

Veterinary Medicine
Advisory Committee Meeting

Pathogen Load

January 23 ~ 24, 2002

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PATHOGEN LOAD**Procedural Comments****by Aleta Sindelar**

(1:01 p.m.)

MS. SINDELAR: Mr. Chairman, members of the committee, invited guest speakers, FDA staff, and public participants, I would like to welcome you all to the second issue for discussion, Pathogen Load. I am Aleta Sindelar, the exec. sec. for this committee, for those of you who have just recently arrived.

I would like to share with you some information about the background materials available at the back of the room. First, let me say that the meeting has been and will be open in entirety, so all information and materials and presented at this meeting will be shared publicly.

At the back of the room, you will find a spiral bound book containing all of the information that has been provided to the VMAC members in anticipation of this meeting. All comments provided for review to the committee prior to this meeting are also made available.

A new agenda reflecting the speakers for this meeting has also been copied for your review. The Powerpoint slides of the speeches presented today, as well as all of the aforementioned materials, have been

transmitted for posting on the CVM website.

The conflict of interest statement has been already made for the record. So, please, at this time, let me turn the meeting over to Dr. Sundlof, the director, Center for Veterinary Medicine. Thanks, Dr. Sundlof.

Welcome/Regulatory History

by Dr. Stephen Sundlof

DR. SUNDLOF: Thank you, Aleta. I just read on the agenda that I am supposed to give some regulatory history. And I just found that out, so I will start. Four-and-a-half billion years ago --

(Laughter)

DR. SUNDLOF: Actually, I am really not qualified to do that, but we have with us a number of folks who are very well qualified to talk on the subject. But on the regulatory part of pathogen load studies, we have required pathogen load studies for antimicrobial drugs that are administered sub-therapeutically in feed for a number of years.

Dr. Gilbert is going to talk about the history of that, and just a little bit of insight about what we have learned from those studies. This should help to give folks a little bit of background when we get into the next part of the discussion, which is looking at the

potential for using these kinds of studies for therapeutic drugs as well.

So I am going to turn the podium over right now to Dr. Jeff Gilbert.

Salmonella Shedding Studies: Regulatory Perspective

by Dr. Jeff Gilbert

DR. GILBERT: Thank you, Dr. Sundlof.

Everybody hear me okay?

I am going to talk a little bit about the, as I titled it here, "558.15 Studies, A Brief History." Before I get going, I wanted to say a word of thanks to my friends on the VMAC Committee internally, David White, Aleta Sindelar, Burt Mitchell, Mark Robinson, Karen Lampey, Charles Easton, and Bill Flynn; also to my colleagues in 157 who helped me with this, Dr. Catherine Well, Dr. Karen Lampey, Steve Yen, and Gary Sherman.

And, last, but not least, a very special thanks goes out to Dr. Jean Cooper. Now, Jean is no longer with us. I mean, she is still here on earth. She is just not with us at CVM. She is at CVRH. And, Jean, before she left, did a very lengthy retrospective analysis of all of the 558.15 studies.

I used to rail on her for carrying around all of these papers in the hall. It always looked like she was going to a construction site because she taped all

of these spreadsheets together and had a big overview of the 558.15 studies.

I have had to call on those recently, so now I appreciate them a bit more. But what I am going to give you today is basically excerpted from Jean's retrospective analysis. So I want to make sure she gets due credit.

(Slide)

If we think back to the '60s, one of my favorite decades, that is when the issue of the use of antimicrobials in animals, and their impact in the human community in medicine really came to the forefront.

A lot of meetings and gatherings of scientists occurred and began focusing down trying to come to the salient question which is, "What is the impact of antimicrobial drug use in animals on the potential development of antimicrobial resistant food-borne pathogens and their subsequent transmission to humans as food contaminants?"

(Slide)

Then, at the dawn of the '70s, we really began to get down to taking a hard look at this. And in 1970, we had the Antibiotic and Animal Feed Task Force formed here at FDA. This task force was formed to address the safety and effectiveness issues associated with

antibiotics administered in animal feed.

(Slide)

What the task force found was that therapeutic antibiotics used to relieve animal disease were thought to pose a small risk, because they are usually delivered at a high dose for a short period of time in young animals. So they thought that there probably wasn't going to be an impact on resistance or pathogen load with that use.

The benefits to animals were thought to outweigh any potential risks to humans. There was an identifiable need for using antibiotics in animals to relieve pain and suffering. And, in general, we have always felt that animals equal a safer food supply. So the task force sort of reaffirmed that.

(Slide)

What they concluded was that preapproval studies would be needed or are needed to support microbiological safety of antibiotics in food-producing animals intended for sub-therapeutic uses only including growth promotion and feed efficiency, the low level uses.

(Slide)

From that came the codification of Title 21, Code of Federal Regulations, Part 558.15, or

affectionately known as 558.15. And, in there, if you read it -- I do not know if you have read it. It is fairly short. It is easy to read.

The back end of it is mostly tables that have to do with interim marking agreements, but the front end is the real meat of the resistance and pathogen load issues. So if you have not read it, please do. It is only a few pages long.

Sponsors of antibiotics are required to submit study results demonstrating that their product does not promote bacterial drug resistance only when their product is intended to be administered for greater than 14 days, and for non-prescription use in food producing animals. That is the first major tenet.

(Slide)

The second, and this is codified in B-1 through B-3, sponsors are required to submit all information to the agency on the impact of their drugs on the salmonella reservoir, a.k.a., the pathogen load in food producing animals by specified dates depending on the drug class.

I am going to go through these dates now. They are all way back in the '70s. And, generally, most of them were hit, but a lot of the stuff trickled in well into the '80s, and we are still looking at it now.

(Slide)

But, by July 19, 1973, sponsors were to have submitted records and reports of completed, ongoing, or planned studies including protocols on tetracyclines, streptomycin, dihydrostreptomycin, penicillin, and the sulfonamides.

(Slide)

That same year, by October 17, 1973, were to be submitted records and reports of completed ongoing or planned studies including protocols on all of the other antibiotics not mentioned in that first slide.

(Slide)

The following year, spring of '74, the deadline was for records and reports of completed ongoing or planned studies including protocols on the nitrofurans -- our old friends, nitrofurans, which are now gone.

(Slide)

Later on, by April 20, 1974, there was a requirement to have submitted data from completed studies on tetracyclines, streptomycin, dihydrostreptomycin, sulfonamides, and penicillin, assessing the effect of the sub-therapeutic use of these drugs on the feed on the salmonella reservoir -- this gets to the pathogen load part -- in the target animal,

as compared to that in the non-medicated controls.

(Slide)

Then we had the same sort of requirement addressing the salmonella reservoir for any antibiotic or sulfonamide drug approved for sub-therapeutic use in animal feeds. Those are the ones that were outside the first group.

(Slide)

And the same requirement for the nitrofurans, to look at the salmonella reservoir, and this was by September 5, 1975. So that sort of wrapped up the series of dates for the requirements to be submitted.

(Slide)

So that gets us into the study design that came out of these requirements, what sort of study was going to be done. The studies were designed as a set, and which would include a shedding component, and a resistance component. So we sort of had two studies within one.

The studies included a negative control and a treaty group. And the animals were inoculated with a lab strain of salmonella typhimurium, a test strain that had been encoded with analgesic acid resistant marker. The strain had to be shown to be free of any transferrable resistant elements.

(Slide)

Again, study design continued. Salmonella were enumerated and tested for susceptibility. The E.coli, resident E.coli in the animals were just tested for susceptibility. They were not necessarily enumerated. though I think some people collected that data.

Studies were generally about eight weeks in length, and the test animals were not required to be near the market age or weight. We had the major test animals, be it beef cattle, or poultry to swine, but they were not always in or around market age or weight when the studies were conducted.

(Slide)

Some of the parameters that were looked at in these studies were the drug effect on the pathogen quantity, prevalence and duration of shedding organisms to salmonella. What was the drug effect on the salmonella antibiotic susceptibility? And also, what was the drug effect on the resident E.coli antibiotic susceptibility?

I do not have all of the details right in front of me, but some of these were just testing back against the drug, or several classes of drugs, just sort of a standard antibiogram.

(Slide)

As far as guidance on these studies, if you have not read these, there is our old guideline 18 and 19, the human health safety criteria, and animal health safety criteria. You can find these in our website listed at the bottom. It is www.fda.gov/cvm.

Go into our section on guidance documents, and you will be able to find them there and print them off. They are fairly short to read too. These guidelines were a product of the task force.

(Slide)

Getting back to the studies, what were the integrity measurements we took a look at. Did the product have antibiotic properties? That was one thing that was important. We wanted to make sure it actually -- you know, at those low levels, what sort of effect was it having on resident bacteria there.

Was there any cross-contamination in the studies picking up bacteria from outside or from cross pins? Were the animal numbers sufficient? Was enough drug consumed to test the highest proposed dose over the course of the study?

Were there any concomitant therapies given, in conjunction with the main drug being tested? Were there any naturally occurred salmonella present came into the

test system?

(Slide)

Was the salmonella marker stable? We generally found that to be true. Could the salmonella receive any resistance factors? Could the salmonella even colonize the animals? Was the appropriate microbiology methodology used for the time that the studies were conducted?

What tissues were examined? Were we just looking at cecal droppings, or scrapes from the intestine, or taking out liver, spleen, those sorts of things? How often were the samples taken? And was the study link adequate?

So these were, more or less, all of the integrity measurements that we looked at.

(Slide)

All right. This is the results slide, if everybody can see. There is a lot of numbers on there, and I think everybody has a corrected one. If not, I have put on the back table back there this most current copy, which basically reflects the final decision made on these studies.

There is another one that has 24 passing. That was sort of straight out of the shoot. That was the first cut of what came through, 24 pass. But when

you look at it and go back through -- we went back through and looked at every one of these studies, the final tally, and came up with the new numbers, where a total of 44 studies were conducted over those classes of antibiotics on the left.

That is the macrolides and lincosamides, 9 studies; 13 studies with ionophores; 15 studies with unclassified gram positives; streptogram is one; glycopeptide is two; bambarmycin is two; and broad spectrum is two; so that totals up to 44.

You can see within each drug class how many drugs were tested in what animal species. Most did the poultry, swine, and cattle. A couple of them just did maybe the poultry, because that is all they were focusing on for the approval.

You can see in our pass, fail, and reject columns the numbers there. If you can add those up, the pass, fail, and reject, they will add up back to the total. This basically gives you a broad overview.

Now, a lot of the studies that fail on the first pass maybe were accepted later on. There may have been some mitigation, or maybe they ran the study over. I have got some of those numbers as far as a repeat column I think in the hard copy, but I did not put it here.

