UNITED STATES DEPARTMENT OF AGRICULTURE

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THE PUBLIC HEALTH SIGNIFICANCE OF NON-O157 SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*

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PUBLIC MEETING

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October 17, 2007
8:30 a.m.

George Mason University
Arlington Campus
3401 North Fairfax Drive
Arlington, Virginia 22201

MODERATOR: DAVID GOLDMAN, M.D., M.P.H.
Assistant Administrator
Office of Public Health Science
Food Safety and Inspection Service
U.S. Department of Agriculture

PARTICIPANTS:

DR. RICHARD A. RAYMOND
DR. ROBERT BRACKETT
DR. DAVID WARNOCK
DR. PATRICIA GRIFFIN
DR. PHILLIP TARR
MS. SHARON HURD
DR. MOHAMMAD KOOHMARAIE
DR. HUSSEIN HUSSEIN
MS. CHERYL BOPP
DR. PETER FENG
DR. FLEMMING SCHEUTZ
DR. MARTINA BIELASZEWSKA
DR. RANDALL HUFFMAN
MS. JENNY SCOTT
MS. NANCY DONLEY

Free State Reporting, Inc.
1378 Cape St. Claire Road
Annapolis, MD 21409
(410) 974-0947

PARTICIPANTS: (CONT.)

MR. PHIL DERFLER
DR. ROBERT BUCHANAN

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P-R-O-C-E-E-D-I-N-G-S

(8:30 a.m.)

DR. GOLDMAN: Good morning to everyone. My name is David Goldman. I'm the Assistant Administrator for the Office of Public Health Science at the Food Safety and Inspection Service, and my job today is to be the moderator for this meeting on the Public Health Significance of Non-O157 Shiga Toxin-Producing *Escherichia coli*.

I want to welcome everyone to this meeting. I'll have a few more words to say after the official welcome from those who are seated to my right here.

I will let you know, just for some housekeeping purposes, that this meeting is being transcribed. So if you come to the microphone and speak and want to make a comment or ask a question, please identify yourself and your affiliation or organization. The transcripts usually take two to three weeks before they get up onto our website.

Also our agenda is quite tight. So I will ask for everyone to keep your comments to the point and short, as short as possible, so that we can move

through our agenda today.

I will let you know that as of late yesterday, we had 150 people registered for this meeting. You'll look around the room and won't see quite that many because we have folks who are joining by phone. There is a phone line open. So we'll invite them to ask questions or make comments as we move through the meeting agenda.

Also, only a few of the presentations are on our website, but we will make them available on our website as they become available to us from the speakers.

Now we'd like to welcome you from the sponsoring agencies, and so we will ask each of the three agencies' representatives to provide an official welcome to this meeting.

Dr. Richard Raymond was appointed Under Secretary for Food Safety in July of 2005. He is responsible for overseeing the policies and programs of the Food Safety and Inspection Service, and he chairs the U.S. Codex Steering Committee which provides guidance to U.S. Delegates for the Codex

Alimentarius Commission. He has extensive experience in developing and implementing policies and programs designed to improve health.

Prior to joining USDA, Dr. Raymond served as the Director of the Nebraska Department of Health and Human Services, Regulation and Licensure Division, where he oversaw regulatory programs involving healthcare and environmental issues. He also developed several anti-bioterrorism initiatives and a statewide healthcare alert system.

Dr. Raymond also played a major role in the development of local health districts in Nebraska that now serve Nebraska's 93 counties. Please welcome Dr. Raymond.

(Applause.)

DR. RAYMOND: Thank you, David, and good morning, everyone. Thank you for coming out today. It was kind of short notice when we were able to put this together, and it really is rewarding to see 150 people signed up and most of our food safety partners are here or are with us on the phone today, people we've been working with for the last couple of years

on other issues to improve the food safety of the United States.

The meeting today, of course, is very focused as some of our meetings have been. This is to discuss the public health significance of non-O157 Shiga toxin-producing *Escherichia coli* and whether certain non-O157 STEC should be considered as adulterants as *E. coli* O157:H7 currently is.

In particular, I want to thank Dr. Bob Brackett and Dr. David Warnock for finding time in their schedules to join us here. It's not easy to do, but to get them here is also very important as their agencies are co-sponsoring this particular morning with us.

Now as everyone knows, I've already mentioned, there's only one strain of *E. coli* that's considered an adulterant in meat. Even so, research and experience are coming in and showing that it's not the only strain of *E.coli* that's caused foodborne diseases. The great state of Nebraska, which used to have a football team did us, the public health lab there just recently finished a study and they

published the results of all *E. coli* infections, and they broke it down further and found that nearly 50 percent of *E. coli* infections in Nebraska were non-O157:H7. Those kind of reports that are coming in as the science gets better and the interest gets better and healthcare providers, public health folks begin to understand that not everything that causes *E. coli* infections is O157. They begin to spread their search. We continue to see continuing evidence that this pathogen is out there. It's out there in numbers not previously thought, and we need to keep that in mind as we set policies.

It's not easy. Some of the laboratory challenges are there. Not all of the non-O157s, of course, cause illnesses. Those are the things that you are going to hear about today. Those are things we need to sort through as we decide what, if any, future steps are necessary to control this particular pathogen.

Since most of you are here because you do have serious concerns about *E. coli* and particularly O157 historically, now the expanding universe, I want

to let you know that, I'm going to digress for just a minute from the morning's topic to tell you some of the things that Food Safety and Inspection Service at the USDA are doing to try to get the recent state of positive food products from our plants, increased recalls, increased outbreaks, attributed to *E. coli* and I want to give you a couple of ideas of what we're doing. We're trying to be as accurate as we can to get this under control. This meeting may be part of that.

We had renewed our emphasis this Summer about mid-June when we began to see an increase in product samples being positive, and we started to see a few very small recalls as a result of foodborne illnesses caused by O157:H7, and it wasn't the Topps recall. It wasn't, you know, the media. We started this activity back in July when we doubled the amount of samples on products that we were testing trying to figure out how widespread this problem was.

We just recently announced that we are going to do a training for our workforce, our inspection workforce and have them do surveys of the

1500 or so plants that either slaughter and/or further process beef, to find out if the guidelines that were issued in 2002 are actually being followed in these plants. We may or may not take further regulatory action depending on the results of that survey, but when someone asks how many plants have these guidelines in place, I cannot answer that. We need to get that information so we can take a stance of our position or regulation, if that's what's necessary.

Hopefully, the plants will recognize the importance of these guidelines. Most of them probably already have them in place, and those that don't, we'll work with them to get the guidelines more intact and more effective.

We've also asked our Agency to do more in depth testing after a plant has a positive product. We used to be able to go in and test once, and that was it. Our surveys, our studies show that that plant has a much bigger chance of having a second positive within the next 120 days than in the plant that doesn't have a positive. We will be doing 14

tests over the next 3 to 4 months in those plants to make sure they are maintaining the policies in effect, to not have another product come out positive. We're going to try to encourage plants to voluntarily hold product when we do test it. So many of the recalls we've had this year have been recalls brought about by routine testing by FSIS but the product was released to the public, and if we can break that cycle, we'll have fewer recalls and fewer exposures, simple things, little things, that we can work together on.

We also have a new policy that when a plant does have a positive sample for *E. coli* O157 or a link because of a patient illness, our EIAO officers will be sent as soon as possible into that plant to do a full food safety assessment. We tried to do that in the past, and we did it quite a bit, but we didn't do it 100 percent, and we will be doing it 100 percent from now on. That's our most effective way to get a better handle on that plant's activities.

We also in January will begin a more targeted sampling for *E. coli* O157 in the plants that

we do regulate. In the past, big plants, small plants, very small plants, all had about the same possibility of getting a sample to test positive. We will take a look at the plant's production. We'll also take a look at the plant's record from past samplings, and we will do targeted samplings which hopefully will be more effective than the current policy that we use.

We also announced in September when we do pull a sample, that sample will be sent to the lab that day. We will no longer be waiting to see if a plant has a sample that day is positive, and then they in turn would destroy the meat or cook it, and we would discard the sample. That policy has also changed. That will give us a better handle on the number of positives. It will also give us a better database at CDC with PulseNet, to look for PFGE patterns that may or may not show up later in public.

However, today is not the day we want to talk about steps any further about what we're taking for O157. We want to talk about the non-O157s, and I'm dedicated to the idea that the actions that we

take today to improve public health should and will be conducted transparently and openly. That's why we're having this meeting, to hear your input, to hear from our scientists, to hear from the consumers, to hear from industry, so we can make decisions that will reflect the feelings of all that are in this room. We need to have as much information as we can available at our fingertips to make the right decisions for the future.

I think this meeting will help ensure that any further steps, any future steps that we take as an Agency to reduce the prevalence of pathogenic non-O157 STECs will be better understood by all of our food safety partners. And I know the FDA and the CDC are also very interested in what comes out of this meeting for their own respective agencies and their policies.

So once again, I want to thank everybody for coming. I look forward to what we're going to hear today. I look forward to the dialogue during the comment period, and I encourage you all to participate actively during that time period so we

can hear from those of you who are not on the agenda. And once again, thank you all for coming.

(Applause.)

DR. GOLDMAN: Thank you, Dr. Raymond.

Dr. Bob Brackett was appointed Director of Center for Food Safety and Applied Nutrition at FDA, on January 1, 2004. In this capacity, he provides executive leadership to the Center's development and implementation of programs and policies relative to the composition, quality, safety and labeling of foods, food and color additives, dietary supplements and cosmetics.

Prior to coming to FDA, Dr. Brackett was a Professor of Food Science and Technology in the Center for Food Safety at the University of Georgia, where he was an active researcher in the area of food microbiology, specializing in the microbiological safety of foods.

Dr. Brackett was also previously on the faculty of North Carolina State University where he served as an Extension Food Safety Specialist and Assistant Professor. Dr. Brackett received his BS

degree in Bacteriology and MS and Ph.D. in Food Microbiology all at the University of Wisconsin in Madison. Please welcome Dr. Brackett.

(Applause.)

DR. BRACKETT: Thank you, David, and good morning to all of you and as well as to Dr. Raymond and Dr. Warnock, who are partners in putting this together.

One of the things I'd like to first do is give my thanks to David Goldman for actually being the point person on this whole idea, and it's something that when he came to us and said are you interested in joining with us in putting this public meeting together, we were quite -- saying yes because I do think that it's something that needed to be addressed. Typically what happens in the food safety community is you wait for some catastrophe to happen before the scientific community gets on board to start answering questions and by that time, you've had people ill or have died, and this is a case where I thought that one could get sort of ahead of the curve in this way. One of the habits that we have in

food science, and in the food safety arena, is to be much more reactive. And in this case, I think this is an opportunity for us to become a bit more proactive in understanding what this group of organisms is, what it does, how one can go about detecting it, and really understand its role in food safety, and so I think it is important for us to get ahead of any future outbreaks and hopefully minimize what impact they might have

As mentioned by Dr. Raymond, this group of organisms is not new to us, but it's something that we've sort of been watching on the side. In the case of outbreaks we've had from FDA regulated products, most notably leafy greens, we've noticed this group of organisms in amongst the isolates of both patient cases as well as the samples of the product itself, not understanding really where it fit within the outbreak, but nevertheless knowing that there was something going on. Likewise, we have seen this group of organisms appearing in cheese products specifically and other dairy products.

So it's something that we do have a great

interest in, we think it's important for the scientific community, the food industry and the regulatory agencies to get a much better understanding of this whole group of organisms and really what it causes and likewise, this is an opportunity to engage the consumer groups early on in the discussion of an issue so that their perspectives can be taken into consideration with any new policies as well as with any scientific direction that might be going forward to address them.

I think this is an issue that we at FDA are very interested in following. We're interested in what your views are. I think this meeting itself was not meant to be a sharing of scientific data because there really isn't a whole lot of data on non-O157 STECs as compared to other organisms but it is an opportunity to try to ask the right questions. And we expect and hope that you would ask the right questions, give us your perspective as well. I think what we don't know is as important as what we do know in terms of directing not only policy but some of our research directions that might go forward as well as

thinking about how we're going to deal with this issue in future years.

So I do welcome you here again this morning, and look forward to hearing not only what you have to say but what our presenters are hearing throughout the day. And so I look forward to a great day. Thanks.

(Applause.)

DR. GOLDMAN: Thank you, Dr. Brackett.

David Warnock is the Director of the Division of Foodborne, Bacterial and Mycotic Diseases at the Centers for Disease Control and Prevention in Atlanta, and is also an Honorary Professor of Pathology and Laboratory Medicine at Emory University School of Medicine.

Before moving to the CDC in 1999, Dr. Warnock was the head of the Mycology Reference Laboratory, Public Health Lab Service in the United Kingdom. He is also a former President of the International Society for Human and Animal Mycology. Dr. Warnock is a Fellow of the American Academy of Microbiology and the Royal College of Pathologists.

He has published extensively on the epidemiology and laboratory diagnosis of fungal infections and on anti-fungal chemotherapy.

Please welcome Dr. Warnock.

(Applause.)

DR. WARNOCK: Thank you, David. Good morning. On behalf of the CDC, I would like to add my welcome to those of Dr. Raymond, Dr. Brackett, to this public meeting.

On the subject, as Dr. Brackett just said, there are more questions than answers. Shiga toxin-producing *Escherichia coli* or STEC infection has been very aptly described as a developing world infection that occurs in the developed world. It is feared, and rightly so, because it kills. Even when it does not kill, it leaves some of its victims damaged for life, in particular, young children and the elderly.

STEC O157, as everybody in this room knows, was first identified in the 1980s, but it was not until the 1990s that we began to see large and dramatic outbreaks of infection. The increasing prevalence of O157 carriage in ruminant farm animals,

its low infectious dose for humans, it's ability to survive in food, water and the environment, and the concomitant industrialization of the food production system that was taking place at that time, were together factors that created the perfect storm and how to explain its emergence as a major human public health problem.

As my compatriot, the Scottish microbiologist, Hugh Pennington has so aptly phrased it, a little uncooked manure can clearly go a long way.

Although O157 is the most common and most widely recognized cause of sporadic outbreak associated STEC illnesses in the United States, infections with non-O157 isolates are becoming more common. Indeed, it's now well-established from data worldwide that these non-O157 strains can cause severe human illness that is comparable with that caused by O157. The low infectious dose of some of these non-O157 strains and their ability, their potential ability to cause severe or life threatening illness among young children in particular, made

these agents an important public health concern.

Estimates from the United States in the 1990s suggested that O157 strains caused somewhere in the region of 70,000 illnesses annually. To illustrate the lack of data that we have for non-O157 strains, estimates for the importance of these strains suggest they may cause as few as 1/3 of the number of infections as O157 to as many as slightly more than O157. Clearly, there is a need for more work in this area.

STEC O111 has emerged as the second most common bacterial cause of HUS in the United States, one of the most severe complications of STEC infections.

In the decade between 1992 and 2002, O111 was identified as etiologic agent in three of seven reported outbreaks of non-O157 STEC infections. Two of these outbreaks included cases of HUS, an association that has also been seen in countries other than the United States.

Improved surveillance and awareness of non-O157 strains, as important pathogens, will without

doubt in the next few years lead to increased detection and reporting of these under-recognized agents.

Now growing awareness of the burden of serious illness caused by non-O157 STEC is attributable at least in part to the changes that have occurred in clinical lab practice, to the more widespread use of non-cultured based methods to detect Shiga toxins in clinical samples.

Although clinical testing, non-cultured based methods, might seem very desirable in terms of expediting clinical diagnosis, I will point out as will be emphasized later in the day, that it is still important to culture these pathogens to confirm that you do, in fact, have an accurate diagnosis, and also to obtain isolates to allow further testing to be done, including molecular subtyping which is obviously a great importance for public health surveillance.

I'm sure that you have all been aware over the last week that we are at the time of year when the winners of the Nobel Prizes are announced,

particularly with the announcement of the winners of the Peace Prize, but let me finish by observing the fact that I have not appreciated until recently, that more Nobel Prizes have been awarded for work on *E. coli* than on any other species except the human. And it is rather ironic that those who worked on *E. coli* to win the Nobel Prize, did not work on it because of its medical importance and, in fact, many of them chose to work on it because they had been told that it was harmless. They simply chose it as an ideal model biological system. How times have changed over the last two decades! Thank you.

(Applause.)

DR. GOLDMAN: Thank you, Dr. Warnock, and thanks again to Drs. Raymond, Brackett and Warnock for their welcome to all of you to this meeting.

For those who have just come in, I want to remind folks that this meeting is being transcribed. Most, if not all, the presentations will be posted. The transcript will be posted in a couple of weeks now. We do have a fairly tight agenda, and I want to make sure everybody has an agenda, and if you don't,

there are agendas out on the table there as well as a few of the presentations already.

I want to begin by thanking people. I don't want to end. This meeting took a lot of effort to put together, and I want to acknowledge briefly those people who were heavily involved in this. First and foremost, Dr. Denise Eblen -- she's stepped out of the room.

(Laughter.)

DR. GOLDMAN: Denise Eblen is a staff microbiologist at FSIS and she was the principal author of the White Paper that many of you may have seen posted on our website which was designed to stimulate thinking in advance of this meeting.

Other contributors were Elisabeth Hagen, Scott Seys (ph.), Bonnie Kissler, Kristin Holt (ph.), Peter Evans and Mildred Rivera Bentancourt. So I want to thank all of them for their participation. I also want to thank Sheila Johnson and Janice Schechter for some of the logistical efforts of pulling this meeting together.

I certainly also want to thank all of our

speakers. You can see it's rather impressive that the first several rows are filled with speakers. There is a lot to say, and I would perhaps differ a little bit with Dr. Brackett. I think there is science to present but I think you will, in fact, find that there are more questions than answers, so in agreement with the other introductory speakers.

And I want to thank all of you for participating and again welcome your comments and ask that you come to the mic and identify yourselves if you make a comment.

Both of the Federal food regulatory agencies, FSIS and FDA, are public health agencies. As such, we view this meeting as part of our assessment function done in collaboration with the many partners that are gathered here to be part of this agenda today. Specifically FSIS and FDA depend on our post-collaboration with CDC as well as with State Departments of Public Health and Agriculture, to bring emerging public health food safety issues to our attention. I think the shared sponsorship of this meeting reflects that.

Now in terms of what we hope to accomplish, the sponsors of this meeting hope that this audience, you, will leave today assured that you've heard a thorough, if not an exhaustive discussion of the scientific issues and the stakeholder perspectives so that the regulatory agencies can make informed decisions about the appropriate course of action to take.

As has been said already, the time is ripe to address the public health issues raised by the presence of non-O157 STEC in the environment, new reporting guidelines, better methods of surveillance, and the availability of better laboratory methodology have prompted us to gather together the experts that you'll hear from shortly.

You will hear from the public health and human health world about the wealth of data that exists on the emergence of non-O157 STEC as a human pathogen. You will hear that there is a relative lack of non-O157 STEC studies compared to the wealth of information and studies that exist about O157:H7. Consequently, there is a need still for more targeted

research so that strategies for control of these organisms in the food supply can be identified. We hope that the research community can focus on developing testing methodologies targeted at these organisms or refining the methodologies that already exist.

Simply, we are interested in knowing the extent to which these other STECs cause human illness, how well they can be identified in clinical isolates and in food and whether interventions can be developed that decrease the contamination of foods and ultimately decrease the risk to public health.

Our agenda today is ambitious for several different reasons. One is that we are limited by time. If this sort of room feels familiar, it is, in fact, the college auditorium and there's a class in here at 4:00. So we do have to vacate the room rather promptly right at 3:30 or soon thereafter. So it's my job to try and keep us on track.

The agenda's ambitious in another way because there are so many complexities to the issue, and you will certainly appreciate that when you begin

listening to the presenters. And as has been said already, ultimately I'm not sure that you will hear clear answers, but rather raise more questions.

As FSIS endeavors to do what all of its public meetings, we have invited wide ranging perspectives on this issue. You will hear highly technical, microbiological and epidemiological discussions. You will hear, because of the international nature of food safety, the global perspective, so that we might learn from the experiences of other countries.

You will also hear from those who will be directly impacted by any decisions that the regulatory agencies might make, namely the consumers and the regulated industries.

The regulatory agencies, FSIS and FDA, will need to decide after what we hear today what approach to take to address this group of organisms, and although FSIS and FDA will speak about regulatory considerations at the end of the meeting, you shouldn't expect to hear decisions about how each Agency will address STEC. You should expect to hear

how each Agency will take what is known, what we hear today, and what we may still need to learn, into consideration of the proper approach to take.

With that, one final note, as the moderator and the timekeeper, I reserve the right to shorten breaks, cut the lunchtime which I hope I don't have to do, and try to keep our speakers and commenters on time, and also reserve the right to cut out the last item on the agenda, which is my summary --

(Laughter.)

DR. GOLDMAN: -- if the time should push us in that direction.

With that, we will transition. We'll ask our welcoming party to move off the dais and we'll invite our first panel to the podium and the dais here. And what I would like to do is, in terms of the panel discussion and public comments, after each panel, you'll see the agenda is divided into three or four sections. After each, there will be a relatively short period of time for the participants in this meeting to ask questions of the presenters, to make comments, and we'll ask the panelists for

each of the panels to remain here on the dais to address any questions you might have about the presentations that you've just heard from them and, of course, they may want to make comments about some of their co-presenters as well.

So at this point, I would ask Dr. Patricia Griffin, Dr. Phillip Tarr and Ms. Sharon Hurd to come join me here.

The first panel that we have will discuss the epidemiology and human health burden of non-O157 STEC, and we're very pleased to have this group of panelists here. I think you will be quite impressed with both the depth of their knowledge as well as the extent of their experience in this particular area.

We will begin with Dr. Patricia Griffin who received her MD from the University of Pennsylvania School of Medicine, trained in internal medicine at the Hospital of the University of Pennsylvania and then later in gastroenterology at Brigham and Women's Hospital and then finally in the CDC's Epidemic Intelligence Service. She holds appointments in the Emory University School of

Medicine and Emory School of Public Health. Currently she is the Chief of the Enteric Diseases Epidemiology Branch in the National Center for Zoonotic, Vector-Borne and Enteric Diseases. This branch includes teams that work on national surveillance, FoodNet, OutbreakNet, the National Antimicrobial Resistance Monitoring System, and the Safe Water System.

Dr. Griffin has supervised epidemiologic investigations throughout the U.S. and overseas, and has authored or co-authored over 150 Journal articles or chapters and other publications.

Please welcome Dr. Griffin.

(Applause.)

DR. GRIFFIN: We're already a little late. So I'll start right in.

E. coli that causes GI illness includes Shiga toxin-producing *E. coli* also called enterohemorrhagic, enteropathogenic, enterotoxigenic, enteroinvasive and other types less well characterized.

Today we're focusing on the Shiga toxin-

producing *E. coli* which include the O157 serogroup and we're focusing today on the non-O157 serogroups.

Animals are the reservoir for STEC. That includes cattle, other ruminants and other animals especially those that have contact with cattle.

The major modes of transmission of STEC to humans, that is how the fecal matter gets to the mouth, include food especially cattle products and food contaminated with food or human feces, drinking water, recreational water, animal contact with farm animals or with their environment, and person contact with the feces of infected persons.

This scheme shows the sequence of events in *E. coli* O157 infection. So first, somehow the person ingests the O157 and it takes three or four days while it multiplies in the intestine before the person develops non-bloody diarrhea and abdominal cramps. In about 80 percent of people that come to medical attention, bloody diarrhea develops in another day or 2. And then 92 percent of people go down to the left side of this to resolution within another 5 or 6 days, but 8 percent develop HUS, and

the number varies. It's higher in children and the elderly.

So I'll show you the same sequence for non-O157 STEC and it looks very much the same except for those highlighted in yellow that rather than 80 percent developing bloody diarrhea, it's more like 40 percent, and rather than 92 percent resolving, it's more like 98 percent, and HUS is more rare.

So compared to persons with O157 infection, persons with non-O157 STEC have less severe illness, but non-O157 STEC include many serogroups, over 100 serogroups with varying virulence. And some of these typically cause only mild diarrhea but others can cause the full spectrum with HUS and death.

Let's talk about clinical lab testing for STEC. *E. coli* O157 has a very unusual feature. It does not ferment Sorbitol at 24 hours. So the lab can streak a stool specimen onto a culture plate that has Sorbitol in the MacConkey medium, and then the lab selects clear colonies, most of the other organisms are pink, and the O157 strains agglutinate when O157 Antisera is added to that clear colony.

So it's pretty easy to find an O157.

But the non-O157, the vast majority lack unusual clinical features and they look just like the good *E. coli* in our bowel. So for a long time, it was harder, almost impossible for clinical labs to find these, and that brings us to the timeline of public health recommendations for STEC.

In 1994, O157 infection was made reportable. In 1995, a commercial Shiga toxin enzyme immunoassay was introduced, and in 2000, non-O157 STEC infections were made nationally reportable.

So let's go back now and talk about testing for non-O157 STEC using the Shiga toxin EIA. So the clinical lab can culture the stool specimen in broth, and then the lab tests the broth for Shiga toxin using the EIA, but a positive test could mean either O157 or non-O157 STEC. Well, the clinical lab can send the Shiga toxin positive broth to the state health lab, and the state health lab can then isolate the STEC organism that's producing the toxin from that broth, and then the state health lab sends the STEC to CDC and CDC determines the serogroup. So

there's a lot of steps here.

So there are some challenges arising from use of the Shiga toxin EIA. After adopting the EIA, some clinical labs actually stop testing for O157 using selective media, and so O157 outbreaks can be missed when that happens. Some other clinical labs discard the Shiga toxin positive specimen without obtaining an isolate. So they simply report Shiga toxin positive to the doctor. The serogroup is then not determined. So O157 strains are not identified and subtyped for outbreak detection, and non-O157 outbreaks are less likely to be identified.

So how do we learn about non-O157 STEC. These are some of the ways. I'll go through them one by one, starting with FoodNet that conducts active surveillance. So this map shows the FoodNet catchment area. It contains 45 million people, 15 percent of the U.S. population. This is our pyramid of surveillance in talking about what it means when we get a positive result. So when a person is exposed to STEC, they may become ill. They may seek healthcare. A specimen may be obtained. The

clinical lab may test for STEC. If they test, they hope the STEC gets isolated, gets found, and then we always hope that it gets reported to the state health department and CDC. So we're focusing here on the clinical lab testing for STEC. Is this done?

Well, FoodNet conducts clinical lab surveys to try to figure out how often that is done, and FoodNet conducts active surveillance near the top of the pyramid finding pathogens that are isolated. So it's important to remember that what food net does, all FoodNet does is make sure that CDC and the states gets the report if an organism is found. FoodNet doesn't make any clinical lab or any doctor do anything. If the doctor doesn't order the test, it doesn't get done. If the lab doesn't routinely or doesn't on request look for STEC, it doesn't get done. All FoodNet does is collect what's already there.

So this graph shows the percent of clinical labs screening all stools for *E. coli* O157. So you can see that testing increased gradually, and then I want you to look at the right side of the graph at

our results from FoodNet sites. We've done surveys at every place you see a yellow bar, and you can see that for the past 10 years, including our new data from 2007, which is preliminary, about 2/3 of the clinical laboratories in the United States test all stools for O157, and that's just in FoodNet sites that we did the survey.

So the comparable graph, the percent of clinical labs that ever conduct on site testing for STEC using an EIA is much different. So here we're not talking about routine testing. We're talking about whether these labs have an EIA that they can use on request.

In 2003, three percent of FoodNet labs said they had the EIA that they could use, and in 2007, our very preliminary data is nine percent. This number is likely to change.

So here's some FoodNet data. On the human isolates of non-O157 STEC by serogroup in FoodNet sites, in 2000 through 2006. Of the close to 600 isolates that we heard about, most of them fell into 6 serogroups. That's 83 percent fell into 6

serogroups, and the rest were in 42 different serogroups but less than 1.5 percent in each of those serogroups. So I'll mention the big six again later.

This graph shows the number of non-O157 STEC identified in FoodNet sites. The message here is don't look at the numbers. Just look at the graph and you can see that testing is increasing. We're identifying more not because we think that there's more going on. We really don't have any way to measure that, but we know that testing is increased. Labs are testing more, and so they're finding more.

