen load studies in the approval process?

DR. MCEWEN: I do not believe so, no.

DR. WAGES: If you were asked to comment would you recommend that they do so, based on the information?

DR. MCEWEN: I was afraid I would get that question.

DR. WAGES: Based on the information that you have given to this committee?

DR. MCEWEN: Well, I very well may be asked to comment. I think, based on what I know, what I have seen and heard at previous meetings, and today, and read in the literature, I think preapproval studies are problematic from a design point of view.

My preference would be to make sure that we

have a fairly capable post-approval surveillance program looking at both resistance, pathogen load, and antibiotic use. That is a big undertaking.

DR. WAGES: Thank you.

DR. LANGSTON: Any other questions?

(No response)

DR. LANGSTON: Thank you.

DR. SUNDLOF: Our next speaker is Dr. Richard Isaacson, who is the with the University of Minnesota, but formally from the font of all knowledge, University of Illinois. So, Dr. Isaacson.

### **Factors Affecting Pathogen Shedding**

**by Dr. Richard Isaacson**

DR. ISAACSON: For those of you on the panel, the slides that I have here are handed out to you separately. So you do not have them in the notebook, as far as I know. And that is because we are using different computer systems. And, apparently, the FDA's version of Powerpoint could not translate my MacIntosh version.

(Slide)

I have asked to talk about some of the factors affecting pathogen shedding, particularly, with salmonella. I was also told that there has been no levity in this meeting yet, so I thought I would start

off with a little bit that might state probably some of the situation that we are going through right now which is stated here.

We are all visionaries. I am a visionary. You are a visionary. But what do you do? And so, we all have different occupations, and so we look at these things in a slightly different way.

I am a microbiologist, and we work primarily with swine systems. And everything I tell you about pretty much will be relative to swine. And I presume it is translatable to other systems, but I do not have data to support that.

(Slide)

So I want to talk about a number of things. And, really, this is the outline, and these are the points I want you to leave with. One is that, with regard to swine, most farms have salmonella. And one question is, but is it the right salmonella as a food-borne pathogen question?

Shedding of salmonella is sporadic. In healthy pigs, which is what we are mainly concerned about, either cecal or fecal concentrations of salmonella, post-challenge, that is after things have kind of equilibrated, is usually very low.

We are talking about between about 10 and 100

cells per gram of feces. And one question that goes through my mind is that enough to cause disease? Is that enough to impact resistance transmission? What is the relevance of that?

That, apparently, healthy pigs can be carriers, and that there are a number of factors that can be used to express the shedding of that organism from the animal; and also want you to come away with the fact that detection is difficult.

We have seen a number of presentations already this afternoon on detection, and even on some quantitative measurements. And let me tell you that those are not the simplest things to do. You cannot just go and plate it out. So I will discuss that.

Growth promoters, and I will give one study that we have been involved with, do not necessarily affect salmonella shedding.

And, finally, is that salmonella can also be rapidly acquired. So you can do all of the nice beautiful studies, and it can all be for not because rapid transmission at the every end of the study.

(Slide)

So let me just start off by telling you, this is some data that we got from a study that we have been doing with pigs in Illinois. And it is just to show you

the serotypes that we have identified from healthy animals, healthy, mature animals in Illinois from 11 different farms.

And the point is, is that these are the serotypes that we have been seeing. Jeff Gray showed you some of this data as well. But if one looks at this from the standpoint in this particular study, you will notice that there is no typhimurium there, and that was a bit of a surprise to me.

(Slide)

But if we look at what is important in humans -- and you will not be able to read this, I am sure. But this is a 10-year summary of data from CDC samples, and this is typhimurium; this is enteritidis; it is Heidelberg. It gets all the way down here to, I believe, agona becomes the first on my list.

So the question is: When we go out just in the field and look for salmonella, are we looking for the right ones all of the time, or do we pick out the right ones?

I have no doubt that salmonella typhimurium is common from pigs, and that pigs are a common source of contaminant, or food contamination. So it is part of the time is that we are going to be looking at things that are not necessarily related to the most prevalent

or food-borne pathogen.

(Slide)

What about the actual prevalence of salmonella on farms?

So we did a study looking at initially 141 farms using slaughter plant samples. And, in fact, we looked at lymph nodes. And when we looked for salmonella, any kind of salmonella in these samples, what we found initially, out of those 141 farms that were surveyed, only 65 percent of them showed at least one positive salmonella, and we looked at 30 pigs per farm.

And we even could divide those up into what we called high prevalence and low prevalence farms, thinking that this might be a way in which we could start looking for risk factors that might be involved in their being there.

In a follow up the following year, we looked at a subset of this 141, almost 100 of them. And what happened is that now many of those that were negative were now positive; some of those that were positive are now negative; some of those that were high prevalence are now low prevalence, and all of the combinations that you could think of.

And, at this point now, if we took a two year

running total, 90 percent of the farms that we looked at were positive. And then, in a subsequent third year of sampling, it starts approaching 95 percent of the farms that we have looked at are positive.

And I think Jeff Gray said, you are just not looking hard enough. These are single sampling times, so we are not going back and repeatedly doing this on a specific farm sample other than once a year. So the answer is, yes, if you look long enough, and you look hard enough, you will probably find salmonella.

And so, the idea is that -- one is that most farms have salmonella, swine farms, and that shedding is sporadic. One day you can find it, and the next day you cannot.

(Slide)

Okay. So question about concentration of salmonella in feces. I have been quoted at times to say I would rather eat a piece of meat contaminated with salmonella, say,  $10^2$  or  $10^1$ , than  $10^7$ . And not that I do not know how to prepare the food to kill the  $10^7$ , but I do not want to be eating that amount of fecal material.

