AT

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

# ANTI-INFECTIVE DRUGS ADVISORY COMMITTEE

# IN JOINT SESSION WITH THE

# PEDIATRIC ADVISORY COMMITTEE

Thursday, April 12, 2007

8:30 a.m.

Committee Conference Room 1066
5630 Fishers Lane
Rockville, Maryland

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GUEST SPEAKER:

Martin Bitzan, M.D.

PARTICIPANTS (Continued)

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Thomas Smith, M.D.
Janice Soreth M.D.
Yan Wang, Ph.D.

C O N T E N T S

C O N T E N T S (Continued)

P R O C E E D I N G S

**Call to Order**

DR. RELLER: Good morning. I am Dr. Barth Reller. I will be co-chairing with Dr. Rappley this morning=s and this afternoon=s meeting. I should like to begin this advisory committee meeting by reading the following statement that will apply to our proceedings:

Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision-making. To ensure such transparency at the open public hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual=s presentation.

For this reason, the FDA encourages you, the open public hearing speaker, if there be any, at the beginning of your written or oral statement to advise the committee of any financial relationship that you may have with any company or any group that is likely to be impacted by the topic of this meeting. For example, the financial

information may include a company=s or a group=s payment of your travel, lodging or other expenses in connection with your attendance at the meeting.

Likewise, FDA encourages you, at the beginning of your statement, to advise the committee if you do not have such financial relationships. If you choose not to address this issue of financial relationships at the beginning of your statement it will not preclude you from speaking.

Today=s meeting will have a lot of discussion which will result in recommendations at the end of the day from the committee for the Food and Drug Administration. We are aware that members of the media are anxious to speak with members of the committee and the FDA about these proceedings. However, both the committee members and the FDA must refrain from discussing the details of this meeting with the media until its conclusion. At that time, FDA will hold a press briefing for members of the credentialed media to discuss the recommendations from the committee and take any

questions they may have.

Next, I should like to introduce Lt. Sohail Mosaddegh, who is the staff program officer for the advisory and consultant staff assigned to this meeting. At the conclusion of Lt. Mosaddegh=s comments, I will then for the record ask each of the committee members to introduce themself and their affiliation. We will start, after Lt. Mosaddegh, with Dr. Patricia Griffin. Lt. Mosaddegh?

**Conflict of Interest Statement**

LT. MOSADDEGH: Thank you, Dr. Reller. The following announcement addresses the issue of conflict of interest and is made part of the record to preclude even the appearance of such at this meeting. The matter coming before the Anti-Infective Drug Advisory Committee and the Pediatric Advisory Committee is a particular matter involving specific parties.

Based on the submitted agenda and all financial interests reported by the committee=s participants, it has been determined that all

interests in firms regulated by the Center for Drug Evaluation and Research present no potential for an appearance of a conflict of interest at this meeting.

We would like to note that Dr. Samuel Maldonado has been invited to participate as a non-voting industry representative, acting on behalf of regulated industry. Dr. Maldonado=s role on this committee is to represent industry=s interests in general and not any one particular company. Dr. Maldonado is employed by Johnson & Johnson.

In the event that the discussion involves any other products or firms not already on the agenda for which an FDA participant has a financial interest, the participants are aware of the need to exclude themselves from such involvement and their exclusion will be noted for the record. With respect to all other participants, we ask in the interest of fairness that they address any current or previous financial involvement with any firm whose products they may wish to comment upon.

Thank you.

DR. RELLER: As each speaker is introduced, remember that if one taps the Atalk button@ the red ring will come on the microphone so that you can be heard by all, as well as recorded as part of the proceedings of the meeting. We have a seating chart and number one on my chart is Dr. Patricia Griffin.

DR. GRIFFIN: Hi. I am Patricia Griffin and I am Chief of the Enteric Diseases, Epidemiology Branch at CDC.

DR. KOCIS: Good morning. Keith Kocis. I am a pediatric cardiologist and intensivist, from the University of North Carolina in Chapel Hill.

DR. HILTON: Joan Hilton, professor of biostatistics, UC, San Francisco.

DR. WIEDERMANN: Bud Wiedermann, pediatric infectious diseases, Children=s National Medical Center and George Washington University, Washington, D.C.

DR. GORMAN: Rich Gorman, general pediatrician, Ellicott City, Maryland, representing

professional healthcare organizations on the Pediatric Advisory Committee.

DR. REHM: Susan REHM, adult infectious disease, Cleveland Clinic.

DR. RAPPLEY: Marsha Rappley, developmental and behavioral pediatrics, Michigan State University.

DR. EDWARDS: Jack Edwards, adult infectious diseases, Harbor-UCLA Medical Center.

DR. SMITH: Margo Smith, adult infectious diseases at Washington Hospital Center, here in Washington, D.C.