So some of the studies, even though they failed on that first go round, they may have come back later and either did it again or fixed something in the study. I am going to talk a little bit there in a minute about what was wrong with these studies. That gives you an idea about the number of studies we are talking about over the past 25 years.

(Slide)

Why did we reject these studies? There were a number of reasons that we tallied up. The salmonella or coliform susceptibility results just were not submitted. That is always not good to deal with when that was a major part of your study.

The quality controls within the experiment, within the microbiology were not adequate. Sometimes the shedding was just too long to measure any prevalence or duration. When we gave that high dose of salmonella, it just took off and stayed up there, and by the end of the eight weeks it was still -- you know, you just could not make any determination about it.

Environmental control animals were often contaminated or they were not included. These are some of the things that would cause us to reject the study, and then maybe they came back and repeated it and they got a pass later.

(Slide)

Some of the animals failed to meet the inclusion criteria. Maybe they were not shedding an adequate number at the beginning or too much. The data was too disorganized to interpret. We had a couple of those where we just could not make heads or tails about what came in. We sent it back for people to work on again.

And, finally, there were just too few animals in the study. A lot of the studies were run in what I would consider sort of a facilities confining mode where we can only do ten animals, because we only have the size for that or whatever. Today, we are trying to get that to be a little more realistic and move to locations where you can have adequate animal numbers.

(Slide)

Some other problems were identified following this retrospective analysis when we looked back at all of this after all of these years. The drug spectrum matched the salmonella and E.coli in only 2 out of the 44 study sets. This we thought could be an issue.

There is limited information on susceptibility changes in the naturally occurring flora that were there. We took a look at some of the susceptibility changes in the resident E.coli.

But what about all of the other bacteria were there, and what was their significance to public health at the time? So we really did not get a whole lot of information on those.

Artificially high inocula. If you have to give in the ten to the 9th, or 10th, or 11th, or 12th, you know, salmonella, is that a sort of challenge that you are going to come in contact with out in the field with these animals?

The lab strain of salmonella is really not representative of any salmonella that we might encounter. It is hard to say just how viable and how good the salmonella was as a representative there.

Small numbers of animals tested. We are not talking about a lot of animals. I mean you can almost count them on a couple of hands there on some of these studies. They were done in isolators often, just very, very confined sort of work.

So, to summarize, what I can tell you, if we think back to the data slide, especially the one where I had 24 passing the first time, nine of those failed, and seven of the nine had failed because of pathogen shedding. Rather than the resistance part, it was the pathogen shedding that they could not overcome.

There were problems with the design and

interpretation of the studies. Obviously, they were designed a long time ago. Science is science, but there are better ways to do studies. And also, with the interpretation we probably could have figured out what to do with the information a little bit better.

Everything that was done was based on the policy and regulation of the time. Salmonella is still important obviously. I can remember in the early '70s, there were TV commercials about salmonella. They were really harping on it.

But is that still the major bug? Do we need to look at other ones? What is it that we are doing now that we need to worried about, as far as the pathogen load and the resistance?

Finally, overall history I think will be helpful in steering any current and future efforts on this topic. I know studies that we have helped design recently or take a look at, we are trying to focus more on the field situation and give us the answer in the animal right before it is supposed to go to slaughter, so that we get a little bit better indicator of what is going on.

So, with that said, that sort of gives you a brief history of what was going on with 558.15 studies from the beginning up through the '70s and '80s, and

really until now. And I will take any questions, answer what I can, at this time, to see if I can answer anything else about the history.

Questions and Answers

DR. HASCHEK-HOCK: Could you go back to the table? I had a couple of questions on that.

DR. GILBERT: Sure.

DR. HASCHEK-HOCK: Okay. First of all, you said two of the -- only two of the drugs matched the spectrum for salmonella. Which classes were they in, do you know?

DR. GILBERT: I cannot remember offhand exactly which ones would have been --

DR. HASCHEK-HOCK: Okay.

DR. GILBERT: The ones. It could have been the broad spectrum that had fallen into that. I cannot remember.

DR. HASCHEK-HOCK: Okay. Then of the ones in the previous table that we received prior to the meeting, there were like nine that had failed.

DR. GILBERT: Right.

DR. HASCHEK-HOCK: Now that we only have five that failed.

DR. GILBERT: Right.

DR. HASCHEK-HOCK: So could you tell us how

many of those five were actually because of the pathogen load?

DR. GILBERT: Originally, out of the nine, it was seven out of nine. I am not sure how many out of five. My thought was that it was five out of five had failed on the pathogen load, as I recall from the data. I cannot be 100 percent accurate, but I am thinking that it was all five of those. The other two I think had gotten mitigated some other way, or had another issue.

DR. HOLLAND: What was an acceptable challenge dose?

DR. GILBERT: On the salmonella, we saw different doses. I think what happened a lot of times were that there was like a pilot study. Every time they broke out their isolate, they did a pilot study to see what was going to be like the current infected dose, or would give you the best shedding, and what have you, so it varied. You saw anywhere from 10^7 , on up to 10^{10} , something like that.

DR. LANGSTON: Is it correct that these animals were given the drug right up to the point of slaughter, in other words, there was no withdrawal period where they were subsequently tested?

DR. GILBERT: I cannot say for 100 percent, but I am pretty -- yes, that is generally the case with

them. They fed the drug the duration. They did not pull it with five days and take any measurements, per se, for those last five days to see what happened.

DR. GLENN: Jeff.

DR. GILBERT: Yes.

DR. GLENN: What are the dates for guidance 18 and 19?

DR. GILBERT: You got me there.

DR. GLENN: I looked on it and I did not see it, and I am just wondering.

DR. GILBERT: Right.

DR. GLENN: Thank you.

DR. GILBERT: I can look that up for you and get back. Well, we can just pull it off the web page. It should have the date stamp on the very front sheet of that guidance document. It should have the date on it, but I do not have those with me.

DR. GLENN: Okay. And then let me back up and ask some very simple questions. I think I understand what pathogen quantity is in the unit of measure, but please give me a definition of prevalence and shedding in terms of the criteria you are measuring exactly.

DR. GILBERT: We were looking at like the numbers of animals out of -- you know, what percent out of 100 were shedding, and the duration was just how long

they would go. A lot of times you would see if the drug had some antibiotic effect, even at its lower use level, they may shed for a few days, and then drop off, and you just would not see anymore.

You know, we see that all over the board with some other drugs where they shed all throughout. Generally, what we saw was, you know, it would drop off and go down. And then, at some point, they would say, we are not going to take data anymore because we cannot collect it again.

We cannot pick up anymore salmonella. We have been doing this now for three weeks and cannot find any, so they would stop the study. That is sort of the duration. And then the prevalence was just the number of animals total shedding.

DR. GLENN: And shedding relates to fecal grab samples, or total fecal collection over a period of time? And how is that modeled? I mean, I do not need all of the details, but just give me a little sense.

DR. GILBERT: Right. There were a variety of tissues collected, also feces collected, grab samples in the bigger animals; with the chickens, it might have been just to scrape the lining, and that sort of stuff.

DR. GLENN: Okay. So there was actually slaughter and scraping the lining and the gut?

DR. GILBERT: Yes.

DR. GLENN: As well as what is in the tract itself, the fecal mass?

DR. GILBERT: Yes.

DR. GLENN: Okay. And then, another question, regarding the integrity of the measurements evaluated, were there specifics relative to replication and statistical analysis way back then when these were being done?

DR. GILBERT: They did statistical analysis and they were looking for some log difference, so there were some stats run on this.

DR. GLENN: Okay.

DR. GILBERT: The statistics, I guess, compared to the bigger studies, I do not know what you would get out of having ten animals, you know, and what not, the number of experimental units, and what have you. I guess it is sort of flaky when you get down very small.

DR. GLENN: Okay. So that gets me to replication.

DR. GILBERT: Right.

DR. GLENN: And, presumably, we did not run a study if we did not have adequate replication, but you have alluded to the fact that we did so. And that was a

point for rejection.

DR. GILBERT: Right. A lot of these you would see maybe individual animals housed, and maybe you would have 10 control and 10 treated.

DR. GLENN: Okay.

DR. GILBERT: So there was some replication, but it was not replicated over locations or over time or anything.

DR. GLENN: Okay. And then the last question I had is, in the table regarding failed studies, is the definition of failed related to one of these procedural things on replication, or is it related to an assessment of pathogen shedding, or one of the criteria that were measured?

DR. GILBERT: Right, failed was failed. It failed as things. And a lot of times what they would do is they would come in with what appeared to be a flawlessly run study, but it just failed. It did not work. So that is what we categorize as failed.

The rejected ones were the ones that we rejected for some of these sort of technicalities, that maybe they were able to overcome later and get back. Sometimes they did not. Sometimes they walked away, or did not want to repeat it, or things just changed over time.

DR. GLENN: Okay, thanks. Thank you.

DR. WAGES: There were no standardized studies that were recommended by CVM for the species indicated? It just seems like -- I mean, well, was there or was there not?

DR. GILBERT: Right. Dennis, I tell you, not being there at the time, but being here now, there is -- I do not know what they did back then. I think probably, based on the guidelines, probably everybody came up with a very similar study.

And the fact that these were -- a lot of these were run at one particular location, I think, between the CRO, and the sponsor, and us, you sort of ran into almost a standardized trial for these things, but there was no, here, follow this protocol and do it.

So every one of them was a little bit different, but they did have a lot of similar characteristics too.

DR. WAGES: Were the salmonella strains used for a particular species standardized?

DR. GILBERT: Standardized?

DR. WAGES: Well, as in the same strain used?

DR. GILBERT: I think probably that knowing a little bit about the situation, I think there were probably two, three, four strains of salmonella. And,

again, as I alluded to, and we still see this, you pull them out and maybe run them through in a pilot to see what is going to work the best under that phase of the moon without water on that day or whatever.

And so, they were able to pick out the one strain that they thought was going to give them good results, and they would go with that one. It may change in the next study for the next company, but there were a couple of strains.

So there were, you know, maybe a handful at most they were looking at. It is not like they were pulling these out of the field and generating them every so often.

DR. ANDERSON: I notice on your table up there, your third one now is unclassified gram positive. Did you require them to tell you what class of antibiotic they were using?

DR. GILBERT: I do not have that list in front of me; otherwise I could tell you all of the drugs that were in there, and I do not know what was required at the time. They generally told us up front what they were -- you know, what they thought they were doing.

We did not always make them go after and tell us exact mechanism of action on everything. So sometimes -- you know, at the time, I do not know

exactly how they classified them. They just fell into the unclassified category.

DR. PARKHURST: One more time please. What prevalence is a percentage or a number?