So, in this study, what we did is we challenged pigs orally with the salmonella typhimurium. It is a wild-type strain that we know we will persist in the colonized pigs, and we followed it over time. And

what you see here is the concentration and log ten, and you cannot just take and plate this out, as I mentioned.

We have got to go through a very laborious double enrichment procedure, as well as doing dilution, so that we can get a most probable number calculated, which is not the same as taking a sample, doing a dilution, and plating it on a plate, and then coming back the next day and counting.

And what we find is that post-challenge is that this is really about a week post-challenge, 56 pigs challenged, is that the level is up in the about  $10^3$  range, so a couple of thousand cells per gram of feces that are sampled.

It goes down, and then it popped up here, because we did a second challenge. We were a little concerned that we did not actually get all of our pigs, so we did it again. And you can see after that it goes back down.

In 120 days post-challenge, the actual average concentration is extraordinarily low. We are talking about less than .1 percent of the -- or .1 colony forming units per gram of feces. That is the sum of all 56 pigs.

And what that represented is about three or four pigs that were still shedding, and the rest of them

that did not appear to be shedding at all. And those that were shedding were in that  $10^1$  to  $10^2$  range, and that was it.

So the concentration is very low. And I would question whether or not that is an infectious dose for a human disease without an adulteration or a breakdown in the processing procedure post-slaughter.

(Slide)

Okay. Apparently, healthy pigs are in fact carriers of salmonella. A similar type of experiment, what we did is we took, in this case, 46 post-weanling pigs. We challenged them orally with  $10^8$  salmonella typhimurium.

It is a strength 798 that was, in fact, isolated from a pig, and was obtained from NVSL some years ago, and we had marked it with nalidixic acid resistance just so we could identify that we had the right strain when we were done, orally challenged them, and then at four weeks interval -- actually, four weeks later we rechallenged them because we wanted to now go with the same protocol that we had established and did not want to start changing it.

All of these animals are then reared sort of conventionally, that is they were not isolated -- they were isolated from the outside, but they were grouped

together in pens, so that they had access to each other during that period of time.

And they were reared until they hit about market weight, about 240 pounds; and then they were divided into four different groups. Those four groups were listed here:

Group one simply remained on feed. They were segregated off. They remained on feed. Group two had feed withdrawal. In fact, two, three, and four had feed withdrawal of 6, 12, and 24 hours; and then each group was separately transported, put on a trailer, and trucked around for about three hours, brought back to the lab, and we necropsied them and asked what was there.

Because we did feed withdrawal, we looked at cecal samples in this case. And just to show you what happens over time is that, again, post-challenge, one month post-challenge, you can see about 80 percent of our pigs are shedding, and it goes down to the five month period almost none.

I believe this is only three pigs out of 60 that were actually shedding at that particular time. This is percent positive now, instead of the actual concentration.

(Slide)

But when we did looked at the pigs in these different groups what happens is that the least -- these are the feed withdrawal times. The zero feed withdrawal pigs had a somewhat increased elevation in the number of pigs that was now positive.

As the time of feed withdrawal increased, the number of pigs that were now shedding, or that we could detect it in cecal content, went up. And this represents actually about 83 percent of all of the pigs.

So we can have apparently healthy pigs that are not even initially shedding -- appear to be shedding salmonella, that ultimately can be shown to still be contaminated. And, in fact, if they are stressed appropriately, they can be brought back to the shedding status.

(Slide)

Detection of salmonella is difficult. In fact, the question is, is culturing is what is the sensitivity of the assay? We do not have an idea of what the sensitivity of even culturing is for sure from in vivo samples. From a lab grown sample we can tell you, but not from in vivo samples.

What are the conditions? What culture conditions affect detection? Is it reproducible? What is the impact of repeated sampling?

(Slide)

And there was a very nice paper that was published last -- two years ago. It was a collaborative effort by Peter Davies at North Carolina State and Paula Fedorka-Cray at ARS looking at the two labs' different methods of culturing.

And at North Carolina State, Peter Davies used this method, which was to take ten grams of feces, put it in buffered peptone water, do an overnight pre-enrichment, then put it into Rappaport's or RV broth, as a second enrichment, followed by plating on indicator plates, XLT4, and then analyzing what was there. And that was their standard method.

And so, they decided they would compare it to what Paula was using, which was to use instead one gram using either one of two enrichment procedures, either using GN Hajna as a first enrichment, or tetrathyanade broth, followed either then by again the RV, and plating on XLT4.

Well, the first comparison was to simply look at the effect of sample size. And I am talking about numbers of grams of feces to see what happens. And this was just using method one, so that is the Davis procedures.

(Slide)

And, as you can see, one gram of feces gives you about 6 percent of all positive animals that were looked at were positive. But if you increase ten-fold, it now was 20 percent of the samples were positive.

So in trying to figure out how much, or whether salmonella is there, it really depends upon what your sample is, and how large that sample size is. But there are other confounding issues here such as: What method do you use? And which lab does it?

(Slide)

So here is a, in fact, a matrix of the different laboratories; laboratory one, which was the Cray lab I believe. Laboratory two is the Davies lab. Method one is the Davies method; two and three are the Cray method; and this is the summary of them all.

And you can see that if you just compare, say, method one, is that the lab that developed it got more positives with the same set of samples. Now, I should tell you they used the same set of samples, as did laboratory one -- or did -- excuse me -- yes, as laboratory one did.

Laboratory one's method, you see, they did better than did laboratory two. And, in fact, the point is is that the methods that you use can generate different results. And, in fact, some of the positives

that the Cray lab found as positive were different than the positives that the Davies lab found.