So another way that we learn about non-O157 STEC is that some clinical labs isolate non-O157. Clinical labs, not just in FoodNet but throughout the United States, there are labs who decide to do testing for their own purposes or because of a clinician recognizes it, and just about all those isolates eventually make their way to CDC because CDC is a reference lab that does the serotyping. And so Nancy Strockbine's lab at CDC, has human isolates of non-O157 STEC that they serotyped between 1983 and 2002, and we wrote a paper on this that we published

just two years ago, and of the 940 isolates in the lab, you can see that 6 serogroups comprise 70 percent of the isolates and those are O26, O111, O103, O121, O45 and O145. Fifty-five O groups comprise less than one percent of isolates each.

This map shows human non-O157 STEC submitted to CDC by the states. The message here is simply that you find these organisms throughout the United States and the numbers in the state boxes really are just a measure of how much testing, how much people look rather than a measure of the incidence of disease.

The seasonality of human non-O157 isolates is very similar to that for O157 with a peak in the summer months.

This is a bit of a complex slide. I'll walk through it very slowly. Again, looking at the same isolate group from Nancy Strockbine's lab, persons with HUS rarely have a non-O157 STEC strain that produced only Shiga toxin 1, and we did this analysis on isolates with clinical information that were submitted to CDC. So we looked at two toxin

profile types. One is those that produced only Shiga toxin 1, and the other is those that produce Shiga toxin 2 with or without Shiga toxin 1. So among the 21 people with HUS, five percent, that's 1 person, had a strain that produced only Shiga toxin 1. The vast majority had strains that produced Shiga-toxin 2, and among the people without HUS, most, 68 percent had a strain that produced Shiga toxin 1.

So overall, in our database, 61 percent of human non-O157 STEC produced only Shiga toxin 1, and yet those strains were less likely to result in HUS.

Another way we learned about non-O157 STEC is that some health departments are doing studies. For example, Minnesota has surveillance for STEC in all diarrheal stools. So this is where they're working with the clinical labs and they said to these clinical labs, you may not want to look for it, but give us your plates from every person with diarrhea and we're going to look for all the STEC. They have a lab in the urban area and a lab that services the semi-rural area with agriculture and dairy farms. And the proportion of STEC that were O157 or non-O157

in these human diarrheal stools is shown here in these graphs. So look first at the urban area and you can see that about half and half were O157 and non-O157, but a higher proportion were non-O157. And again look at the semi-rural area. Again, it's about half and half, this time with more that were O157. So overall, it was about half and half, and you'll see similar numbers from other studies from the United States.

Another way we learn about non-O157 STEC is from outbreak investigations. This graph shows outbreaks of non-O157 STEC in the United States. We've counted 23 of them, and you can see more since the Shiga toxin EIA became available. This table shows the serogroups of non-O157 STEC outbreaks. What's striking here is the vast majority of outbreaks were caused by the top one *E. coli* O111. The green highlights show the most common serogroups of the sporadic cases. So you can see that there's a lot of overlap between those big six I showed you earlier and the ones that are causing the outbreaks.

This table shows the modes of transmission

in the non-O157 STEC outbreaks. Most common was food, then person to person, lake water, animal contact and undetermined. And these modes are very similar to what we see for O157.

And the food vehicles in these outbreaks have included salad bar, salad and ice, berries, milk, cider, punch.

And this map shows the sites of non-O157 outbreaks reported to CDC and sort of like the other map site I showed, the message here is that you see them all over the United States. Finding them is probably as much related to efforts to look for these organisms as anything else, but you may notice that there's a bit of the northern tier phenomenon that we also see with O157. We are seeing more outbreaks at least reported from northern states.

One of these outbreaks was STEC O111 infections that occurred at a cheerleading camp in Texas. Fifty-five persons had diarrhea and most were teenage girls. Eighteen had bloody stools, and two developed hemolytic uremic syndrome. No one died in this outbreak. It was transmitted by a salad bar and

ice. So another way we learn about non-O157 STEC is studies of HUS. We did a national perspective of diarrhea associated HUS study in which we enrolled adults and children with HUS and we requested a stool sample and some serum to measure antibodies to O157 lipopolysaccharide.

In looking at those patients who had both stool culture and serology results, 18 percent had no evidence of STEC infection, and I'm not going to go into the reasons for that. We'll focus on the 82 percent that had evidence of STEC infection. While 98 percent of these had evidence of O157 infection, and 3 of the 4 with non-O157 STEC isolated from stool also had antibodies to O157 LPS, which suggests that O157 may have caused their HUS.

So the results of the national study suggests that the proportion of HUS cases in the United States caused by non-O157 STEC was small.

There have been other studies of HUS with stool cultures among HUS cases tested within six days of the onset of diarrhea. The proportion with O157

isolated in the United States was 96 percent in Phil Tarr's early study, and in Canada, it was 87 percent.

There have been other studies of HUS with serology, and the proportion of HUS cases with O157 LPS antibodies was 73 percent in England and Central Europe, and 67 percent in France.

So other studies in the United States and other countries have also reported that O157 is the major cause of HUS.

So CDC has done some work to improve the diagnosis of STEC infections. We began a clinical diagnostic working group that includes CDC clinical labs and others, and we had meetings in 2006 and 2007, and we published a MMWR with guidelines last September, a year ago. This is the MMWR called "The Importance of Culture Confirmation of Shiga Toxin-Producing *E. coli,*" and we had a box that had specific recommendations for clinical laboratories. I'm not going to read all these.

So, in summary, non-O157 STEC are a diverse group but about 75 percent of human infections in the United States are due to 6 serogroups. Clinical

illness due to non-O157 STEC includes diarrhea, bloody diarrhea and HUS, but it's overall less severe than O157. Most non-O157 STEC infections are not diagnosed. Few clinical labs test for Shiga toxin, but use of EIA has increased and more non-O157 STEC illnesses and outbreaks are being detected. And there are challenges in testing for STEC by the EIA. Shiga toxin positive is not sufficient. Serogrouping is very important, and rapid identification of O157 is important for outbreak detection.

STEC diarrhea, O157 and non-O157 STEC are isolated with similar frequency in many places in the United States. And about STEC associated HUS, we estimate that less than 10 percent is caused by non-O157 STEC. Strains that produce only Shiga toxin 1 are much less likely to cause HUS than strains that produce Shiga toxin 2, and 61 percent of human non-O157 STEC strains in our collection produced only Shiga toxin 1.

So the contributors to much of the data that was used in this talk came from state and local health departments, from the Enteric Diseases

Epidemiology Laboratory. We have many other collaborators including the current and former members of the Enteric Diseases Epidemiology Branch. Thank you.

(Applause.)

DR. GOLDMAN: Thanks very much, Dr. Griffin, for that review of the epidemiology and for highlighting some of the issues that difficulties in testing, isolation have on knowing what the burden of illness really is. I think you've highlighted that very well, and we will be able to ask her questions in just a minute.

Next, we want to hear from Dr. Phil Tarr who has already been referenced in this discussion. He's a physician who graduated from Yale University School of Medicine, entered residency training in pediatrics and had post-residency training in gastroenterology, infectious diseases, microbiology, all at the Children's Hospital and Regional Medical Center and the University of Washington in Seattle.

In 2003, Dr. Tarr moved from Seattle to St. Louis and joined the faculty of the Washington

University School of Medicine where he is the Melvin Carnahan Professor of Pediatrics, Professor of Molecular Microbiology and Director of the Division of Pediatric Gastroenterology and Nutrition. He maintains his research interest in the field of diarrheagenic *E. coli* including aspects of prevention, diagnosis, evolution, path of physiology and disease management.

Thank you very much, Dr. Tarr, for joining us today. And it will just be a minute while we load his presentation.

DR. TARR: Thank you very much, Dr. Goldman, colleagues.

I'm going to present a variety of different data that converge 100 percent on what Dr. Griffin just reported. I'm going to present this largely from the perspective of point of care, point of diagnosis, much more community based, perhaps state or local health department based studies. This convoluted title reflects the complex problem that you're trying to get a grip on now, and I applaud USDA and related agencies for attempting to address

the problem of non-O157 Shiga toxin-producing *E. coli* lest they bloom into something like *E. coli* O157:H7, which is today still the greatest threat to North American public health in terms of diarrheagenic *E. coli.*

I'm going to present this largely from the United States perspective. There will be excellent speakers from overseas to demonstrate what is happening in other countries. I must say that there is a pediatric bias to some of the data that I will present, by virtue of my studies and my background, but I am also going to try to bias my reports towards systematically collected specimens and cohorts of subjects all in the context of patient care.

You know the ground rules here for this organism. There are many different *E. coli* serotypes out there, and a large subset of them, 100 at latest count, or over 100 at latest count, will produce Shiga toxin 1 or its variants, Shiga toxin 2 or its variants or both. However, only a small subset of those organisms that produce Shiga toxin has really been demonstrated to be pathogenic to humans, and

it's those we want to keep at bay.

I choose to categorize Shiga toxin-producing *E. coli* in four different groups. First and foremost is *E. coli* O157. This needs little introduction in 2007. It's a global pathogen, causes epidemics and severe disease including hemolytic uremic syndrome. There are a variety of sources and, and it is of enduring importance. It is not going away. It is not just a bloom that came out and then receded. It is easily detected in human specimens using what I consider to be very good microbiologic practices, namely plating stool. All stools, not at physician request, but all stools that come into microbiology laboratories, on Sorbitol MacConkey agar, and you can see that pale colony over around 9:00, that's an *E. coli* O157:H7, easily detected. Ten minutes later, the microbiologist can call the physician and say, I think I've got a suspect colony. You better look at that patient again more closely.

The second group of organisms, I think that are just as virulent, fortunately they've not yet come to this continent, is represented by *E. coli*

O157 nonmotile. This is a group of pathogens described in the early 1990s from Germany, from Professor Karch's (ph.) group. These organisms very closely related to *E. coli* O157:H7 ferments Sorbitol. You will miss them on the Sorbitol MacConkey agar. They are found increasingly in a few other countries, largely in Europe, recently in Australia. Dr. Bielaszewska will be describing this in greater and exquisite detail later today.

Now the sources, despite their best efforts, are often elusive, and these organisms require toxin assays or gene probing to detect.

The third group that you also want to keep out of the food supply and out of your children are pathogenic non-O157 Shiga toxin-producing *E. coli.* Not O157:H7, not O157:H7 minus, which, of course, you want to keep out but these are the serotypes, the big six that Patty just described, O26, O111. They are global. They're found in many different countries over many different decades. The distribution of serotypes varies from country to country and year to year. They are usually not causes of epidemics, and

their epidemiology and sources remain unclear at least for at least for sporadic cases. These two are less easily detected. You need toxin assays to find them. They may be transmitted by food, and we need to be vigilant.

And finally, there's this big background of non-pathogenic, at least in humans, Shiga toxin-producing *E. coli,* organisms containing toxin genes. They are ubiquitous. They are probably not major causes of human disease.

We really want to nail the first three. The reason we want to nail these is they are demonstrated or theoretical causes of the hemolytic uremic syndrome, when defined stringently, and this will come up a little bit later why it's important to define HUS, it is a potentially fatal disorder consisting of severe anemia, low platelet counts and acute kidney failure, and it occurs between 1 and 2 weeks after the first day of diarrhea.

About 15 percent of children under the age of 10, in the United States and Canada, who are culture positive for *E. coli* O157:H7 will meet that

stringent case definition of HUS. From a regulatory and surveillance standpoint, this is a good disorder to target because you're very unlikely to miss a case of HUS, and you can multiply the number of cases of HUS in children by seven and determine approximately how many positive cultures are out there or should be out there if you want to try to use this as a surrogate marker.

Some non-O157s will clearly cause HUS. O157 Sorbitol fermenting nonmotile strains from Germany are clearly a group of pathogens. O111 is clearly an important cause of HUS, and the small subset that is not attributable to O157, O113, we'll get to in a minute, seems to be rare, but also are quite virulent and has been found in Canada and Australia.

There are problems, too. Once a child develops hemolytic uremic syndrome, about two-thirds of them are culture negative for *E. coli* O157. You've got to get them in the week before they develop HUS. That's why it's critical to back up your analysis, where did the child present, what did

the laboratory do, what tests were applied at the point of presentation? If it is not found, when a child has HUS, if this pathogen is not found in North America when a child is HUS, it doesn't mean it wasn't there. It just means you didn't find it and may have already been cleared when they come in with this index case.

So let's look at the timeline and the thought processes and the technology applied to children with diarrhea. As you can imagine, this is a massive problem. Centers for Disease Control estimates that there are two and a half episodes of acute diarrhea per annum per child in the United States. When you look at all the people in the United States, all ages, there's over a half a billion episodes of acute diarrhea caused by a wide diversity of agents, very few of which are Shiga toxin-producing *E. coli.*

There's only about 15 million cultures performed per annum in the United States, and the technology to find a pathogen is quite cumbersome. And also the vast majority of diarrheas stay at home,

patient gets better, a self-limited illness, but in the subset that do enter the medical system, they can go to one of three places. They can go right to a doctor's office. They can go right to an emergency room. Some patients can be directly admitted to the hospital, usually via the emergency room or doctor's office, and what's going to bring most patients into a medical setting will be painful diarrhea, bloody diarrhea of hemolytic uremic syndrome, and not severe dehydration if they're infected with an *E. coli* that produces Shiga toxin.

Now once they're in the setting, you've got to do something and most people would agree that a child hospitalized for severe diarrhea, painful diarrhea, bloody diarrhea, or seen in an outpatient setting, should undergo a stool culture, physician write stool culture. The physician really has no idea what that means, but they request it. They really don't know what the panel of microbiologic tests will be performed once they write that down. And there are biases. Bloody diarrhea in HUS patients are probably disproportionately cultured.

Most people go to their doctor with diarrhea will not get a stool culture.

Furthermore, it seems to be emerging that sporadic cases, at least *E. coli* O157 are predominantly rural and the microbiologic resources needed to thoroughly work up an enteric culture are often not available close to the point of presentation. This is what it takes to work up a stool culture in 2007. This technology is from the '90s, the 1890s. If Louis Pasteur were to be reincarnated today, you could put him to work right here with very little reorientation. This is what it takes to get the panel of pathogens that end up on Patty Griffin's databases, and it's complex, it's expensive, it's labor intensive, and it's low yield. We have to face that.

But let's assume that it is done appropriately, my opinion appropriately testing involves plating all specimens for *E. coli* O157 immediately, as well as performing a toxin assay represented by the word signal there. Once the signal is positive, then you might look harder for an

E. coli O157 or you could just send the broth off to the state lab and be done with it, but in any event, this is the flow once it gets into a lab, if it gets into the lab, if your doctor orders it.

And then once it's ordered, most labs will at least make a digital report to the state, frequently submitting the isolate. There's a bias at this point because non-O157s may be disproportionately sent onto the state for typing because you've now got an isolate that's not an O157 producing a toxin. You don't know what it is. If you've got an O157, there's really no clear need at the point of presentation or point of diagnosis to send it onto the state.

Let's look at some of the studies that have been performed in the United States over the past 10 years or so, and try to look at each of these points as a patient progresses. There was one very good study, it's limited by the problem, actually a pair of good studies from Wernicke, et al., looking at diarrhea that didn't come into a doctor's office. This is a home-based diarrhea study, 494 episodes

over about six months in about 2,000, and only 1 patient had Shiga toxin-producing *E. coli* in that group. None of the control specimens had that. It's hard to know if that was related to the patient's illness, but this is the only pre-office visit or non-office visit assessment that I'm aware of.

There have been a couple of studies reported in a single manuscript by Donna Deno (ph.) looking at patients who came into a private pediatric practice and an urban ambulatory practice in Seattle and none of 225 children with acute diarrhea had Shiga toxin-producing *E. coli* in their stool. If you want to find Shiga toxin-producing *E. coli,* ambulatory and low acuity settings are probably not the right place to go.

ERs are probably a better place to study these organisms. If we think that they cause serious disease, we should go to where serious disease presents. Eileen Kline (ph.) has assembled two cohorts, one 1998 to 2001, the second 2003 to 2005, where she examined all children coming into the Seattle Children's Hospital Emergency Room with

diarrhea who would allow stool to be obtained. Now only about a third of all patients who are coming to the emergency room for diarrhea could we get a specimen from. They didn't produce the stool, and then only a subset of those allowed Eileen to even get a swab. Stool is a very hard analyte to get and to study. But despite this, about 0.7 percent of all children were infected with a non-O157 Shiga toxin-producing *E. coli.* 1.7 percent in these cohorts were infected with O157.

The most intense analysis was from her initial study where she looked at 1,626 stools and 39 of them gave a positive signal in that broth. Of those 39 positives, and I see people taking a lot of notes, I will have handouts ready by the end of the day or send it or post this on the web. So don't worry about trying to get all the numbers down.

Of those 39 toxin positive organisms, 25 of them were *E. coli* O157. Ten were not O157. One was a non-O157 co-isolated with *Campylobacter,* hard to know which was the pathogen, and three signals yielded no Shiga toxin-producing *E. coli.*

These are the serotypes in Eileen's study. Most all of these were on the list that Patty just mentioned. So we're starting to show that large databases and focused databases are converging on similar serotypes.

Of the 39 Shiga toxin-producing *E. coli,* of the 11 children with non-O157, none developed HUS. Eighteen percent of the children with O157 developed HUS. Half of the children with non-O157, bloody diarrhea, almost all of the O157 children, had bloody diarrhea. Similar data emerged in our follow up study.

Let's look at children with bloody diarrhea and adults with bloody diarrhea. A recent study came out of Michigan which used a network of approximately 20 laboratories soliciting all bloody stools and looking at what was in those organisms, not unreasonably thinking this would be a good place to find non-O157. However, of the seven STEC from grossly bloody stools, six were *E. coli* O157. In the expanded portion of that study, where they did not use the cut point in blood versus no blood, 177 *E.*

coli O157 were found, 18 non-O157s, O45, O103 predominated.

In this study, as in our other studies, about 5 to 10 percent of toxin assays, failed to detect *E. coli* O157:H7. So relying purely on Shiga toxin assays at the point of care, we'll miss some O157s.

If you are going to focus on bloody diarrhea in further studies, remember don't ask the laboratorian, is there blood? Ask the patient or the family. Laboratorians cannot detect visible blood. Blood should not be the index in stool for screening for *E. coli* O157:H7. And furthermore, when there are no barriers to culture, many, perhaps most non-O157 Shiga toxin-producing *E. coli* are associated with non-bloody diarrhea.

Let's focus on HUS. Multiple studies, this is really just a subset of them worldwide, demonstrate that *E. coli* O157:H7, easily detected with Sorbitol MacConkey agar plate is the biggest threat to children and to their kidneys. Maybe somewhat different in adults, maybe somewhat

different distribution in some other countries, but O157 continues to predominate. You want to find it. Don't wait for the child to get HUS. You need the pre-HUS cultures and as Patty just showed you, if the organism isn't there, the antibodies frequently are, but get them quick. They won't be around three or four months from now. If a child comes in with HUS today, they are short lived. Get them within a month.

And remember, that absence of proof is not proof of absence. If you didn't get an O157 out of a child with HUS, doesn't mean that something else caused it. We probably missed the O157 for whatever reason.

Conversely though, this target population is probably the best place to assay or has the highest yield for finding non-O157 Shiga toxin-producing *E. coli.* My estimates are that between one and five percent of childhood HUS in this country is caused by non-O157 Shiga toxin-producing *E. coli.* If you look at any other population, your yield in finding non-O157s are going to be under one percent.

So if you really want to get a collection, HUS is really the place to go.

If you want to go to the lab, these are some studies that have been performed over the past 10 to 15 years. The first study was before the Meridian toxin assay became available. We probed nearly 500 stools in Seattle. Approximately one percent had non-O157 Shiga toxin-producing *E. coli.* Only one of those children had bloody diarrhea. Four did not. However, three of the five were sufficiently ill to be hospitalized. So it is clearly another kind of illness that these organisms cause. *E. coli* O157 predominated in the early 1990s as it continues to do.

Closer to here in Falls Church, Virginia, several studies have been posted by Choong Park in the 1990s and in Milwaukee by Sue Kale (ph.) and colleagues, O157 was similar to non-O157 or exceeded the non-O157s in these toxin-based assay studies, once the Meridian toxin EIA came on line.

Same way with Children's Hospital over the last four years, approximately 25 percent of children

with O157 developed HUS. There was about a two and a half to one predominance of O157 to non-O157. Again, almost all the serotypes that Patty talked about are found in this group. Using toxin assay alone, we do both, but using toxin assay alone, we would have missed three O157s.

A recent study from a consortium of microbiology labs in Falls Church, Atlanta and Salt Lake City, studies 711 specimens using a new toxin amino assay. Nineteen were *E. coli* O157. Eight were non-O157s. Serotypes were not demonstrated.

Finally, let's look at state level data. This is a two-year study in Montana. There all the microbiology laboratories in Montana in a study funded by the Centers for Disease Control were asked to submit their specimens for toxin assay testing, and for *E. coli* O157:H7. O157 is usually bloody. Non-O157s about half the time were bloody. O157s caused more ER visits and were associated with more procedures. Again, the usual suspects of serogroups is emerging.

In Connecticut, a recent report in MMWR,

demonstrated again the predominance of O157 and again the usual suspects at a state level of serogroups of four non-O157s. HUS was not defined in this study but it was about 10 percent in children with O157, none with a non-O157s. It doesn't mean that it cannot occur. One needs to be careful though when defining HUS because unless you use a stringent and easily referable definition, you can skew your data one way or the other as to whether or not these organisms cause HUS. I think we need standardization. Similar data from Nebraska published seven years ago in emerging infectious diseases.

To wrap up, over the past 15 years, I've been involved in approximately 100 cases of children with hemolytic uremic syndrome. We've gotten better with the microbiology at the point of presentation and we've gotten very aggressive about getting the plates sent to us with the patient when they develop kidney failure. We've gotten *E. coli* O157:H7 out of about 90 percent of those patients. About five percent of them, we never got *E. coli* O157 from their

stool, but they shared a household or an outbreak with a child who was cultured positive for *E. coli* O157 and in four percent we found nothing. I had found only one child in these past 15 years infected with a non-O157 and that was an O111 that developed post-diarrheal HUS.

So to summarize, human exposure to non-O157 Shiga toxin-producing *E. coli* is probably common. Association with disease is relatively rare. Exposure to O157 Shiga toxin-producing *E. coli* is probably less common. Food is not very contaminated with this organism fortunately. The burden of disease still remains greater but we cannot be complacent enough that non-O157s will not emerge here and now is the time to try to get ahead of it.

In the meantime, diagnostic resources should still focus on *E. coli* O157 in the United States and in children. We need to address the non-O157s that predominate that Patty described. I think we need to be on the lookout for *E. coli* O113. It's potentially quite virulent and has been found in Canada. And fortunately, the Sorbitol fermenting

O157s are not yet in the United States.

We need to determine what is the HUS rate for individual serotypes. We're sort of converging on a 1 to 5 percent likelihood from a variety of data but remember series might be biased by focusing on HUS. And we also need to determine with greater certainty what is the source of pathogenic non-O157 Shiga toxin-producing *E. coli* especially outside outbreaks.

Others today will talk about this locus or this assay. I will defer to them, but as you listen to their data, remember the toxin assays, while they might be okay clinically will bring up a lot of background if we start testing food in the environment. Organisms that contain this gene are ubiquitous. I certainly am much more worried about Shiga toxin 2 but Shiga toxin 1 positive, Shiga toxin 2 negative strains have caused diarrhea, bloody diarrhea, and HUS. Intimin, which is encoded by eae must certainly be included in any sort of a definition of these organisms, but *E. coli* O113, quite virulent, does not contain intimin. And if one

wants to think about O antigen targets, the ones that Patty mentioned, should certainly be on the list of focusing either by antibodies to pull these bugs down out of polymicrobial outgrowths or RFB low side, but I would also like to add in view of its virulence some attention be paid to *E. coli* O113.

So I think that's it. Thank you very much.

(Applause.)

DR. GOLDMAN: Thank you, Dr. Tarr, very much for adding to the earlier presentation on the epidemiology of these non-O157 STECs. Again, we'll have our panelists come back up here after we have this last presentation here.

Sharon Hurd comes to us from Connecticut. She's one of our FoodNet partners, and she's going to bring us the state perspective. She has a clinical microbiology background and has worked in a number of Connecticut's clinical and hospital labs throughout her career. She has been the Project Coordinator for Connecticut's Emerging Infections Program and FoodNet Program since 2001, and in that role is responsible for coordinating the activities related to active

surveillance and epidemiologic studies. She's the liaison for the clinical labs and the public health lab in Connecticut. She's also served as a reference for other EIP projects outside of FoodNet as well. And we welcome Sharon Hurd to the podium. Thank you.

(Applause.)

MS. HURD: Good morning. Today I'm going to present some Connecticut data on the trends and epidemiologic features of Shiga toxin-producing *E. coli* infections.

As we've already heard, STEC infections are an important public health problem with *E. coli* O157 being the most widely recognized STEC in the United States. Clinic based studies have suggested that infection caused by a non-O157 STEC may be as prevalent as O157. Importantly though, as we've heard, standard culture methods do not detect non-O157 STEC, and laboratories do not routinely culture for non-O157. As a result, the incidence and trends of non-O157 STEC infection are not as well established. Increasingly, we've found that clinical laboratories are using assays to detect Shiga toxin.

This provides an opportunity to evaluate the occurrence of non-O157 and to monitor the trends over time.

The objectives of this presentation are to describe the Connecticut Shiga toxin surveillance system, and then present an analysis of our first seven years of data.

I'm going to begin with a short summary of an outbreak in Connecticut that immediately preceded the addition of Shiga toxin related disease to the State Reportable Conditions List. Then I'll continue to describe the frequency of non-O157 STEC compared to O157 STEC, describe some trends in the incidence of STEC infections over our past seven years of surveillance, describe some clinical and epidemiologic features of non-O157 versus O157 infections, share some preliminary data from the STEC lab survey and make some recommendations based on our findings.

By 1999, it was noticed that several clinical laboratories in Connecticut were using Shiga toxin testing in place of culture for O157. At the

time, Shiga toxin positive results were not reportable and isolates were not available for further testing, such as serotyping or PFGE. This was an EPI problem since the ability to both detect or investigate outbreaks could be severely limited.

In July of 1999, follow up of routine surveillance reports of children with hemolytic uremic syndrome identified a small cluster of three cases of HUS, all of whom had spent overlapping time at a Connecticut lake community. Further investigation led to a cohort study and an environmental investigation. In total, 11 cases were identified, including the 3 cases of HUS. The diarrhea illness was found to be associated with swimming in the lake and swallowing during a specific time period in July.

The isolation of *E. coli* O121:H19 from a toddler who swam in the lake, prompted health officials to test for *E. coli* O121 antibodies in the other cases implicated in this outbreak. Six of the cases had significant antibody titers to *E. coli* O121. This outbreak might have been detected sooner

had Shiga toxin screening been routinely conducted in HUS cases. This was the first outbreak of non-O157 STEC in Connecticut and to date the only cases of HUS attributed to non-O157 STEC in our state.

In Connecticut, *E. coli* O157 has been reportable since the early 1990s. In 2000, Shiga toxin positive tests were also made laboratory reportable. Clinical laboratories doing Shiga toxin testing are required to submit their positive Shiga toxin broths to the state laboratory for confirmation and culture.

At the state lab, the broths are plated on SMAC, or Sorbitol MacConkey agar and CT-SMAC agars and incubated for approximately 18 to 24 hours. At this time, Sorbitol-negative colonies are tested with an O157 agglutination test and if positive, further testing is done to determine the H antigen. However, if O157 negative Sorbitol-positive colonies and a sweep of the plate are also tested for Shiga toxin. All non-O157 isolates are sent to CDC for serotyping. Of note, our state lab does have the capacity to do some preliminary identification of the most common

serogroups.

Between 2000 and 2006, a total of 478 STEC confirmed infections were identified in Connecticut. Of these, 214 were identified from O157 culture isolates and 264 were identified from Shiga toxin positive broths submitted to the state lab. Among the 264 Shiga toxin positive broths, 40 percent yielded O157 and 60 percent yielded a non-O157 STEC Isolate. We found 24 different serogroups identified from the 159 non-O157 STEC isolates.