DR. GILBERT: Well, I guess it could be both. We were looking at overall how many animals were shedding on the day of the collection. If there were 10 animals in the treatment group, we would get an idea, you know, yesterday it was 1 out of 10; today it is 10 out of 10; tomorrow, 10 out of 10, and we kept tracking that. So, depending, it could be a number or a percentage, I guess, depending on how you calculated it.

DR. PARKHURST: Different studies have different numbers, right?

DR. GILBERT: Yes, different studies have different numbers.

DR. PARKHURST: So, in one case, you could be looking at 1 out of 10, and in another case you could be looking at 1 out of 100?

DR. GILBERT: Sure, sure. Again, these are fairly small numbers. We were not talking thousands of animals, or even hundreds of animals. They were more like into the tens or even single digits in some cases. So we, you know, took a look at that best we could to see is it significant that eight out of eight animals

are shedding today on any given day?

DR. PARKHURST: So was it a percentage, or is it a number?

DR. GILBERT: I do not have that exact data in front of me. I do not know how it was reported in all of the studies. I think we got probably a little of both.

On this day, a 100 percent of the animals were shedding. Many animals were there. On this day, maybe only three animals out of five were shedding, so we got three out of five.

DR. PARKHURST: And could you just give me a sense of the sample size on these studies that ranged from say the smallest to the largest?

DR. GILBERT: Sample size?

DR. PARKHURST: Number of animals in the study.

DR. GILBERT: Maybe an entire experiment done with, let's say, ten pigs or ten calves versus a study done with maybe 40 versus 40 chickens, 80 total, something like that. So they were fairly small numbers.

DR. PARKHURST: Thank you.

DR. KOICHEVAR: You have already mentioned that the design was probably pretty similar just because the same maybe groups were running the studies. Were the

conditions under which the animals were held and handled pretty much the same as well, in other words, the same time of the year? Or were some of them in the dead of winter, and some of them were -- I mean.

DR. GILBERT: I do not have the exact start and finish dates for all of the studies, but they were probably run throughout the year. Obviously, some of the CROs will ramp up or ramp down depending on when they want to take vacation, or what time of the year is, but they were spread throughout the year. They were not always down in the spring time.

DR. KOCHEVAR: I guess what I am getting at is just were there more stressors for some animals in the study groups versus others?

DR. GILBERT: I think the conditions at the CRO locations are fairly well-maintained.

DR. KOCHEVAR: Okay.

DR. GILBERT: So they are not going to be out in the freezing cold, and then brought in and out, and in and out like that. So they are fairly well taken care of, and I think the conditions are fairly standard.

DR. LANGSTON: Was the issue of pathogen shedding you mentioned, number of animals that were shedding at a time, did this also include the number of colonies from each of those animals at the time? Was

that also considered?

DR. GILBERT: All of that information was collected, coliform units, and all of that is collected, yes, and taken into consideration. I have not looked at any of the data analysis going back to the old -- you know, the actual data analysis to see what they -- you know, but they generally would come in and give us as much information as they could.

And, besides, just the numbers and that, they gotten all the way down to the CF used per gram of feces, and that sort of stuff, so that was reported.

DR. GLENN: Jeff, I have another question.

DR. GILBERT: Sure.

DR. GLENN: I need to go back to a better understanding for myself on the issue of a failed evaluation for a study. I know that we are measuring pathogen quantity, prevalence, and duration of shedding.

DR. GILBERT: Right.

DR. GLENN: The fact that you have a failed interpretation means that you have set parameters for each of those criteria. If they are above the bar, they win; and if they are below, they lose. Is that right?

And then, are those all itemized out and available?

DR. GILBERT: Yes, obviously, the review staff

that reviewed them at the time had their bars and knew what they were looking for.

DR. GLENN: Yes, okay.

DR. GILBERT: And when you look at each one of the studies, we had actually tallied it up on the sheet with, you know, pass or fail for those three things, duration, prevalence, and what have you.

So we know that maybe they failed this, failed this, but passed this. And, you know, I do not know what other considerations were taken in, but then some sort of overall conclusion was reached and it either passed or failed.

DR. GLENN: Because it seems on the issue of assessing the salmonella reservoir that we cannot fail something on these three parameters in the absence of a whole lot of other information. So I am just asking that as a general question. And you mentioned other considerations must have been taken in, but it is hard to quantify that.

DR. GILBERT: Yes, when you look at any study, besides just the prevalence, and duration, and quantity, there is going to be a myriad of other things that we take a look at that again may have said, well, okay, they failed this one of the three.

Was there anything that we can say, oh, well

obviously they would have failed because this went wrong also? There are a whole lot of other criteria that go down through the review process.

But it does all go back to those three things, and somebody had to make a cut on statistically they were -- you know, little a did not equal little b, these were different. So it was, you know, a plus/minus sort of thing there, and they just checked them off as failed.

DR. GLENN: Okay.

DR. KOCHEVAR: This is probably kind of a dumb question. But was the bar the same? I mean the bar did not move? In other words, the standard for all these studies for pass/fail was held constant in a given species?

I am assuming species may vary, as far as where the bar was set.

DR. GILBERT: Right.

DR. KOCHEVAR: Once it was set, that was it.

DR. GILBERT: From my perspective, I think the bar was probably the same. Obviously, different people at different times, as time went by, were judging these. But the basic criteria, you know, in conjunction with statistical analysis, you just had to get down to, you know, it either passed or it failed.

And, again, we try to look at each study to see was there anything in there that was real obvious that we could go back to the company and say, you failed, but did you think about this, all the animals were outside overnight in minus 20 weather, you know, or something like that?

And that may have spurred them to, okay, we are going to do it over again. So there was a lot of mitigation and stuff that went into the review.

DR. HASCHEK-HOCK: So, of the ones that failed, were any of those repeated? I mean, if they failed, they failed completely, whether they repeated or not? Is that --

DR. GILBERT: Yes, if they failed, some of them might have been repeated. I do not know exactly how many out of that were done, but those were the ones that -- that column of failed were the ones that we absolutely judged.

These here reflect, this actually says that five failed, and that was the end of them. Those five did not come back. When it was before, when it had the 24 and the 9, I guess 4 out of the 9 either were repeated or were mitigated, and they made it into the pass column.

DR. HASCHEK-HOCK: So, in theory, then, I

mean, it is possible that these five could have come back if they wanted to repeat the studies?

DR. GILBERT: Sure.

DR. HASCHEK-HOCK: And so, some of the ones that were judged as failed could be repeated and potentially pass?

DR. GILBERT: Yes, I am not sure what the policy on the time was, as far as the sequential experimentation, you know, just keep doing it until you get a pass. I do not think that was allowed.

But there may have been a very obvious reason that they failed, and that we could not help them, you know, as far as a mitigation. They could not come to us and say, well, yes, we know why we failed.

So, they went away, and for whatever reason did not come back. It may have been an economic decision, or, you know, the drug just disappeared.

DR. LANGSTON: I believe I recall from the reading that some went back through and were accepted after lowering the dose or --

DR. GILBERT: Changing the conditions of use, those sorts of things.

DR. LANGSTON: Yes, were any allowed just to repeat numbers and see if it came out the same?

DR. GILBERT: I am sure they were allowed to

do that, yes. I am not sure which ones of the ones that passed, again, or went through it a second time. The four that I know that I can attest to, you know, I am not sure exactly what happened with them, but they would have been allowed to try again.

DR. LANGSTON: When it was those same, if it was the same, were the two studies pooled?

DR. GILBERT: That I do not know. I would probably pool them, but I do not know what they did back in the '70s.

DR. WAGES: So we do not know. This is kind of the same question I guess. So, in theory, if I had a product, I could have failed three times, and on the fourth time pass, and be kicked over in the pass category. Is that --

DR. GILBERT: Gee, Dennis, I --

DR. WAGES: You do not know?

DR. GILBERT: You know, anything is possible, but I do not know that that would have been the case. I think -- you know, I do not know what the review atmosphere at the time was, but now we really do not like to enter into sequential experimentation like that. Three strikes you are out I guess.

DR. GLENN: I have another question. I did not pursue this enough. One of the criteria measured is

the duration of shedding, so duration relates to time. What is the unit of measure, let's just say, in a fecal grab sample?

Tell me. We are measuring the pathogen? Are we measuring the number per gram of dry matter per minute, or per hour? What is the unit? Tell me what I am measuring.

DR. GILBERT: Well, some of those, they were probably collecting samples like every two or three days over the period of like eight weeks.

DR. GLENN: Right.

DR. GILBERT: So that is what they were basically picking up. So, you know, we had this giant eight week timeframe.

DR. GLENN: Okay.

DR. GILBERT: And, you know, at the very start, you may have high numbers; and then after a few weeks, it trickles down to almost nothing, or they become indistinguishable from controls, and that may run out to the end of the study.

DR. GLENN: Okay.

DR. GILBERT: And that is basically what they were taking a look at.

DR. GLENN: Okay. Now, the physiological stage of growth of this animal impacts digestive

passage, and I assume got shedding. And so, I assume that ideally these would all be -- you know, within a species they were all conducted within the same physiologic stage of growth and level of intake of diet.

DR. GILBERT: Well, I think we saw a little bit of everything. I do not think it was extremes where we had 300 pound hogs versus weanlings in two different studies. But, you know, for the time, early mid-'70s,, eight weeks probably was not too far off on broilers was it, Dennis? I do not know. What do you think?

DR. WAGES: No, I mean, if you are looking at

--

DR. GILBERT: Yes, so eight weeks probably would have been okay for broilers. And the hogs -- you know, I do not have it, obviously, right in front of me every single study.

But, generally, I am thinking that knowing the CROs and the sources of the animals, they were probably very similar each time as they went by. I do not think, like I said, there were the extremes in having small pigs in one, and huge ones in the other.

DR. KOCHEVAR: Am I correct in understanding that if there was cross-contamination that that was a criteria for exclusion, that that was a problem, so that there really was not an attempt to measure transfer of

resistant bacteria from the test population to a bystander population, that was not part of the design?

DR. GILBERT: No, cross-contamination would probably have been a criteria for failure or rejected, you know, depending on what it was.

DR. KOCHEVAR: Right.

DR. GILBERT: That was the problem. Yes, we would have told try again.

DR. KOCHEVAR: So you really do not get any insight from these particular studies into how easily that was going to move from?

DR. GILBERT: Right.

DR. KOCHEVAR: Okay.

DR. GILBERT: Yes, these studies were not designed to like follow the salmonella and see where it is going.

Any other questions?