So methods are important, as well as who is actually performing them. So there are variations that can occur. And trying to standardize an assay then becomes, as you can imagine, quite a headache.

(Slide)

Okay. We did then a study, and I want to talk then about the growth promoter aspect of it. We did not do this to find out if -- what we were really trying to find out was whether flavomycin or bambarmycin had an impact in reducing salmonella shedding. And I can tell you that it did not.

But we took 60 pigs, challenged them again orally in our model with  $10^8$  nalidixic acid resistance, salmonella typhimurium strength 798, divided them after that, a couple of weeks after into two different groups. One got flavomycin, one did not; raised them again conventionally.

But now the two groups were separated from each other, did our feed withdrawal, 24 hour feed withdrawal followed by three hours of transportation because we knew that would be one way of getting an increased shedding rate.

(Slide)

And what we found was this kind of data. The red is the bambermycin, or flavomycin. The blue in the back is the non-treated group. This is the shedding over time. And you can see that initially, actually, this treated group was slightly lower.

But I can tell you that there are no statistical differences here. Even though there is some apparent differences visually, they were not statistically different.

And, at the end, you can see that indeed, if anything, the treated group was slightly higher; but, again, statistically not different, so that there was not really any impact of flavomycin on the shedding of salmonella in this study. However, we did show that flavomycin actually was doing something because we did have parameters of performance.

(Slide)

We looked at weight gain and feed conversion. You can see the treated groups had a slightly higher, 1.7 grams daily -- pounds, excuse me, of weight gain average per day compared to the non-treated group was slightly lower. And the conversion rates, feed conversion rates were slightly in the benefit of animals that receive flavomycin.

So, in fact, it was working as a growth

promoter, but it had absolutely no impact on the salmonella shedding.

(Slide)

Okay. Then the final point that I was going to make is that salmonella can be rapidly acquired. And Jeff Gray, again, mentioned some of this earlier, and I am going to take it just a little bit deeper, is Paula Fedorka-Cray did do some initial studies, really elegant studies in '95, showing that pigs could be challenged, rather than orally, intranasally instead, and that within hours after intranasal installation that you could find salmonella in the gut of these pigs, and not just necessarily in the tissue, but actually in the lumen, so that there is a very rapid transition from respiratory tract into the gut, and they can be secreted.

(Slide)

Taking that a little bit further, Scott Hurd at NADC has taken, and using pigs that were challenged intranasally using the feces from those, and then putting them in the pen with naive pigs showed that if there was about  $10^3$  colony forming units of salmonella typhimurium in a gram of feces from these initially challenged pigs, and naive pigs were exposed to that, within two hours you could start seeing that pigs are

becoming positive.

Now, these are the samples that were looked at. Some of them are lymph nodes; some of them are gut samples. And it just took one positive sample to be called positive in this.

The point though is that by six hours after exposure to these feces, all six animals that were exposed in that case were now positive for salmonella. And the point was that when animals are transported and brought into a start or a holding pen at the slaughter plant, lairage, that those animals can very rapidly pick up salmonella even if they were not carrying out.

So, even if the herd that it came from did not have salmonella, which is highly unlikely, they could still pick it up, or they could pick up a different serotype. And it can happen within hours.

(Slide)

So, the points were, most farms have salmonella, check; shedding is sporadic, yes; very low concentrations, yes; and even those low concentrations are enough to get it into a new pig.

Healthy animals can be carriers. And, in fact, apparently, non-shedding animals can oftentimes be positive. Detection is difficult and the culturing systems are very complex. At least one growth promoter

had absolutely no impact on salmonella shedding, and it can be in fact transmitted and acquired very rapidly.

(Slide)

The studies that we did were in conjunction with a number of individuals. These are the people, and these were the sources of funding. And I will take any questions you have at this point.

### **Questions and Answers**

DR. KOCHEVAR: I was interested, when you talked about the effect of withholding feed on increasing the number of animals that were shedding; and then I remembered what they talked about with the FSIS presentation that -- maybe I misheard this -- but that they encourage withholding of feed in order to limit the production of feces to limit contamination. So which one do you go with?

DR. ISAACSON: Well, it is a bit of a catch 22 because if you withhold feed, then the gut is empty. And that is why we did cecal contents, because we could not reproducibly get feces. And so, the potential for nicking a gut and having it contaminate the surface becomes reduced.

On the other hand, the potential for salmonella being there is slightly higher. If we looked at it from another pathogen standpoint, it may be

absolutely the exact opposite.

In fact, some of the studies that have been done with E.coli, and doing changes in the feeds that they are getting, take them off without the high growth feeds and putting them onto grasses just prior to shipping, which we think would actually reduce salmonella.

In fact, I think that the studies actually showed that that would reduce salmonella -- actually, no, increase salmonella, but reduces E.coli, so but that is in cattle. So I think that the answer is going to be it depends upon what you are looking for that particular day.

It may have a positive effect for one thing, and a negative effect in the other. And so, the net result is what ends up on the carcass. I would just sort of add though from a food safety standpoint and a question of the impact of resistance transmission to humans, is whether a  $10^2$  contamination still is a relevant number, or whether we need to be thinking about something higher than that.

I think one of the problems that I have seen in the literature, and one that I have worried about a lot, is are we worrying about the wrong phenomenon?

Are we worrying about these low level

contaminations as being the source of food borne transmission, or is it the occasional, very high animal that actually is the one that contributes to the real problem?

And no one is looking at that, and even with the FSIS rules, it is a yes/no. It is not a quantitation. So I think it is another question that certainly needs to be thrown into the mix and addressed.