Overall in Connecticut, incidence of all STEC infections declined 45 percent from 2.9 cases per 100,000 population in 2000 to 2.2 cases in 2006. Incidence of *E. coli* O157 has also declined 52 percent. However, the incidence of non-O157 STEC increased 150 percent.

This table shows the trends over time in the percentages of STEC infections that were O157 and non-O157, and the percentage found by Shiga toxin testing. The overall percent that were O157, which is highlighted in pink, tended to decrease over time, from 87 percent in 2000 to 55 percent in 2006. Most

importantly, the percentage of all STEC found directly as a result of Shiga toxin testing, which is highlighted in yellow, has significantly increased over time. However, among Shiga toxin broth isolates, the percentage that were non-O157 has been consistently greater than 50 percent since 2001, but no significant trend over time. Another significant trend, which is highlighted in blue, is the overall percent of O157 isolates that we have gotten from broths.

To assess whether the increase in non-O157 incidence over time, which is shown in red, may be related to an increase in the number of laboratories performing Shiga toxin testing, we also examined trends of O157 found through Shiga toxin testing. The yellow line shows the percentage of all O157 found through Shiga toxin testing, increasing significantly from 23 percent in 2000 to 56 percent in 2006. This suggests that the increase in non-O157 incidence is likely due to the increase in Shiga toxin testing.

As we've heard from the two previous

speakers, this table shows the top 6 non-O157 STEC serogroups and in Connecticut, group O103 and O111 together account for 40 percent of all non-O157 isolates. The top six serogroups have not changed over time in our state.

I'm going to focus now on a little bit of the epidemiology of STEC infections in Connecticut. STEC patients reported between April 1, 2004 and December 31, 2006, were interviewed regarding symptoms and potential exposures. Differences between patients with non-O157 and those with O157 STEC were assessed.

In terms of the relative severity of disease, O157 patients were three times more likely to be hospitalized. This was a significant finding. Additionally, O157 cases were more likely to have developed hemolytic uremic syndrome or HUS. There have been no cases of HUS or deaths associated with the isolation of a non-O157 STEC. The outbreak in 1999, those HUS cases were identified serologically.

This table shows a comparison of symptoms reported by patients who were interviewed. When

compared to O157, non-O157 patients were significantly less likely to have bloody stool. This is also true for both nausea and vomiting. There are no significant differences among the two groups regarding fever or other gastrointestinal symptoms.

No significant differences were observed between the non-O157 and O157 patients interviewed with regard to known risk factors for *E. coli* O157, such as eating hamburger or ground beef or visiting the farm or petting zoo. Neither eating out at a restaurant or international travel was significant. Interesting enough, there was a significant difference between the two groups when place of residence was examined. Those living in suburban areas were more likely to have O157 as opposed to non-O157. While we can't explain this, it's important to note that the place of residence is self-reported by the case and not verified by census track data.

As Patty mentioned earlier, an STEC lab survey was conducted in all the FoodNet sites earlier this year, to determine clinical lab practices

related to STEC testing. The survey addressed practices related to both culture and non-culture based testing and included media used, methodology and circumstances for testing. Questions were asked as to whether the specimens were tested routinely upon physician request, whether the laboratory noticed blood in the stool, seasonality or by age of patients.

668 labs reporting in FoodNet sites were surveyed, and the analysis only included those labs that reported testing on site for STEC. Preliminary data showed that 65 percent of the labs surveyed do on site testing for STEC and that the majority, 92 percent, still do culture based testing. It's interesting because most of the labs do EIA testing and a few of them have mentioned that in the preliminary data, that they're very interested in the new test that has just come out, a lateral flow method test that can distinguish between Shiga toxin 1 and Shiga toxin 2 and I think other lab people may be able to expound upon this later.

Because Connecticut's a FoodNet site, we

also conducted the same lab survey in our 32 clinical labs. Eighty-four percent of our labs do tests on site for STEC and 55 percent do only culture based testing, and 45 percent do non-culture based testing for STEC. All use EIA methods, and many expressed interest in using the new lateral flow test.

In looking at both the lab survey in all the FoodNet sites and Connecticut, one thing that was interesting to note which has been touched upon here this morning, is the use of both culture and non-culture based testing simultaneously, and we found that in Connecticut, as well as in all the other FoodNet sites, very few labs do simultaneous culture setting up both non-culture based and cultured based methods at the same time.

Since 2000, when the clinical labs in Connecticut were required to begin reporting positive Shiga toxin results, and submit all the positive broths to the Connecticut Public Health Lab, we've seen an increase in the number of labs in our state that are doing Shiga toxin testing. Since the survey was completed, two additional labs began using a non-

culture based EIA method, bringing the total number of labs in Connecticut performing some type of testing which would capture non-O157 STEC to 52 percent of the labs.

In conclusion, an increasing number of clinical laboratories are conducting Shiga toxin testing, and we found that by 2006, 56 percent of all O157 isolates in Connecticut were found through Shiga toxin testing.

Second, in Connecticut, the overall incidence of O157 has declined while the incidence of non-O157 has increased. This increase in non-O157 incidence is likely due to an increase in the Shiga toxin testing.

Third, positive Shiga toxin tests are consistently more often associated with non-O157 STEC than with O157.

And finally, while the severity of illness from non-O157 appears to be somewhat milder, there also appears to be no differences between the non-O157 and the O157 in frequency of exposure to known cattle-beef risk factors associated with O157.

We've also learned that diagnostic testing has an impact on public health activities. Surveillance activities are an important component for outbreak detection and disease prevention. Isolates are extremely important to success investigations, and clinical laboratories definitely are increasing their use of non-culture based methods.

Based on our seven years of surveillance, we would like to make the following recommendations. Clinicians should consider non-O157 STEC infection when evaluating patients with diarrhea. Continued education regarding the ordering and interpreting of these test results is necessary. Clinical labs currently only culturing for O157 should definitely consider also using Shiga toxin testing.

Given trends in clinical practice, public health departments must assess and assure that all labs doing Shiga toxin testing follow up positive Shiga toxin tests with either a culture for O157 and/or shipping the broths to the public health lab for isolation of an organism.

Based on our experience, we feel that it is also feasible for public health labs to conduct surveillance for non-O157 STEC assuming that CDC has the capacity to provide supportive serogroup identification. Ongoing surveillance for both O157 and non-O157 STEC is needed to better describe trends and the epidemiology of STEC infections. And periodic surveys of clinical laboratories are also necessary, to follow changes in the testing methods that could explain trends in STEC isolation.

I'd like to acknowledge the following, the Emerging Infections Program and FoodNet, the Connecticut Department of Public Health and CDC's Foodborne and Diarrheal Diseases Reference Laboratory for their help in this. Thank you.

(Applause.)

DR. GOLDMAN: Thank you very much, Ms. Hurd, for that very detailed and in depth state perspective, and again contributing to the overall understanding of this group to the epidemiology of non-O157 STEC.

Our panelists are back here on the dais and

we have 15 or 20 minutes to entertain questions to individuals here or comments, and I'll remind those of you here in the room, we do have people on the phones. So I'll go around to them after we take a few questions here in the room. And please identify yourself and your affiliation. Sir.

DR. PARK: Choong Park from Inova Fairfax Hospital, just 10 miles away from here.

I'd like to share our experience with this organism in the past 11 years in our clinical lab. Since 1996, we started testing a -- on all stools, regardless of physician's order, and these are our findings. I hope that this might interest you. We isolated, recovered 134 unique patients during 11 years period and 48, approximately 36 percent were non-O157. They were confirmed by CDC, and half of them had a bloody diarrhea, but no HUS. Today, since January to October 2007, we recovered 13 -- 13 patients, 5 out of 13, about 38 percent, were non-O157. Another interesting phenomenon was three patients from non-O157 had concomitant pathogens, two *Salmonella* and one *Campylobacter.* Thank you.

DR. GOLDMAN: Thank you. Nice to have a report on the local experience here. Any comments? I think that's consistent with what we heard, perhaps a little higher percentage of non-O157s than the ratios that were reported here a minute ago.

MS. WARREN: Wendy Warren, Food Safety Net Services. A question for Dr. Tarr. You indicated in your presentation that there were several samples that would have been missed just by looking for toxin alone. If I'm understanding the diagnostic tools correctly, that would be a protein-based test. I wonder if you could comment or have any information as to whether they were possibly missing the gene or if they did contain the gene, just not expressing the protein.

DR. TARR: Very good questions. Two of those isolates were tested intensively and they were quite toxinogenic. The other two have not -- one just came out last week, and the other one was not collected in a protocol that enabled us to continue testing it.

MS. WARREN: Okay.

DR. TARR: So I think that it was performance failure inside the polymicrobial broth.

MS. WARREN: Okay.

DR. TARR: Gene was present. Toxin was made. Test was negative.

MS. WARREN: Another question, too, I'm wondering what everybody's thoughts are about the non-H7 O157.

DR. TARR: We'll both give our opinions. About three to five percent of O157 -- a lab gets a culture, Sorbitol non-fermenting O157 antigen positive, North America, you probably have a pathogen. The H7 testing is optional. That can be done in due course. About three percent, five percent of isolates nationwide from the CDC's study and I think local experience also bears that out, will not have a detectable H antigen. They're nonmotile. These are different than the German Sorbitol fermenting nonmotiles. In my personal opinion, the H typing has no bearing clinically.

MS. WARREN: Thank you.

DR. GOLDMAN: Caroline.

MS. SMITH-DeWAAL: Thank you. Caroline Smith-DeWaal with the Center for Science in the Public Interest. First, I just wanted to alert people that our database, the Outbreak Alert Database is up on the website. Dr. Goldman and I were trying to get the capacity to actually demonstrate it during the break, but unfortunately George Mason doesn't have that, but we do have fliers for people who want to access the data.

I wanted to ask the panel and, Patty, in looking at our data, we had come up with somewhat more food related outbreaks from non-O157 *E. coli* than you had mentioned in your presentation. But the most striking thing to me in looking at the data that we have derived which is largely from CDC sources, but also they have to have a known identified food and an identified pathogen as part of our methodology for analyzing the data. But in looking at the data, what I've observed is that the size of the non-O157 *E. coli* outbreak seems to be larger, much larger. The average outbreak size is about 100 percent versus about 20, 24, 25 people for *E. coli* outbreaks at

least among the outbreaks we've identified. Have you observed that, and do you have any comment on what might be causing that? Generally when we see larger outbreaks, we tend to suspect that the public health community, the local public health community is having more difficulty identifying it as an outbreak. And so it goes on longer and causes more illnesses. But do you have any thoughts on that?

DR. GRIFFIN: Yeah, as usual, you're right on, Caroline, and as far as you having more outbreaks due to food, we just presented the outbreaks that have been reported to us. There may be some that health departments haven't reported to us, and we'll be soliciting them and reminding them that we want them all reported to us, not just the ones due to food, but the ones due to any source. And as far as the size of the outbreaks, if you look back at the first 10 years of O157 outbreaks, we had all these big, scary outbreaks and those were just the ones we found. The smaller outbreaks were going on. We just weren't that good at finding them, and I think we're in that same stage with non-O157 outbreaks.

MS. SMITH-DeWAAL: Thank you.

DR. GOLDMAN: We have a couple of more folks in the room coming to the microphone, but I want to turn to the phone and see if there are any calls or questions from the callers.

OPERATOR: Thank you, sir. If you have a question on the phone line, please press star 1.

Go ahead with a question in the room --

DR. GOLDMAN: Sir.

MR. WALLACE: Morgan Wallace from DuPont Qualicon. Please correct me if I'm misinterpreting the data, but it looks like from the Connecticut data, there was a 100 percent culture confirmation rate from the EIA positive cultures that were sent to you guys. Is that correct? And if so, is that typical of the state public health labs? In other words, it looked like you got an isolate on a plate of the STEC for every EIA positive culture that was sent to you.

MS. HURD: No, those numbers that I gave you were just the culture confirmed numbers. Occasionally we do get a positive broth that comes

into the state lab that we cannot get an isolate from, in which case we send those down to CDC for PCR and confirmation. Whether they may be false positives. If our state lab cannot confirm them as Shiga toxin positive, you know, we use our state lab as the goal standard, but if they confirm it as a Shiga toxin positive, with no STEC isolate, then it does go to CDC for PCR.

MR. WALLACE: And what percentage or just a gut feel of how that breaks down in terms of proportion?

MS. HURD: It's probably I would say probably less than five percent. We don't get that many that we do not get a culture isolate from. But again, as more labs are doing the Shiga toxin testing, we're finding that we are getting, you know, it is progressively increasing, and that is something that, you know, needs to be addressed.

DR. TARR: We find that when we apply this to an emergency room population, we have about a five percent, seven percent can't get an isolate rate. When it's applied to patients presenting at the

doctor's office, the proportion of signals that go up, that an isolate is never produced is much higher.

MR. WALLACE: Thank you.

DR. GOLDMAN: Dane.

DR. BERNARD: Thank you. Dane Bernard with Keystone Foods. First, thank you, to all of you for your presentations, a very informative session.

As we go forward and try to develop more rapid testing platforms for this, one of the factors that we're particularly interested in is whether there are other attachment factors other than those coded for on the eae gene that are significant here. I wonder if any of you have any comment on that?

DR. TARR: I don't think that current data can lead us right now to a sensitive and specific formula for detecting these organisms prior to ingestion by humans. I know that at least two of the talks later today will discuss pathogen specific virulence factors including the adhesines.

DR. BUCHANAN: Just a quick question. I think this will go to Phil or maybe Patty or any of you really.

DR. GOLDMAN: And this is Dr. Buchanan from the FDA.

DR. BUCHANAN: Dr. Buchanan, FDA. What's the carriage rate of STEC in stools of otherwise healthy patients? What's your baseline?

DR. TARR: We have a little bit of data from a USDA sponsored study where we collected approximately 600 stools from children without diarrhea, and one of them had a non-O157 toxin producing *E. coli.* I don't know what serotype. None of about 600 baseline stools in the Wernicke studies had non-O157 or O157 toxin producing *E. coli.* So it's low.

DR. GRIFFIN: I reviewed all the literature and I did that review probably 15 years ago and published it. I'm not sure I exactly remember but at that point, it was less than one percent but you do find them in healthy people. Many of the reviews at that time at least didn't serotype the STEC. So you didn't know what they were finding in those healthy people. You have to expect that it's common in the food supply, and what's in the food supply, we eat

and comes out in the stool. So you expect to find some STEC in healthy people.

DR. GOLDMAN: Let me ask the operator again if there are any questions on the phone?

OPERATOR: We have a question, sir. Pat Buck, Center for Foodborne Illness, your line is open.

MS. BUCK: Hello, my name is Pat Buck, and I'm from the Center for Foodborne Illness Research and Prevention, and I have a general question for the panel. Listening to all of the discussions which I greatly appreciate, it seems to me that there is a higher prevalence for the non-O157:H7 pathogens in the food or exposure to the public. If we have the capability to declare one of the big six an adulterant in food, would that be helpful or would it be more helpful to put in place a requirement that the lab test for the Shiga toxin? Do any of you have an opinion on that?

DR. GOLDMAN: The last part of the question was whether it would be more helpful to just have labs test for the presence of Shiga toxin?

DR. TARR: In food, in product or clinically?

DR. GOLDMAN: Pat, was your question about food testing or clinical human testing?

MS. BUCK: Well, I'm trying to get at what is it that other people can do to be helpful in getting the information to the agencies that can actually conduct the research. Would it be more helpful to have one of the non-O157 strains declared an adulterant so that we could have more testing done on it, or would it be more useful to simply say that we are now going to test at the labs for Shiga 1 or Shiga 2? Because it seems to me that the Shiga 1 is not as concerned with as the Shiga 2.

DR. GRIFFIN: I don't have the answer to the question, and I think what would be more helpful would require an analysis of the cost of testing, what would need to be tested, what would be found. We haven't listened to data on what's typically found in ground beef samples and what are the Shiga toxin profiles that you find in ground beef samples. So that's a big question and it involves a lot of work

to go into that answer. I think the data that you've heard presented this morning indicates that we're much more worried about strains that have Shiga toxin 2 than about those that have Shiga toxin 1, and that there are certain particular serogroups at this point that, and O111 in particular, that causes a lot of illness and causes more HUS than many of the other non-O157 serogroups.

DR. TARR: I concur. I think that if you use toxin as your canonical index prior to an organism getting into a human, you're going to find an awful lot that doesn't show up in the human populations. The big six are what we're seeing clinically.

MS. BUCK: In other words, you really don't have the capability of knowing the answer to that question?

DR. GRIFFIN: I wouldn't say that we don't have the capability of knowing the answer. I think part of the answer lies in what are the virulence profiles and the serotypes of the organisms that you find in ground beef, what sort of testing would need

to be done, and therefore what tests would be most sensitive and specific to find any organisms that are most likely to cause human illness. A lot of that data may be available. I don't have it.

DR. GOLDMAN: Are there any other questions from the phone?

OPERATOR: Yes, sir. Lora Dawson, Food Physics and Body Dynamics, your line is open.

MS. DAWSON: Thank you. Good morning, ladies and gentlemen. It's a pleasure to be able to ask my questions. I was an individual that contributed with the United States Department of Agriculture, Center for Policy and Promotion for the food guidelines distributed in 2005 to the American public. So I'm very concerned about food consumption at a table level.

One of the three questions I have to address are first of all, has anyone considered the use or misuse or microwave cooking since it began in merely the 1980s, and the elevated consistency of statistics associated with *E. coli* infection seems to have inflated since that date, and I realize that a

number of people that I have witnessed, do not know the proper use of reheating or cooking in the event that they are using microwaves?

And then secondarily, I'll ask the two questions combined so that the person most able to address can choose. Second of all, the Shiga toxin, either non-Shiga toxin or non-toxin or the Shiga 1, do they respond to chlorine baths at the time of storage or pre-usage if they are on produce, say lettuce and ice, as was mentioned in the cheerleader evidence?

And also, the last item, there is a -- light being distributed by -- Company and it is fairly good at releasing toxins, negative ionic toxins through feces and urine. Has anyone taken a look at that as a treatment protocol for this particular Shiga toxin? I think that's the end of my questions.

DR. GOLDMAN: Thank you. That's three questions in one. Any comments on the issue of the contribution of microwave use to this problem?

DR. GRIFFIN: Yeah. I appreciate the

thoughts on this. We do have concerns about microwave cooking. For example, we just had a big *Salmonella* outbreak due to pies in which part of the problem was that the directions for microwaving didn't require enough time in the microwave to kill the *Salmonella.* So I think it's a legitimate concern.

When we've looked at outbreaks, we do ask about how people cook things, and we haven't found many and perhaps -- I can't think of one outbreak due to O157 that was associated with inadequate microwave cooking, not to say that this hasn't occurred, but we don't have any indication that this is a big problem and is in a large part responsible for our non-O157 or O157 problem. I think that a lot of what we see with contamination of foods relates back to factory farming.

MS. DAWSON: Thank you.

DR. GRIFFIN: As far as the chlorine bath, studies that I haven't done but I've read, that have been performed by food microbiologists, indicate that almost anything that you can do to a food including

putting it in chlorine and washing it in running water or putting any of these commercial sprays on it, will decrease the contamination by about 1 log but that's about all you can do other than cook it. So if you're planning to eat something raw, you can reduce your contamination by just about a log.

And as far as the light or ionic treatment, I don't know about that.

MS. DAWSON: All right. Thank you so much for addressing my questions, and I don't recognize your voice.

DR. GOLDMAN: That was Dr. Griffin speaking.

MS. DAWSON: Thank you so much. Have a good day.

DR. GOLDMAN: Okay. Thank you very much. I think we've come to our break time. We will have a 20-minute break, and we will reconvene promptly within the next panel. Thank you. And thanks to our panelists for the first session.

(Applause.)

(Off the record.)

(On the record.)

DR. GOLDMAN: Okay. In this next session, we will turn our attention to some of the research that's been underway in ruminants, ruminant animals as well as the session has two sub-components. The other sub-component will be attention to some of the testing hurdles that exist, testing and isolation and detection hurdles that exist in labs. So we will begin this with the focus on ruminant animals and we're pleased to have a panel of four experts in this area, in the two areas I mentioned.

We'll begin with Dr. Mohammad Koohmaraie, who is the Director of the U.S. Meat Animal Research Center in Clay Center, Nebraska. This Center of ARS is one of the largest of the 100 laboratories of ARS and has an operating budget of $23.8 million.

Dr. Koohmaraie received a BS Degree in Animal Science from Pahlavi University in Iran, and his MS from Texas A&M and a Ph.D. in Animal Science from Oregon State University. As a scientist, Dr. Koohmaraie's research efforts for the last 13 years have helped focus on the control of foodborne

pathogens in the red meat supply in this country, and under his leadership, the U.S. Meat Animal Research Center's Safety Team has made numerous scientific contributions such as demonstrating that steam vacuuming can replace knife trimming in the plants and also that enumeration methods, he's contributed to the enumeration methods for *E. coli* and *Salmonella.* So please welcome Dr. Koohmaraie.

(Applause.)

DR. KOOHMARAIE: Thank you, David. Good morning, ladies and gentlemen. I'd like to thank our colleagues from FSIS for inviting us to be here and share with you some of the work that we've been doing for the last, over a decade with respect to non-O157 Shiga toxin-producing *E. coli.*

For my time, what I'd like to do is give you a brief introduction, give you our perspective on non-O157 Shiga toxin-producing *E. coli,* give you the highlights of some of the work that we have been doing over the last decade with respect to prevalence of non-O157 STEC. In that context, talk about the efficacy of the interventions that are used by the

processing plants and finish up by summarizing the highlights of the presentation and some concluding remarks.

First, I was hoping someone would have it done by now, but since it was not done, unless for the one person in the room that does not know how we come up with these names, this is an illustration of *E. coli.* The main body is the cell. There's a component, a cell called lipopolysaccharide. We characterize that for the O antigen and there's a component, the flagella, which gives bacteria some mobility. We characterize the protein in that. That's how we come up with H typing. There's some 170 O type and some 57 H type. So the combination of O and H gives you all these weird names that we come up with O111, O157, et cetera.

With respect to our perspective in the Agricultural Research Service, we work very closely with industry to solve problems, at the same time collect data so that the regulatory agency, which our customers, whenever they make policy, those policies are rooted in science. So for any pathogen, what we

do, essentially three things. We do a lot of studies to determine the prevalence, assess the prevalence. Then we ask the question is what the industry is doing controlling the pathogen, and the last thing we do, we do national surveys of the product, and I'll go through the same set of data for you with respect to non-O157 STEC.

Again, non-O157 STEC maybe hit the news media lately but we have been working on this as I mentioned to you for over a decade, doing a lot of prevalence work, determining the efficacy of interventions, et cetera.

As has been said repeatedly, there are lots of *E. colis,* a lot of O157 STEC but only a fraction have the ability to cause the disease, and I'll describe for you how we assess for that.

It is very important to recognize that STECs in general are part of the normal microflora ruminants and they pose no threat to the animal. The intervention that we use intuitively, one would think it will be equally effective with non-O157 and O157 because they're both *E. colis* and I'll show you some

data with that respect.

There's been a lot of talk about the methodology. It's certainly very complex. We have made great improvements at Clay Center and the scientific community as a whole, and whatever we have done, we'll be obviously more than happy to share with anyone.

I hate to bore you with methodology, but I think the audience needs to understand the complexity of the methodology. Again, I was hoping this would be done by now, but since it was not done, I will go through it very quickly. Most of these pathogens that we deal with in our product, whether it's carcass swab or feces or hides, except feces and hides, or ground beef, there is such a low concentration that current methodology cannot detect them. So what we have to do, we have to give them the proper environment to increase the concentration of bacteria, bring it to the level that our detection methodology can detect them.

Now this is what we call enrichment. So essentially we add food the bacteria likes, we put in

environment the bacteria likes, and make the bacteria happy. They'll grow, and after proper growth, now we can detect them.

After we have done the overnight enrichment, we take an aliquot of that enrichment, we test for Shiga toxin. We ask the question, is any bacteria in that culture has the ability to produce Shiga toxin, and we use something called a multiplex PCR which has all these pathogenic determine and we focus on STX1 and STX2.

Now if you find STX1 and STX2, the next question is there are hundreds of bacteria in there. How did we go about finding out which one is producing STX?

So what we do next, we do the so-called colony hybridization. We take an aliquot of that enrichment, plate it on agar media, and after overnight growth, we make a duplicate of that plate on a nylon membrane. Now we go and screen that nylon membrane for the presence of Shiga toxin. This plate had only one colony, and this plate was loaded. So we go back to the same plate, we matched this nylon

membrane with the plate and remove an aliquot and remove an isolate that we think has STEC. We do it again. After we pick the colony, we purify the culture, we reconfirm that what we picked actually still has the ability to produce Shiga toxin.

Once we got that, then as Phil said, there are a whole bunch of other bugs that has the ability to produce Shiga toxin. We're interested in *E. coli.* So we do by a chemical characterization to make sure that the bug that we have in hand is an *E. coli.* Once we got that, so we got a bug, it produces a Shiga toxin, it is *E. coli,* we then do serotyping. This is what they used to do until 2006.

We then became aware that you can use sheep blood agar to reproduce colony hybridization for a screening by colonies for Shiga toxin production. So this part of the test, which we were able to do 100 tests a week, we now have replaced it with the sheep blood agar which we now can do 100 tests a day. As many samples as we process in our laboratory, the faster the methodology, the more we can process. So only this part of it has changed.

So these are all series that goes through the process to come up with a serotype in hand. If you work continuously, it's at last 62 hours, but in reality, it's about a week or 2 to get the results. And again, I wanted to make sure you understand the complexity of the assay.

My colleagues at CDC and others have mentioned top six CDC. So whenever we do prevalence, we do a number of things. First thing we do, we serotype and then we ask the question, what's the frequency of seeing top six CDC. Then we also do virulence factor determination.

We have done a whole bunch of studies last few years. We have been in commercial fed cattle processing plant. Fed cattle processing plant is a function of season of the year, and the cow/bull slaughter facilities, lamb processing facilities. We have looked at the microbial quality of the imported meat, and we're right now doing a national survey of the ground beef with respect to these pathogens and a whole host of others.

I'd like to take this opportunity to

formally thank all the members of U.S. Meat Industry for giving us their opportunity to get into their facilities and use their facilities as our laboratory. Without their help and their generosity, we would not be able to collect the data that them, FSIS and us we think has been really instrumental for making some significant changes, and I just wanted to thank them for giving us this opportunity to do that.

First set of data that I'd like to describe for you is what we did in 1999. At that time, our information about O157:H7, for those of you who have been in the field, was very rudimentary. We did not know where O157 is coming from. How does it get on the carcass? How does it find its way into ground beef?

So we went to four commercial processing plants, and we sampled feces and hides to tell us about the status of cattle as it's presented for slaughter. Then we sampled the carcasses sequentially throughout the process and we used DNA fingerprinting, pulse field, to determine the source of O157.

But then we went back to these same samples and asked the same questions about non-O157 STEC. For folks that are not familiar with the processing plant, it is important to recognize the process so you can put some context to the data that I'll present for you.

Cattle is presented for slaughter. It's humanely stunned and then they go through bleeding process. We usually take a sample right here, right before the hide is removed. That way we know the status of the cattle as it is presented for slaughter. And we then take a sample right after the hide removal. That way we know how good a given process in plant is in removing the hides, and we have done a lot of benchmarking, industry data comparison, they've learned a great deal from each other, to improve how they remove the hides.

So the data that I'll show you here doesn't show -- it's called before or pre-evisceration. When you see that, don't be alarmed. The numbers will be higher, but the reason for that is there's absolutely no intervention has been done at this point. Then it

goes through a whole bunch of process, whole bunch of intervention, and then we finally get the final which is after full complements of all the interventions.

So the data again, 1999, at that time we were not, neither us nor the industry were aware of the hide to carcass transfer. Forty-four percent of the carcasses were positive for *E. coli* right after the hide was removed. Right after full complements of all the interventions, there were six, and actually all of these six were from one plant in one trip. Non-O157 STEC, because there is so many of them, you expect it to be higher. It's 54 percent. After full complements of all the intervention, there were 27 carcasses, or 8 percent. This is very crude data but let's dig a little bit deeper.