(No response)

DR. GILBERT: All right. I am going to turn it over now to Tom Shryock. He is going to come up and I think give us what an industry perspective, or maybe a further history on this from the other side as it were. So give it to Tom.

DR. LANGSTON: Before we do that, there is something I just wanted to mention that perhaps I should

have covered just a tad earlier. It is probably not necessary to mention, given the audience is, I am sure, well-informed, as is the panel.

But, of course, the designation "sub-therapeutic" means different things to different people. And there is I think a perception among many lay people in the press that sub-therapeutic refers only to growth promotion, which I believe I am correct in saying that it also refers to some disease prevention uses, and I simply wanted to point that out. This is not necessarily dealing with just growth promotion.

Conduct of Salmonella Shedding Studies

by Dr. Tom Shryock

DR. SHRYOCK: I would, first of all, like to thank the CVM for the opportunity to be here today. It is not often that one has the opportunity to talk about research that you have been involved with from time-to-time in such an arcane field as salmonella shedding studies.

So I am grateful for the opportunity to share some of my insights and experiences, and also to relate to you that I have worked with Diane Fagerberg at Colorado Animal Research Enterprises where much of this work has been conducted over the course of the years.

As far as what I will be talking about this

afternoon, I have put a quick agenda outline here before you just to kind of order through this. And Dr. Gilbert's did an admirable job of reviewing the history of the 558.15, so some of my initial slides may be a bit redundant with his.

Some of the comments you will see, however, are going to vary markedly from his perceptions. So we will have to see how we can best address those as we go through.

But, at any rate, what I want to do is go through the protocol, talk about some of the limitations of the study, and then the lessons learned, which may be then applicable and relevant to the proposed pathogen load studies which is the issue before you today; and, finally, to give you some conclusions.

(Slide)

This is one of the slides that Jeff had already showed to you, but I would like to highlight for you that the FDA Task Force in 1970 was the driver behind these studies, and specifically called for certain kinds of data.

With regard to the salmonella reservoir, prolongation, the carrier state, and the prevalence of R factor containing bacteria, just that R factor is kind of an historical term at this point in time, the

resistance plasmic, so things have progressed from that stage. But these were the considerations in that task force report.

(Slide)

This was codified into the 21 CFR 558.15 with the following objectives directed to sponsors conducting studies. Basically, a sponsor had to show two things: (1) that the drug did not adversely impact the quantities, the prevalence, or duration of salmonella shed, and this was in comparison to a baseline non-medicated control group; and then, secondly, that the drug did not increase the salmonella or coliform, meaning E.coli resistance, again, over baseline, to drugs either human or animal medicine.

So, really, for the pathogen load section of the discussion, we are really concerned with just that top bullet point, but I will mention these studies in passing as we go through because they are interrelated.

As we found out, there really was no specific study protocol that was outlined in these regulations. Rather, sponsors were asked to consult with CVM on protocol design.

(Slide)

So, taking these regulations then, and trying to actually implement them, became kind of a work in

progress over a period of time. As I alluded to, most, if not all of the studies were done in some way, shape, or form in conjunction with CARE.

There were a few that were not. The sponsors did themselves, and then submitted. So, Dr. Fagerberg is quite the guru of the studies, if you will. Generally, these 558.15 studies started out originally as a single study which could assess all parameters.

But then over time they started to evolve into a salmonella study and a separate coliform study, and that evolution of study continued such that currently -- and I say currently somewhat advisedly, because the last time these studies were actually run to my knowledge was probably a decade or so ago.

But right now we would have a quantitation study, a prevalence and duration study, and then a separate coliform resistance study. So you can begin to see that things are expanding in terms of the numbers of studies that would fall into this 558.15 category.

(Slide)

Let me show you the time line, general protocol sequence here, which may address some of the questions that the committee was posing to Dr. Gilbert. There may be some slight differences between the experiences as I have captured them, and as he has

related them, and we will try to reconcile those as best we can.

Again, this is somewhat of historical perspective, if you will. There is not a lot of people that are still around that have the hands-on experience and were there when these things were being done a couple of decades ago.

In general, we would have a situation here where, at least two weeks prior to an oral challenge, we would have cultures, weights, feed intakes, clinical observations being taken.

The animals, be they chicks, be they pigs or calves, when they had an acceptable baseline of coliform resistance, which I will talk about momentarily, the study was begun with a fasting period at day minus one. The fasting period could last from 12 to 24 hours depending on the study.

This was to help them establish the salmonella challenge which would occur at day zero. The animals were allowed ad lib feed for a short period of time, a couple of hours, then fasting was reinstated and a second oral dose challenge was administered.

It is at this point that the feed was administered, and the 56 day or eight week observation period began. The feed was consistent throughout. It

was the same lot of feed. The only difference here would be one had medication incorporated, the other did not.

The challenge dose could either be a high challenge, 10^{11} total CF used in a quantitation study, or 10^6 total salmonella in a prevalence duration study.

Again, taking cultures periodically once a week, feed intake, so that you can actually confirm that animals were being satisfactorily medicated. In the clinical observations, necropsy tissues were taken for tissue sequestration analysis. There were some variations as far as the length of time. The one that I am using here is the eight week period.

(Slide)

Some other factors that were very critical to the study design are that the animals that were used were either specific pathogen-free, or, at a minimum, salmonella-free.

What was much more rigorous criteria, however, was the 20 percent or less baseline resistance in E.coli, and there were 12 different antibiotics which were tested by MIC broth dilution to make that determination.

This particular criterion was extremely problematic to find animals that had met those criteria.

And, in fact, one of the anecdotal reports initially was that the clinical research organization went to lengths that included hiring a trapper in Florida to acquire feral pigs. So this was an extremely rigorous requirement in some of the early studies.

Dr. Gilbert mentioned the salmonella strain. And, to my knowledge, there was, for the most part, a single strain specific for cattle, a specific strain for swine, and one for poultry.

The strain had to be susceptible to as many antibiotics as possible, because we were looking for resistance transfer into the salmonella, and also into the E.coli. We will see some of that data a little bit later. But this is not a component of pathogen load study, per se. Nevertheless, it is mentioned here.

The salmonella strain, as far as virulence, had to be, well, somewhat of a wimpy strain, I guess you could say, in terms of virulence. You did not want to cause disease in these animals. That was not the objective. Nevertheless, as we will see, some of the doses could actually cause that to happen.

I have listed feed here with three parameters that may seem a little boring, that you need a validated feed assay; you need a specific dose which you are going to use, and that has to come from efficacy studies; and

you needed a formulated pre-mix product.

And I will show you why this is so important in just another slide. As far as the study groups, and the numbers of animals that were required, basically, it was about 10 to 12 animals per study group. There were three study groups; the medicated, the environmental control, and the non-medicated group.

The environmental control was placed, physically located between the medicated and the non-medicated groups. These animals themselves in these groups were housed individually, generally, in either isolation, in cages of some sort for poultry, or for swine, in isolation or rooms for the calves.

One reason for that, not only the physical separation, but to minimize coprophagia, so that the animals did not reinfect themselves. The environmental control served as the cross-contamination piece, so that basically you could determine if, in fact, there was some sort of introduction of salmonella from an outside source.

Separate caretakers were used for each one of these groups throughout this study duration, and strict biosecurity was maintained. These studies were done according to good laboratory practices, so there was a very emphasis on data quality.

(Slide)

Let me share with you as far as when these studies were done, and why those assays for feed were so important. This would represent kind of a pipeline diagrammatic for generally any industry product, where you would have a discovery phase, find your new candidate, optimize that with final chemistry selection, formulation, and do some toxicology studies.

Then you would take your molecule out and do clinical studies. You would get your manufacturing or the CM&C package put together, and begin your nonclinical studies at that point as well.

This is what 558.15 studies then basically were a late phase situation. You needed to have a dose. You needed to have an analytical assay and a formulated product in the manufacturing. So, basically, you are very late into a pipeline situation because there is a lot of investment. Timing, of course, is dedicated to getting to this particular point.

Why is that so important? It is important for the following reason shown on the interpretation issues. These studies, the 558.15 studies, were on a pass/fail basis. You could go all the way through your development pipeline to come up against this particular criteria, which, on the basis of a total of 30 animals,

could basically stop in its tracks the development of a particular product.

So you are kind of rolling the roulette wheel a bit at this point in time. You do not know for sure whether you are going to pass or not.

Here are some of the criteria, as I was able to obtain them through Diane Fagerberg's experience. And, certainly, the sponsors collectively did not totally have access to these pass/fail criteria. And it is my understanding that Larry Rollins with CVM was very heavily involved in drafting just that.

(Slide)

But it goes on to say that that alone cannot be the basis for determination of a public health hazard. That is why this concept of a biological significance came into play. This was an attempt to understand some of the biological variation that you might see in a small group of ten animals.

So, as best as I understand it at least, these would be the criteria for a pass/fail determination for the quantitation, prevalence, duration sections of the shedding studies. And you can see that there is some wiggle room, some wobble here, in terms of the range that you may come out with, in terms of the data.

In the next couple of slides I will show you,

actually have some data that will help you to I think picture what this is really trying to convey for this pass/fail. So this may be one source of complication in terms of why a particular study failed and another one did not.

Now it is not clear, at least to me, whether you had to pass as a sponsor all three of these pieces, or whether two out of three was a majority of two to one, if you will, in the past. That was never really made clear to me. Perhaps, somebody else has information on it.

No matter how you look at this, the bottom line is that there is really no evidence that I am aware of that these criteria can actually be related to the on farm or commercial situation to contaminate meat, or even to human health. These are simply study criteria that were outlined.

(Slide)

So here is the first of two data slides, as far as what actually happened in a particular study outcome. This would have been the quantitation study in the medicated group represented by the very thin dashed line, and non-med in the solid line here.

So you can see that after the two challenged doses, you would have a fairly high degree of shedding,

about 10^5 per gram, that went down fairly quickly, about by day ten or so; and then maintained itself at this red line, which is an arbitrarily drawn line just to help you visualize, and about 10^2 CFUs per gram is where that stabilized.

And you can see that there is a lot of standard air between the different animals that were in the study. But, by and large, there was no adverse effect on salmonella shedding in medicated relative to the non-medicated controls. So this is the kind of data that would be generated for the quantitation study.

(Slide)

For the prevalence and duration study -- and I recognize this will be a little bit hard to read -- there were, to set the table for you, treatment groups here, the medicated, and then the non-medicated groups with the individual animals, in this case, pigs, that were listed out.

In the post-challenge sampling day, ranging from 2 up through day 56, these zeros were ones indicated a presence or absence of salmonella, and this was done with an enrichment broth. So you either had it or you did not. There were some cases where there was not a sample for some particular reason.