DR. GLENN: A question regarding the same study with the time of feed withdrawal increasing -- as time of feed withdrawal increased, study increased. These animals were challenged two times, I believe, with the same dose probably both times, ten to eight

Did you have a control group? What would be your speculation on just a regular ole' healthy pig?

DR. ISAACSON: Do you mean would there be any salmonella there?

DR. GLENN: Would there be this interrelationship between time of feed withdrawal and shedding?

DR. ISAACSON: Well, we --

DR. GLENN: What do you think?

DR. ISAACSON: When we selected our animals from a university herd that we did not have -- we could not demonstrate the presence of salmonella there. And

we never did find any salmonellas in these -- at any other serotype, any other non-antibiotic now resistant strain there.

So, if we had done that, we did not do that, if we had done that, we expect that we might not have seen anything. But it is an interesting question because, you know, maybe it was there hiding and we did not find it.

DR. GLENN: Yes, my concern is if the animals are sick upon challenging, then I think there is a lot confounding effects occurring relative to gut motility and the shedding rate. And so, you get this confounding effect of, you know, if you are sick, you know -- I realize you went through the feed, so that is good. That was controlled. But, still, I was just wondering about that.

DR. ISAACSON: And so, we did not look at a non-challenge group.

DR. GLENN: Okay.

DR. ISAACSON: But you mentioned sickness, and I think I should mention that if the challenged dose that we took caused about 50 percent or so of animals to have a transient mild diarrhea. None of them were ever dehydrated. The other 50 percent never showed any clinical signs.

And, interestingly, on the second challenge, as you might expect, there might be some protective immunity; the second challenge, we saw no illness at all associated with it.

DR. WADDELL: I was wondering, in light of Scott Hurd's study on the lairage, did you guys clean and disinfect the vehicle that you transported those pigs with, and hold them in a different area before they were euthanized?

DR. ISAACSON: We did not hold them. So the vehicle was actually large enough that we could put two groups on there. And there was a good solid partition, so there was no cross-contamination. We did two groups one day; and then the next day, we did the next, and it was completely disinfected during that timeframe.

When they came back, we tried to do our necropsies very quickly. So, within about an hour, we were able to do 15 of them. An interesting question is we asked whether there was anything, any difference between the ones at the very first to be slaughtered versus the 15th animal in that group, and whether there might be a pattern there, and there was none.

So we did it quick enough I think that we did not see probably the lairage issue that Scott has mentioned. But it is possible that we see some of that

because it is a three hour transport, that there could be some of that going on in the truck.

But, remember, these animals, particularly the ones that were 24 hours, which were the highest, there was nothing to defecate, so there was -- if that was going on, it was -- would be a pretty interesting -- well, it would be hard to rationalize how that might be occurring.

DR. WADDELL: Were you surprised on your survey that you did not find a cholerasuis in pigs?

DR. ISAACSON: No, because when you find cholerasuis, you generally find sick pigs, and these were healthy pigs. And, generally speaking, out in the field it is hard to find cholerasuis. And that is something that kind of comes and goes. I do not think we have seen too much cholerasuis in disease settings recently. It has declined quite a bit.

DR. WADDELL: Has the avery live vaccine had an effect on long-term shedding?

DR. ISAACSON: I think there are some claims to that, but we have not looked at that. So I could not really comment on it. I think there has been some claims that it has some impact. I am not sure how much.

DR. PARKHURST: In that rapid infection study by Hurd, it appears that after six hours there were only

six pigs left. Is that correct?

DR. ISAACSON: Right.

DR. PARKHURST: What happened to them?

DR. ISAACSON: I have to tell you. I pulled this out of the literature, so I mean it is his study. I think they only had six animals at that point in their group. Actually, I am thinking -- no, because they were actually -- they had three different separate -- you know, three separate groups in there.

And I guess I am just assuming their group size was not sufficiently large. They have replicated this several times, and they still see the same kind of effect.

DR. LANGSTON: This is more just kind of a point of interest question. I have been reading a little bit about quantitative PCR techniques. And, given the difficulties, would that hold any potential for this sort of thing?

DR. ISAACSON: Well, I think that is a good question because you could start asking the question about whether or not it is there. It is very difficult to do quantitative PCR from fecal samples. There is just all sorts of inhibitory substances there that makes it very difficult to do.

However, we have done some work, and others

have done some work of doing either pre-enrichments, or doing enrichments, and then working from that to at least move the timeframe forward a bit.

I mean, to do what we were doing, the standard procedure, it takes almost a week or more, a week to ten days to get confirmation of what you are working with. That is a long, long process. For research it works okay. If it was to be invoked in the field for any purpose that would be inadequately.

So we have looked at PCR, and we have actually had some pretty good, albeit, preliminary data by rather than using the tetrathyanade enrichment, we can go right into RV, do an overnight incubation, pull a sample out of that, and then do the PCR, and it works reasonably well.

I know that Randy Singer, who is at the University of Illinois, is doing similar kinds of work with cattle samples, and he has been able to go into tetrathyanade, initially takes a sample out after overnight growth, takes a sample out, dilutes it into BHI, lets it grow for about three hours, and then does the PCR. And, again, he gets pretty decent results with that as well.

DR. WADDELL: Are programs that would be looking at elimination of salmonella from swine farms,

is that a practical goal?

DR. ISAACSON: Do you want my opinion? No, I do not think it is everywhere. We have looked at pigs. We have looked at mice. We have looked at insects. We have looked at birds. We have looked at people bringing it in.

Unless you could completely isolate the animals from any contact on themselves, as well as any type of wildlife, and that includes the insects, it is going to be really hard to get rid of it.

I think it is really ubiquitous. And for a long time as a microbiologist, I would say that salmonella was a pathogen because it is inherently invasive. It likes to find M-cells and go into M-cells. It goes into enterocytes. It likes doing that.