The first thing we did, we did serotyping. So let me orient you with these slides. The title is not shown for these, but that doesn't matter. These are the serogroups that we identified in the study. The number of isolates represented each serogroup, their distribution before any intervention and after all intervention. And then the arrow shows where the

top six CDCs are.

First of all, we see the tremendous efficacy of the intervention that are used because there's a tremendous reduction as we go from pre to final. And second most important observation is that none of the top six CDC, we found them in these samples.

Again, the second state we look at, after we did serotyping, we asked about the virulence factor. I don't have to tell any STX1 and STX2 but, in fact, in reality, we don't really know what it takes for a bug to cause disease. You can't do the experiment. The experiment is to get the bacteria that have all these characteristics, give it to humans in this case, see who gets sick. Well, we can't do that. What we do, we learn from our colleagues from CDC and Phil and others, when people get sick, they go look at the bug, what was the characteristics that cause disease?

It is commonly believed that if a bug, if *E. coli* has STX1 or STX2, the eae which is the gene for producing intimin has the maximum likelihood of

causing disease. And again, as Phil mentioned, like in any area of science, there's always exceptions, but this will catch all of them.

So the next thing we do, we do virulence factor and see how frequently we see what. This is from the same data set. Again, these are the virulence factors. Those that can cause disease will be shown in a box, okay. So there was only 4, and these 4 actually came from 2 carcasses and 2 out of 326 carcasses had bugs, that had the proper virulence factor that has the ability to cause disease. That's about .6 percent and remember this number.

The next study that I'd like to mention to you is again the same thing. We went to commercial processing plant, and this time we looked at it as a function of season of the year. Again, this is the prevalence, a lot of it in feces, a whole bunch of it on the hides, gets onto the carcasses and then interventions are extremely effective.

There were literally thousands of isolates. We could not afford economically to serotype this. So we did the virulence factor. Again, we see

there's 39 isolates. Those 39 isolates came from 22 carcasses that has the ability to cause disease. Again, that's about .6 percent.

We also did enumeration. You have to have, be the right bug, have the right machinery to cause disease. It also has to be the right concentration. This is the duration for spring season, summer, fall and winter, the number sample we had and the concentrations. Spring, for example, 66 of them were less than 3 CFU per 100 square centimeter. One was 3.6, et cetera. So when we find them, it's extremely low concentration.

Lamb processing plant, we just published that data. Again, we did the exact same thing. The exact same model as beef, and we did that at the request of the lamb industry. We isolated and we came up with 846 isolates. There were 288 carcasses per plant, and we had 3 plants. We had 846 isolates, 488 isolates, from the carcasses after full complements of all the interventions. When we do virulence factor profile, none of the 488 had the ability to cause disease in human. When we serotype

them, none of the top six CDC serotype was identified in these 488.

The next one, again we were asked to help the industry determine the microbial quality of imported meat. We have done a whole host of it, but this one I'll talk to you only about non-O157 STEC. Currently, if it's changed, I'm not aware, but we import meat from Australia, from New Zealand, Uruguay. Typically we bring lean mean to give us the proportion, the right proportion of lean for domestic consumption. These are the number of samples that we process. Again, we looked at a whole host. That's published, but I'll only talk to you about the non-O157 STEC part. Nine samples from Australia which we got ten isolates, we get as many isolates from a given place as we can get. So 10 means one of those plates, we took 2 isolates instead of one, 4 isolates, 52 isolates, and 32 isolates.

When we serotyped them, again there were some serotype that is associated with causing disease in human, but only one isolate, one isolate was in the top CDC.

The last one that I want, we're in the midst of that actually. We are not finished. We have set it up with the industry, what they do is, this is the so-called BIFSCO map, Beef Industry Food Safety Council, and if you're not familiar with that, I encourage you to go to BIFSCO. That organization is an extremely effective organization. We have identified plants in each of these regions. They take a weekly sample, they put it in the freezer, and at the end of the month, at their own expense, they send the sample to us and we look at a whole host of bacteria.

So, so far, we have received 4,136 samples. Of those, we have processed 3668 sample, 960 of those were positive for Shiga toxin. There's 962. We have processed with 285, we were able to recover 1 or more isolates. Of the 285, we have processed 223. From the 223, we have been able to isolate 13 isolates that is top six CDC. These are the top six CDC that we found in these samples. One is O26, five O103, and seven are O121.

The next thing we did, we looked at the

virulence factors. Again, these are the virulence factors. There's only 4, 4 out of 13, that have the ability to cause disease, and it's actually STX1 and no STX2 in these samples.

In terms of summary, for this data, if you want to screen for STX, which I'm glad to hear everyone before me said don't do that, that means you're going to have to throw 26 percent of the product away and not needed but it's really irrational anyway. 5.8 percent for top six CDC and only 1.8 percent had the ability to cause disease but they only had STX1. Again, you know the STX1 versus STX2 by now.

In terms of summary, STX are the natural part of animal microflora. Some non-O157 STEC can cause severe disease in human. Non-O157 STEC are found at a very high frequency, the same or higher than the O157 STEC. Again, they are just as prevalent. I hope the data that I showed you convinced you that intervention used are equally effective. Why wouldn't they be? If they're effective against O157:H7, they're the same bacteria,

you would expect them to be equally effective.

A very small proportion of the non-O157 STEC, these numbers, 11.3 percent, 7.3, .4 and 2 percent from different studies that I mentioned to you, have the combination of virulence factor that provide the maximum likelihood of causing disease.

In 10,159 samples that we have processed, we have seen top 6 CDC in only 15 cases, and a small fraction of those have what it takes the machinery to cause disease.

To the best of our knowledge, there has never been a meat-borne non-O157 STEC outbreak in the United States and I hope someone will correct me, and if this is wrong, research of the literature have not been able to determine that.

Again, this is a highlight of the stuff. We have a lot more data obviously, and if anyone's interested, we'll be more than happy to share whatever data we have with everybody. Thank you.

(Applause.)

DR. GOLDMAN: Thank you very much, Dr. Koohmaraie, for that introduction to the research

that you've been doing, and I think people can tell from your presentation, that there's a lot more research that you could have reported on had you had more time. I think that's a nice overview of the research that your lab has been doing on these pathogens or these organisms in ruminant animals, and cattle in particular.

We're going to hear now from another researcher who's done a significant amount of work in some of the same area. Dr. Hussein Hussein is an Associate Professor of Nutrition and Microbiology at the University of Nevada Reno. His research focuses on the nutritional and microbiological interactions that support human health through improving quality and safety of farm animals, and he has specific emphasis on pre-harvest factors that affect the prevalence of STEC in ruminants.

Dr. Hussein's research has been supported by federal and private funding, totaling approximately $3 million, and we welcome Dr. Hussein.

(Applause.)

DR. HUSSEIN: Thank you, David, very much

for the introduction. Before I get started and talk about *E. coli,* I'm sure you need a break of that, I'd like to start by something personal, and that's really related to the meeting today.

When I came to the U.S., the first time was July 15 of 1984, it took me about 13 hours from Cairo to Washington, D.C. Yesterday and today it took me 22 hours to come from Reno, Nevada to Washington, D.C.

(Laughter.)

DR. HUSSEIN: I also had a suit and tie, but unfortunately they're still in the air somewhere. So --

(Laughter.)

DR. HUSSEIN: But regardless of all of that, I'm not here about what, you know, it's not important what I wear today, but what's important really is the meeting which you can see I'm excited to be here, despite what happened yesterday and today, but the reason for the excitement is really very important, as a person who has been studying STECs for almost 10 years or more and has been

spending lots of effort in that area.

One of the things which have been on my mind during all of that time is why we are running behind the rest of the world on looking at all Shiga toxin-producing *Escherichia coli,* because the answer is very simple. The first outbreak in the U.S. started by O157:H7 in 1982. Since that time, all our interest has went on looking, every time somebody has diarrhea or cramps or any problems, we look for O157:H7. That's one reason.

The other reason is it's very easy to find O157:H7, very easy. Chemically you can just, biochemically characteristics are easy to follow and it doesn't cost much money and doesn't cost much time.

But the picture with regard to the remaining isolates or the remaining serotypes is very complex. And maybe that's one of the reasons we don't want to get there. And that gets me to the excitement about being here because I look at, as a student of studying *Escherichia coli* or pathogenic *E. coli,* I look at this meeting today with full

appreciation to the folks who put that meeting together because this is the first step in my opinion in the right direction. This is the first time we get people involved in the problem and sit down and talk about non-O157 STECs. So I appreciate their efforts in that direction and I believe that that will be a good start for all of us to work together and hopefully we can establish a database on non-O157 STEC in the U.S., highlight how important they are and hopefully find solutions to this problem.

But also very important issue is how can we work together to find methods which we can standardize and follow because I tell you right now, I listen to Dr. Koohmaraie and the way he does his isolation and detection, and I'm familiar with many other labs around the country and around the world, what they do. What we do in my lab is completely different than anybody else. So everybody has his own method of enrichment, detection and so on. But regardless, I'm sure we are working in the right direction.

So with that, I need to get started to talk

about what I'm here today. You heard about humans and related issues in the morning, and you heard some issues related to the source, the ruminant animal. So I'm going to focus on that today.

Here it says since the first outbreak, 1982, all the efforts have been just going in that direction with regard to O157:H7. So we need some progress with regard to understanding its prevalence. We also did need some progress and understanding the issues related to the infection, pathogenic factors and also there's lots of work has been done with regard to identification of pre-harvest or post-harvest methods to control this foodborne pathogen. Some of them have been successful and some of them have not. But we are still working in that direction.

But if you look around the world, obviously I can go on for hours talking about the outbreaks of non-O157 STEC around the world, but I just give you six examples today.

Look at Argentina. We find that these are -- you can see that these guys here, these are

serogroups which have been identified because this large size outbreak. There are larger size outbreaks worldwide in Japan and Italy and other countries, especially Europe, and Australia, too. All of them are non-O157 STEC based.

Italy, you can see O111:H-. That's a nonmotile isolate of O111. Canada you can see six cases and the pathogens involved, and none of them is O157:H7. You also can see that U.S., we had in Montana one time, 1994, four cases. Raw milk was the source, and O104:H21. Australia, you can see they have been working on that for quite sometime, and Karl Bettelheim has been really the leader in that direction. Spain, without I need to mention, Georgia Blanco there has been working in that direction for long time, and you can find a really good solid database with regard to non-O157 STEC in Australia and also in Spain and Germany, Beutin's work in that direction.

But you can see here beef, ground beef, beef sausage, raw milk, those are the vehicles again like O157 for these foodborne pathogens.

One of the things we need to keep in mind is they are pathogenic because they have some virulence factors. So they do produce Shiga toxin 1, Shiga toxin 2, alpha hemolysin, EHEC-hemolysin or intimin for the attaching -- for the intimin attachment to the intestine mucous. And all of these are produced or recorded by various genes.

I'll get to how important these are later on. But you heard today that some of these pathogens, you know, we always say O157 is pathogenic. These guys are less pathogenic. We can't really say that because there are too many factors involved in the pathogenicity. Lots of us get the worst nightmare of *E. coli* everyday and we don't get sick because our immune function is fine. That doesn't mean that it can't hurt somebody else. They can.

And the research has shown that you don't need all of these virulence factors to cause human illness. You can have one and that can do it. But it comes down to where they're coming from. Obviously STECs have been isolated from cattle,

sheep, goats, swine, horses, rodents, poultry, obviously humans. So they have been isolated from most animals.

But research has shown also that they are more prevalent in ruminants than any other animals, and among the ruminants, they are more prevalent in cattle. We can't also say that with 100 percent because we didn't test sheep or goats as much as we did test cattle.

The other thing here is the infection has been traced in most cases to the edible products from cattle or from water or leafy vegetables contaminated with cattle feces. So cattle remain as the key source in this problem.

We did summarize the published research on O157, non-O157 in the past 25 years worldwide. And all of that work has been published in five different reviews in the last -- four in 2005 and one in 2007. But the results from that summarization is you can see that non-O157 STEC in beef cattle, the prevalence raised from less than 1 percent to 70 percent, and the number of STEC serotypes isolated from cattle

hides or cattle feces, were about 341 and that's based on our calculations at the end of 2005. So I'm assuming there are more by now.

But the key issue here is looking at the three large databases with regard to pathogenic STEC around the world, that's one from the World Health Organization of the United Nation. The second is Bettelheim's database and the third is Blanco's database. You'll find that 36 percent of the 341 serotypes are pathogenic. So that's a very large number. O157 is just one. So you can tell here there's more than 100 other non-O157 as bad as O157:H7.

With regard to dairy cattle because, you know, beef and dairy, the range also with regard to prevalence went from less than 1 percent to 74 percent. The number of serotypes was much less, about 152, but 50 percent of those were pathogenic. So that's really important.

With regard to our work, our work was funded or got a big boost from the National Integrated Food Safety Initiative, and we are

appreciative to that, and our work was done between Nevada and California, but really most of our database was created from California as they do have larger number of animals, and that was key to come up with some kind of information we can count on. And I appreciate the work of our collaborators in California who made that possible with regard to the organization and collaboration to get everybody involved, and I also appreciate the work of my graduate students who did most of the lab work.

Our goals, the main goal was really to identify on-farm factors that influence prevalence of O157 and non-O157 STEC. To do that, we first needed to assess the prevalence, the human health risks with regard to the isolates we can find, and also identifying pre-harvest control measures in terms of how can we manipulate the prevalence.

The second objective was to really start or initiate a kind of transfer of the information to the people who are raising those animals, to help in minimizing the problem.

The work in Nevada, you can see the herds

are very small. But I'm not going to get into details about these but these are the isolates we were able to find in Nevada. You can see O157 and you can also see non-O157. The different color, the orange color is showing you isolates or serotypes which are known to cause human illnesses but especially HUS. So those are lethal in a way because they can cause death in humans. The ones in yellow can cause other human illnesses like bloody diarrhea, vomiting, cramps and such. So they're not as bad as the first set.

With regard to California cattle, we were able to do the work with larger operations. The number of cattle in total was about maybe or more than 3,000 cattle tested over one year, and we covered all the production systems. So beef cattle in feedlot, beef cattle on the range, beef cattle in grazing irrigated pastures and dairy cattle also. The most important piece of information here is the Shiga toxin-producing *E. coli* isolates from beef cattle in the feedlot, beef cattle on pasture, beef cattle on range and dairy cattle, belonged to 14, 13,

35 and 16 serotypes, respectively.

So the point here is you can see a large number of serotypes have been isolated from those cattle. And here you can get really the picture. You can see the same scenario. You can see very large number of non-O157 STECs. The ones in orange color again are the ones caused hemolytic uremic syndrome. So those are the bad ones or the more serious ones. The ones in yellow color are the ones that cause all various human illnesses.

So what you can see here is really a serious problem.

A very important piece of information here, you can see the beef cattle on the range had the largest number of serotypes, and those are cows and there those cows, you know, after they're done with the production cycle, they go to make hamburgers. So we need to be aware of that. The same for dairy cattle. Dairy cattle all go for hamburger making because their meat is not good anymore. So we need to keep that in mind in terms of how serious these things are. We found O157:H7 in all the production

systems, but really one out of a larger population.

With regard to looking carefully at the pathogenicity of these kind of these kind of serotypes or isolates, we found 161 STEC isolates. When I say isolates, they are not the same. They can be from the same serotype but they are different with regard to their genotype, phenotype or by chemical characteristics. 134 were non-O157 STECs. 83 percent of our isolates were non-O157 STEC, and now we get a feeling about very large number of those were pathogenic, or known to be pathogenic but, you know, that's kind of what the literature say to us.

So looking at what we have in terms of virulence factor and whether it expressed those genes or not, that's really the most important issue in determination of how potential pathogenic that pathogen or that serotype is.

The first thing we need to know here is based on our results, all the 161 isolates were lethal to Vero cells. Those are the African green money kidney cells which are very sensitive to Shiga toxin 1 and Shiga toxin 2, and those are used for

culturing or for testing, the first way of testing STECs.

Seventy-eight had and expressed only Shiga toxin 1 gene. Sixteen had and expressed only Shiga toxin 2 gene. Forty had Shiga toxin 1 and Shiga toxin 2, but out of those forty, three expressed only Shiga toxin 1, two expressed only Shiga toxin 2 and thirty-five expressed both genes. So that's serious because you know Shiga toxin 2 is worse than Shiga toxin 1. Ten had and expressed alpha hemolysin, and eighty-four had EHEC-hemolysin, but only fifty-six expressed that gene. We also looked at the attaching effacing gene, and we found that 53 of the isolates had the gene.

With regard to testing, most of them also expressed that gene but we have more work to do because eae is not a symbol, and they're the other ones, there are some other variations with regard to attachments. So we are looking at that right now.

One main message I want to bring to your attention is, that's a quote, it says "Because STEC strains lacking the attaching and effacing gene or

the hemolysin genes have been shown to cause human illnesses," that's an old quote, 10 years ago, "it was suggested that these genes are not absolutely required for pathogenicity, and each STEC strain should be considered a potential EHEC."

So the point I'm trying to make here is we don't want to say it never causes human illness and this is never known because its never been reported. Like the fact of the matter here is, people who are saying that we don't have, we don't have to worry about non-O157 STEC, but here you saw the data, and in a very large population of potential pathogenic STECs in our cattle, but we never tested for those before. So we need to keep that in mind.

Another piece of information is 29 serotypes we had from our samples were not reported previously in cattle, and that's again based on the published report that we have a database on.

The other thing that we looked at is trying to identify solutions because talking about the problem is not going to help. So we looked at different factors. Those are management factors or

animal factors, which we hope that we can find some potential to manipulate or ask the farmers or rancher to do something about them, and we looked at those.

With regard to dairy, obviously the list is a long list, some of them have high potential and some of them have potential to do something good but I start with only ones which are significant and be value, less than .05. For dairy cows, feeding soybean meal for example, as the main protein supplement was helpful in reducing the carriage and shedding of this foodborne pathogen.

In feedlot cattle, for example, we found that having heavier cattle was reducing the risk, cleaning the feed bunk also was helpful in reducing the risk, and interestingly, increasing the forage diet from 10 percent to 15 percent was also helpful to decrease the risk. The reason for that is most people feed 15 percent or 10 percent, depending on the situation or what people do, but you don't want to go less than 10 percent in a feedlot situation. Otherwise, the animal life will be at risk.

For irrigated pasture, we found that

offering running drinking water like streams or spring versus ponds or ditches was very helpful in reducing the risk, and shortening the calving season was also helpful.

With regard to range cattle, we found that decreasing the stock density was helpful. Early separation of the calves is also helpful because calves are always more susceptible than cows to carry the pathogen. Increasing the size of calving pasture is also important, and the absence of any diarrheic calves was also helpful. With regard to the diet, we found that supplementation of those cows with molasses was also helpful in decreasing the risk.

With regard to outreach efforts, we have been trying, with Dr. Atwill really taking the lead in that because he's an extension veterinarian in taking that information and others and sharing those with the farmers and ranchers throughout California because he's covering the whole state but, you know, today I'm standing here with you, I will tell you publication, meeting with the folks and all of that stuff is helpful but really the future is going to be

having a website or websites addressing those issues, so everybody can access and everybody can learn and those sites can be updated periodically, so people will be on the top of things, and don't wait for things to be published and to be public domain.

With that, I'd like to stop and I'll be glad to answer any questions later on.

(Applause.)

DR. GOLDMAN: Thanks very much, Dr. Hussein, for sharing your perspective and your research into this issue. I think that the two presentations may raise some questions for the panel as we continue here.

We're going to shift a little bit now to two experts in the field of microbiology who have spent many years working with these organisms in the lab and will share with you some of the definitions they've encountered in trying to isolate these organisms and do some of the work that you've heard already alluded to in some of the previous presentations.

And first we have Cheryl Bopp, who is the

Chief of the Epidemic Investigations Laboratory, a unit in the Enteric Diseases Laboratory Branch at the CDC for the past four years. In her role as a CDC microbiologist, she has about 30 years of experience with laboratory investigations of outbreaks of foodborne pathogens including *E. coli* O157, the non-O157 Shiga toxin-producing *E. coli* and various other pathogenic bacteria. She participated in the investigations of the *E. coli* O157:H7 outbreak linked to raw spinach last year and the *Salmonella* outbreak associated with peanut butter. And we're very pleased to have Cheryl Bopp make her presentation. Thank you.

(Applause.)

MS. BOPP: Good morning. My talk today is going to be focused on the difficulties that are facing clinical diagnostic laboratories and public health laboratories to isolate and detect non-O157 Shiga toxin-producing *E. coli.* So this obviously is going to be linked to clinical, to human diagnosis.

I'm going to talk briefly about the nomenclature for these organisms and some

abbreviations, and then I'm going to talk about the various challenges that face labs today.

I don't know if it's necessary in this roomful of experts to talk about this, but a clinical microbiology colleague of mine at CDC reminded me that, you know, there's these three sets of nomenclature for these organisms and this alone is confusing to clinical microbiologist. And we need to realize that -- she said we need to have a brand message for this, and so we need to keep that in mind. So we may have to move away from our Canadian and European colleagues and strictly limit it to STEC nomenclature.

And then these are some abbreviations that I will use. I think they're pretty standard. Other folks here have used them. I am going to use the abbreviation Stx EIA for all of the commercial Shiga toxin amino assays even though there are some that are not really traditional ELISA formats.

Okay. This slide shows a typical protocol for isolation of O157 STEC. The clinical lab receives a stool specimen and they plated it on a

selective agar and most of the time this is SMAC but there are several other very specific isolation media that are available. After an overnight incubation, the clinical microbiologist looks at the plate and among all of the pink colonies which represent the more or less normal *E. coli* or coliform flora, they'll look for these colorless, non-pigmented colonies, and you can see one there. I've circled it in red.

As it was mentioned before, *E. coli* O157, we're fortunate that it has a rare characteristic of not being able to ferment Sorbitol and that differs from about 90 percent of other *E. coli.* So this is a very good way of differentiating O157 from background *E. coli.* This is a colony. The microbiologist selects a portion of this colony and does an agglutination and specific antiserum for O157. You can see, I've got a slide there. You can see the little agglutination particles, and this immediately provokes a phone call from the clinical microbiologist to the clinician saying I think that this could be O157.

The clinical microbiologist hopefully does take this O157 isolate and send it to the appropriate state or local public health laboratory where they confirm this to be *E. coli* O157:H7 or O157 nonmotile producing Shiga toxin, and they do a PFGE, and I've shown a pulsed field gel electrophoresis there, and the public health laboratory will then upload these PFG patterns to PulseNet, and it is in this way that we are now able to detect outbreaks of *E. coli* O157.

Now this is a typical clinical diagnostic laboratory protocol for detection of non-O157 STEC, and this is even simpler and more streamlined. The laboratory will take a stool specimen and inoculate, usually will inoculate it into a special broth, frequently GN broth or a MacConkey broth. This broth is incubated overnight and a broth supernatent is then tested in an Stx EIA. This whole process takes, you know, 24 hours or so. A positive is then reported as Shiga toxin detected to the clinician. Of course, it could be *E. coli* O157:H7 or non-O157 but no one knows because there is no SMAC plate and no colony and therefore no serotyping can be done,

and then no isolate can be sent to the public health lab. So there's no PFG, and there's no patterns to be sent to PulseNet. And even though it affects non-O157, it also affects O157.

The main obstacle to isolation of non-O157 STEC, there's no good isolation medium available. Non-O157 STEC are typically *E. coli* but they tend to be heterogeneous. There's many serotypes, well over 100 have been identified from humans, and they also, while they're typical *E. coli* they don't share all of the same biochemical characteristics and no one has been able to identify a single biochemical test that would be useful as Sorbitol is for O157. For this reason, Stx EIAs are the only practical method right now for clinical diagnosis of non-O157 STEC infection.

There are some disadvantages to using these Stx EIAs, and the most important one is that they cannot differentiate between O157 STEC and other STEC serotypes and there is quite a bit of agreement that O157 tends to cause more severe illness and more HUS. Most of these EIAs cannot differentiate between the

two toxins although there are some new ones, the new lateral flow devices which can. I'm not sure how useful this information is to the average clinical microbiologist, but it could be that perhaps we could educate clinicians to recognize that the presence of Stx2 could be a more ominous diagnosis. These Stx EIAs, false positive and false negative reactions are not uncommon. Public health laboratories tell me that they have more problems with false positive reactions than with false negatives, and the false positives are generally due to inadequate laboratory techniques such as inadequate plate washing, not using a micro titer reader and just reading these plates visually, testing inappropriate specimens and there have been some anecdotal reports of some cross-reactions with Pseudomonas or norovirus infections.

There are at least five commercial Stx EIAs that have received 510K approval, and they're listed here. The first two, the Premier EHEC and the ProSpecT Shiga Toxin are both traditional ELISA formats and have been around the longest. The Verotoxin GLISA and the ImmunoCard Stat! EHEC are

very, very similar tests. They're lateral flow immunochromographic tests, and then the new test which has just been published BioStar Optical Amino Assay.

This is a table of all of the non-O157 STEC isolates that have been received at CDC from 1983 to 2005. And I just wanted to show you this to see that before the first Stx EIA received 510K approval, the CDC was receiving maybe, you know, less than a couple of dozen of these isolates a year but after the approval, within a couple of years, we started to see rapid increases in the numbers we were receiving. So I think this means that clinical labs are using them, and public labs are getting these specimens.

This year, interestingly enough, we are well on the way to receiving over 1,000 non-O157 STEC isolates at CDC.

So if it's difficult to isolate non-O157 STEC, then how can we detect outbreaks? This is the big issue. When a clinical laboratory sends a specimen to a public health laboratory for isolation of these strains, this is a typical protocol that the

public health lab would use. The specimen is usually a Shiga toxin positive broth but it may be a stool specimen. The public health lab will usually plate the specimen on SMAC or CT-SMAC or some other special medium for O157 because that way they can, you know, kill two birds so to speak. They can identify O157 colonies if they're Sorbitol negative, but they will also have the other typical colonies to test for non-O157 STEC.

Public health labs select usually somewhere between 3 and 10 colonies, at least what I hear from them. This is a source of some controversy and I have received many, many questions about what is the optimal number of colonies to pick, and I don't have a good answer. Three or four is probably reasonable but I know that at CDC in our laboratory, that we have actually had to pick up to 50 colonies to identify a single STEC isolate.

These colonies are selected and are tested either in an Stx EIA or by PCR. Many states do not have PCR technology, but increasing numbers of them are using it for this purpose, and they're trying to

encourage them to do that. And if one of these colonies is identified as positive for Shiga toxin genes or Shiga toxin production, they then have an isolate which they can confirm and test for PFGE, and hopefully send to CDC for serotyping.

As you can see, the procedure for isolation is very tedious, time consuming, expensive. So they don't attempt to isolate non-O157 STEC. The vast majority of clinical labs who send, who send specimens to public health labs are sending just the broth rather than isolates. Public health laboratories have really stepped up to the plate. Most of them do attempt to isolate non-O157 STEC from these broths. There are a few that do not. They simply do not have the resources.

This influx of broths is creating problems for public health labs. It's expensive to try and isolate non-O157 STEC. You can't select a single colony and then test it or not. You have to test multiple colonies. The reagents involved are expensive. The Stx EIAs, it can cost up to 15 or $20 per colony to test these. So this is a big drain on

public health lab resources.

Laboratory personnel time, there's a lot of that, and that's scarce and expensive for public health labs. And, you know, there's a lot of other big issues out there in public health that labs have to deal with.

This is a list of outbreaks of non-O157 STEC that my laboratory is aware of, and I just wanted to show this briefly to show you that public health labs are identifying outbreaks. Clinical labs are sending these broths to labs and the labs are increasingly identifying outbreaks.

However, just besides the resource issues, public health labs have another big issue, and this is an issue also for clinical labs. If the clinical lab gets a positive result on a broth and sends it to the state, and the state retests it and gets negative results, either they failed to isolate non-O157 STEC or they failed to identify Shiga toxin in the specimen, they report it back to the clinical laboratory. This creates much consternation on both the clinical lab and the physician because they had

reported this as Shiga toxin positive but now the state lab is saying it's negative.

And then there's other issues, health issues. What if a child has been excluded from daycare based on this test or a food handler excluded from work?