So what you would look for here, in terms of

prevalence and duration, is the proportion of days positive. And you can see that that would vary depending upon the animals in the study, and it would vary between the treated and the non-medicated control groups.

The duration shedding simply was how many days did the salmonella continue to be shed before you had a consistent pattern of no recovery. And that varied tremendously again between the animals, even in the treated group.

In this case, it was fairly consistent in the non-medicated group. So these are the kinds of data that were generated for the prevalence and the duration studies for salmonella.

(Slide)

So let me summarize some of the industry experience with these studies. As we know, most of these drugs that were tested were gram positive active. They are all in feed. It was very difficult originally, and probably still would be, to get coliforms at a 20 percent baseline of resistance.

And I used Jeff's original figures here to make my 73 percent calculation. That may now vary a little bit. But, at any rate, the majority of the studies, I guess we could say, passed these studies.

But, more importantly, it is a variety of antibiotic classes. It was not that one single class failed. It was that there was a pass in each one.

And if you were to even look at the framework document, categories 1, 2, and 3, there were representatives from each category that were tested and passed: virginiamycin, category one, passed; category two, we would have say like swine that passed; ionophores, category three, those passed.

So there is no particular pattern to what passed and what would fail. The failure of these studies -- this might be something to discuss further. Maybe this is due more to the interpretation than anything.

Those ranges for the different study components, how much of that was borderline versus an outright obvious effect on shedding. I do not know. I do not have the ability to look across studies other than the linco studies.

We did mention that failed studies could be repeated and passed. If a sponsor did want to play again, you could put more money on the table and roll the roulette wheel one more time. It is interesting to note that if you passed in one species, say, chickens, you would usually pass in another species, say, swine.

So that is an interesting concept if you think of what I call study creep here, where we started with that initial one study, that soon became three separate studies; and then for each species. So you potentially could have nine separate studies that you are having to do towards just salmonella shedding component.

(Slide)

As far as the limitations, we are trying to take these model studies and say that there is a relationship to commercial farms, to carcass contamination levels, and even to public health that has not been done to my knowledge. I am not sure how you could do that.

So far as I am aware, there really was only one strain that was tested for the majority of the 558.15 studies. And if you recall that time line, the salmonella was given prior to the medication, which would allow basically the salmonella to slip by the normal flora, if you will. Those animals were fasted.

So the salmonella are already intracellular. They are established by the time the medication is then administered. So that may have some important ramifications later on.

We mentioned high challenge dose. That is not realistic. There is not too many animals that bump into

10¹¹, colony forming units of salmonella. That dose itself can overcome a protected flora. It has caused disease in some of these animals as I understand.

So you not only have a situation that can compromise the study integrity where sick animals do not eat as well, but there is also some potential for welfare concerns as well.

(Slide)

And then trying to take the model study and compare that to the field situation, what are the similarities? What are the differences?

But I think, you know -- I won't go through all of this, but there is a lot in terms of the challenge, the housing, the natural exposure proportion of animals that have seen or not seen salmonella.

I guess the bottom line is that in the model studies, this was a research grade status. Everything was ambient temperature, well-controlled, best of feed that you could get, great biosecurity, on and on, not very reflective of the real world situation.

So the model study really could not factor these particular real world parameters in, because you needed to control them so carefully.

(Slide)

So what lessons can we apply from this 558.15

experience to pathogen load studies? Well, really, all we have got is an in-feed medication database on a multi-week basis with salmonella challenge. That is all we have got to really draw from and try to extrapolate further.

There really is no experience with higher doses, other routes, shorter durations, post-medication withdrawal effects, et cetera. And as you consider therapeutics and how they are administered today, how does that play into this whole situation? When should we sample? Is it even relevant to do these studies?

We learned that there was some study creep that was beginning to enter in. Will this also come back and we will need a separate study for salmonella, one for campylobacter, one for E.coli 0157? How many studies will actually be required?

There is little experience with broad spectrum drugs, say, flora quinoline, or a broad spectrum cephalosporin. Those are likely to actually decrease shedding. What are you going to do with that?

I am not particularly sure how that is going to be address. We also know there is a lot of biological variation. Ten animals is what was being used here. That is a small number.

(Slide)

I would just like to refresh your collective memories here as far as some other events that led up to this particular meeting. There was a preapproval workshop held here at the Double Tree, where these groups found no value to conducting pathogen load studies.

So that is on the record. It is on the CVM website, as far as transcripts and presentations. The exponent report, which we will shortly be hearing about, again, found no consistent evidence to indicate an association of salmonella shedding with antibiotics.

And, again, going back all the way to the 1970 FDA Task Force recommendation where therapeutic drugs were excluded at that point. So right now we are in a situation where there is not a pathogen load shedding protocol, per se.

And it would be a shame to try to go back and modify this on a pay-as-you-go basis, so which we did with the 558.15 studies. And, to be frank, I do not think that is an acceptable way to do business.

I will draw your attention to the fact that already some sponsors, to my knowledge, have been asked by the CVM to do some of these pathogen load studies. Whether those have been done or not, I do not know, but I am aware that some people had been asked to conduct

those studies to pursue some of their registration claims.

(Slide)

So the relevance of the pathogen load studies, just a couple of items for your consideration. If you look at the swine population, about 8 percent could be considered salmonella positive, HACCP pork carcass baseline.

We actually get to the meat and it is about an 8 percent positive rate. The feed additive use is 90 percent. That is data from the NAMS. So is there a relationship there? Have these studies done their job? Just something to think about perhaps.

So, in terms of trying then to apply a pathogen load situation to therapeutics, how is that going to really impact the real world?

We also have to consider that if you look at human salmonella serotypes, 6 of the top 10 are different from those that are in food animals. So what strains are you going to use to predict impact on public health? A difficult question in study design.

(Slide)

We also know that salmonella load can be affected by a variety of external factors, not just antibiotics. For example, transport stress, feed, and

environment, all sorts of things can cause a shedding non-specifically.

We also know the contamination of meat can occur post-slaughter, but one could assume that it is a farm origin instead. So there are some issues there as far as how does all this apply?

Already there is a number of interventions that are in place, and there are some even on farms, such as vaccination against salmonella, that are being utilized. But HACCP, and cooking, and a variety of other interventions already are serving to minimize food-borne pathogen contamination.

If you think about it really, what the producer is delivering is muscle to the slaughter plant and it is sterile. The muscle would be sterile. It is only upon the cut up that you would have contamination occur on the surface of that meat.

So that is a real important critical control point, which is perhaps outside the purview of a preapproval study. But it is something that does occur, and does go on.

(Slide)

So, to conclude here then, keep in mind that these 558.15 studies are in place now for in-feed antibiotic products. Those have not changed. They are

still on the books.

They are still required for response or wanting to take a product through that particular pathway. The new twist to this is that the pathogen load studies would be required for therapeutic antibiotics.

And, to my knowledge, I struggle with having to try to design one of these that it has got relevance to an on farm practice, to meat contamination, and, ultimately, to subsequent human illness. To try to link those is for me a stretch.

And, finally, you know, to be frank about it, I think these are basic and unnecessary impediment to the new animal drug process for therapeutic antimicrobials. The more burdens put on the sponsors along these lines provides additional disincentive to come out with new products.

In terms of safeguarding public health, I think there is sufficient other avenues that are already in place that will provide that assurance. So, with that, I will close. Once again, I will thank CVM for the opportunity to share my thoughts on this. And I will be happy to entertain questions from the panel. Thank you.

Questions and Answers

DR. WAGES: Tom, do you have any information on if this drug sponsor is being requested to actually do some of these pathogen load studies? Are they a specific designed study to be performed?

DR. SHRYOCK: It is a situation where you would need to consult with the specific individuals involved and discuss a protocol design. I really cannot speak for other sponsors as to what they may or may not have agreed or not agreed to do, and just mentioning that there is some discussion ongoing between the agency and a sponsor. So I cannot tell you specifics, Dennis.

DR. KOCHEVAR: Just to clarify the statement in your slide that the preapproved workshop group found no value in conducting pathogen load studies, that was specifically related to therapeutic use, not sub-therapeutic use?

DR. SHRYOCK: That is correct.

DR. KOCHEVAR: Okay.

DR. WOOD: Have any optimum models been developed for a therapeutic study? I mean you have pointed out problems with the current dealing with sub-therapeutic antibiotics. But have any models been developed that this might be an approach that would work?

DR. SHRYOCK: I am not aware of any models along salmonella shedding lines that have been developed. There may be some research reports that are out there that are semi in that vain.

But to have them with the rigor and the control that meet the 558.15 study criteria, I am personally not aware of those. And I do my utmost to keep current with the literature at this point and on these topics.

So I would be welcome, open to other inputs to change my mind on that, but I am not aware of any such study designs.

DR. GLENN: Tom, I am sorry.

DR. SHRYOCK: Yes.

DR. GLENN: I wanted to reiterate something that you said that I think -- I realize we are getting your perspective. But it is somewhat disturbing that a standardized, validated protocol with clear interpretation of results does not exist for pathogen load studies.

That implies that there is no reason to do a pathogen load study, that we are wasting our time. So that is a big mouthful there, and I am just making that comment. We will continue to assess that, and I would hope that the committee, you know, gets enough

information to get our arms around that one.

DR. PARKHURST: What is the status of the historical data? Are there raw data that are available?

DR. SHRYOCK: All of the information, as far as I am aware, are proprietary and would reside at some point with the CVM or the sponsors that submitted them. I am aware that there are studies that have been done, but not submitted, because an initial evaluation by the sponsor would have been such that they felt they probably would not have passed an approval situation.

The only other way to find these bits and pieces would be to look in the literature as the exponent report has done. And they certainly did not capture all of the studies, but some people have reported 558.15 studies in the literature.

So there are some examples there. But, certainly, not all of the compounds and studies are out there for ease of access.

DR. ANDERSON: I got the feeling that you think the -- what you said is that you feel like the pathogen loading should be done away with because other avenues already in place to safeguard public health are available. Can you describe those avenues?

DR. SHRYOCK: Certainly. For each and every pathogen that you can consider, there is probably a

specific intervention on farm that could be considered for a given species group. So that would take up more time than I think is appropriate.

But there is on farm activities as a start that could be implemented, adequate nutrition, housing management, vaccination, et cetera. So I will leave it as a general on farm there.

When you actually bring these animals to a slaughter or processing facility, again, there are interventions in place there that we collectively rely upon such as HACCP and other mechanisms to assure that the contamination is held to a minimum.

And products do make it to the marketplace with salmonella on them. These are baselines. They are not zero levels. So anything that comes out of that plant has met those criteria for HACCP.