It likes to get into even leukocytes. And I thought, well, that is pathologic. It has got to be pathogen. But the more I looked at it from a farm standpoint with these healthy animals, believing that it is an indigenous part of the microflora.

And I do not think that it would be any easier to get rid of salmonella than to say that we want to eliminate all E.coli as well. I think it is asking for something that would be really, really difficult.

Could you reduce it? Yes. But I do not think

it is elimination as a practical approach.

DR. LANGSTON: Any other questions?

(No response)

DR. LANGSTON: Thank you, doctor.

DR. SUNDLOF: Okay. Our next speaker is Dr. Mark Robinson, who is the director of the Division of Human Food Safety in CVM. And Mark spoke yesterday, but for those of you who are here for the first time today, here is Mark.

### **Human Food Safety Evaluation of Animal Drugs**

**by Dr. Mark Robinson**

DR. ROBINSON: Thank you, Steve, and good afternoon, good late afternoon.

As Dr. Sundlof mentioned, for those of you in the audience who were not here yesterday, the committee has already heard my schpill with regards to human food safety evaluation, as it relates to chemical drug residues. And I won't suffer that upon you today.

Hopefully, my talk will be more on the order of food for thought. And before I get to it, I would like to make a couple of comments with respect to issues that have come up, which the committee may have questions.

The first is that the 21 CFR 558.15 is still extant. It is in the regs. But in the last period of

about three to four years, we have really been operating in the evaluation in the preapproval mode, with respect to the framework document and guidance 78, which, in particular, guidance 78 causes us to look at the effect of the us all classes of antimicrobials, so the therapeutics come in partly as a result of that.

The second comment is that there has been reference to a workshop that was held in February of 2000. And I would like to give my observation of that workshop. This was what has been referred to as the preapproval workshop.

The central focus of that workshop, from my perspective, and I was new. I was on-board for one week, having returned from five years in Europe. So I think I had the newest eyes in the FDA group looking at this workshop.

And the central focus, it appeared to me, was to look at the types of studies that would be revealing with respect to the rate and extent of resistance generation. And because the framework document includes both antimicrobial resistance and pathogen load as subjects of concern or subjects of evaluation, pathogen load kind of came along for the ride.

I did not hear one unanimous voice with respect to pathogen load studies. I heard a number of

voices. One voice that I heard was the repeated statement that 558.15 studies and pathogen load studies in general really could not provide much, if any, information with respect to the question of the rate and extent of resistance generation, which I think is logical.

Another voice that I heard was that pathogen load studies, in the generic sense, just were too difficult to perform. There were too many confounders, too many variables, therefore, we should not be in that business.

And, as Dr. Shryock has mentioned earlier, he pointed out that the types of studies that have been done to date trying to draw any inference with respect to those studies to on farm practices, to contamination at the slaughter house, or to public health effects, is very difficult.

The voice that I did not hear at that meeting, because there was a an FDA public workshop, and the FDA was there to listen not to drive the direction of the workshop, the voice that I did not hear was the regulatory voice. And so, that is what I will try to speak to now in this presentation.

(Slide)

I am going to drag you through just a little

bit of yesterday, which is to remind you that in the preapproval human food safety evaluation, we look at the sponsor-generated data demonstrating the effects of defined concentrations of an active ingredient, formulation component, metabolite, or drug product in relation to specific endpoints of relevance to public health, in contrast to the evaluation of chemical drug residues.

If we are talking about pathogen load, changes in bacterial drug susceptibility, or even competitive exclusion products, we are talking really about the drug product, and the potential effects of the whole gamish, because we are involving biological phenomenon not just chemical phenomenon.

We do this, again, because, in part, because of Section 512(d)(2) of the Act which states, "In determining whether such drug is safe for use under the conditions prescribed the secretary shall consider, among other relevant factors, the probable consumption of the drug and of any substance formed in or on food because of the use of such drug."

As I mentioned yesterday, that is a pretty wide open field with respect to interpretation.

(Slide)

The point again is to identify any potential

adverse human health affect that may be caused by the consumption of the animal drug residue and edible tissues from food animals.

Now, we can get into a long drawn out discussion as to whether a bacteria in any form in the gut of an animal could be construed as a residue. But if you hark back to 512(d)(2), we are really looking at the effects of the use of a drug, and how those effects might be translated to the food.

We also, if we find a problem, we would like to see if we could mitigate this.

(Slide)

So why ask about pathogen load?

Well, there is a lot of data presented today, which, I think if -- well, there is a tremendous amount of data, and one interpretation of that data would suggest that maybe we did not quite get it right with 558.15 in the salmonella shedding studies.

I think that to take that a step further, and to attempt a meta-analysis of all of the data, both public and proprietary, that is out there, and to draw conclusions would be extremely problematic.

When we think about the safety of a drug product from the human food safety public health perspective, in the last couple of years we have come to

asking a couple of fundamental questions.

This is actually something akin to a HACCP analysis. We want to specifically identify the hazard associated with a particular drug/bug combination; also, with respect to the production environment, the species, and the proposed conditions of use.

Because, for those not familiar with the system, we do not approve a drug product. We approve a particular proposed condition of use of a drug product, so that the condition of use is important, the production environment becomes very important.

So some questions on any of these microbiological issues that would apply to one species in one production environment with one drug just may not be applicable to another situation. And it really drives the experimental construct that you might come with to answer the critical questions to almost a case-by-case basis.

Now, I know that this was spoken to earlier today, and that we need to have some universal standard. I think we do need to have universal principles with respect the evaluation of the drug. I do not know that we could ever approach a universal experimental construct that would be appropriate to all situations.