So this brings up the subject that one more challenge we have is to develop some guidelines for laboratories and physicians. We need specific guidelines for diagnosis and detection of these across the board, all the stakeholders. I think it's been brought out quite eloquently, physicians, they need to act quickly. They need to know that they need to order the appropriate diagnostic tests because in many instances, laboratories do not automatically look for STEC, either O157 or non-O157 STEC unless it is explicitly ordered by the physician. Physicians also need to understand the difference between the two tests, whether it will only detect O157 or whether it will only detected Shiga toxin. And they need to understand the limitations of these tests.

Clinical labs have also been asking for some specific guidelines for diagnostic testing. Which specimens to test, which test methods to use, how to interpret and report results and hopefully also to send these isolates and positive broths to public health labs.

CDC attempted to, you know, do this back in September in the MMWR. We did issue some recommendations to clinical labs and public labs. We asked clinical labs to consider adding an Stx EIA to their routine stool culture, but to not eliminate the culture for O157.

What else do clinical labs need to know? They need to know that neither SMAC is enough or Stx EIA is enough. They need to realize that these commercial assays can produce false positives and negatives, and for this reason, they should participate in proficiency testing programs, which these programs are not widely available for Shiga toxin or O157. They need to know the importance of promptly communicating positive results to the physician and another thorny issue for labs, which I

can't really help them with too much is they have to figure out how they can be reimbursed for this testing, and apparently this is a very big problem.

Public health labs need to realize that timely culture of these broths for non-O157 STEC is important. Outbreak detection certainly is crucial and is the only way it can be done. Submitting clinical laboratories and physicians both also appreciate this feedback, the confirmation from the public health labs or to point out perhaps some conflicting results.

Public health labs need to somehow find the personnel and train them to do this isolation. This is a big need which there's not a lot of this type of training available, and CDC right now is not able to do this training either.

And finally, we want public health labs to send these non-O157 STEC isolates to us so that we can serotype these and do confirmation and do some surveillance on the prevalence of serotypes.

And finally public health labs need to get this message, is that what I hear from large

commercial, nationwide diagnostic labs is that there are many different -- every public health lab has their own specimen submission rules. Some public health labs will not accept Stx positive broths. They will only accept isolates or fecal specimens, and that this is confusing and frustrating to the labs because they're trying to do the right thing by public health.

So we're trying to encourage the Association for Public Health Laboratories and public health labs themselves in collaboration with clinical diagnostic labs to develop some consensus guidelines for submission of Shiga toxin positive broths and specimens for STEC testing.

CDC also has to go back to the drawing board and improve on our MMWR black box. We are working currently with our partners, stakeholders in this. The APHL, ASM, public health labs and clinical labs, clinicians, to develop some consensus, specific guidelines and recommendations for isolation and identification of STEC. We also hope to at least have some preliminary, some interpretation guidelines

for Stx EIA.

And to finish very quickly, the challenges are daunting, but I am encouraged by the quite remarkable cooperation among all the stakeholders in this issue, commercial diagnostic labs, public health labs, APHL, clinicians, and CDC, and I'm encouraged that in another year we will see progress. Thank you.

(Applause.)

DR. GOLDMAN: Thank you very much, Ms. Bopp, for telling us about some of the practical limitations that clinical labs face and in their interactions with public health labs which are critical to our characterizing this issue.

Next we have a research microbiologist, Dr. Peter Feng, from the Center for Food Safety and Applied Nutrition at FDA. Dr. Feng has worked for 18 years on genetic characterization and the evolutionary emergency of O157:H7 and atypical variants in the Division of Microbiology at FDA CFSAN. He's focused on rapid detection methods for foodborne pathogens. Prior to his joining CFSAN,

Dr. Feng was the Post-Doctoral Fellow in Molecular Biology at Perdue University, and a Program Manager for Assay Development, IGEN Incorporated. He received his Ph.D. in Microbiology from Iowa State University, and he's also on the Editorial Board of the Journal for Food Protection, and a member of the American Society for Microbiology.

Please welcome Dr. Feng.

(Applause.)

DR. FENG: Good morning. Thank you very much.

I've been asked to address the aspect of food testing for non-O157 STEC, and this is basically an outline of my talk. Some introductory material you have probably heard from other speakers, but I'm going to be coming from the standpoint of testing from food safety aspects. We're going to look at some of the problems in testing for pathogen and toxin in general, in testing for foods, and then some strategies on how to test for non-O157 STEC in foods. You can test after Shiga toxins or Shiga toxin genes, using different kinds of antibody and DNA assays, or

you can go after testing for the organism itself, namely the STECs.

And then you're dealing with a whole bunch of other complicated factors, such as what enrichment media to use, what inhibitors, antibiotics and so forth. And then finally to wrap up a little bit, by talking about our work on testing for seropathotypes.

Okay. This is a slide you seen before. These are the major pathogenic *E. coli* groups that have been recognized. The top four, of course, are the ones that are commonly transmitted through foods, okay. Now all of these organisms are truly *E. coli.* So what categorizes them as different pathogenic groups, of course, the different virulence factors that are carried by each group, okay.

From the testing standpoint, you can do it two ways. You can test the food directly for the virulence factors and then go through the labor intensive task of trying to isolate the organisms that carry the virulence factor or you could go the other way and test the food for *E. coli* first, identify it as an *E. coli* and then test the different

virulence factors that are carried by these organisms.

Now the ones that we're interested in today, of course, are the Shiga toxigenic or Verotoxigenic *E. coli,* okay, which have the acronym STEC or VTEC, but we also have an acronym EHEC which aside from Shiga toxins carries other virulence factors. And this has, like Cheryl says, caused some confusions. So I want to look at, clarify this confusion a little bit and also look at some of the definitions that have been used that are also a very controversial topic.

Shiga toxigenic *E. coli* and Verotoxigenic *E. coli* are essentially the same thing, and the only criteria or the only virulence factor that are produced by these organisms is, of course, the Shiga toxins.

Now some statistics say there are 100 serotypes. Some I've seen say 200 serotypes. I think it's safe to assume that it's more than 100 serotypes of *E. coli* that will produce Shiga toxins, okay. The problem, of course, is that not all of the

serotypes have been implicated in human illness so far.

Now EHEC on the other hand, defined as Enterohemorrhagic *E. coli* is a subset of Shiga toxigenic *E. coli* and the type strain, of course, is O157:H7. In addition to the production of Shiga toxins, they carry a number of other virulence factors. One of the principal virulence factors, of course, the locus of enterocyte effacement pathogenicity island, or the LEE Island which includes factors such as the translocatable intimin receptor, the intimin itself which allows the bacteria to adhere to the cells. There's at least 15 different alleles of intimin that have been recognized. Some of the common ones, such as alpha and beta are carried by O26, O111, O157, O145, tend to carry the gamma type intimin. You also have a large plasmid called pO157 which includes for things like enterohemolysin, serum proteus, catalase peroxidase, but all these are punitive virulence factors so far.

Now there's been many definitions proposed

for EHEC. One of the very simplest ones, of course, is a STEC strain that also carries intimin, but as Dr. Tarr showed not all of EHEC strains that cause illness would carry eae. A more complicated definition is that it's an STEC strain that's implicated in clinical illness, namely hemorrhagic colitis or HUS. Okay. And then a more complicated definition is an STEC strain that have the same clinical, epidemiological and pathogenic characteristics.

Now the definition of EHEC has been very controversial at the last EHEC meeting in Melbourne because unlike the other pathogenic *E. coli* which are named after the virulence characteristic it has, such as enteroinvasive, enterotoxigenic and enterohemorrhagic is actually named after the illness it causes which is very different. So there's been a proposal to change the nomenclature for EHEC or to modify the nomenclature for EHEC but in the meantime, I would like to use the term EHEC to distinguish for STEC. EHEC, namely those that have clinical infection symptoms.

Okay. So the big dilemma we have is basically summarized by this diagram, where we have the large circle, which represents the large group of STECs that we know of, and the criteria, of course, is a production of Shiga toxins. On the blue circle, we have eae, the intimin gene, and part of it's outside the circle because enteropathogenic *E. coli* also carries eae as its virulent factor. Okay.

So the overlapping is where we have the EHEC which carries both Shiga toxin and eae.

But there's also exceptions, of course, in that we have enterohemolysin which is still kind of a putative, but most people recognize as a virulence factor, okay, and in the middle of all this, you have O157:H7, the majority of which seems to carry Shiga toxin, eae and enterohemolysin.

So the dilemma we have in testing for O157 or non-O157 in foods is how are we going to distinguish this large group which has not been implicated in human illness so far from these guys that are known to cause human illness?

Now this is statistic that's -- dated, but

what I want to show is the trend, okay, of non-O157 infections and the trend is, of course, that non-O157 infections are going up in the last several years, and also interestingly, the strains that are not typed have also drastically increased. But I think part of the problem with these non-typed strains is the lack of methodologies. We really don't have very good methods to allow us to detect these non-O157 strains, and also we don't have very good methods to allow us to identify these non-O157 strains.

Now to test for these strains in food is truly a very challenging task, and this is not only O157 and non-O157, this is to test any pathogen and toxins in foods. It is a very challenging task. And the problem, of course, is that the food matrices are very complex, not only in its physical form. It could be solid, liquids, gels, powder, you name it. If you start throwing ingredients, you have proteins, fats, carbohydrates, oils, everything, and all of this is going to interfere with the assays and that causes a lot of problems, okay.

A lot of the raw foods, of course, contain

very high levels of normal flora, ground beef, sprouts, easy to contain 10 million bacteria per gram and, of course, your target that you want to detect are often found in much, much lower numbers. And the problem we have with foods is processing. A lot of foods will go through processing such as heating, refrigeration, heat treatment, whatever, and those tend to cause stress injury on the organisms and if it does not allow this organism to resuscitate, oftentimes it's very difficult to detect them.

The solution we came up with, of course, is to enrich the food samples in different culture medium, okay. We have different enrichment schemes, some using antibiotics, inhibitors, to allow to select for the organisms that we want to detect. Enrichment works pretty well. The only problem is that it takes a lot of time.

Now when you're testing for toxins, some of these problems also apply and, of course, the solution we came up with, you have to do extractions, a lot of times you have two concentrations. If your toxin has been denatured, you have to renature before

detection.

Now this situation probably does not apply to Shiga toxins because, as we know, these organisms have very low infectious dose, usually around 10 to 100 organisms. So it's pretty well understood that you have to ingest the organism before it will make you sick. So this is not analogous to a situation with staph enterotoxin or clostridium where it's a case of intoxication. The illness is caused by ingestion of preformed toxins in foods.

Now there's been some studies to show that Shiga toxin will be produced in foods, okay, such as ground beef, sausages and dairy products, but the food has to be incubated at 37 degrees and with very good agitation and aeration, to induce the organisms to produce toxins. So it's pretty much understood or, you know, logical to assume that on the most normal food handling and storage conditions, which is not going to be at 37 degrees, toxins is probably not going to be made in foods.

So what are the strategies we can use to test for STEC or Shiga toxin in foods? Well, there's

two pathways you can take. One, you can test for the toxins or one, you can test for the organisms. Okay. Let's address the toxin one first.

Now I've already mentioned that to test food directly for Shiga toxin is probably not very useful because the toxin is most likely not produced in foods, but you can certainly put the food in broth media, incubate this broth media at 37 degrees and be able to detect toxins in that food homogenate. So the question is, what type of test do we use?

Well, obviously the choices are very narrow because we'll have no good microbiological assays that will detect either the toxin or the genes, okay. Tissue culture cells can be used to detect for toxin 1 and toxin 2, the cytotoxic effect like in HeLa cells or Vero cells but tissue culture assay certainly is not a very practical means to use to test for foods.

So the choice we're down to is basically serology and DNA, and there's certainly plenty of assays, commercial or non-commercial that are available to test either for the toxin genes or the

toxin itself. Some of these have already been mentioned. There's many ELISAs. There's some immunoprecipitation assays. There's some PCR assays and, of course, there are a ton of non-commercial PCR assays that have been published to test for Shiga toxin genes in these strains.

Okay. So what if your toxin test comes up positive? Well, the testing aspect is the easy part. If it comes up positive, that's where the bulk of the work really starts because as Cheryl mentioned, sometimes you have to pick 50 colonies in order to isolate the colony you want. And isolation of a culture, as mentioned repeatedly by many speakers, is very important not only in a regulatory standpoint but also in EPI, okay. So once the test comes up positive, you essentially plate out the positive sample, you pick the isolates, you pull and you retest, okay, and you repeat this process until eventually you end up for a pure culture, okay.

Once you end up with a pure culture, you're still not done. You have to serotype because serotyping information is important, and then after

that, you still have to figure out whether this is just simply an STEC strain or an EHEC strain that's going to cause illness in humans.

Now when you're going after the organism itself, namely STEC, the factors of the parameter that you have to consider are a lot more complicated because, first of all, you have to do, you have to think about the selective or the non-selective enrichment media you're going to use. If the cell or the food has been thermal processed or injured, and your cells are injured, you probably have to consider some sort of non-select medium. If not, you can go directly with the selective medium, but the things you have to consider is what type of inhibitors to use, how inclusive are these inhibitors, okay, what kind of incubation temperature are you going to use? Are you going to use an elevated temperature like 44 degrees, 43 degrees, or are you going to incubate at 37?

The differential, once you have to incubate, you have to plate these organisms out, okay, and it's very useful, you know, you don't want

to have to screen hundreds and hundreds of organisms. So it will be very useful to have some sort of a selective medium, okay, that detect some sort of unique traits that allows you to pick the colonies you want for further testing. So the trait here is a crucial issue in differential and selective medium.

Now when testing for O157:H7, we're very fortunate because you have several definitive traits, namely the absence of Sorbitol fermentation and also the absence of beta-glucuronidase activity. There are, of course, exceptions like everything in Mother Nature. You have the Sorbitol fermenting strains that are Sorbitol positive, Sorbitol fermenting O157 and they're also O157 strains that are glucuronidase positive. So there's always exceptions in nature, okay.

Serotyping O157 is easy because you have the O1 antigen 157, you have the H antigen 7. So very easy to identify, very easy to detect.

Non-O157, the situation is more complicated because Sorbitol is basically useless. These organisms are like a typical *E. coli.* So most of them

are Sorbitol positive, same thing. They're atypical *E. coli.* So the glucuronidase activity from most of these strains are positive, okay. Absence of rhamnose fermentation has been identified as a pretty useful marker for testing O26s but for the other EHEC serotypes, it's not as reliable. Same thing with -- activity, O11 has been identified as having negative -- activity but for the other serotypes, it's not as reliable.

Serotyping, of course, is a nightmare because you have so many different serotypes of STEC that can be considered EHEC. So about the only thing they have in common are the virulence factors.

So because of this no unique phenotype that you can use to do your selection and for enrichment, a lot of the selected pressure inhibitors used are general. It applies to a lot of enteric organisms. Some of the common culture enrichments that have been used are things like modified TSB, modified EC, modified buffer peptone water and so forth. None of these were select only for O157:H7. They're meant for non-O157. They're meant for just generic *E. coli*

in general. Some of the common inhibits, antibiotics like Novavax, Acriflavin, Vancomycin, Cefsulodin, Cefixime, Potassium Tellurite, elevated temperature and so forth, okay.

None of these factors again are specific for the non-O157 STEC group. Things like Potassium Tellurite have been shown to work pretty well for O26, O145, O157:H7, except for the Sorbitol fermenting strains but it doesn't seem to work well for O111, or for the other serotypes. Same thing with Cefixime, it works well for O26, O111, O157s but the other serotypes, it's not as responsive.

Now in the selective and differential plating aspects, most of this medium were developed for O157:H7. So they look at things like Sorbitol and glucuronidase, okay. But some of this media has been found to be possibly useful for testing other non-O157 STECs. For example, I've read that Chromocult made by Merck seems to be a pretty good selection medium for O111s. Rainbow agar which used beta-galactacyte (ph.) and beta-glucuronidase activity, okay. They have reported that their media

can actually be used to differentiate O157 glucuronidase positive O157, O26, O48, O11s, okay. Cefixime tellurite MacConkey -- Rhamnose MacConkey agar, like I said, have found to be pretty useful for selection of O26 STEC.

And then recently, this summer I had a talk in England about the group in Belgium, Posse, et al. University at Ghent, and these folks did a huge, a tremendous amount of work by looking at a very large panel of different serotypes of STECs, a tremendous large amount of carbohydrates subseries and antibiotics and they came up with a combination of carbohydrates and inhibitors and with a medium that will select and differentiate O26, O103, O111, O145, both Sorbitol positive and Sorbitol negative O157:H7s. So I think this type of medium are going to become much more abundant.

Okay. Immunomagnetic separations, of course, is a tool that's very useful to allow us to select out the targets we want, okay. So immunomagnetic separation can be applied at different stages to try to fish out the organism you want but

the complexity there is, you're going to have to use a cocktail of organisms and the key question is what serotypes they're going to use in these cocktails. It certainly is not a shortage of antibodies because there's plenty of antibodies that are available from many, many old types including many of the key STEC O types. Denka Seiken and Statens Serum Institute certainly have a very complete collection of O antigen serums. So antibodies are available but the key question is what panel, what cocktail are you going to use to try to fish the organisms out.

Okay. When it comes down to the bottom, when you have a pure culture, you still have to do serotyping, and once you do the serotyping, you have to figure out whether it's just the plain STEC or whether this is truly an EHEC that's going to cause disease. This is the big dilemma. How are you going to distinguish STEC for EHEC?

Now some people have attempted to try and make that character distinction, and one of those is published by Dr. Mohamed Karmali of Toronto, who came up with this classification called seropathotype

classification, and he looked at various factors such as incidence, frequency, severe disease, serotype and listed here are things like virulence factors, like intimin, O Island 122 and various factors. And he classified five major seropathotypes, okay. Seropathotype A, high incidence, common, severe disease, O157:H7 and it's nominal variance. Then you have seropathotype B which incidence is moderate, okay, also cause severe disease and as you can see, you have five of the major six that CDC has been identified. O45 is not listed there. And then you have Category C which includes some things like O104, O113 and so forth, okay. And then D and E which are typically are not considered to be human pathogens.

Now this classification is not etched in stone. It is not a one size fit all because some country will have problems, more problems with O113. Even in the U.S., you will tend to isolate more O45s from clinical specimen. But it's not meant to be a one size fit all worldwide, but it allows us a pretty good handle to try and fish out the pathogen and strains of STEC.

Now we don't have any regulations for dealing with a lot of these non-O157 STECs, but I thought it would be interesting to try and develop some sort of assay to try and fish out or to identify these organisms. So in my lab, we started this little project and we came up with a multiplex assay that uses nine primer pairs on a single reactions, okay, and the nine primer pairs consists of number one is again -- DNA to serve as an internal amplification control. We use 1 primer pair that will pick up all 15 different alleles of the eae gene. So we will pick up alpha, beta, gamma and all those, okay. We use one primer pair that will pick up both Shiga toxin 1 and 2, including many of the other forms of Shiga toxin 2, and then we'll use the WZX gene that's responsible for transporting all the lipopolysaccharide to the surface of each one of these serotypes, O26, 103, 111, 121, 145, 157. This is the actual gel. This is the bioanalyzer scanner of the same gel, okay.

The purpose of the assay is that if these strains contain Shiga toxin gene eae and one of these

serotypes, it's probably a good chance that it is going to be a EHEC strain because it carries Shiga toxin eae and it's going to be of that serotype.

So we looked at a bunch of different strains and you can see that lane number one is O26 nonmotile strain. It has the eae gene but no Shiga toxin, and here's the O26 band. Now this could either be an EPEC strain because it doesn't have the Shiga toxin gene or it could be a EHEC strain that simply lost the Shiga toxin gene, because the toxin gene is encoded by phage, so they can pop out, okay.

Lane two, you have O103, carry Shiga toxin eae, O103. Here you have the O111 strain. You have both virulence factors, O111.

Lane 4, you have an O121 strain which has neither virulence factor but it has O121 antigen. So this turned out to be just a simple generic *E. coli* that has the O121 antigen but it's neither STEC or EHEC.

And then you have here O145 and then finally O145, both toxin and the eae and the O157 antigen gene.

So this is some of the assays that we're working on, and hopefully -- now it's not meant to apply for testing for foods, but at least once you get down to the isolate levels, hopefully with this type of assays, it will allow us to recognize this virulent strains of STEC more easily. Thank you very much.

(Applause.)

DR. GOLDMAN: If we could have the panelists come back up. We'll take about 15 minutes to see if we have any questions here in the room or on the phone.

OPERATOR: On the phone lines, if you would like to ask a question, please press star 1.

DR. GOLDMAN: Okay. We have a question in the room. Nancy?

MS. DONLEY: Nancy Donley from STOP. Thank you very much for your great presentations. I have two questions of very different natures and one is for Dr. Koohmaraie and the other one is for both Ms. Bopp and/or Dr. Feng.

And not to leave Dr. Hussein out here, I

thought your presentation was fabulous and I know we're going to be hearing more this afternoon about more of the global STEC of what's happening and where you've pointed out some of these things that happened in other countries. Argentina was really quite eye opening. Thank you.

My question for Dr. Koohmaraie, you said that you are doing the prevalence survey of the national ground beef supply for non-O157 STEC. I have two questions. Did I get this correct that BIFSCO is sending -- they're taking the samples and sending the samples to you at ARS? Is that how the process is being done?

DR. KOOHMARAIE: BIFSCO is not really -- it's partially involved. You say BIFSCO map. That map has been used --

MS. DONLEY: Right.

DR. KOOHMARAIE: -- for doing *Salmonella* monitoring. The processing plant, the private companies, those are the ones that takes sample after the grinding and they send it to us.

MS. DONLEY: So plants are choosing their

own samples to send in.

DR. KOOHMARAIE: Right. We're not doing retail sample. At the processing time, they take a sample and they send it to us, that's correct.

DR. DONLEY: Okay. And do you have kind of an approximate date of when you expect to have your --

DR. KOOHMARAIE: I showed you the data. We have another 7 or 800 to process and we're doing *Listeria* on those. We're doing *Salmonella* on those. We do multi-drug resistant *Salmonella,* a whole host of stuff. That's why it takes a long time to do it. I would say, we're doing our best but few months probably.

MS. DONLEY: And we can expect to hear -- get some sort of report from you in a couple of months?

DR. KOOHMARAIE: Sure.

MS. DONLEY: Fabulous. Great.

DR. KOOHMARAIE: You bet.

MS. DONLEY: And then my questions -- it's the same question really for Ms. Bopp and Dr. Feng.

Can you give us kind of an indication in some general form of where we are today with non-O157 STEC technologies and where we were back when O157 became a concern and we were really there starting from where it just kind of burst on the scene, if you will, and where are we positioned today with non-O157 for testing in both the foods and in humans versus compared to where we were with O157?

MS. BOPP: I think that we do have adequate technology for detecting non-O157 STEC infections in humans. What we do lack, we're missing the connection for outbreak detection. So I think that, you know, increasingly as clinical labs use these commercial tests, which are excellent, they're expensive but they're excellent, I think that the potential is there to diagnose clinical infections. But until we can find, improve the technology for detecting outbreaks and getting an isolate, I think we're going to have a hard time detecting non-O157 outbreaks in humans, unless they're very large.

DR. FENG: I tend to agree with Cheryl, that the technology is there but the dilemma is to

try and isolate a non-O157 STEC in foods. In the foods that are regulated by FDA, we don't find O157:H7. So the chance of finding non-O157 STEC is probably a little better than the O157 but again even if we find it, you know, because we have no regulatory position for these organisms, it's hard to say how we're going to proceed even if we find them.

MS. DONLEY: Thank you very much.

DR. GOLDMAN: Okay.

MS. WARREN: Wendy Warren, Food Safety Net Services. My question is for Dr. Feng regarding the use of PCR specifically related to Shiga toxin genes. As you pointed out, there are multiple commercial and non-commercial PCR assays that are available for review. My concern is related to the selection of the primers. So if there's not a universal set of primers, what sort of concern might we have as far as artifacts in the data go, that type of thing? Are we all getting the same messages?

DR. FENG: That's an excellent question, and there needs to be a lot of standardization and validation in the selection of primers simply because

the specificity in a PCR rationale are dictated by the short or legal nucleotides, and I've seen PCR assays that don't work simply because there's a snip within the primer binding side and the primers simply miss it, okay. So that is certainly one of the problems, and also the other problem, of course, is that just because it's detected by PCR, there's no guarantee that the toxin is actually made because they do have mutations and sometimes the toxin genes are not expressed.

MS. WARREN: Okay. Thank you.

MR. BURNS: Frank Burns, DuPont Qualicon. As these presentations were going on, Dr. Koohmaraie pointed out that we really don't have a disease model that mimics what happens in humans, an animal disease model, and for every virulence factor that is highly associated, there are exceptions as Dr. Feng pointed out. And as we look geographically across the world and we've got different O serotypes involved in different places, and also historically these have changed in some countries over several years, at what point does human health get protected better by

looking at generic *E. coli* levels in keeping those down as opposed to chasing down more and more serotypes to try to exclude from the food supply.

DR. FENG: Who was your question addressed to?

MR. BURNS: Anyone that wants to juggle that one.

DR. FENG: I can give you an example. One of the commodities that the FDA regulates is cheeses, and we used to have -- well, you know, we still test cheeses for enterotoxigenic *E. coli* stemming from an outbreak that happened, you know, almost 30 years ago, involving enterotoxigenic *E. coli.* And at the time, the limit was set was that, you know, the level of generic *E. coli* allowed in cheeses was 10,000 per gram, and if you find 10,000 per gram generic *E. coli,* you will test for ETEC, okay.

As times have changed, and we've realized that the rest of the world has much more stringent generic *E. coli* level in cheeses, we're attempting to bring those levels down to 10 per gram. Okay. And if we can implement that level of 10 per gram for

generic *E. coli,* it obviates the need to test for enterotoxigenic *E. coli.*

MR. BURNS: Thank you.

DR. SCHEUTZ: Flemming Scheutz from Statens Serum Institute in Denmark. I was intrigued by all of the fascinating work done by Mohammad Koohmaraie and I have one question for clarification and also an observation, that I'd like to share with you from Denmark, from our food safety agency.

The samples that you were taking post processing, were they taken before chilling?

DR. KOOHMARAIE: Yes, sir.

DR. SCHEUTZ: Before chilling.

DR. KOOHMARAIE: After full complements of all the interventions, the carcasses go into chillers, and we sample them as they enter the chiller.

DR. SCHEUTZ: And how long are they chilled for?

DR. KOOHMARAIE: In the U.S., typically they chill for about 36 to 48 hours.

DR. SCHEUTZ: Okay. Because my Danish

colleagues have observed that after chilling of I think it's about 6 days, we have exactly the same findings as you've presented, 60 percent of positive for Stx, about 4 percent positive for O157, and after 6 days of chilling, everything comes out negative. So that might be another step of reduction that you want to look into.

DR. KOOHMARAIE: Thank you. And let me make it clear. There's about 15 scientists, that we work as a team. I represent the work of the group. I want to make sure, so.

DR. GOLDMAN: Caroline.

MS. SMITH-DeWAAL: Thank you. Caroline Smith-DeWaal, CSPI. I always have to bring things back to, you know, what do we tell consumer a problem here, and so I really have two questions. One is can you give me some clarity on the difference between the STECs and the EHECs and the ETECs and the EPECs, because we're, you know, in our database we identified I think 21 outbreaks, which included both we think STEC and ETEC, and we want to know what the difference is there.

Also, is there a way eventually, because basically what we're going to end up getting to is the concept of pathogenic *E. coli* and, you know, you guys will need to know all these nuances, but really when it comes to consumer communication, we're going to be talking about pathogenic *E. coli.* But what would be useful for me is having some context of the virulent factors, not in great specificity but in some general categories where we can say, you know, HUS is these pathogens but here are the other virulent factors we're looking at.

DR. KOOHMARAIE: It sounds like it's for Peter.

DR. FENG: As I mentioned in my talk, these pathogenic *E. colis* are categorized based on the unique virulence factors they carry. Enteropathogenic *E. coli* strain, the main virulence factor is the intimin gene. It's the common cause of infantile diarrhea in third world countries. Enteroinvasive *E. coli* is essentially like a Shigella. It carries a large plasmid which allows the cells to invade into gastrointestinal cells.