A final safeguard as I see it would be basically good kitchen hygiene, cooking, preprocessed meat products, et cetera. So each of these steps is designed in a HACCP mode, if you will, to reduce the risk throughout the entire food chain continuum.

So those are the safeguards that I perceive are in place and are serving us effectively. There is room for improvement, but there always will be. But those are the ones that I would see as key.

DR. KOCHEVAR: I had two questions. Back on about slide 10, you make the statement, "Antibiotic resistance, moot point, no difference is ever observed. Those really were not part of these studies." Is that true?

DR. SHRYOCK: Yes, thank you for pointing that out, because I did gloss over that. In terms of the salmonella that were recovered in the studies here, either in the quantitation or the prevalence duration aspect, those were actually tested against a panel of 12 antibiotics representing different classes of antimicrobials which were important to both animal and human medicine.

The finding was that there was no change in the antibiogram of the isolates relative to the initial challenge strain. So the coliform study would have been additional reinforcement of that.

And my understanding from Diane Fagerberg, at least, is that she had not seen any changes across the board for all of the sponsors products that she had tested. So, basically, there was never an issue, as far as I know, when the CVM reviewed that that a product was failed because of antibiotic resistance.

Again, keep in mind that part of the reason for that as well that the products tested are gram

positive active. Salmonella, being gram negative, you would not expect it to do anything. So, from that sense, it is good that nothing happened if you were to expect that.

DR. WOOD: I just want to make sure though that you were not implying, were you, that on farm intervention is -- I see it the other way. We have always understood that on farm intervention is very important in this whole process, and also in terms of reducing pathogen load.

A good case and point was in the supreme beef, you know, it was determined that the problem could be traced back to the farm in terms of the salmonella pathogen load at that point; and, certainly, in other studies, like by Robert Tuckson, and some European studies as well that sees the connection.

So is your premise to question the need for on farm controls, or is it to question the appropriateness of there being therapeutic studies for pathogen load?

DR. SHRYOCK: I, in no way, meant to imply or denigrate on farm controls and interventions. I think those are very important, and they serve a very critical role.

Where I am coming from is that in the context of the drug approval process, pathogen load studies are

done in a preapproval mode situation, in a very artificially done model situation that would be very difficult to use the data to predict an affect, either in a commercial situation, a commercial production situation, meat contamination situation, and then ultimately to a potential impact on public health disease incidence.

That is the disconnected that I find difficulty with. Does that help?

DR. WOOD: Yes, it does, thank you. Is there any post-approval role for measuring pathogen load?

DR. SHRYOCK: There are no requirements to do so that I am aware of. Many research investigators at academic institutions might well choose to look at that. I know that many production and producers would consider those kinds of things if they do monitor salmonella in their plants. And if they saw a blip, they would ask the question why, and try to look at it from that direction.

DR. KOCHEVAR: I guess I am still worried about the antibiotic resistance part of this.

If, basically, these studies contribute nothing to our understanding of whether or not antibiotic resistance occurred because the antibiotics were not matched to the bugs that were looked at, I

mean, you would agree in these studies?

DR. SHRYOCK: That would be an accurate assessment, yes.

DR. KOCHEVAR: And so, in the workshop on therapeutic use of pathogen load, we really do not have any data to evaluate that, since the data from these studies is no use for that.

DR. SHRYOCK: No, the preapproval workshop study discussions. And I only was in one of the four species groups, so I cannot speak to the others. But my perspective in understanding just when we had the summary statements was that these kinds of studies in general would not lend a whole lot of value to a drug approval process. And a resistance component is covered yet in another dimension within the framework document. Thank you.

MS. SINDELAR: Our next speaker is Jeff Gray.

Pathogen Shedding Study Design Considerations

by Dr. Jeff Gray

DR. GRAY: Committee members and meeting attendees, I appreciate the opportunity to discuss some things about pathogen study design considerations. I think when you are discussing salmonella and pathogen load, there are volumes of data out there to consider.

I think one thing from discussing briefly with

the speakers ahead of this meeting, you will see some overlap between the talks of areas that we all think are important issues for you to consider. And you may well want to pay attention to those.

(Slide)

But I am going to discuss study design considerations, and I have the talk divided up into four areas: Organism characteristics, where I will discuss a little bit about host range and clinical status of the host animal; study design, measuring the effects, and confounding factors.

(Slide)

The first slide I want to discuss a little bit is this one in which we list. I have the top isolates from a given year, and I believe this is 1995/1996. And these will vary year-to-year, but there are some things here that are useful to point out.

When we consider swine and human isolates of salmonella, you can see that some of the serotypes on the list overlap and some do not. And you also have to consider that when we are considering cattle, turkeys, and chickens that the list on your left-hand side are going to differ a little bit.

The other things that is true about these pathogens is all of the salmonellas on these lists are

not created equal. They differ in their ability to invade in the whole species, as well as in humans, from serotype to serotype. And I will discuss that a little bit further as we go on in the talk.

(Slide)

Now salmonella is a broad host range organism in general, and we generally term it as being ubiquitous. We can find it all over the place.

And, oftentimes, if a colleague or a student comes to me and says, well, I have a herd that I believe is negative for salmonella, a convention production herd, I tell them you have not looked hard enough because salmonella is there. It is very ubiquitous in the environment.

And the next point that clinical status is questionable. In animals, especially, as I noted in that first slide, we have a broad range of salmonellas that can infect animals. Not all of those salmonellas will cause clinical disease.

In fact, a good majority will not, so there is no way to visually measure whether an animal is sick with salmonella. So they are going to carry that organism whether or not they are sick.

Now, I will mention a few other organisms as we go on in this talk just as a comparison.

Campylobacter is much the same as that group of salmonellas in animals. We have a lack of clinical signs in food animals.

We know that campylobacter jejunae in-coli exists in cattle, and swine, and chickens, but we have a lack of clinical signs. E.coli 0157:H7 we know is the same way. We have a much narrower host strain here with cattle being a known host, but we have no clinical signs in food animals.

So these are things to consider when we are trying to figure out what in the field is going to be positive and how to measure what is going on in a specific study for salmonella, or another food-borne pathogen.

(Slide)

Now, something else that is interesting about salmonella is that it can be invasive in the host species. So, in other words, it is not an organism that will stay in the tube, that is, the intestine. It does not necessarily stay in the lumen.

It can invade lymph nodes, and it can go septicemic, in which case, we would see clinical disease. In that case, we are going to see invasion of other tissues. But we clearly know that salmonella likes very much to hang out in lymph node tissues.

And that brings us to the shedding designation. Oftentimes, shedding is viewed as we can take feces out an animal and we can isolate the salmonella. Well, we have carrier animals that are indeed very much positive for salmonella, but in fact are not shedding at that given time.

So, given the right stress, a change in diet, the change in environment, we may in fact induce shedding, because they are positive in their lymph nodes.

The other thing about shedding that we need to take into account is how to measure that. And I will discuss sensitivity of sample taking in a bit. But how we measure shedding, whether it is by fecal swab, whether it is by a sample out of the pen, or whether it is by a large fecal sample from that animal can be very different in the sensitivity of that test.

Now, again, as was mentioned in the previous talk, we have strain differences among salmonella isolates, not only do the serotypes differ between their ability to cause disease, the level that they are going to shed, and how long a duration that they may shed, individual strains within a serotype can differ very greatly in their ability to do this.

So, if we take five salmonella typhimuriums,

there can be large differences in what you see in the outcome after you dose a group of animals. I am going to move on here to infectious dose, and some discordance that exists between shedding and infectious dose that we see.

(Slide)

Now, if we look at challenge studies, and we look at challenge dose, and this is an experimental situation where we take and we either give 10^3 CFU, 10^6 CFU, or 10^9 CFU salmonella in swine. With 10^3 , what we typically see is no detectable shedding, and we have seen no deep tissue infection.

So, in other words, when those studies were done, we could not easily go back and isolate the organism. However, if we took 10^6 CFU, we got a 10^1 shedding peak, as was measured by the techniques used, and we got carriage in deep tissues out to eight weeks.

If we go on to a 10^9 -- and this would obviously be a curve of doses, we go on to 10^9 , we have a 10^3 shedding peak, and we have long-term carriage, well over 12 to 16 week carriage in these animals.

So, now, if we look at controlled natural infections, these are infections done in experimental environment with previously known salmonella negative animals. However, the infection process would be done

differently where a seeder animal is infected and put in with a group of negative animals.

(Slide)

In those situations, what we see is a 10^3 shedding peak of the seeder animals. However, all of the naive animals that came in contact with that seeder animal also had a 10^3 shedding peak in those "naturally" exposed animals.

So, if you look at that previous dose study, in this one there are some measurements that do not add up, and those are some things that really have yet to figure out whether what we took out of the lab and gave to the animals was indeed not in the right state, the salmonella was in the right state to infect, or whether the salmonella that the level or the sensitivity of the tests were able to measure using the same technique study-to-study really does not give us an accurate measurement of what is coming out of the pig.

But, in fact, if we had -- in this situation, if we had a 10^3 shedding peak, and we are assuming that all of these animals were exposed and had a long-term carrier state, the level of coprophagia that would have had to occur there are indeed thousands of grams of feces, and that probably is not occurring.

So there is some questions there that in the

literature have not yet been answered.

Now, if we also look to sort of further on that point, swine infected with infected desiccated feces. And what this is is taking a controlled group of animals, infecting them with salmonella, collecting the feces, and allowing it to dry out for a three month period.

Then we go back and we infect new animals with that feces. We can again go into a state where all of the naive animals were infected, a 10^5 dose was given. There was indeed no shedding.

But if we look at the deep tissue infections in these experiments, we had a 10^3 level of salmonella in the liver, the spleen, and the ileocolic lymph node. So, again, there are some questions there that with infection dose and shedding that are difficult to add up.

(Slide)

Now, if we go on to look at measuring of the effect of your experiment and maybe a treatment that you are given, you have to consider the sensitivity of the measurement you are using on the back end.

If we looked in 1998 and 1999, some of the papers that were put out, we believe that E.coli 0157:H7, prevalence and feedlots was 5 to 8 percent

based on the techniques that were used.

In 2000 and 2001, we now believe that the prevalence is 23 to 25 percent. The only difference in those studies was the technique used to detect E.coli 0157:H7. So it does not mean either study was wrong. It means that the sensitivity of the methods changed over time, and therefore the bar changed over time.

And so, here differences are based on detection methods. So when you are looking at a study you have to ask, are the detection methods used sensitive enough to detect treatment effects? And there needs to be a measurement of that when you are looking at a study. That has to be demonstrated.