So the question arises first, what do we

really care about?

Well, we have heard from the FSIS folks about slaughter contamination and control of that contamination. We have heard from the production specialists about various levels of prevalence of infection on farm and in animals during transport.

I think that really if we focus down on the issue what we would care about is the bugs that are making it to the food commodity. And, yes, the meat is sterile when it is cut. But it is becoming infected, or it is becoming contaminated in the plant.

Now, one of your questions to the committee is, can you draw any kind of a line between drug use and this contamination?

And I would suggest that, in one sense, you can because that contamination is coming from the gut of the animal. I would also suggest that maybe the intercellular infection is only important to the extent that the intestinal mucosa ends up also being a contaminant in the slaughter house.

The FDA does not have the ability to control or prescribe evisceration techniques. That is not in our domain. But we do have the ability to control the events leading up to what happened to that if a drug is used in that process.

And so, I think it is important to consider that there may be production situations in which a drug is used, either therapeutically, prophylactically, or as a growth stimulant, in which the animal may not suffer other events.

We have seen corporate farming emerge in this country where animals do not go on trucks. They go from pen to slaughter. They do have stress certainly. There is the animal behavior. But we do not necessarily have that disjunction of several days with transportation that is incurring in every production environment.

So the point that I am getting to here is that we have, in chemical drug approvals, we have in the past considered inherent production withdrawal periods, and incorporated those into our evaluations.

We have come to regret that in some instances. And I think that it is important to consider potential worse case, and then to evaluate how significant is that worse case with respect to any public health problem.

The second area where I think that there is a potential for a problem is in environmental recirculation. We have heard about fecal contaminants, and their ability to infect naive animals with salmonella.

And, Dr. Wages, I am not going to pick on

poultry just to pick on poultry. But I think that there is a good body of evidence that suggests that whatever is in the litter in a broiler house, regardless of top dressing or any other treatments, that what is in the litter is a major factor in what ends up in the guts of the birds that follow into the house in the next round.

So, we may want to know, we may want to ask a question. In that case, we would want to know about shedding rates, not about necessarily about what is in the gut of the animal at the time they are slaughtered, but we might want to know about shedding rates. And we would want to know that coupled with other information.

You could speculate that, in this case, campylobacter, which is not particularly viable under any circumstances, probably is not going to be there. So, even though campylobacter might be a public health concern with respect to chicken, you could maybe wipe that off your list with respect to an environmental recirculation question.

So, what I am saying, what I am trying to describe here is an approach that might be taken with respect to focusing in on what questions need to be asked. It is not within my competence to address exactly the mechanisms by which those experimental constructs would come about. I think that there are

plenty of experts here who could deal with that, and have already spoken to the issue.

Finally, I would like to go back to the Act, and the requirements that we have placed on all other drug approvals. We do not prejudge any of the assays, the experiments that we request of sponsors, as to whether they will reveal something that is good, bad, or indifferent.

We are asking for data, the principle being that the sponsor needs to demonstrate to the FDA that a particular proposed condition of use is safe. I would find it very difficult to make a universal assumption that all uses of all classes of antimicrobials would be safe with respect to pathogen load in the absence of any data.

I think that there is a fundamental need to describe the effects, probably most of which will be beneficial to the sponsor, but we need to make an effort to approximate the proposed conditions of use and provide data which would support a conclusion of a reasonable certainty of no harm. Thank you.

Any questions?

#### **Questions and Answers**

DR. WAGES: Did you just not, in some of your comments, try to persuade this committee how to address

these questions?

DR. ROBINSON: I tried to provide a perspective that I think has not been heard. I am just making suggestions. I am not trying to persuade. But these are concepts that I have not heard, and I think that they are important to bring out.

I am very interested in your comments with respect to this issue. And I am very willing to accept if you propose that it is a non-issue.

DR. WAGES: I just found personally some of your comments inappropriate coming to this committee in the format it was made.

DR. ROBINSON: I am sorry.

DR. LANGSTON: Any questions?

(No response)

DR. LANGSTON: Thank you very much.

DR. SUNDLOF: All right. Now, here to wrap this whole thing up on pathogen load, the session that you have heard this afternoon, we have Dr. David White, who is with our Office of Research, but is more recently on detail within the Office of the Director, dealing with many of our antibiotic issues. So, Dave.

MS. SINDELAR: This is our real computer expert right here. Thank you, Dave.

DR. WHITE: Thank you, Aleta.

## **Summarize and Adjourn Discussion**

**by Dr. David White**

DR. WHITE: First of all, I would like to thank everyone for spending the whole day here. I know we have kept you over, did not realize we would stimulate this much discussion along with VMAC. And it has been a very good discussion.

I would like to take this time as well to thank the speakers for coming here, for the VMAC, as well, for coming here, and for all of the attendees for taking time out of their busy schedules to come here and talk about this subject.

We all know time is a very important commodity, and there is no price on that, and I appreciate everyone coming for that.

My objective today at the end is to summarize and adjourn, so I am going to do this quickly. I do not want to keep you here longer than we need to be.

(Slide)

Let's get a little history on this issue with CVM. It has been around a long time, the pathogen load. As Dr. Gilbert mentioned in the beginning, the CFR 558.15 studies were developed in the mid-1970s. They were focused on sub-therapeutic uses, and there was a shedding and resistance component to the studies.

As Dr. Robinson alluded to, in 1998, guidance for industry 78 was put into play, and it looks at all uses of antimicrobials, so not just sub-therapeutic, but therapeutic as well. And there is two components: One is a resistance component, where you look at the rate and extent of resistance; the second is pathogen load, and they are different.