Enterotoxigenic *E. coli* produces two toxins. One is a labile toxin and one is a stable toxin, and these toxins -- the infections of those of ETEC tend to be very high around 10 to the 8th and to the 10th, and these toxins are often produced in the foods that people ingest and they get sick. Enterohemorrhagic *E. coli,* of course, has a very low infectious dose. It's key virulence factors are Shiga toxins, intimin, possibly enterohymolysin and maybe others.

MS. SMITH-DeWAAL: So is it correct to say then that the ETECs are more like Staph. Aureus or *Clostridium perfringens* in terms of the toxicity, the toxins are there at the dose to cause illness at the point of ingestion?

MS. BOPP: ETEC is not caused by preform toxin.

MS. SMITH-DeWAAL: They're not.

MS. BOPP: The organism itself must be ingested.

MS. SMITH-DeWAAL: Okay. Again I think what I'm outlining here is a problem we're not going to solve with this panel, but that at the end of the

day, we do need to be able to communicate effectively to the public on what the virulence factors are in a way that's very clear. So I appreciate your presentations, and I just ultimately needed dumbed down for me so that we can explain it.

DR. KOOHMARAIE: Carol, the problem to that is no matter what we say, there's going to be one exception to it and Dr. Hussein did a good job mentioning those exceptions.

MS. SMITH-DeWAAL: Yeah.

DR. KOOHMARAIE: So if you go with that, you say this is the maximum likelihood, that's where we look at the Stx1, Stx2 and eae, to help capture maximum likelihood but there will always be an exception. So if you want something that covers 100 percent, it will be very difficult to do unless we can give it to humans.

DR. GOLDMAN: Thanks, Caroline. Dr. Tarr?

DR. TARR: Yes. Dr. Hussein, you stated quite rightly that early on there was an ascertainment bias towards finding *E. coli* O157, the flagship of the toxin producing *E. coli.* But since

1991, there have been about a dozen studies in about eight or nine states that have yielded about 1,000 isolates and the data are remarkably similar to what Dr. Park related earlier. Using technologies that will detect O157 in humans and non-O157s, there's still about a 2 to 1 predominance of O157s. Are you aware of any additional studies that would support your contention that we are now missing this massive part of the iceberg?

DR. HUSSEIN: Not from the U.S. but, you know, in other countries that's really the case.

DR. TARR: Which country?

DR. HUSSEIN: I'm talking about Australia, Germany and also Italy and Spain.

DR. TARR: Well, the number of cases cited were rather low, and if you look at the plurality of the serotypes, it's still O157:H7 even if it isn't the majority.

DR. HUSSEIN: But with regard to Germany, in particular, there are some studies that I have seen, many of the cases were non-O157.

DR. TARR: Many, but the plurality --

DR. HUSSEIN: Yes.

DR. TARR: -- the chief serotype is still O157:H7, is it not?

DR. HUSSEIN: Yeah, that's correct.

DR. TARR: Okay.

DR. KOOHMARAIE: Over in Australia, we tend to think it's, you know, they tell me it's still O157, the plurality.

DR. GOLDMAN: Dr. Park.

DR. PARK: Choong Park from Inova Fairfax Hospital. Just suggestion to colleagues from CDC, being in a clinical lab and knowing the many clinical laboratories, unless we, the clinical laboratories, isolate this non-O157 and send to state laboratories or CDC, you didn't know whether there's outbreak or not. Unfortunately not many clinical laboratories do perform the toxin test and not many of them are aware of this subject. Then, so my suggestion is, CDC or local health organizations should be more aggressive addressing the awareness of this organism.

Now CDC published this in the MMWR. How many people read that? In the clinical laboratories,

there's very few people that read that. So you have to be more aggressive, and this is my suggestion. Thank you.

MS. BOPP: Dr. Park, to put you on the spot, we are actually trying to decide what is the best form for disseminating information. What do clinical microbiologists, where would they see this?

DR. PARK: Well, the ASM, the news, now they call them Microbe, also in ASM general meeting probably representatives from CDC can have a forum or some discussions sessions. I know you did that several years ago. That was a very small portion of it. So there is May 2008, and it's in Boston.

MS. BOPP: Thank you.

DR. GOLDMAN: Okay. Before we go to lunch break, let's see if we have anybody on the phone.

OPERATOR: We have a question, sir. Felicia Nestor, Food and Water Watch, your line is open.

MS. NESTOR: Thank you. Dr. Koohmaraie, I'm just wondering if you know of any studies where the efficacy of the interventions has been tested

under normal operating conditions? I mean the study that you spoke about, it sounds like everybody knew that the study was being done and it was time limited situation, but it's my understanding that a lot of those interventions unless they're used properly, you can have a real disparate effectiveness rate.

DR. KOOHMARAIE: Thank you for the question. That's an excellent question, and that's precisely why I tend to look at the intervention the way we report it to you because that data is in a day-to-day operation of the plant. If those interventions were not operated properly, we would have seen it. For your information, we also have published data in the year 2000 I believe that we looked in a laboratory setting on the efficacy of interventions, but that would be laboratory settings. Our data I think is far more relevant, and again, the one case that I mentioned, there were six O157:H7, we had, it was in one trip and in one plant. Clearly there was something going on wrong with that data. We caught it, that we would not have caught if it was in the laboratory setting.

DR. GOLDMAN: Are there any other questions on the phone?

OPERATOR: At this time, there are no further questions.

DR. GOLDMAN: Great. Okay. Please help me thank this panel for their presentations.

(Applause.)

DR. GOLDMAN: And we will try to get started at 1:30. There are some eateries within a short walking distance and the folks out front can probably help you with that.

(Whereupon, at 12:44 p.m., a luncheon recess was taken.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

(1:35 p.m.)

DR. GOLDMAN: A little bit of several things. You've already heard mention in earlier presentations, allusions to the international experience, the fact that other countries have had experiences with non-O157 STEC/EHEC and we have two very experienced presenters who will share their experience both from kind of the truly global point of view as well as from one country's experience.

Then we're going to ask, as I mentioned earlier, those who would be impacted by changes in policy to speak, that is the regulated industries and we'll get a consumer perspective as well very importantly.

And then we'll end up with what you've all been waiting for, and that is the policy considerations, and we'll have both regulatory agencies, FSIS and FDA, provide some thoughts about what we might do with the information we've heard and where we might go next in terms of policy considerations.

So to begin this afternoon's session, we will have the speakers come up one at a time. We'll begin with Dr. Flemming Scheutz, who is the head of the International *Escherichia* Centre of the World Health Organization. He has an MS in Molecular Biology and a Ph.D. in VTEC. He's the head of the International *Escherichia* Centre as I mentioned at WHO. He's published more than 50 papers primarily on the typing, clinical features and epidemiology of *E. coli* infections. His areas of interest are typing *E. coli,* detection and subtyping of specific virulence factors in relation to zoonotic diseases or infections and international standardization. He was an active participant on the EU funded surveillance and research programs and has been in charge of five trials of serotyping, virulence typing and PFGE of *E. coli* for the EU's dedicated surveillance network called Enter-net.

Please help me welcome Dr. Scheutz.

(Applause.)

DR. SCHEUTZ: Thank you very much, Dr. Goldman. It's a great honor to be here, and I

was very, very proud to receive this invitation. I've been looking forward to this meeting very much.

I've got about 18 years of experience with *E. coli* and non-O157s. So the 20 minutes given here is a very short time. Forgive me if I'm rushing over a few issues. I hope that there will be time for questions later on then.

If you look into the scientific publications listed by PubMed and search for O157 or non-O157 in the titles and abstracts, it's by far dominated by O157. So no reason to question the fact that we do know more about O157.

Interestingly enough, VTEC and STEC, which have been explained earlier to you, are listed but also Shiga-like toxin publications are still being published even though one would think that they were banned after the discussion in '96.

When you look at studies of non-O157 studies, there's a nice review by Kristine Johnson listing 16 countries, comprising 32 studies and if you add them all up, they're covering the countries listed here. About 48 percent of these studies will

list non-O157 strains. There are a couple of recent studies done in the Netherlands which was presented, a study presented at the EU Enter-net meeting in Vienna earlier this summer. Eighty percent of their STECs were non-O157, in Australia, it's 64 and so on. You can see that some of these studies have a very, very high prevalence of non-O157 strains.

If you look at the surveillance data that I have access to, we have 27 countries that have submitted data on STEC since 2000 through the Enter-net database, and you will see that it is highly skewed. There are some countries, the numbers are listed here. For example, in Great Britain almost 6,000 isolates, no non-O157 isolates. Whereas if you go to Germany, you will see more than 4,000 isolates and you have 80 percent as non-O157 strains. Denmark falls into this category. We have 872 isolates in the database and I'll draw a little upon that data to illustrate some of the issues today.

If you look at the groups, they're very similar to what you see in the States. You have what we used to refer to as the gang of five, that is the

O157 and then the O26, O103, O145, and O111, but please notice that O45 is not represented. There's only three isolates in this database which covers more than 6,000 isolates in Europe. So there are certainly differences in epidemiology.

One thing is the percentage of non-O157 but we'd like to look at incidences and if you compare these incidences from 2000 until 2006, I'm sad to say that Denmark has a prevalence which is either the second highest or the third highest in Europe. However, one has to interpret these data with caution, and I'm going to use and show you a map of Denmark to illustrate how cautious you have to be when you analyze incidence data.

As you see here, the incidence in some of the Danish counties in 2006 are rather low, whereas in other counties they are rather high. The counties where you see an incidence above 2 up to even 12 per 100,000 inhabitants per year, are the counties that are covered by molecular detection methods. The other counties do not use molecular detection methods which we've been using since 1997. And this is from

2006, but if you look at the ratio between counties that have used molecular detection methods versus other methods, you will see that they are in the range of three and up to eight times higher in counties using molecular detection methods. If you narrow that down to children, we're sometimes up to 25 times higher detection ratio in counties using molecular detection methods.

So I think the methodology plays a great role in our understanding of non-O157 and STEC in general because these are just STECs in general. They cover O157 as well.

A few words about outbreaks. I'm going to go into a little detail about two of these outbreaks but some of the serotypes you've seen previously, I'm going to focus on two.

The first one is the Norwegian outbreak of 2006. The interesting thing about this outbreak apart from the date of onset of disease that you see here, is that it was actually notified as an outbreak of HUS, and what's interesting about this, is that HUS is not notifiable in Norway. But one clinician

began worrying a little bit when he had three cases of HUS admitted in a very short period of time. Usually in Norway you'd have one or two cases per year. So he notified the public health laboratory in Oslo and very soon, over two days, they were able to detect another six cases.

What's interesting about these cases is that only two of the HUS patients were actually positive for Stx2, whereas all the remaining cases of HUS and diarrhea, were negative for Stx2 but positive for the causative organism O103. The remainder of these cases were identified by serology. The source was identified as sliced, dried fermented lamb sausage which is often served to kids in kindergartens and so on, and sheep meat was also identified.

What's interesting about the findings in the cured meat products and sheep meat, and they were not very high numbers, but they were definitely indicative of the right source, is that all of these isolates were Stx2 negative and eae positive. They were O103:H25. So you have a -- marker there and

they clustered by MLVA which is DNA fingerprinting that the Norwegians are using extensively as a supplement or replacement for PFGE.

So, in summary, in the Norwegian outbreak, we had 17 cases, 15 of which were children, 10 with HUS and 1 child died, and it was notified by a clinician seeing cases of HUS.

Earlier this year, we had a very similar outbreak but also different with an O26:H11 strain in Denmark. It was Stx1 positive and eae. We had 20 cases, all of which were children, median 2 years, very mild symptoms. Actually some of these patients were not examined because of diarrhea. The outbreak was discovered by real time PFGE of all STEC strains in Denmark, and it received very little media attention. When I asked friends around, they have never heard about this outbreak.

So what are the lessons learned in Norway. Well, the outbreak was discovered due to the notification of cases of HUS. Also methods in the clinical laboratories were inadequate in five out of six cases of the first HUS case. So they were not

even able to detect this non-O157 bug. Stx2 negative isolates dominated. So additional subtyping of the isolates were used in order to find the incriminated source and confirm the source. In this case, it was MLVA.

In Denmark, real-time pulsed field gel electrophoresis of non-O157 strains detected this mild outbreak, similar to an outbreak of O157 that we detected two years earlier, which was associated to milk. And it was only possible because all the clinical laboratories submit their isolates for typing at the Statens Serum Institute. The source was identified using access to purchase records and supermarket specifying exactly which product was purchased by the families that we interviewed. When we interviewed them, they were not able to point to a specific brand of sausage, but when we looked at the purchase records, we could point out the exact source. And cooperation with these supermarkets in searching of their central computers was absolutely important in identifying this source.

We were aware when the outbreak occurred

that it would not be a very serious outbreak, but we used this as an exercise to test these new methods in epidemiology and were successful.

Let's have a look at which countries have HUS notifiable. The 27 countries that I have data from, only 7 of these countries will have HUS and STEC notifiable. An additional 13 will also have STEC but in 7 countries, neither are mandatory.

And the clinicians will tell us that this is very difficult, and that was the reason why HUS was not notifiable in Norway, and I certainly see an interface and a lot of problems associated with how HUS may be notified in terms of having case definitions and Phil Tarr mentioned this earlier. And I think this is crucial in outbreak detection and surveillance, that we have clinicians made aware that they may be part of something which is going on that we normally wouldn't detect. And I think that's one of the main messages that I'd like to present you with.

Now which types are associated with HUS. We've seen a lot of data on that, and in 2004, we

presented this paper where you can see that the odds ratio for Stx2 here is 32.5 times higher and also higher than Stx2c which is a 4.7 for development of HUS.

I should point out, that all our HUS patients here are eae positives. So that's certainly a feature that we've seen previously this morning.

In this multi-variate analysis, O157 does not come out as statistically associated with HUS. So clearly, there is a huge difference between the data that we've seen here in the U.S. and Denmark. Again, differences in epidemiology may be the explanation.

The first study that I showed you covered about 205 patients, but I'd like to show you some unpublished data including data from 560 patients where still when you look at it, the compilation of eae and Stx2 is there in cases of HUS, and then you've got persistent bloody diarrhea, bloody diarrhea, persistent diarrhea and diarrhea and other features here. This is covering all the different Stx variants.

So we've been focused on trying to subtype and find out which are these different Stx2 variants, and in summary, there are about four subtypes of Stx1 covering seven or eight variants. I'm not going to take you through all these. Just notice the suffixes here. There are about 7 subtypes of Stx2, and there are about 35 variants.

I'm alluding a little bit to share Bob's presentation, how well have the different detection kits and methods that are being used? How have they been validated against this panel of strains?

Well, we've done some subtyping and it was published in June of this year. Basically we use sequencing and we use partial sequencing of some of the variable regions, the last part of the A subunit of the toxin and most of the B subunit.

The good news is that in Danish patients, out of the 35 possible variants, we only found 12.

The bad news was that at the time we found new variants that had never been described before that were quite common, and we found some types that were found in humans for the first time.

As an illustration here, the C variant found in the O157 strain, which is usually referred to, was never found in Danish patients. So that may be an exotic finding in this particular laboratory strain or that may reflect differences in the epidemiology.

We've looked at the attack rate of Stx2 variants, and I know this is a bit complicated, but basically what I'm telling you here is that if you've got an Stx2a variant here, you will have an attack rate of approximately 20 percent. So this is at least 5 percent above the data that Patricia Griffin presented you with this morning. And in non-O157 strains, again if you have a variant in the strains, you'll have an attack rate of approximately 20 percent. So 20 percent of patients infected with these different types here, they will develop HUS.

It's a little similar to the Sorbitol fermenting O157 but a little less maybe because usually that's up to 50 percent. In a recent Scottish outbreak, 20 patients were identified and 10 of those developed HUS.

In conclusion, we see these two variants associated with HUS. They're either the EDL933 here or these two which are identical, the Sorbitol fermenting clone will have the same as the O148 type here, and we have asked the question of how can STEC be classified? Is it certain virulence cocktail genes that are associated with severe disease rather than the serotype? We're not saying there's a direct causality between the Stx2a variant and HUS, but it's certainly a very good marker.

And Peter Feng presented this slide earlier which is one way of classifying STEC into five seropathotypes, which is certainly a step in the right direction. But I see several problems associated with this classification.

First of all, it's associated with serotype and not virulence profiles. And we've seen many bids on how many STEC types there are but there are more than 120 O:H serotypes listed in the second edition of Bergey's Manual of Systematic Bacteriology. And many of these O:H serotypes, such as the O113 that has been mentioned, the O145 strain that has been

serotyped, they display extensive heterogeneity. Even the O111s that we've heard about earlier. So within the same serotype, you see a lot of variation.

The involvement in outbreak which goes into the definition here is problematic because in the Norwegian outbreak, we saw a strain that we had never seen before. So we couldn't identify it until 2006.

And then the relative incidence, well, I showed you data that it certainly is skewed by lack of efficient detection methods, and it will vary very much I think according to the epidemiology. One of the dominating type in our Enter-net database is the O91 serotype and that's partly because Germany reports it and France, but we don't see it in Denmark very often. So there are problems, and there are certainly epidemiological questions that needs to be answered.

We have come up with some alternative classifications. So now you have Phil Tarr's, you have Peter Feng's and you have that of Mohamed Kamali, and here's a fourth one.

We think that HUS inducing STEC and/or

epidemic outbreak potential strains are best defined as eae and Stx2a, subtype positive, and we see from some German studies, that there are a small proportion of strains that are eae negative, the O113 strain has been mentioned, which carries the Stx2d activatable subtype. And then we have a few serotypes with eae in Stx1.

We have a lot of strains that will induce diarrhea in humans. They have many different virulence profiles, but their common feature is the capacity to produce Shiga toxin and association with human disease.

And then we have these animal associated STECs. They are very, very prevalent. We saw data from carcasses just this morning. About 54 percent of carcasses would have STEC on them before slaughter. They're found extensively in the reservoir, and they seem to be the natural habitat of these STEC types, yet we don't see any human cases.

Our Swedish colleagues have requested that we list Stx2e associated with edema disease in pigs as one of these types. So that's up for discussion.

We have some questions that we have had to answer ourselves in terms of management and treatment. Since 2000, both STEC infection and HUS have been notifiable and all patients with STEC are excluded or quarantined if they are children in institutions and day care, if they're staff of health care facility, workers, if they hospital staff or food handlers. And they are not allowed back into the institution or workplace until they have two consecutive STEC negative stool samples.

Now somebody was asking about the carry rate of STEC. We have carriers with STEC that have carried STEC for more than year. Can you imagine what kind of social problems that will induce in families and so on? So we've had to address this issue and especially in families where the kids are infected.

So we are currently revising our guidelines according to treatment, and they may include antibiotic treatment of asymptomatic patients if they have eae negative STEC identified, and we have eae plus Stx1 identified. There are some serotypes that

are a little hesitant on O103:H2, yet we have not seen any serious disease within this, after the acute phase. So we have treated actually some of our STEC patients with O103:H2.

And asymptomatic patients are likely to be allowed back into the institutions and day care and so on, after treatment. So I know this is a very controversial issue, but this is an issue that we have imposed upon ourselves. Out of prudence, we asked that everybody be quarantined in 2000. We are revising this currently.

Our recommendations are that adequate detection methods should include the isolation of bacteria so they can be subtyped, and the typing methods should be standardized. As I said, there are a lot of different variance of Stx2. Peter was telling us there are about 15 different kinds of eae. Are they associated with severe disease or not? And then subtyping methods for Stx2a variants associated with HUS should be implemented. We could even take that a bit further and ask the question should we look for these particular variants in the animal

reservoir and study the ecology of these variants.

And then I see an urgent need for standardized nomenclature, not only speaking of STEC, VTEC, EHEC and so on, but also that we speak a common language in terms of toxin and virulence factors.

So how much is detection and surveillance skewed by these differences?

Can we obtain case definitions for HUS to be notified within the public health system so that if we only have cases of HUS in an outbreak, we will be notified?

Will management and treatment of STEC patients depend almost on a case-by-case based assessment because this field is constantly changing, on an outbreak-to-outbreak approach? We were definitely more leaned back during the O26 outbreak in Denmark than the Norwegians were with their O103.

And are the differences in epidemiology? Even as I see it in the States, there are differences between the different States here in the U.S.

And then to answer the question of today's meeting, should non-O157:H7 STECs be considered to be

adulterants as *E. coli* O157:H7? And I'm saying the good news is yes, but the bad news is only some.

Thank you for your attention. I'd like to acknowledge my coworkers at Statens Serum Institute but in particular, my co-authors on the nomenclature of these toxins, Lothar Beutin from Germany, Denis Pierard from Belgium, and Nancy Strockbine from CDC. Thank you for your attention.

(Applause.)

DR. GOLDMAN: Thank you very much, Dr. Scheutz for clarifying the problem and even introducing new challenges in terms of further classification of these various groups of organisms.

We're now going to turn to Dr. Martina Bielaszewska who is a Research Fellow at the Institute for Hygiene and the National Consulting Laboratory on HUS at the University of Munster in Munster, Germany. Prior to this, she was a Research Fellow at the Institute for Hygiene and Microbiology at the University of Wurzburg, Associate Professor at the Institute for Medical Microbiology at Charles University and a visiting scientist at the Department

of Microbiology at the University of Toronto and Hospital for Sick Children.

Please help me welcome Dr. Bielaszewska.

(Applause.)

DR. BIELASZEWSKA: Good afternoon to everybody. I first of all would like to thank the organizers for inviting me here and to be able to share with you our German experience with non-O157 STEC. And actually, I would like to speak here about the non-O157:H7 STEC because as Flemming and Phil already told, we have a big proportion of infections in Germany caused by Sorbitol fermenting STEC O157, and these strains are example in important epidemiological and diagnostical features to this non-O157 STEC.

So in Germany, since 1997, STEC belong to notifiable microorganisms and HUS belongs to notifiable diseases. And according to this German Protection against Infection Act, there is so-called dual communication which means that HUS cases are reported by physicians and STEC isolates are reporting by microbiology laboratory, and this

insures that all infections are documented. These reports are first collected at the levels of local public health offices and from here, they are sent to the Central Public Health Office of Germany and this is Robert Koch Institute where they are analyzed and necessary interventions are implemented.

Between 2001 and 2006, there were between 927 to 1250 STEC infections per year and between 55 and 115 HUS cases per year reported to Robert Koch Institute. And approximately 80 percent of reported STEC belonged to non-O157 serogroups.

A study from Robert Koch Institute investigated risk factors for STEC infections in Germany. This was study performed during 2001 to 2003, and included 202 cases of STEC infections with different clinical manifestations. Five of these patients developed HUS. And 86 percent of patients had by culture non-O157 STEC strains.

And this study interestingly demonstrated that the risk factors for STEC infections are age specific. In children younger than three years, the major risk factors were direct contact with

ruminants, playing in sandbox or drinking raw milk. So this was the major way of transmission of infection in this age group is fecal oral transmission. In contrast, in children older than 10 years, the major risk factors were different kind of foods.

And here are the serogroups which were isolated in this study. So as I told already, 86 percent of isolates were non-O157 STEC and the most frequent serogroup was 113, and this was followed by O26 and O91. And as you can see here, the majority of this non-O157 STEC were isolated from patients with uncomplicated diarrhea, non-bloody diarrhea, whereas 1/2 of these 13 person of O157 were isolated from patients with bloody diarrhea and HUS. So it means that the non-O157 were associated with milder outcome of the disease.

And now I come to hemolytic uremic syndrome because we are German national consulting laboratory for HUS and we investigate most of stools from HUS patients in Germany. I would like to show you the serotypes which we identified in patients during

study covering 1996 to 2006. You can see that the situation in Germany is different from that in what was reported in the United States. *E. coli* O157:H7 accounts for half of isolates from HUS patients, whereas the other half, these are non-O157:H7 strains, and the most frequent here are the Sorbitol fermenting STEC O157 nonmotile strains. They account for approximately 1/3 of this non-O157:H7 and for 17 percent of all STEC associated with HUS. Additional frequent serotypes are O26:H11 or nonmotile, 145, 103 and the last here is 111. So this is also a bit different.

But the message of this graph is that half of the isolates from HUS in Germany are non-O157:H7 STEC and their association with HUS is somehow prove that these strains are really pathogenic.

Sorbitol fermenting STEC O157:H- in addition to being the second most common cause of -- cases in Germany, cause the largest outbreaks in Germany. Between 1996 and 2006, there were four outbreaks of HUS. These included between 6 and 38 cases and I think that the 2002 outbreak with 38

cases is probably the largest outbreak of HUS which case caused by STEC worldwide. In this 2002 outbreak and in the first outbreak, 1996, the mortality was approximately 10 percent. As you can see regarding the source, in two outbreaks the source remained unknown. In two outbreaks, case control studies implicated certain kinds of food as a possible source but Sorbitol fermenting STEC O157 were never isolated from these foods or from the environment.

The common features of these outbreaks were that most of them, it means the first three, all but the last, occurred during cold months of the year, and they were all detected by increased frequency of HUS cases. There were no according or parallel increasing in number of cases of diarrhea which is the feature which is typical for outbreaks caused by *E. coli* O157:H7. So that's why these outbreaks are called HUS outbreaks only.

This observation in outbreaks and also in sporadic cases of infections caused by Sorbitol fermenting STEC O157 demonstrate that there are certain differences between STEC O157:H7 and Sorbitol

fermenting strains. And this is not only in phenotypes, what is well known, but there is also different epidemiology of these infections such as by different seasonality. The Sorbitol fermenting mostly occurred during the cold months, and also in each of the patients, which are -- affected. Sorbitol fermenting STEC O157 infection occurred predominantly in children younger than 3 years, and these strains have this in common with non-O157 STEC. Also the majority of non-O157 STEC associated with HUS are isolated from patients under three years.

And there is probably also different risk for HUS development between STEC O157:H7 and Sorbitol fermenting strains. This study from Robert Koch Institute established the risk for HUS development after infection with *E. coli* O157:H7 to be 10 percent but this seems to be higher in Sorbitol fermenting strains as suggested by observations from the outbreaks. And also in the large outbreaks caused by these strains in Scotland last year, 50 percent of patients which were infected developed HUS. So it seems that this risk is really high.

And this is just to demonstrate it was also said here already that Sorbitol fermenting STEC are not anymore problem only of Germany but they spread to several other European countries and they were also isolated in Australia and Asia, in South Korea.

And here are some examples of outbreaks caused by non-O157 STEC in Germany. In contrast to outbreaks, caused by Sorbitol fermenting O157 strains, these outbreaks were usually small. They occurred in families or in institution for young children like day care centers or kindergartens, and they usually involve only cases of uncomplicated diarrhea. Only in some of them was also HUS detected and one outbreak caused by STEC O26, all strains produced Shiga toxin 2, there were only three HUS cases. We were not able to detect any cases of diarrhea.

STEC O26:H11 in this case, strain which produce Shiga toxin 1 also caused the largest outbreak caused by non-O157 STEC in Germany. This was in 2002. This was multi-state outbreak which affected children in three states, but there are only

cases of diarrhea, no HUS. And this outbreak, one of exceptions were food was implicated as source of the infection but again only based on epidemiological study. This beef containing product which is in German called Seemerrolle couldn't be cultured, by culture shown to contain the strain. The only outbreak in Germany were cattle and contaminated milk was shown to be the source of infection was this first 1989 outbreak caused by STEC O22:H8.

Now I will shortly characterize the major STEC, non-O157 STEC which we have in Germany. STEC O26 are the most frequent cause of HUS is non-O157. They are responsible for more than one-third of these strains. There are two serotypes, O26:H11 and O26 nonmotile type strains, but all these strains possess fliC gene and encoding H11 and -- so this one clone, O26:H11. STEC O26 produce Shiga toxin 1, Shiga toxin 2 or both these toxins, but since late 1990s, there is a shift in Shiga toxin genotypes of these strains from Shiga toxin 1 to Shiga toxin 2. It means that the Shiga toxin 1 gene is replaced by Shiga toxin 2 gene, and this Shiga toxin 2 only clone is

significantly associated with HUS and with ability to cause outbreaks.