Continuing on, if we look at temporal measurements of salmonella shedding, if we have a salmonella high dose challenge, and we look at the shedding curve -- and Dr. Shryock showed you some data like this -- if we look at CFU per gram of feces, we have a fairly predictable shedding curve that goes from day 1, on this slide, goes out to week 7.

And, as you can see, it goes up very rapidly, and then declines slowly over time. And at what point do we want to intervene on this shedding curve? At what point is a treatment given?

We have to consider all of those issues

because where we intervene is indeed going to effect the outcome in this shedding curve. And I think that is yet another point you need to consider is that all is not created equal when we look at the shedding over time of salmonella.

(Slide)

If we look at times of intervention, if we intervene prior to infections, what we would probably have to assume here is that we are altering the flora in the intestine so that we are either taking away binding sites, or we are promoting the growth of bacteria that are harmful to the salmonella.

If we look at if we intervene during peak shedding, we have to decide when is that peak shedding. Are we basing it on clinical signs? Because in the field we certainly cannot do that.

And we have to then take into account are we going to cause the induction of a carrier state if we intervene during peak shedding or late in the production cycle. Are we going to drive that salmonella into the lymph node and create a carrier animal?

(Slide)

So, again, what measurements are used, method sensitivity? Are we looking at feces? Are we looking at feces off the floor? Are we taking an adequate

sample out of an animal?

If we are taking swabs out of the rectum of an animal, there is data out there that indicates the sensitivity of that, and that is not -- the sensitivity of those methods really are not really very good.

So at what time point are we measuring that? For how long are we measuring the shedding? The idea of no salmonella in the field is not currently feasible, okay. We have to assume that salmonella is going to be there. If salmonella is in many animals, we have to assume it is clinical.

Normally, what we see is it is detectable in a few animals at low levels. So is that the state that we should be looking at when we consider pathogen load and its effect detectable in a few animals at low levels? And that is a hard situation to reproduce. And then what is the food safety risk in those situations?

(Slide)

Now, seasonal changes, this is also important. Winter seasonal prevalence of E.coli 0157:H7, we know to be 3 to 5 percent; summer, 23 percent. Salmonella, we have species and serotype differences to consider, and we have a peak season prevalence of 8 to 12 percent.

That does not lead very much leeway to create a real -- to look at your method sensitivity, and look

at what effect you are having on pathogen load. When you are only considering an 8 percent prevalence, you are in your peak season.

Diet can be a confounding factor. Composition of feed stuffs can effect prevalence in transmission of food-borne zoonotic pathogens. So you have to consider when you are looking at a study what was the diet used? Is that reproducible? And is it useful to use that diet in a study?

(Slide)

Stress on the animals. The first thing you have to consider is the manner in which stress is measured. We know that we can have an effect on pathogen shedding with stress. However, stress in a lot of these studies has been used in a very general way.

And if you look at a classical, which has often been used with salmonella, put an animal on a truck and ship it, which is what happens to all of the animals, but that does not create a reproducible stress to cause predictable shedding.

And I think that is going to be touched on a little bit later, so I won't go any further. But Dr. Isaacson, as well as a few others, have done those studies, and that unpredictable stress, while it can have an effect on pathogen shedding, it is hard to

predict what effect.

Lairage exposure. Recent swine data indicate that lairage may be an important exposure point. In other words, what has been recently shown by Scott Hurd and his colleagues is that when animals -- swine are held in lairage.

The number of animals that are infected at that point before lairage is much lower than those during lairage and post-lairage directly into the packing plant. And, therefore, that late in the process, we need to consider how are we going to effect pathogen load at that point.

Deep tissue infections. Again, we have salmonella serotype differences here, and just to reiterate that point. Some very much like to be in the lymph node, and we need to consider that. And we are looking at the actual salmonella used.

(Slide)

Environment. Treatment effects may be environment-specific. We know that to be true. The mechanism of action of treatment, remember the temporal relationship of the shedding curve? Are we having an effect on flora or directly on the pathogen? And that is going to effect our outcome. So you need to consider the mechanism of action of the drug.

Long-term survival and infectivity of salmonella. We know that salmonella survives very well in a desiccated state, and it will survive in the environment long-term. So when you consider pathogen load, that can indeed be an important issue, how much salmonella are we putting into the environment?

(Slide)

So to wrap things up here, organism characteristics. Studies must adequately account for specific characteristics of the food-borne zoonotic pathogen. One thing to consider is while studies probably cannot do 25 different isolates within a single serotype, we need to have a measurement.

A real time today measurement is, did the isolate used carry the appropriate virulence factors? When we looked at it, can we do the molecular analysis of that isolate? And is it normal compared to what we expect to see in the field?

Study design. We need to model a realistic infection cycle and a target host species.

Measuring the effects. The measurements must be robust enough to show differences under realistic circumstances.

And confounding factors. They must reasonably account for factors in production systems and pathogen

strain differences.

And, with that, I will give you a view of the challenges of sampling collecting and take any questions.

Questions and Answers

DR. WAGES: Dr. Gray, if you were going to, how would you go about in a flock of birds, or a herd of cattle, or a farm of swine, how would you determine, go about determining their salmonella status being positive or negative?

DR. GRAY: Positive or negative in that situation would probably -- the best case scenario would be to sacrifice animals and look at deep tissue infections.

DR. WAGES: How many of those animals do you need to sacrifice?

DR. GRAY: It would depend on the species. It would depend on the flock situation, how many birds were in that barn, and what serotype of salmonella you are hoping to get a positive or negative status on.

DR. WAGES: Okay. And you maintain that there is no such things as a negative flock or herd, is that what you said?

DR. GRAY: I maintain that it is probably more difficult to find a negative flock than it is a positive

one.

DR. WAGES: Because that is different from what you stated earlier that there was no such thing.

DR. GRAY: What I said was -- would be an anecdotal conversation between a student and myself if they tell me a herd is negative simply by going out and taking fecal samples. What I would say is you probably have not looked at that hard enough. Salmonella is probably there in one form or another.

DR. HOLLAND: This holds true for cattle herds too, beef, dairy? Are you just talking about pigs and poultry?

DR. GRAY: Are you talking about a pen, or a herd, or cow calf, or feedlot? I mean, those things all differ.

DR. HOLLAND: I am talking about cow calf and a reasonably well-restricted dairy herd, where you do not have animals coming and going.

DR. GRAY: What type of dairy, dry lot?

DR. HOLLAND: No, not a dry lot, free stall, close, relatively close, except for feed materials coming and going.

DR. GRAY: Those kinds of measurements are not in the literature. But if you look at what NARMS shows, herd prevalence hovers around 8 to 12 percent. So you

predictably do have negative herds. But, again, you have got wildlife in the area, and that is going to effect whether or not you can find salmonella.

DR. HOLLAND: I guess I have seen too many naive beef and dairy herds to say that you have not looked hard enough to find it, both serologically and culturally, culturing for it.

DR. KOCHEVAR: Given the confounding nature of changes in assays where the percent prevalence of a particular pathogen has changed over the years in the literature because the analytical method has changed, do you think we have any basis at all to say that the load has gone up, in general, in herds -- herds, flocks, whatever? Or, at this point, do we have no way over time to have really an insight into that?

DR. GRAY: I am not sure I have the information to answer that question. But I think over time, if you look at NARMS studies, typically, the methods that have been used to measure salmonella prevalence has changed from year-to-year because those studies are not done on a yearly basis, and not done by the same lab. It is not a criticism of the study. It is a reality of those type of studies when they are done.

DR. KOCHEVAR: So, given those realities, do

you think we have a basis upon which to say it has gone up or down, or we just do not have the data to say it?

DR. GRAY: In my opinion, we do not have the basis to say.

DR. WOOD: With regard to shedding, do all serotypes of salmonella not shed all of the time? I mean, is that a general characteristic of salmonella that a bird or an animal could be carrying salmonella and not shedding it?

DR. GRAY: Are you asking are there serotypes that --

DR. WOOD: Are more likely to shed than other serotypes?

DR. GRAY: It depends on the host species, and what serotype you are talking about, and those studies are hard to come by. We have to really go by field data. And an awful lot of the data we have in animals, is clinical data, because there really are not that many non-clinical infection studies done.

But one would often expect things like typhimurium to be shed more frequently than some of the other serotypes if you simply look at the surveys that have been done.

DR. WOOD: But, still, even given these characteristics, the bug of choice, if there were to be

a choice, is still to be salmonella as opposed to campylobacter pathogen for measuring pathogen load?

DR. GRAY: Yes, I think that that is a true statement.

DR. WOOD: And then, in terms of confounding factors and stress, you said that there would be a need to address stress in a predictable way. I am getting feedback here. But what kinds of elements then would have to be put in place to measure stress in a predictable way?

DR. GRAY: Well, I am not a stress physiologists. But I think that Dr. Isaacson will show some interesting data on stresses that have been done, and how their outcome, what their outcome is, and that the way in which oftentimes stress has been measured in salmonella studies does not create a predictable stress, and therefore is not necessarily a reproducible way to do it. And I think if that were a factor to come into play, a stress physiologist should be consulted on that.

DR. WOOD: But stress could confound then the findings of a pathogen load studies ---?

DR. GRAY: Sure, absolutely.

DR. WOOD: Right.

DR. LANGSTON: I noted that you mentioned something that I quite often hear relative to

antibiotics promoting the carrier state, and I presume that means an antibiotic to which the organism might be susceptible but has grown resistant or otherwise.

And I oftentimes quote that, but I have never actually gone back and looked at the evidence of that. Can you summarize that? Are you familiar with it?

DR. GRAY: You know, most of the studies that we use, there are some studies in swine that are quoted in the diseases of swine book that look at that. But the main ones that are used are in humans, and we know that antimicrobial treatment in humans can indeed induce a carrier state. And, oftentimes, we are assuming that that same effect is going to occur in animals with a similar serotype of salmonella.

DR. LANGSTON: And that is, in fact, when the organism is susceptible to that antibiotic, it still promotes the carrier state?

DR. GRAY: I would have to go back and look at the studies directly. But, yes, I believe that to be true.

DR. PARKHURST: Could we look at your slide number nine, the second one on measuring effects?

DR. GRAY: Which slide was it?

DR. PARKHURST: I think it was slide number nine, way back in the begin -- at -- okay, come forward

a little bit.

DR. GRAY: Okay. Is this slide one?

DR. PARKHURST: Yes.

DR. GRAY: Okay.

DR. PARKHURST: That one.

DR. GRAY: Okay.

DR. PARKHURST: Could we just start off, as a matter of curiosity, what is the Y axis?

DR. GRAY: The Y axis is CFU of salmonella per gram of feces.

DR. PARKHURST: Okay. So that is a response, a measured response?

DR. GRAY: Yes.