And the VMAC today is looking at the second part, the pathogen load component. We are not looking about the resistance component today. And, in 2000, also as Mark presented, or mentioned, we had a CVM meeting on preapproval studies in resistance and pathogen load.

The focus really was on resistance, but pathogen load was part of the meeting agenda. That is on our website, if you all need to access that. And I believe that some people requested transcripts, and I hope that you have received those. If not, let us know, and we can get those to you.

(Slide)

One thing that is important to remember is that consideration of the potential microbiological effects of antimicrobial new animal drugs has been identified as a significant component of the animal drug safety evaluation process.

Also, the lessons learned from the 558 studies, along with 20 years of advancements in scientific knowledge, are important factors for helping to develop appropriate methodologies for evaluating the relevant microbiological effects associated with the use of drugs in food producing animals. Those are just two things to put out front.

(Slide)

I just wanted to mention again the speakers we have had today and summarized their talks. I am not going to reiterate what they said, just kind of some of the major bullets.

Dr. Sundlof started off the meeting introducing the subject. We had Jeff Gilbert talking from CVM, talking about the history of the studies. We had Tom Shryock talking about the conduct of the studies from the industry perspective.

Jeff Gray, from USDA, who has been very involved in these types of studies, talked about the design considerations. We had Dr. Mike Goodman from Exponent, who actually wrote -- was involved in the Exponent review that is on our web page as well.

Dr. Scott McEwen, who was able to travel from Great White North down here, talked about epidemiological evidence related to pathogen load. Dick

Isaacson, from the University of Minnesota, talked about the other confounding factors that may affect pathogen shedding in food animals.

Dr. Barbara Masters and Karen Hulebak, from USDA FSIS, we thank them for coming out and talking to the VMAC with regards to what PRH, pathogen reduction HACCP, means; and also Dr. Robinson explaining from his perspective on the human food safety evaluation.

(Slide)

So just some bullets to remind you. Dr. Gilbert presented the brief history. He mentioned study design and examined parameters. He talked about the integrity measurements, the study results, also identified problems at CVM recognized, but he also mentioned that it was based on the policy and regulation of the time which was the 1970's.

(Slide)

Dr. Shryock also presented industry experience with these studies. He also talked about the design interpretation, the results. He also identified problems and limitations, and he questioned the relevance of such studies.

(Slide)

Dr. Gray presented information regarding the studies, and he mentioned some ideas about the organisms

used, again, the study design, measuring effects, and some confounding factors.

(Slide)

Dr. Goodman, as a CVM contractor with Exponent to conduct a review of the literature, and Dr. Goodman tried to summarize that, and I said it is located at this web page. As well, it should be in the notebooks if you want to access that as well. They looked at 33 literature databases, and tried to break it down into challenge, or observational, or observational studies, and realized that there was limited data on the subject.

(Slide)

Dr. McEwen presented an overview of the relevant epidemiological information related to food animal studies, and he talked about human studies, animal studies, food animal studies, and he talked about some more study results. These are things that you have already said. I was just trying to summarize for you all.

(Slide)

Dr. Isaacson presented information relevant to shedding of pathogen food animals. He talked about the study designs, bacterial detection, culture methodologies, confounding factors, and some take home points.

(Slide)

Dr. Barbara Masters and Dr. Karen Hulebak talked about the information with regards to pathogen reduction HACCP. They gave us a brief history. The regulatory requirements, the implementation, performance criteria, and performance standards related to salmonella.

(Slide)

Dr. Robinson presented information describing evaluation of a sponsor-generated data. He talked about relevance to public health, identify and mitigate potential adverse human health effects, and he talked about the safety of the drug product.

(Slide)

Here are some key components to end on. The CVM recognizes that scientific information in this area is limited, and acknowledges the concerns raised at the February 2000 public meeting. Those are on record in the transcripts.

In response to that, we did contract with Exponent to try to get a grasp on the literature looking at these studies. And we feel that today's VMAC represents our ongoing efforts to look at the issue of pathogen load, and to develop appropriate policy in this area.

And to the VMAC, we are seeking recommendations on the issue evaluating potential antimicrobial drug effects on pathogen load in food producing animals, as part of the new animal drug application process. So, it is important to remember these approval studies that we are looking at.

(Slide)

Lastly, for tomorrow morning, just to give you an idea what is up, or an heads up, we have an open public session in the morning, and that's a.m. We have the presentation of the questions to the VMAC, committee deliberations, and the meeting summary at the end.

And, lastly, I would like to thank the members of the working committee on pathogen load. And that is Bill Flynn, Pat McDermott, Charles Easton, Karen Lampey, Jeff Gilbert, Aleta Sindelar, Mark Robinson, and Burt Mitchell.

And I thank you for your time. I do not know if there anything else you would like to say.

DR. SUNDLOF: Thank you. Thank you for that excellent summary in such a brief time.

And, at this time, I will turn it over to the chairman of the VMAC to adjourn. And, from myself personally, I hope everybody has a relaxing evening. Enjoy the Washington area. And, Mr. Chairman.

DR. LANGSTON: Thank you very much. I will just make a comment relative to Dr. Robinson's presentation. I think it is important that we know what the FDA is thinking, but I also understand Dr. Wages comment as well.

And, remember, from the committee standpoint, it is the science and whatever FDA thinks in the past/present, it will have no bearing on what we decide. So I will just end on that. Yes?

DR. PARKHURST: Before our experts leave, I do not know whether you will be here again tomorrow or not, but could I ask one question?

And maybe either Dr. Gray or Dr. Isaacson would be, or anybody else would be willing to address this, are there some pathogen shedding levels that are more hazardous than others, or would that be a relevant question to ask and have answered at some point?