STEC 145 are the second most frequent non-O157 STEC associated with HUS. The motile strains belongs to serotype O145:H28 or H25, but the majority of these strains are nonmotile. So you cannot determine H antigen by classical serotyping and that's why we performed the fliC typing, and this demonstrates that there are two fliC types which agree with the serotypes and this is fliC H28 which is the major type and fliC H25 which is only in 2 percent of the strains. And interestingly, each of these fliC types is associated with a particular kind of eae gene.

In contrast to O26, we see only three different Shiga toxin genotype. There are five different Shiga toxin genotypes in STEC O145, and the most common is Shiga toxin 2 only which again these strains form the major number of strains which are isolated from HUS patients.

Also the majority of STEC 111 are nonmotile strains and therefore here we need the fliC typing to

really determine the serotype of the strains and here are three different fliC types, H8, H11 and H10, and some of these strains with H8, they have -- sequence in the fliC gene. So these results, of course, in different -- pattern.

Strains with fliC H8, it means the serotype 111:H8 are the most common of these 111 isolates. It is responsible for approximately 80 percent of the strains isolated from HUS patients. And all these three fliC types or also serotypes, you can differentiate further by Shiga toxin genotypes and by a specific combination of the presence of eae gene, of -- genes located within O Island 122 and the presence of cad genes which encode Lysine Decarboxylase and also by the ability to express this phenotype. And as Peter Feng already told, this most common 111:H8 strains are losing the Decarboxylase negative.

And again, in STEC 111, we see the shift in Shiga toxin genotypes. This started approximately in 2000, and there is the shift from strains which contain Shiga toxin 1 only to strains which contain

Shiga toxin 1 plus Shiga toxin 2. So here is the current situation, that in O26 were Shiga toxin 1 was replaced by Shiga toxin 2. This is not possible 111 because Shiga toxin 1 is encoded by a defected trait which is fixed in the gene and cannot be lost. So here Shiga toxin 2 has been introduced in addition to Shiga toxin 1. And again, this acquisition of Shiga toxin 2 is significantly associated with the ability of these strains to cause HUS.

The majority of STEC associated with HUS in Germany, and these are all these serotypes I was speaking about until now are eae positives, -- the gene encoding intimin. Only three to four percent of STEC associated, HUS associated STEC are eae negative, but as you can see here, the majority of these strains possess Shiga toxin 2d activatable, but this is the variant which is activatable by -- and which are highly biologically active in mouse motile. So eae negative STEC are -- by HUS but most of these strains are deactivatable Shiga toxin and the serotypes of these strains are mostly O91:H21 and 113:H21.

And this is the diagnostic scheme which we use in our laboratory to detect STEC and this allows to detect *E. coli* O157:H7 and non-O157 strains. This scheme includes enrichment of the stools in GN broth. This is for all STEC. Then specific enrichment using immunomagnetic separation for *E. coli* O157 and the enriched stools are plated on Sorbitol MacConkey agar and EHEC hemolysin agar.

After incubation, the whole growth from the plates is harvested into saline and this suspension is used as a target for PCRs, targeting Shiga toxin 2, eae, rmbO157 and sfpA genes. Just to shortly explain why we use this last PCR, this sfpA PCR, this is a PCR which we use to look specifically for Sorbitol fermenting STEC O157 strains because this gene which is located on the large plasmid of Sorbitol fermenting strains is not present in *E. coli* O157:H7. It is not present in other diarrhea enterogenic *E. coli,* in common saw *E. coli,* -- pathogenic *E. coli* and also not in other -- bacteria. So it's seen until now that this gene is really specific for Sorbitol fermenting O157 strain. So

that's why we use this in our diagnostic scheme.

So the results of PCR give us the preliminary hint of what is probably in the stool. Then to isolate the strains, we plate this PCR positive stools again on SMAC, CT-SMAC and enterohemolysin agar, and the strains are isolated based on their characteristic phenotypes or if this is not possible, then by colony hybridization and further characterized for molecular and phenotypic features, and this is just to show what is well known. This is *E. coli* O157:H7 which is very easy to be detected on Sorbitol MacConkey agar. In contrast, Sorbitol fermenting strains and all, or not all, but the majority of non-O157 STEC look like normal flora. So they are clearly missed if only SMAC is used for culture, and this is the reason why we introduce enterohemolysin agar to diagnosis the majority of the strains of these major serotype of non-O157, both eae positive and eae negative, express hemolytic phenotype and that's why it can be detected from enterohemolysin agar.

The problem are Sorbitol fermenting strains

which although they possess the gene encoding hemolysins, they don't express the phenotype. So it's very difficult to isolate them but not impossible. It is possible from Sorbitol MacConkey agar after immunomagnetic separation using either slight agglutination, usually more colonies, 5 to 10 or if this is not possible then by colony hybridization and here the big help is really the sfpA PCR which gives you information that the strain is present, and then it's possible to isolate or it must be possible. But, of course, there is clearly need for selective diagnostic medium for these strains.

And this is very shortly to stress that the culture is really necessary. It shouldn't be abandoned in favor of this non-culture method because isolation of STEC from stool is necessary for correctly perform epidemiological studies for monitoring of virulence of the strains which are in the population, and by this highly pathogenic clones which are emerging can be identified.

Just very shortly, to thank all colleagues

from Robert Koch Institute who performed the epidemiologic work in Germany, and to Professor Karch, the head of National Consulting Laboratory in Munster and to my colleagues, Alex Freidrich and Alex Melimann for their work. Thank you.

(Applause.)

DR. GOLDMAN: Thank you, Dr. Bielaszewska. Thank you very much for sharing the German experience. I'm sure some of the differences that have been highlighted in both the last two presentations will prompt some questions.

We will now move, shift a little bit to the industry perspective. We have two different speakers, and we'll talk about the meat perspective first, since you've heard a lot about the reservoirs for these organisms.

Dr. Randy Huffman is the Vice President for Food Safety Programs at the American Meat Institute Foundation. He joined AMI in January of 2000, and manages the Foundation's Food Safety Research Agenda, assists members in finding solutions to food safety and quality challenges and serves as a liaison

between AMI and various scientific organizations.

Among this various responsibilities, he's been part of an AMI Foundation led *Listeria* Intervention and Control Task Force and the Beef Processing Best Practices Task Force, that have developed and conducted multiple in depth training workshops for industry and government.

Prior to joining AMIF, Huffman was the Director of Technical Service at Coke Industries in Wichita, Kansas, where he managed food safety and product development issues. He received his BS in Animal Science at Auburn University and a MS and Ph.D. in Animal Science from the University of Florida.

Please welcome Dr. Huffman.

(Applause.)

DR. HUFFMAN: Thank you, Dr. Goldman, and I appreciate the opportunity to address this group today. Thank you to FSIS, CDC and FDA for hosting the meeting and listening to the industry's perspective on this very important issue.

I'll start by saying that for much of the

last decade, if not longer, our industry has taken this issue of food safety and specifically control of *E. coli* O157 extremely seriously. We've invested a lot of time and a lot of effort and a lot of money in trying to address this problem because at the end of the day, the numbers that I represent, which are beef processors, meat processors in general, specifically today I'll talk about beef processing and the slaughter industry, those members are concerned with selling safe food. Selling safe food is good for business, and that's what we'll strive to do every day.

So with that as a background, I want to provide our perspective and specifically Dr. Goldman presented me with a single question to address today and I really could get us a little bit back on schedule by making the presentation very short and answering your question with a single word. We think yes.

But the question was, do existing interventions for *E. coli* O157 work as well against non-O157 STEC? And at least at this point in time,

and I'll address this in more detail toward the end of the talk, but we believe that with the existing data that we have to evaluate, we believe the answer to that question is yes.

But given that I do have 15 minutes to talk, I'll use every bit of that to explain to you some of the things that we have done to improve food safety in the beef processing sector. I think it's important for many of you in the audience who are probably not familiar with the intricacies of beef slaughter and beef processing, and so we'll go into that in a little bit more detail and I'll talk to you about some of the things that we have done as an industry working jointly with our many stakeholders throughout the process and other groups within our industry such as the National Cattlemen's Beef Association, NAMP and NMA and other trade associations that represent the industry. And I'll end with just a little bit of what we think represents some progress that our industry has made.

So a brief history, and this shouldn't be news to anyone in the audience. So I'll keep it

rather short but obviously we became very engaged in this issue as an industry in the early nineties as a result of several large outbreaks specifically associated with undercooked ground beef.

In about '93, FSIS announced the zero tolerance policy for fecal contamination on carcass and began enforcing it rather strictly.

Then in '94, in somewhat of a surprise announcement, then Administrator Michael Taylor announced at the AMI annual convention that *E. coli* O157:H7 would be an adulterant in a raw product, raw ground beef in this case, and that would have been the first time that we're aware of, from a regulatory standpoint, that a pathogen would be declared an adulterant in a raw product at least in the meat industry.

So that was probably a watershed event for our industry, no question about it, and certainly led to over the next several years, numerous larger recalls, a couple of large outbreaks and certainly a lot of action in our industry to try to address this problem.

One of the things that we were challenged with at this point in time was somewhat of a lack of information. Today, we've heard so much about the science of this issue from presentations today, and a lot of this we take for granted in this decade, but in the early nineties, there was a real dearth of information about the prevalence of this organism and about methods that we could use to control it.

So the industry generally reacted somewhat reluctantly to this policy but over time, as we collected more information, we learned, we improved, we implemented validated intervention technologies, and I'll go into some of those in a little more detail.

You could argue that that initial policy created maybe a delay in progress but over time, it certainly did lead to an industry that produces a safer product today than probably a decade ago.

Now one of the aspects, and we've heard a lot of discussion today about testing, and one of the experiences we had with *E. coli* O157 with respect to testing, at least initially, was somewhat of a false

reliance upon the idea of end product testing as a means to ensure safety. And I think we'd all recognize, all the scientists in this room today, and certainly many organizations have pretty well established, that testing finished product is not going to be 100 percent effective at insuring food safety, and I'm certain that I'd get no argument from many of you in the audience today. But testing certainly does provide us with a lot of information and allows us to validate and verify our processes, and that's how we use it today.

The industry has conducted, and we don't have an accurate count, but I think it's safe to say millions of tests for *E. coli* O157 over the last 15 years or so, and certainly that's helped us understand the problem and make improvements. So testing, as I'll summarize later, as well, testing should be used to verify the effectiveness of the interventions and process.

So we've taken on many steps to try to improve food safety, and one of those was the declaration by our Board of Directors at AMI about

2001, that food safety would be classified as a non-competitive issue in our industry. This led to a lot of change. It led to a lot of information sharing among the technical representatives within the companies, a lot of learning from each other and sharing of technical information, not only on activities that worked to improve safety but also on things that maybe were tried and didn't work. And so sharing of information became quite obviously to me. As a facilitator of this industry, I got to see it firsthand, the technical representatives from companies who were otherwise highly competitive in the marketplace, they were able to share information freely, and we think that made some improvements.

Certainly we've invested a lot of money in food safety research. I was talking to Beau Reagan from NCBA earlier this morning, just to get a rough estimate, and we think our two organizations, AMI and NCBA, alone since 2000 have invested over $30 million in food safety research. And certainly a large portion of that comes from the Beef Check Off. AMI has a food safety research program targeted at one of

our primary priorities being *E. coli* O157 control. We continue to fund research. Beau was telling me about $2 million a year through NCBA. AMI has about $500,000 a year that we invest in research projects. So we still recognize that that is an important aspect.

But implementation of interventions in the process is really the key to reducing the prevalence of this organism, O157, and I'll talk in a minute, we think as well for non-O157 STEC.

A couple of the other things that have been affected we believe is this relationship between the suppliers, the members, the processors that I represent and their customers. Customers demand safety as well obviously, and so this relationship within the industry certainly has led to some improvements and some recognition of ways we can improve our process.

And then finally, the implementation of expanded trim testing programs for *E. coli* O157 instituted earlier, probably in 2002, we've seen a dramatic increase in the amount of testing by

industry. So all those things, as well as others, have led to some improvements.

I want to briefly go through sort of a high level overview of some of the interventions that are in place in beef slaughter, and I'll start off with saying that we along with several other organizations have tried to get this information out to the working level folks in the plants, folks that are managing the slaughter floor, managing the processing lines, trying to convey this information which, in many cases, appears rather simple on the surface but that there is a lot of detail that goes into implementing these best practices.

We work cooperatively with our groups in developing what we believe are the best ways to slaughter animals and to do it hygienically. We've implemented several techniques, and I'll talk about each of these in a little more detail, but this is kind of a quick list of many of the factors that are important in the process.

So as Dr. Koohmaraie mentioned earlier, the identification of the hide and the hide removal

process is a key step in controlling the transmission of *E. coli* O157 to the carcass. That was identified as a key step, and we recognize that as being very important.

Here are just a couple of examples, the use of physical barriers, in this case demonstrating that just a plastic or paper barrier that is laid between the hide and the surface of the carcass to prevent any physical contact between the contaminated surface and the essentially sterile surface of the carcass. So simple technologies, it's hard to even call this a technology, but a practice has been effective.

Using 160 degree sterilizer dips and using a two knife system so that workers can trade those out between animals is another simple step that's implemented at the hide removal point.

Dr. Koohmaraie also mentioned the development of the device called a steam vacuum which was developed at the Meat Animal Research Center. These are just a couple of pictures that show the steam vacuum unit in action. And it's use on what we call pattern mark or the area of the carcass where

the hide is opened up, where that's most likely where transmission of pathogens might occur. -- showed that the effectiveness of this in achieving about a 1 log reduction in total bacteria counts.

The use of organic acids such as lactic and acidic acid in a rinse cabinet has been shown to be effective at the pre-evisceration step. Hardin (ph.) et al., Journal of Food Protection published some work on this, again looking at total bacteria count as an indicator of organism.

Probably the most effective treatment on the kill floor would be the use of a thermal treatment, either hot water or steam. So steam cabinets and hot water wash cabinets such as this are implemented in nearly every beef slaughter plant in the U.S., and there's plenty of data that demonstrates the efficacy of this particular method. Again, there's some data in the published literature on APC, which is measured, you know, in in-plant situations since the prevalence of the organism is so low but certainly is an indicator of the effectiveness of this method.

And as I go through these, of course, our target all along has been O157 but we believe that these technologies are broad spectrum, especially when we talk about heat. We're not aware of any data that would show that the non-O157 STECs would have any unusual resistance to heat. And so that's really the effectiveness of this particular intervention is the application of heat, in getting the surface of the carcass to as FSIS recommends here 165 is going to be as effective against non-O157 STEC as it is against O157.

This is one paper that Dr. Koohmaraie referenced earlier. The data that he showed is more extensive, but this particular paper published by Cutter and Rivera specifically answers the question that Dr. Goldman posed to me, and that is do the interventions currently in place work as well against non-O157 STEC as they do against O157. And in this study, published in 2000, really looked at that question using O111 and O26. As well, they looked at *Salmonella* and *Salmonella* DT104. And this was a laboratory-based study. It was not done under plant

conditions. It was done with excised tissue under very controlled conditions, and it did show, basically these are the conclusions in quotations, interventions used currently in the industry and this would have been hot water or steam, lactic acid, acidic acid. We do not use trisodium polyphosphate in normal operations today, but these other interventions are widely implemented.

Hot washing is a new intervention. Before the hide is removed, certain plants have this intervention in place, and it has been shown to be an effective step.

We're implemented these practices as I've mentioned previously. We've continued to look for ways to improve and look for new technologies, but really it gets down to management commitment, employee willingness and the ability to invest capital in these processes. It definitely requires those things to do it properly. And cooperation throughout the value chain is another key step.

One of the efforts that we've done to try to get this word out beyond just the membership of

our organization and the others is cooperation with the organization mentioned, the Beef Industry Food Safety Council. I won't go into detail on these but our group meets frequently and we develop these best practice documents. They're freely available and posted here at this website. We continue to work to develop and improve these best practice documents and get them distributed.

Testing, I mentioned briefly earlier. Again I'll just reemphasize that we view testing for *E. coli* O157 as a method to validate and verify that the process is working and that the interventions that are in place are effective.

I won't talk a lot about pre-harvest because I see I am out of time just about, but we continue to look for opportunities to reduce the carriage and the prevalence of these organisms prior to the animal arriving at the slaughter plant. Certainly if there were effective interventions that could be implemented at that step, we would be very interested in looking for ways to do that, and we continue to work cooperatively with our other groups

such as NCBA to look for pre-harvest interventions that are effective. And certainly we should consider both O157 as well as potentially other pathogens that are out there.

So I want to try to close with at least one indicator of how we're doing. This would be the ongoing routine monitoring by FSIS of finished ground beef, which is one marker, if you will, for the prevalence of the organism in ground beef. And I summarized this last night, with the most recent data, the last positive that's posted at the web was on 9/25. And so for this year, this represents at this point, 95,999 samples, 19 positives so far this year, for a .19 percent prevalence rate. The past two years, it was .17 or .18. So we're very similar in terms of O157 prevalence, the previous three years. Certainly we have had some high profile recalls and a couple of outbreaks this year that certainly have caused us to refocus and try to understand what might be happening. But it is important to use all the data that we have at our fingertips, and this data does show that we've made

some improvements over time and that the prevalence, at least, as it's measured in this particular program, is relatively consistent.

So in summary, I'll close by saying that we need to have rationale and achievable regulatory policies in place that are based on measurable public health outcomes. As an industry, we need a foundation for process control in place, best management practices, good manufacturing practices, however you want to define those, they need to be in place every time, and we can continue to work and strive to achieve that as an industry.

We need reliable and timely pathogen data to understand our processes, and so some of the information that I've learned today is going to be very useful as we continue to evaluate our practices and our processes.

We have to use data to develop valid control strategies. We can't just rely on intuition. We have to use data to make decision and to modify our practices.

We have to continue to share best practices

in a non-competitive fashion. That has been effective in the past. We'll continue to embrace that going forward.

And we think we're making progress as an industry. We're not where we want to be as an industry, and we'll continue to strive to get better. We have to recognize that there aren't any silver bullets, and we'll continue to recognize that.

So to close, do industry interventions for O157 impact non-O157 STECs? That was the one question I was asked, and our answer would be that there are currently no data to indicate that the existing validated beef processing interventions would not be similar in effectiveness against multiple serotypes of *E. coli.* And we do have at least one published study that specifically answers that question.

So with that, I'll close. Thank you, Dr. Goldman.

(Applause.)

DR. GOLDMAN: Thank you very much, Dr. Huffman.

We're going to shift here and I had several discussions with Dr. Bob Brackett, who I don't think is here right at the moment about how we should represent the non-meat industry. There have been, and you've already heard today some associations between illnesses and produce and raw milk in particular. And so we have asked Jenny Scott to represent all of the other industries as best she could in representing their perspectives on this particular problem.

And as many of you know, Jenny Scott is the Vice President of Food Safety at the Grocery Manufacturers/Food Products Association in Washington, D.C., where she's been employed in a variety of positions since 1980. She directs the Association's food safety activities on food inspection crisis management and provides technical assistance and expertise to members and staff on issues and policies related to microbial food safety.

She received her BA Degree in Biology from Wellesley College and MS in Bacteriology from the University of Wisconsin, and a MS in Food Science

from the University of Maryland. She has published widely in various areas of microbial food safety, and she currently serves as a member of the U.S. Delegation to the Codex Committee on Food Hygiene and is also on the U.S. National Advisory Committee on Microbiological Criteria for Foods.

Please welcome Jenny Scott.

(Applause.)

MS. SCOTT: Thank you, David, and it's a pleasure to be here. I think that this meeting is an excellent forum for sharing current information on emerging pathogens, and I'd like to see more of them.

I will start out the way Randy did and give you industry's position that we want food to be safe, and we are concerned about any microorganism in foods that can cause illness.

We also know, and we've heard here today, that some, but not all, of the non-O157 STEC can cause illness.

If an organism presents a significant risk, then companies are going to have to address this in their HACCP plans. And currently, we have

insufficient information to identify non-O157 STEC as a hazard reasonably likely to occur for most foods, and this is the basis for addressing a hazard in a HACCP plan.

So industry needs some answers. We need to know what foods these organisms are associated with, and we need to know which of these foods have been associated with illness from these organisms.

This is a graph of or a chart of all of the *E. coli* O157:H7 outbreaks worldwide from 1982 to 2006. And, thank you, Randy, for providing this to me. This was developed at the University of Wisconsin. You can see that O157 comes not only from beef and other meat, but also from dairy, produce, other foods and other sources, water, person-to-person spread.

We would not expect non-O157 STEC to be much different with respect to where it comes from, at least at this point in time, and I haven't heard anything today that would suggest otherwise.

Food sources of non-O157 STEC are primarily foods of animal origin, from which over 100 serotypes

have been isolated. These come from beef, lamb, pork and chicken, and also from animal products such as milk and cheese.

Also, we might expect it to be in foods that are cross-contaminated from animal products, and I'm not looking at those animal products as the types of products you eat, but read that as feces.

So certainly produce would be source, and we've seen some evidence of that here today. We've also seen illnesses from a variety of sources from milk, from sausage, from salads, and this is worldwide. So we are not seeing these organisms coming from anywhere that we haven't seen O157.

I'm going to look at a couple of studies that have come out of France recently. Pradel, et al., looked at the prevalence and the characterization of Shiga toxin producing *Escherichia coli* isolated from cattle, food and children in a one-year study. They looked at 2143 samples using PCR for the Shiga toxin-encoding genes. They found that 60 of 603 cheese samples were positive for the Shiga toxin gene. They were able to isolate STEC

from 5 of the 603 cheese samples.

In the study, they ultimately had 220 Shiga toxin isolates. Thirty-two of these were not cytotoxic. The eae gene was found in 12 of these 220 strains, and they concluded that the majority of STEC isolates from cattle, beef and cheese, at least in this study, were not likely to be pathogenic for humans.

Perelle, et al., did a study screening food materials for the presence of the world's most frequent clinical cases of Shiga toxin-encoding *E. coli,* O26, O103, O111, O145 and O157.

They used PCR-ELISA tests for the Shiga toxin gene, and they found that 21 percent of 205 raw milk samples and 15 percent of 300 minced beef samples were positive for this gene. So of those 88 samples, when they checked them with another PCR assay, they found 74 of them confirmed as being Stx positive. They then did a multiplex real-time PCR for the specific serotypes of concern, O26, 103, 111, 145 and O157, and from this, they confirmed 18 of the 74 STEC positives were these serotypes of concern.

So they determined that the contamination by the main pathogenic *E. coli* O serogroups of major public health concern were 2.6 percent in minced beef and 4.8 percent in raw milk. But the most probable number of these organisms was very low, 1 to 2 STEC cells of the highly pathogenic serogroups per kilogram.

And they concluded and I'm quoting directly from the paper that, "Contamination of beef meat and raw milk by the highly pathogenic serogroups of STEC is very low," and "Risk of consumer infection by human pathogenic strains of STEC present in these samples is probably very minor."

It also noted that there were both Stx gene positive and Stx gene negative strains present in each O serogroup, and when both Stx and O serogroup genes sequences were detected in food, there was no evidence that these signals were displayed by a pathogenic *E. coli* strain. So they concluded that isolation from food with confirmation is necessary but they also indicated that it was problematic and time consuming.

A New Zealand fact sheet on non-O157 STEC indicates that an isolate possessing the ability to produce either Shiga toxin gene in the absence of other virulence determinants is unlikely to be a major pathogen.

So again, industry needs some answers. How do we detect the pathogenic strains of non-O157 STEC?

Food businesses need rapid tests for short shelf life products in particular, so that they can verify and validate interventions. These tests need to be collaboratively studied.

Currently, we don't have any reason to believe that the interventions that address *E. coli* O157 or *Salmonella* would not be effective against non-O157 STEC, at least to the same degree that they are effective against O157 or *Salmonella*. If there are unique properties or resistances of these organisms that suggest otherwise, then industry needs to know that, and if there are foods that are unique to the non-O157 STEC, again we need to know that so that we can identify these organisms as hazards that need to be addressed in a HACCP plan.

Let me turn to this question of adulteration. What makes a pathogen an adulterant?

Well, we have a lot of definitions of adulteration in our laws, but basically a food is adulterated if it bears or contains any poisonous or deleterious substance which may render it injurious to health. However, if a substance is not an added substance, a food is not adulterated if the quantity of the substance does not ordinarily render it injurious to health.

Ultimately, this gets determined in our Court system, and the U.S. Courts have held that *Salmonella* in raw meat is not an adulterant because the ordinary methods of cooking and preparing the food kills *Salmonella* and that O157:H7 in ground beef is an adulterant because *E. coli* contained in ground beef may be injurious to health when it's properly cooked according to the way Americans consider this product properly cooked.

So where do the non-O157 STECs fall?

Well, there may be instances when it could fall into either category. So with respect to FDA

regulated products, and certainly FDA is going to continue to take action against ready-to-eat foods containing pathogens. They have done that with respect to *Salmonella* in produce, and that's not going to change. If there is a pathogen in a food product and it's making people sick, it will be considered an adulterant.

We need to be able to assess which of the strains of non-O157 STEC are pathogens and at what level they are causing illness. Again, there's no reason for us to believe that the current practices for other pathogens in FDA regulated products such as pasteurization of milk, would not also address the pathogenic non-O157 STECs.

However, at this point there are insufficient data to warrant a change in industry practices or regulatory requirements with respect to these organisms.

In this country often it is a crisis that is the trigger for change. The Chinese ideogram for crisis is composed of two characters. The first one meaning danger, and a second one meaning opportunity.

We don't have a crisis at this point in time with respect to non-O157 STEC. I think with respect to certain strains of non-O157 STEC, you can say we do have a danger, and this does lead to some opportunities.

We need good methods to rapidly detect pathogenic strains of non-O157 STEC, and these need to be collaboratively validated with respect to the food of concern. And, these need to be cost effective for industry to use them.

We need to better assess the risk for non-O157 STEC to determine if changes are warranted, and then any changes that we make, need to be based on science.

And clearly, we don't want to wait for a crisis to happen. But we also shouldn't lose focus that *E. coli* O157:H7 is the *E. coli* of most significance to public health in the U.S. today. And we have limited resources and we need to focus our resources so that they address the issues of most concern to public health. Thank you.

(Applause.)

DR. GOLDMAN: Thank you very much, Ms. Scott.

We will now turn to the consumer perspective and as this Agency and FDA as well, always likes to do, we want to consider the whole range of perspectives and, of course, this one is as important or more important than the others. We want to know how these pathogens, these organisms that are pathogens affect humans, and our jobs collectively are to come up with rational policies for minimizing the danger that they may present.

Nancy Donley is the President of Safe Tables Our Priority, a national non-profit grass roots organization dedicated to reduce foodborne illness and death through sound public policy advocacy, building awareness of foodborne risks and its management and providing victim assistance.

She has served on the USDA's National Advisory Committee on Meat and Poultry Inspection from 1996 to 2002, and she has been recognized as a leading proponent of improvement in both government and private food safety efforts since the death of

her six-year-old son, Alex, over a decade ago, from the consumption of *E. coli* O157:H7 contaminated ground beef.

Please welcome Ms. Donley.

(Applause.)

MS. DONLEY: It's nice you only have to have one consumer perspective, because we all the products.

I'd like to thank FSIS, FDA and the CDC for holding this meeting. I'm especially heartened to see the three agencies working together on the need to address non-O157 STEC in our food supply. As a country, we've learned the hard way, through foodborne illness outbreaks that animal reservoir pathogens are not of concern solely in the possible contamination of meat. Once considered the hamburger disease, *E. coli* O157:H7 and its STEC cousins, are now known to contaminate a wide range of foods including product, juice, sprouts and milk.

It would be unusual, I think to the point of delusional, to think that disease causing non-O157 STEC would veer from the same paths of contamination

that occurred with O157.

That's why I want to commend the governmental agencies today and especially FSIS, Dr. Goldman, for taking the lead, for collectively analyzing pathogenic contamination of foods as a whole instead of through the tunnel vision approach of looking at single product categories individually.

It will be through the pooling of interagency talent and resources that we can most effectively create a proactive approach to food safety, rather than the reactive one we have had in place for so many years.

I think that it's safe to say that leaders in all sectors of food safety, industry, academia, government and consumer advocates, would agree that a prevention strategy to keep disease causing or pathogens from making it into commercial is the best strategy to employ to most effectively protect public health.