DR. PARKHURST: And day one is the first day of challenge?

DR. GRAY: Day zero would be day of challenge; and day one would be first day of measurement of the salmonella shedding.

DR. PARKHURST: So could you just give me a little information about at what point of intervention, what are the pros and cons at different points of intervention?

DR. GRAY: Well, I do not know that I can give you pros and cons. But the things you need to consider is if, for instance, we know that if a drug is given

prior to salmonella ever being induced, what we have to -- and it has an effect on this curve, what we have to assume is that drug is having an effect on the flora in the intestine.

So it is preventing salmonella from infecting or making the intestine more prone to infection by salmonella, if it has an effect on this curve. If we give a drug late in the cycle, what we assume to happen is if we have very few organisms there, and we have a lot of other organisms that the drug can have an effect on, that we may induce carrier state by the salmonella wanting to, in a very general term, hide itself from the drug and go into the lymph nodes, go intracellular.

So, if the drug cannot reach the salmonella intracellularly, it has the ability to do that depending on the serotype. So it sort of depends on where in the cycle -- and, again, this is a clinical shedding cycle.

In a normal herd status, we probably are not going to see this shedding curve among the whole herd. We are probably going to see a low level of shedding throughout the herd over a period of time in non-clinical type state.

DR. GLENN: I have a question regarding in your conclusions you made remarks regarding study design to model a realistic infection cycle which we have been

talking about of course. Then, under Study Design, you were relating these various dose response curves relative to the dose that you were challenging with.

And, as opposed to this control of natural infection, are you advocating that this control using the seeder animal is a way to get at what you call a more natural or realistic sort of scenario? Is that a type of study that --

DR. GRAY: I am implying that that is an alternative because the dose response curve that we see with direct inoculation does, in fact, create problems with -- it is not necessarily what we expect to see in the real world because our doses and our shedding do not necessarily match.

DR. GLENN: Okay. And I wondered if you might speculate on how the naive animals became infected from the seeder animal relative to your remarks on consuming feces? Do you have any idea?

DR. GRAY: I think that there are a number of ways that one needs to look at that, and it is complete speculation. I think that there is everything from the state of the organism being shed from the animal, being different than what we grow in the lab.

I think it could be that a minimal level of coprophagia in that state could occur. We also know

that with salmonella intranasal or respiratory exposure can cause infection. Exposure of the head associated lymphoid tissue can cause infection. And we do not know the doses at which those things need to occur to cause an infection.

DR. GLENN: It is not related to the animal handlers?

DR. GRAY: In this case, it would not related to the animal handlers.

DR. GLENN: One last comment. How many studies of this type have you personally -- have you conducted in this whole area of assessing the salmonella reservoir? Is it predominantly with swine, I take it?

DR. GRAY: Yes, I have done a number, depending on whether they were non-studies or otherwise, sitting here counting, a couple of dozen.

DR. GLENN: Yes, okay. Thank you.

DR. KOCHEVAR: I am just curious on the shedder animal, has anybody sort of done shedder animal curve to see how low the shedder animal has to be before you do not get infection of the surrounding animals?

DR. GRAY: No, that has not been done to my knowledge.

DR. PARKHURST: It is a brief question. But once an animal is infected, does the animal become well

again after a period of time, or is it always a low grade infection?

DR. GRAY: No, what you typically see in normal dose of animals is a number of animals will clear the infection, and you cannot find it again. And it is a relatively low percentage that become true carriers.

MS. SINDELAR: Mike Goodman is our next speaker. But with great thoughtfulness for everyone here, Dr. Langston has recommended a break at this time.

And so, I will passing out background material that has been prepared by Dr. Goodman to the VMAC members, and the participants are more than welcome to pick up copies that are at the back of the room. Thank you.

(Whereupon, a short recess was taken.)

DR. SUNDLOF: Our next speaker is Dr. Goodman from Exponent, who has conducted a literature view for us on pathogen load. And Dr. Goodman is here to present the results from that literature review. Dr. Goodman.

Exponent Literature Search

by Dr. Mike Goodman

DR. GOODMAN: Good afternoon. It is a pleasure to be here today. I should probably start with a previous claimer that I am a human epidemiologist, and probably do not necessarily speak the language that the

guests and the members of the committee speak. So, sometimes, if it seems like it is over my head, please forgive me.

(Slide)

We were contacted back in mid 2000 to evaluate the published literature on pathogen load in food producing animal. And this first slide probably is more for people like myself, who tackle issue which was fairly new to us.

Our group is very experienced in reviewing literature, conducting comprehensive literature review, understanding the body of the literature in a balanced way. But this specific topic was very new and intriguing to us. Therefore, it is more for my own convenience than for members of this audience obviously.

(Slide)

The concern, of course, is the animals carrying increasing amounts of pathogen at the time of slaughter -- may have an increased amount of pathogens due to antibiotics or when they receive antibiotics mixed with their feed.

The task to us was to review the body of literature, published literature available to date and see what are the common themes that emerge from review of that literature.

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We have started it based on an usual algorithm over scholarly review by identifying database of the literature that could be helpful for our needs. We found 33 literature databases from various areas of human knowledge, medical, agricultural, food literature, veterinarian, and, of course, general scientific. Using a variety of terms that, just given here as examples, we are able to identify a total of about 30 articles.

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And then extracted information from each study using the extraction criteria. First of all, with species, we evaluated what was the antibiotic in question, what was the dose, how was the study designed, the bacterial species evaluated, and, finally, what were the findings.

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It is always very useful once you collect a body of literature to try to classify it, to make the thinking process or analysis process easier. This diagram shows our current understanding of what that published literature looks like.

The total number of studies is 29; 22 of them are challenged studies or experimental studies; and only 7 of them what we termed as observational studies. In

other words, there was no challenge with bacterium used.

For those of you who are mathematically inclined, let me point out that it is not a mistake; $16 + 8 + 2$ does not equal 22, but the problem is -- it is not a problem. The explanation is that some studies evaluate more than one species. Therefore, the sum total is more than 22.

And then once we have understood the structure, you know, we have sort of identified the skeleton of the literature that exists, we have analyzed a study using the same box, the same criteria. And some of the important observations are that the number of antibiotics evaluated is fairly limited, less than 10.

The majority of studies that use challenge, use challenge with salmonella typhimurium as inoculum. What is also important that when you look at the years of publication, you identify a gap, and I have no explanation to it. Maybe one of the members of the committee or the audience can help us out with that.

The first studies appeared in 1953; then there was a fairly active research that appeared in peer reviewed literature all through mid-80s or so; and then for about 15 years there was nothing in the literature until '99/2000, when some additional papers started to appear.

Whether that would mean that, for some reason, no work was performed during those years, or maybe the work did not get into a peer reviewed publication, but it seemed like a drop in interest in the topic.

Using classification that I just presented in the organizational chart earlier, let us review the results of studies. The challenged studies -- and we combine them for swine and calves. It may or may not be a legitimate way to combine studies, but there are very few of them.

They really found no evidence of consistent increase in salmonella shedding in these animals with and without antibiotics. What I would like to refer you to is the summary tables that were prepared as an additional handout for this presentation.

These are too busy to be imported into Powerpoint. And, therefore, I would like you just to refer to these tables as we go along. So this slide would correspond to table 1 in the handouts.

The only study that was of interests were the 1978 publication by Williams where they used two types of salmonella challenge species; one resistant; and the other one is sensitive. And they used chlortetracycline as antibiotic of interest.

Using that dichotomy, there seems to be a

discrepancy of results. The sensitive strain showed a decrease in shedding in animals that received antibiotics, and the resistance strain predictably showed an increase in shedding.

To help you understand the keys of this table, please refer to the footnote where the symbols are at the bottom. The less sign means that experimented animals. In other words, those receiving antibiotics had less shedding; the more sign, the opposite; and then when they were similar or no significant difference, there is a little wavy line.

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Moving onto poultry studies, first of all, what we have to point out, the number of study was much higher. It was much more studies conducted with the poultry challenge than with large animals.

There seems to be a consistent story presented by one group of researchers that came out United Kingdom that showed significant increase in salmonella shedding in chickens that received antibiotics.

The numbers were higher, as well as duration of shedding was higher, and the results were particularly strong for avoparcin. However, it seems like these studies were somewhat in isolation compared to similar studies conducted elsewhere, say, in

Scandinavia.

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With regard to avoparcin and salmonella shedding studies, what Scandinavian studies found is that the increase in shedding was observed only with single inoculation in early life. However, in circumstances where series inoculations were used, and in inoculations that were performed later in life, there seemed to be no impact of avoparcin.

Similarly, another later study by Holmberg et al, found no effect of avoparcin on salmonella shedding. However, a combined use of avoparcin with monensin seemed to have resulted in an increase in salmonella shedding.

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There were other studies that looked at a similar design, but for different antibiotics. These are all summarized here. And they, more or less, found no evidence of effect of these antibiotics on pathogen load.

Interestingly, these studies were also used different microbial species for challenge which makes them more interesting. In addition to salmonella, there was a challenge with E.coli campylobacter and clostridium.

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The observational studies, work with term observational studies, is obviously the ones that did not involve a purposeful inoculation of animals; and then the species of interest, the ones that were looked at, were more diverse.

These are summarized in the table number three in the handout. And you can see that an interesting finding is that of effective penicillin. If you flip to table 3 of the table, first of all, most studies are fairly early. They are done in the '50s, and the latest one was done in 1960.

All three were done in pigs; all three used penicillin. And whenever penicillin was used, the shedding seemed to have increased. However, the same results were not observed with other antibiotics. I am referring specifically to two studies by Bridges, '52/'53, and a study by Fuller.

Well, this is, in a nutshell, the results of our findings. I have to say that what we also did, in addition to our own research, we identified authors of the most recent studies and contacted them to conduct a peer review, make sure we have not omitted any major studies, and their input is also incorporated in our final report that we submitted to CVM. I believe that

was late year 2000.

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What are the limitations of the study that we reviewed?

First of all, the only salmonella studied was studied extensively. Other findings are sort of sporadic, and there is no consistent story that emerges from reviewing those studies. As already pointed out here, earlier challenge study may not represent real life conditions.

With regards to animal species, only swine and chickens underwent a substantial number of studies. The data are lacking for other species of animal. And then, an important consideration is variability of genetic lines as diets of animals around the world.

So our understanding is that diets used in Europe may not necessary be the same as those in North American, and therefore their effect on bacterial shedding may be quite different. They create different conditions in the gut.

These are limitations of the study themselves, but it is important limitation of this review. One, of course, is that it is limited to published literature. Therefore, an assumption that published literature reflects the body of knowledge that exists out there may