DR. ISAACSON: I think that is a great question. I do not know that there is an answer to it. We need to establish what is a level that is appropriate to which you do not want to exceed. And it depends upon the specific pathogen that we are working with.

Salmonella, the infectious dose, depends upon again the specific strain, but could be in the  $10^5$ ,  $10^6$  range for humans; whereas, E.coli 0157:H7 is down around

single organism, 5, 10, 20, or cells per infectious dose.

So I think those things are things that we ought to be looking at and trying to get some data to establish some levels.

DR. PARKHURST: So would it be fair to say that it is detrimental to the environment, but not necessarily human health at this point?

DR. ISAACSON: I do not know that I would say that, because I do not think the pathogens in and of themselves are detrimental to the environment, per se. They are certainly not hurting the animals.

And the only time that it is detrimental is when the dose is high enough, exceeds some threshold, and a person consumes it, that it becomes a problem. So I do not know that it is an environmental issue in that sense.

DR. PARKHURST: Would it be true that we could always get rid of it? Or is there some threshold where it makes it just -- the problem is prevalent and it is very hard to get rid of?

DR. ISAACSON: My feeling about an organism like salmonella is that it is ubiquitous. I think Jeff Gray said that as well. You can find it literally anywhere. The idea of being able to eliminate it, is

probably not a realistic goal.

I think that what is a realistic goal is the containing it, so that the levels are maintained below a certain level. And I am talking about within an animal. We can talk about carcass contamination as well. And I think there you can do things to try to reduce the number of carcasses as well that are actually being contaminated.

But I think the ultimate question is the specific level, and I think there are things that we can do to reduce that. But if the question were, can we eliminate salmonella, I would answer no.

Because there is this intimate association over time and evolution between the mammalian hosts, as well as other hosts -- it does not have to be just mammalian -- and salmonella. They have co-evolved and there is a strong association between them. And I do not think that it is a realistic possibility that that bond will ever really be broken.

But, as I was showing you in my data, is that the levels, the concentrations of salmonella per sample is reasonably low. And so, if we can keep it so that the interaction is good, but that it is not excessive, then it would okay.

I have a hunch if we try to do things to

eliminate salmonella, it goes back to the question of antibiotic resistance, is that we are going to create a whole nother problem.

And there have been some epidemiologic associations, for example, between the emergence in poultry of salmonella enteritidis, and the decline, the directed decline of salmonella pullorum, the pullorum eradication procedures, and that what happened is that when pullorum was eradicated that there was a new, there was an ecologic space that was opened for which enteritidis could enter in. That is why we are seeing the epidemic of enteritidis.

So, I mean, I think that the best solution is a control of the population size, but not an eradication, because as soon as you eradicate or attempt to, then something else happens. And if it is not a resistance type thing, then it is going to be the emergence of perhaps a worse strain.

DR. PARKHURST: Tell me why control is important.

DR. ISAACSON: Well, why is control is important?

I think control is important from the standpoint of keeping the infectious dose sufficiently low, so that the risk is reduced. I do not think we can

eliminate risk entirely as a food borne pathogen, but we can reduce the risk of it entering into the food chain, so that the incidence of diseases is lower.

So, if you can control it -- and I think a lot of these things have to do with hygiene and animal health, and processing, and abuse post-processing, and such, temperature abuse, for example, that will be useful in controlling the population size, per se.

DR. HOLLAND: I do not want to turn this into an issue to debate. But if salmonella is ubiquitous and everywhere, then why isn't it a problem in humans? Are humans carriers then?

MS. SINDELAR: Is this directed to Dr. Isaacson?

DR. HOLLAND: Well, we have heard it a number of times today that salmonella is ubiquitous and it is everywhere. Then are human beings carriers?

DR. ISAACSON: It is a good question. And I think the answer to the question is, no one has looked to ask the question. You know, it is hard to find it in animals, although we know it is widely distributed. You find salmonella frequently.

But people who are in the diagnostic labs in hospitals, unless they are looking for diarrhea causing organisms, are not going to be looking for salmonella.

So I do not know the answer to the question, "Are humans carriers?"

We know that there is the famous carrier, Typhoid Mary, who carried typhi, but that was a unique and different kind of situations. Whether there are gut carriers, we really do not have very much data to say one way or another. It is possible; it is possible that they do not.

DR. HOLLAND: One more provocative comment.

DR. SHRYOCK: If I could comment on that just a little bit. I think what Dr. Isaacson was getting at, is if we took a survey of everyone in this room, we cannot answer whether someone in this room is carrying salmonella subclinically.

However, we do know that in cases where people have been infected with a non-typhoid salmonella, that they can carry it for years. The same is true in a sub-clinical sense for E.coli 0157:H7.

We have cases where a fully virulent organism can be carried for months with an individual and not show no clinical signs. So I think directing to the question you asked, no one has looked, but there is evidence to say, yes.

DR. HOLLAND: One last provocative statement. Then, if it is ubiquitous, and it is in humans, then why

are we here?

MS. SINDELAR: I would just like to make two comments: One, that all of the speakers will be available for tomorrow for further questions, as a reassurance; however, Dr. Goodman will not, and Barbara Masters will not. So, if there are questions, then we can make the telephone calls as necessary to answer any inquiries.

And, second, just for transcription purposes, I just wanted to note that, thank you, Dr. Isaacson. You were the Mac user here. And so, we did have copies made, and the VMAC members were provided the copy prior to presentation. All copies will be available tomorrow. So, thanks very much.

DR. LANGSTON: I think my brain is getting too tired to deal with these issues anymore. So, barring any other comments, or an objection to adjournment, we will adjourn and reconvene at 8:30 in the morning.

(Whereupon, the meeting was adjourned at 5:40 p.m.)