Although the association of STEC with human disease dates back to 1982, it was until the 1993 Northwest Pacific O157 epidemic, that the dangers of

foodborne pathogens first made it onto the airways and catapulted the issue of unsafe food to the public's attention. That outbreak alone sickened more than 700 people and killed at least four children.

For those of you unfamiliar with the consumer organization that I'm representing, let me briefly explain who we are. STOP was born in the aftermath of the Jack-in-the-Box outbreak. Our founders include parents of children impacted in that epidemic as well as others impacted by O157 nationwide. STOP is a national non-profit organization whose mission is to prevent illness and death from pathogens in the food supply, and as Dr. Goldman explained, our work involves sound policy advocacy, building awareness of foodborne illness and its risks and its management in providing victim assistance.

Our members include families who have suffered illness and loss from a broad spectrum of food, including contaminated meat and poultry, produce, juice and ready-to-eat processed foods.

As you know, I became involved with STOP shortly after its inception, after the death of my six-year-old son, Alex, from *E. coli* poisoning in 1994. Alex's case was an isolated occurrence. He was not part of an outbreak. He suffered from both HUS and TTP.

My goal as President of this fine organization is to put us out of business, by working to see practices and policies enacted that will lead to a significantly safer food supply with a corresponding decline in the number of foodborne diseases and deaths.

STOP has been keenly interested in the topic of non-O157 STEC for years, and we appreciate the opportunity to participate in today's discussion. Over the years, we've had conversations with CDC and both FSIS and CFSAN about the need to expand programs to include the detection and prevention of non-O157 STEC contaminated foods making it into the marketplace.

These discussions were frankly during the prior Administration. We've wasted a lot of time,

but I hope that today's meeting will lead to a fast track of ratcheting up food safety by putting preventative measures in place to keep disease causing STEC out of the food supply.

STOP has been working with foodborne illness victims and their families for nearly 15 years. We are aware of many situations involving victims diagnosed with HUS, preceded by bloody diarrhea, but who were not O157 culture confirmed. Some were never cultured at all. Others were cultured too late, and if they had the O157 strain, the bacteria itself had passed through the body although the toxins remained. And many others, we feel, may have had O157 STEC but were not cultured for them.

I want to share with you briefly the story of a STOP family where it took two years to determine what had taken the life of their two-year-old daughter, Anna, in 2002. Anna was the youngest of three daughters. The Nelsons live in Wisconsin close to the Wisconsin/Minnesota border. The family routinely dined at restaurants and bought groceries

in both states.

Anna fell very ill and was hospitalized in the Minneapolis-St. Paul Children's Hospital where her condition spiraled into HUS and she died in a matter of days. Her culture for O157 had come in after her death as negative. The public health department then did nothing, even though she had died from HUS, a syndrome which is closely associated with *E. coli* poisoning. They were not required to, nor did they investigate the possible cause of her death.

When Anna's parents returned home, Anna's father had the presence of mind to take his toddler's blood soiled diapers out of the diaper pail and store them in the family's deep freeze. While doing some Internet research, sometime later, he discovered STOP and called us for our help and support.

We were able to find a lab willing to conduct tests and Anna's father, an airline pilot, air shipped his daughter's diapers to a lab halfway across the country for testing. Lab results detected Shiga toxin and it was then that the Minnesota Health Department agreed to get involved.

Another round of lab testing went on that ultimately showed that Anna had died from *E. coli* O121, a pathogen that was then in 2002 and still is, off the radar screen for both diagnostic testing in humans and as an adulterant in the food supply.

In Anna's tragic illness, had non-O157 STEC testing been done, and had its findings been reportable, it could have led to an investigation that might have determined the vehicle of transmission and identified populations exposed to that risk. Had O121 been classified as an adulterant in food, perhaps that food never would have made it into commerce at all, and Anna might be alive today.

I've used a lot of perhaps and mights and could haves in what I've just said. I'm neither a physician nor a scientist but I tell you this. I am a very well educated consumer on the dangers of contaminated foods and the tragic consequences that can result. I cannot stress enough the brutal pain and suffering that victims of foodborne illness and specifically STEC infection endure as they struggle to live. Nor can you imagine the pain of the

survivors.

You have heard a lot of information today from doctors and scientists on the subject of STEC, about it's abilities to infect and kill, and I'm not going to reiterate the studies and statistics. One piece, however, actually it was raised, I had written this before I heard Dr. Koohmaraie speak, but it goes to the point of the issue of imported trim that is used in the production of ground beef. Dr. Koohmaraie's study which is titled "The Microbiological Characterization of Imported and Domestic Boneless Beef Trim Used for Ground Beef" compared trim produced in the United States, Australia, New Zealand and Uruguay. Their studies showed about 30 percent of the total samples, from all four countries, were positive for Stx genes, some common, some different. They also identified 11 new STEC serotypes and concluded, "There are many STEC serotypes yet to be identified."

Any discussion and decisions on STEC must also take into consideration meat products that we import from other countries that get commingled in

our domestic food supply. This would apply to non-meat food products that we import as well.

Tests already exist to detect STEC in both humans and in foods. Today's current tests may have some shortcomings but remember that testing for O157 also had shortcomings in the beginning. Testing procedures for O157 have improved and evolved as demand increased and testing became more widely used. I think the technology industry has already identified the need for and exhibited innovation in developing testing methods for non-O157 STEC even before any significant market demand.

And if history can be considered an indicator, it will certainly rise to the challenge of developing even better products as demand for better, faster protocols are expected.

STOP is calling on all sectors of industry and government to make the detection and prevention of STEC in our food supply a priority in order to prevent another foodborne illness epidemic like the one we had 15 years ago. Specifically, we are asking FSIS to declare all pathogenic STEC as adulterants in

ground beef and in beef products destined to be ground under a zero tolerance policy.

We are also calling on FSIS to expand its current O157 random testing program to include all pathogenic STEC and to require companies exporting trim to the United States to do the same.

We urge ARS to conduct research on the possibility of swine being a reservoir for STEC and a link, if any, to human illness. FSIS' White Paper cited a 2004 study that "determined that 70 percent of 687 swine fecal samples tested positive for the presence of Shiga toxin, and found that most of the serogroups isolated have been associated with human illness."

We find this particularly alarming because of the many sausage products, both ready-to-eat and raw that are made from ground pork.

We are calling on FDA to develop a meaningful sampling program for both domestic and imported products to detect pathogenic STEC in foods most at risk of being contaminated. We also ask that whenever FDA is conducting environmental sampling,

when doing an investigation on a product, such as spinach which has a historical link to the O157 strain of STEC, that they look for all pathogenic STEC, and not just the strain that was associated in the product in the past.

We'd like to commend CDC for recommending that physicians and labs routinely screen for all STEC infections when doing stool cultures and to recommend that states adopt mandatory reporting laws for all STEC infections.

We'd like to ask that you take it one step even a little bit closer, and that is to recommend that both, O157 and non-O157 STEC sampling be done together when physicians are doing their testing. Families are in a panic when their children are in hospitals and that *E. coli* word comes up, and you don't know what it is you're looking at. Please conduct the tests simultaneously.

And industry, please take ownership and leadership in working in a proactive way to prevent another major epidemic by an organism that we know today can be in widely distributed products. Please

don't fight this like you did O157. We're sorry if it's inconvenient to you or too costly, but foodborne illness is a lot more than an inconvenience and is very costly.

January 2008 will mark the 15th year anniversary of the Jack-in-the-Box outbreak. What better way to mark that milestone and restore public confidence both in the government's commitment to its citizens welfare than by the USDA's declaration that all potential deadly *E. colis* are to be called an adulterant in ground beef. It would be a win, win, win, for government, for the food industry that has been shaken by a record number of recalls and foodborne illness outbreaks, and by a nation that is better served and protected from deadly bacteria in their food. Thank you very much.

(Applause.)

DR. GOLDMAN: Thank you very much, Ms. Donley, for sharing your perspective, your concerns, and your recommendations.

We will move right along. We're a little bit behind schedule but I want to move us along so

that we do allow you the opportunity to hear the regulatory agencies think out loud. We have done the assessment piece. As I mentioned first thing this morning, we are now moving into the policy development or at least at this point policy consideration. I think you've heard a wealth of information. I don't think you disagree with me that we haven't heard a consensus about some of the scientific issues, but we certainly have plumbed the depths of the literature and studies that are out there.

And, now we want to move considerations by both FDA and FSIS, as they consider what we've heard today and perhaps consider that we need even more information before moving this forward.

Dr. Bob Buchanan will present on behalf of FDA. He is their Chief Scientific Advisor on the significance of new and ongoing scientific developments affecting CFSAN's research programs and policies. His duties include advocate and facilitator of science at CFSAN, including research, planning and formulating aspects of scientific and

research proposals and the training and professional development of regulatory scientists.

He previously served at CFSAN as the lead scientist for the President's Food Safety Initiative, and has served as a research microbiologist for ARS in USDA where he studied the effects and mechanism whereby sub-lethal stresses alter the thermal resistance of foodborne pathogens.

Please welcome Dr. Buchanan.

(Applause.)

DR. BUCHANAN: Thank you, and I couldn't help be struck by a phrase that one of my former bosses used to use, Joe Levitt (ph.) and he had a favorite time of saying that if you're confused, that means you've been paying attention. And in some ways we have a substantial amount of confusion or uncertainty as we like to use in the scientific phrases, and so what I'd like to go through is some of our current thinking about what's going on and our emerging policy on non-O157 STEC, and talk about some of our current thoughts and some of our future directions.

And to do this, I'd like, David's given me all of 12 minutes, to cover our policy, but I'd like to very quickly go through a number of things, a little background on food safety policies for pathogenic microorganisms. Virulence markers versus ability to cause disease, practical aspects of implementing a food safety program for non-O157 STEC and then a few concluding remarks.

And I might note that as I go through this discussion, I'm really going to be focusing on within the family of STEC, EHEC, because this is by all clear indications the highest risk group within that broad family. And so I will also be talking not only about the biology and the policy but also the concept of managing risk.

So let's start off with a little bit, a 101 of food safety policy for pathogenic microorganisms at least within the FDA.

Food safety policies really represent the application of scientific knowledge within the framework of laws that we've been given that define the different risk management options, and I might

note also, not only the options but also some of the limitations, that are available to a regulatory agency to enhance and move public health forward.

And just to remind people that within FDA, this is articulated by the Federal Food, Drug and Cosmetic Act which is the underlying laws that we are charged to enforce.

And there are two very important phrases or subsections within that law that everyone that is dealing with FDA needs to be aware of because this is the two parts of the Code that we use most often to deal with microbiological concerns.

The first is what is referred to as an (a)(1), that defines that a food is adulterated if it bears or contains any poisonous or deleterious substances which may render it injurious to health, and I'm not going to read the rest, but the underlying portion is the important part, is that we have to establish that there is a true impact on public health before we move.

The second is a broader one that says that this is an (a)(4) determination, that a food is

deemed adulterated if it has been prepared, packed or held under insanitary conditions whereby it might become contaminated with filth, or whereby it may have been rendered injurious to health.

And those are the two things that we use in order to regulate foods against pathogenic microorganisms. So if we have evidence and evidence in this case could be either isolation of a pathogen or support of epidemiology of a pathogenic microorganism in foods, that would be the basis of an (a)(1) determination. And so when we talk about enterohemorrhagic *E. coli,* that was primarily what we were talking about, is taking an (a)(1) action as opposed to the use of indicator organisms which would be used to consider the potential for an (a)(4), and typically we would use *E. coli* there, too, but in a different role. We would typically use non-pathogenic *E. coli* as the basis of an indicator of fecal contamination.

So it's good to keep those two in mind because those are two of the major tools that we have.

Then I might note that in any specific pathogen, the stringency of the policies are also supposed to reflect the risks that they represent to public health. And, we start dealing with individual pathogens, and we consider things like the severity of the disease, for example, something that would cause HUS is much more risky or more threatening than simple diarrhea, infectious versus toxigenic pathogens. We deal with the foods that they are present in. So ready-to-eat foods are always considered more risky than non-ready-to-eat foods, and then also we deal with things like dose-response relationships. So for an organism like EHEC that have a very low infectious dose, we would be more stringent than we would for example Vibrio parahemolyticus where you probably need say maybe 10,000. So we take those all into account.

So let's talk now about some of the policy challenges that FDA will be facing as we have an emergence in policy on non-O157 STEC. And I'd like to start off by just saying that FDA recognizes that non-O157 STECs can be an important threat or are an

important threat to public health; that the science related to the ability of any individual STEC to cause disease is highly complex, as was demonstrated over and over again today; that there is a likely continuum of STEC strains in relation to potential public health impact; that they're not all created equal; that there is substantial uncertainty in the science which in turn is going to impact the development of food safety policies for STECs; and then there is a need, in fact, I think it's a critical need, for some unifying concepts that would allow our science to lead us into the new food safety policies.

And, particularly the challenge is going to be able to link the non-O157 STEC to disease, and part of this problem is a problem of definition. Pathogenic *E. coli* traditionally have been characterized by their disease manifestations. Sometimes they're simple virulence markers, but other times they're more complex. So very clearly for the ETEC, this produces a cholera diarrhea. For the enteroinvasive *E. coli,* this produces a *Shigella* type

disease. And for the EHEC, these are ones that produce the severe symptoms that we've heard discussed over and over again today.

Compare those definitions, EHEC, ETEC and the rest of the E words, against the definition of STEC, which is a definition based on a specific virulence marker and not on the ability to cause disease.

And the presence of a virulence marker does not necessarily mean that that isolate, the organism that we're going to have to deal with is capable of causing disease.

The ability of STEC to cause disease is dependent on a combination of virulence factors and based on the current state of the science, and I heard nothing that changed it today, in terms of uncertainty, it is unlikely that a single detection of an isolate with an Stx gene is going to be sufficient to take an action against a food.

Instead, we're going to need additional evidence. Isolation, and the most straightforward, is going to be the isolation of an STEC from a

patient showing atypical EHEC related symptoms by default is a disease causing organism. It has established the criteria for injury.

In the absence of that epidemiological link, there's probably going to be a need for supplemental evidence. And probably the most likely approach is going to be to see if those STEC isolates possess and express the additional virulence genes that will make them EHECs, that will definitively establish them as pathogens and provide the evidence that is needed to make that connection between a simple virulence marker and the ability to cause disease.

Now that does not mean that the absence of one or more of these additional markers makes the organism non-pathogenic. It's just that it's much more difficult for us to prove that it is a pathogen in the absence of epidemiological evidence. It also emphasizes the fact how closely we need to work with CDC and the states to provide that link if it's available to be able to come forward and say, yes, this organism has been associated with disease

outbreaks.

So we have some real challenges facing us as we try to implement risk management programs associated with STEC.

We have some good news. All the evidence we've heard to date is that many of the barriers and interventions put in place to prevent *E. coli* O157 should help us control the non-O157 STEC. Likewise, we have continued confidence that the ability to control *E. coli* as a primary sanitation assessment tool, is continuing to help us drive down the level of all *E. coli* within the food supply and, in fact, still serves as a basis for us to make a determination for an (a)(4) to remove food from the marketplace that is contaminated with fecal material.

However, we are facing some real challenges in the development of food surveillance programs. A lot of these you heard about as the various scientists got up and talked about the methodological concerns, the fact that there can be multiple isolates within a single sample, that there's no distinguishing phenotypes, et cetera.

And from a personal standpoint, I think that this whole area of food surveillance and, in fact, the whole area of non-O157 STEC is going to be dependent on us being able to come up with a clear, relatively simple definition of what constitutes a pathogenic STEC.

Now I might note that this is going to be a risk management decision because we do have this spectrum, and we know at one end, the O157s are highly severe and quite dangerous. At the other end, we probably have some non-pathogenic STECs, and we need to articulate somewhere in that continuum where we're going to be able to take regulatory action.

I also might note that while not quite as complex, in terms of methodological challenges, the ability to do trace backs has specific limitations associated with that.

So a couple of quick concluding remarks because they're flashing a little flag at me, FDA recognizes that non-O157 STEC are an important emerging food safety problem, that it impacts both imported products and our domestic food industry, and

it represents a significant scientific and risk management challenge to us.

And to face those challenges, we have and do remain committed to reducing the burden of foodborne disease including that associated with non-O157 STEC infections, of addressing the challenges of non-O157 STEC to the application of sound science led risk management. We remain committed to seeking the best scientific and food safety policy advice for managing this threat to public health, and we consider today's meeting a very integral part of that activity. We are and have been and will continue to encourage the scientific community to develop the analytical and intervention tools that we need in order to provide practical means for controlling this problem, and then we're also committed to insuring that our investigators, our laboratories and our outreach programs are prepared to address this new and emerging food safety concerns.

And with that I thank you.

(Applause.)

DR. GOLDMAN: Thank you very much,

Dr. Buchanan.

And now for the FSIS perspective. Mr. Phil Derfler is the Assistant Administrator for our Office of Policy, Programs and Employee Development. He's the Agency's representative responsible for formulating policy, establishing and modifying regulations, and for design and evaluation of significant new programs and systems. He has been with FSIS since '97, and before that worked as a staff attorney at FDA, and graduated from the Law School at New York University. Mr. Derfler.

(Applause.)

MR. DERFLER: I had about various things that I wanted to say today, and during the course of today's presentations, I managed to throw in most of them. So let me just sort of say a couple of things that I think are important.

First of all, to take off on what Dr. Buchanan talked about, the question about whether or not non-O157 STEC are pathogens and then whether they're adulterants, one being an essential question for us, and given the factors that he talked about

which we will need to consider, we need to go through that process, because unlike FDA, we really don't have the option of waiting for a sick patient to come back and be presented to us. We put our mark of inspection on the product before it leaves the plant, and that mark of inspection means a finding by us that the produce is non-adulterated.

Given the difficulties we heard today, and how we're going to do testing and how you would sort the various STECs that are pathogens but not human pathogens as opposed to those that are, is a really daunting challenge for us as to how we're going to get to a reasonable regulatory policy on how we're going to address these microorganisms.

So that leaves me with a somewhat different task than what Dr. Buchanan talked about. Instead of talking today about what we're going to do, I need to talk about what you're going to do. As Dr. Brackett talked about this morning, we have an opportunity now to try and get it right with respect to non-O157 STEC but if we're going to do so, there needs to be a sense of urgency that we all feel to do research and

otherwise develop the data that we need to help us find a way to answer the outstanding questions with respect to these microorganisms. Is there a way to distinguish non-O157 STEC that are pathogenic to humans from those that are not, so that FSIS can readily employ action against these microorganisms? Are there species other than cattle, and it's been alluded to a few time today, whose meat may be contaminated with these pathogens and about which we should be concerned? Is an in plant regime that is designed to rigorously be protective against *E. coli* O157 adequate to protect against any other STEC as well and including those of human health concern?

These are just some of the questions that we need to answer in developing our approach to these pathogens. We need any input that you may have on how we can do this. We need you to make us aware of any data, studies, ideas or other information about which you are aware that is going to be relevant to this effort, and we need this input now.

As for next steps for this Agency, assuming that events don't overtake us, that is that we get

confronted with a non-O157 STEC that causes an outbreak, assuming events don't overtake us, we expect to put together a group of Agency scientists that will study the record of this meeting and other available evidence and recommend a set of options to the Agency on how it should proceed with respect to non-O157 STEC.

Another particular action that we're considering is to do a baseline to determine how prevalent non-O157 STECs are in non-intact beef that has been processed and is ready for introduction into commerce. In such testing, we would likely ask that the establishment hold the product pending receipt of results given the possibility of finding non-O157 STEC that may be injurious to health.

It is our hope that once we formulate a tentative plan for how we intend to proceed, we will be able to make that plan public and put it out for public comment and input. So that's where we are.

I want to thank you all for your input. Thanks.

(Applause.)

DR. GOLDMAN: Thank you, Mr. Derfler.

We've covered quite a bit of ground today and we're actually beyond our time in this room. We have just a few more minutes. I realize that we didn't entertain any questions for the last group of panelists. All those presenters I think are still here if you have questions for any individuals, or if you feel that there's one question that's so important that everyone should hear it, I'll entertain one or two now. I see two people interested. Go ahead.

MR. BURNS: A lot has been talked about sort of what I would consider a false positive issue that you'll pick up a lot of STEC that aren't pathogenic, but two of the studies here especially and some previous work that Dr. Tarr had done, really showed a need that these virulence factors have a significant false negative problem, and that is the Danish study wherein the O103 outbreak, they had 62 meat samples that actually had the organism in it that they isolated. None of them had the Stx gene, okay. So the Stx gene jumps in and out all the time,

and Dr. Tarr can tell you all about it. He did a great paper on integration, excisions and truncations of Stx genes several years back which I've always enjoyed.

And the other thing is, looking at the other marker that people look at, eae, it's not only the O113 and the O91 and the ones that don't usually have the intimin that are a problem here, at least the data that I thought I saw from the German study was that out of the O111s that usually do carry the eae gene, 5 out of 72 that were isolated from HUS patients had no eae gene. So anything that's looking at these virulence markers that are on these prophages that can hop in and out, you know, there's a significant false negative issue that really needs to be addressed. And what I heard most people talk about here was false positive, and I just wanted to raise some awareness about that.

DR. GOLDMAN: Okay. Thank you. I don't know if anyone wants to respond to that.

DR. TARR: --

DR. GOLDMAN: Carl.

MR. CUSTER: I have a quick simple one for Dr. Bielaszewska. This is Carl Custer, retired food microbiologist. And in the 1995-96 -- outbreak, was that a pure pork product or was there any ruminant meat in that?

DR. BIELASZEWSKA: The Seemerrolle, this is a product from raw beef. So it contains raw beef.

MR. CUSTER: It did.

DR. BIELASZEWSKA: Yes.

MR. CUSTER: Thank you.

DR. BIELASZEWSKA: But this microorganisms were not isolated from the product. Epidemiological study implicated the food but they were not isolated from the food.

MR. CUSTER: So it wasn't a pure pork product. Okay. Thank you.

DR. SCHEUTZ: You asked for a comment regarding the previous question, and I think there is one very important message today, and that is that in the U.S. we do not have sufficient data on the human side. I mean clinical laboratories are not detecting this. The story of Anna illustrates this very well.

And one of the requirements mentioned by Dr. Buchanan is isolation of STEC from a patient, but this cannot be verified with the present state of detection, and I'm really surprised to see that the food industry, the food administrators here in the country, the veterinarians, they use PCR and they use a lot of very high sophisticated technology, whereas when we're talking diagnostics of ill people with a life threatening disease, the clinical laboratories are not even able to implement this. I'm really surprised about that.

We have developed a commercialized multiplex PCR which is sold in Europe. We've seen the EIA kit here, and if I'm to be frank with you, I think that the most severe problem right now is on the human side.

DR. GOLDMAN: Thank you for that comment.

DR. SCHEUTZ: And it goes back to what strains are really virulent and what markers are you looking for. I was mentioning in my talk that the epidemiology of STEC is very different in the U.S. from other countries, and if you don't have that

data, you will not be able to make those assessments.

DR. BIELASZEWSKA: I just would like shortly comment on eae negative 111. These are only strains with H10. So it means serotype 111:H10. These are eae negative but these are only approximately 10 percent of these all 111, but the most common one 111:H8, they are always eae positive. So just not to confuse the 111 are eae negative. They are mostly eae positive, and that's why I think it's not enough to detect only level of serogroup, but the whole serotype must be determined just to predict some clinical implication or clinical significance.

DR. BUCHANAN: Yeah, David, I'd like to follow up a comment and clarify something that Phil said that I have some concerns about and people leaving this room with that impression.

I tried to provide a feeling for what is the legal requirement associated with us moving against a food, and certainly the identification of an outbreak is an immediate determination that injury has taken place. However, FDA remains committed to

preventing disease, but for that, we're going to need a lot more scientific tools to meet the burden of proof that's required of us. And we have the same exact needs and same exact goals of FSIS of moving this forward, but it's got to be done on the basis of sound science and we're desperate to get more information from this group and everyone else.

DR. GOLDMAN: Thank you. I think that's what I heard Phil say, too, that we need more science. In our Agency, we're going to have a group start looking at what we've heard today and what other science may come to us as a result of this meeting. So I think we share the paradigm for moving forward that you have at FDA.

Before we conclude, I want to ask if there's any questions on the phone, if there's a lingering question?

OPERATOR: Question?

MS. DAWSON: Hello, yes. My name is Lora Dawson. I have a question.

OPERATOR: Go ahead, Ms. Dawson, your line is open.

MS. DAWSON: Thank you so much. I apologize, ladies and gentlemen, I'm on a low battery here today, and at the end of this wonderful meeting with so much information. One of my chief concerns is that we may be accelerating with our regulatory affairs when we might want to also look at the human intake and human input to this in a household environment. I believe the Germany study was speaking about petting zoos and so forth that children are exposed to, and I'm wondering if there's cross-contamination from dogs and pets specifically. So many households in the United States have dogs as pets, and dogs do roam. They do eat animal products, et cetera. And they may be one of the contaminants that's right in the household. Are we creating policy and promotion information based on a disease that may be beginning in the home itself? Can someone respond to that after studying that information?

DR. GOLDMAN: Thank you for your question. I don't -- do you have a comment, Dr. Bielaszewska? Anyone else have a response to that? Dr. Griffin

will respond.

DR. GRIFFIN: Thanks for your question. From our studies, we don't think that household pets are a major source of Shiga toxin-producing *E. coli* although, for example, if there's an animal that's running around on a farm, you might expect that they would pick up the organisms that are in the environment on the farm, and they may be excreting them. Similarly, if the animal eats contaminated food, they could excrete the organism. As we saw recently with an outbreak, we had a few months ago associated with pet food that was contaminated with *Salmonella,* some of the pets in the home were excreting the *Salmonella* as well. So it can occur. We don't think that they are a major source of transmission of the enteric pathogens.

DR. GOLDMAN: Thank you. Any other questions on the phone?

OPERATOR: Yes, sir. David Kerr (ph.), BioControl, your line is open.

MR. KERR: Thank you very much. I appreciate this great forum for discussing these

issues and it's a great first step in understanding the impact of the non-O157 STEC. My question is this. Are there actual plans to establish an actual working group among government and food industry and the diagnostic companies so that food industry will establish the appropriate -- for detection of non-O157 STEC? And similarly, does the current program that's dealing with the same issue with -- between FDA and -- a similar working group would be in the future for STEC?

DR. GOLDMAN: I didn't get all of your question, but your question was to what extent there would be collaboration across the government and beyond the government on this issue. Certainly you heard FSIS say we were establishing a group within our Agency. We might rationally link together with FDA in such an effort, and as we usually do, we invite partners from outside who are interested in this issue. So thank you. I think we will move forward in that sort of way. I'm not sure if we'll replicate the model that you suggested there.

I want to bring this very impressive

meeting to a close by thanking really what I thought was a world class panel of presenters, and I want to have you help me thank them right now.

(Applause.)

DR. GOLDMAN: Just one or two brief comments. I mean we were all here -- we are all here with an interest in identifying to the extent possible contamination that does cause human illness, and therefore devising whatever appropriate policies or approaches there may be to preventing that contamination from causing human illness. I mean just even leaving aside the adulteration issue, we all have that interest.

I think what you heard repeated over and over, certainly in terms of the scientific perspective, is that we are struggling to construct a pathotype, something that's reproducible and reliable as a way of identifying a subset of STECs that we can detect in human isolates, in food products, in the environment and thereby once that's done, create a rational policy to prevent that from contaminating foods.

That was our interest here. I think we heard that it's a difficult challenge. You heard a commitment on the Federal Government's part to continue working on this challenge, and I want to thank everybody for your participation at the beginning steps of addressing this issue. So thank you.

(Applause.)

DR. GOLDMAN: And if I could ask your cooperation, if you want to say hello to someone, if you could, as quickly as possible vacate this room, there are a group of eager undergraduates who need to get in here by 4:00.

(Whereupon, at 3:45 p.m., the meeting was concluded.)

C E R T I F I C A T E

This is to certify that the attached proceedings in the matter of:

THE PUBLIC HEALTH SIGNIFICANCE OF NON-O157 SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC)

PUBLIC MEETING

Arlington, Virginia

October 17, 2007

were held as herein appears, and that this is the original transcription thereof for the files of the United States Department of Agriculture, Food Safety and Inspection Service.

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Dominico Quattrociocchi, Reporter

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