DR. ROSENTHAL: Jeff Rosenthal, pediatric cardiology, Cleveland Clinic.

DR. TARR: Phil Tarr, pediatric gastroenterologist, Washington University in St. Louis.

DR. ACHESON: David Acheson, Chief Medical Officer, FDA, Center for Food Safety and Applied Nutrition.

DR. CNAAN: Avital Cnaan, professor of biostatistics, University of Pennsylvania and

Children=s Hospital of Philadelphia.

DR. TOWNSEND: Greg Townsend, adult infectious diseases, University of Virginia.

DR. HUDSON: Melissa Hudson, pediatric hematologist-oncologist from St. Jude Children=s Research Hospital.

DR. MOXEY-MIMS: Marva Moxey-Mims, pediatric nephrologist. I am at the NIDDK at the NIH.

MS. DOKKEN: Deborah Dokken, I am the patient family rep. at this meeting and I am the associate director of a project called the Initiative for Pediatric Palliative Care.

DR. KASKEL: Rick Kaskel, pediatric nephrologist at Montefiore and Albert Einstein College of Medicine in the Bronx.

DR. FANT: Michael Fant. I am a neonatologist at the University of Texas Health Science Center in Houston.

DR. WONG-BERINGER: Annie Wong-Beringer, infectious disease pharmacist, adults, University of Southern California.

DR. WARD: Bob Ward, neonatologist and pediatric clinical pharmacologist, University of Utah.

DR. MALDONADO: Sam Maldonado, pediatric infectious diseases, industry representative.

DR. DAUM: Good morning. I am Robert Daum, from the University of Chicago, pediatric infectious diseases.

DR. RELLER: Thank you very much. It appears we have a full house. Thank you, and we will next have our first presentation. Dr. Thomas Smith will speak to us about regulatory pathways for products for prevention or treatment of disease caused by Shiga toxin-producing bacteria. Dr. Smith is the first of the FDA presenters.

I started with the list and we did not completely go around the circle but we will next have introductions from the complete members of the FDA team.

DR. COX: Ed Cox, Acting Director for the Office of Antimicrobial Products, CDER, FDA.

DR. SORETH: Janice Soreth, Director of the

Division of Anti-Infective and Ophthalmology Products, FDA.

DR. NOSTRANDT: Amy Nostrandt, a pharmacologist in the Division of Anti-Infective and Ophthalmology Products at FDA.

DR. WANG: Yan Wang, statistical reviewer, Division of Biometrics IV, CDER, FDA.

DR. T. SMITH: I am Tom Smith. I am a medical officer in the Division of Anti-Infective and Ophthalmology Products.

DR. RELLER: Dr. Smith?

**FDA Presentations:**

**Regulatory Pathways for Products for the Prevention or Treatment of Disease Caused by Shiga Toxin-Producing Bacteria**

DR. T. SMITH: I would like to start by thanking Drs. Rappley and Reller and the permanent members of the Anti-Infective Drugs Advisory Committee and Pediatric Advisory Committee, along with our temporary voting members, particularly Drs. Griffin and Tarr who will be giving presentations, and I would also like to thank Dr.

Martin Bitzan who is our guest speaker, and our two industry sponsors for presenting their development programs.

[Slide]

Today we will be discussing two biologic products for the prevention or treatment of disease caused by Shiga toxin-producing bacteria.

DR. RELLER: I am sorry, Dr. Smith, can you speak a little louder and perhaps into the mike? People are having trouble hearing.

DR. T. SMITH: Sure.

DR. RELLER: Thank you.

DR. T. SMITH: The two biologic products that we will be hearing about today are both monoclonal antibody preparations. In my talk I will be discussing some of the regulatory mechanisms for approval of drugs and biologics. We will have other FDA presentations on animal models of infection and on clinical trial design issues. Our topic experts will speak about the epidemiology of Shiga toxin-producing E. coli infections and the clinical course and consequences of some of these

infections. We will hear a presentation about a clinical scoring system for Shiga toxin-mediated events, and then we will have our two industry presentations.

[Slide]

As we proceed this morning, we would like you to keep in mind some of the issues that we will be discussing later on. These involve the role of data from animals models in the evaluation of these products and how they may contribute to an understanding of the effectiveness of these products. We will also consider what the proper primary endpoint for clinical studies for these infections and their consequences should be, whether it would be hemolytic uremic syndrome, some advanced stages of hemolytic uremic syndrome or perhaps there are other clinical and meaningful endpoints that might be worthwhile looking at as primary endpoints. Then, the third general issue is, given our understanding of the epidemiology of these conditions and of the likelihood that there is a very limited window in which any therapeutic

intervention would have to take place, to consider some trial design and enrollment strategies that might help studies to proceed.

[Slide]

In considering regulatory background, I would like to begin with a little introduction to the Food, Drug and Cosmetic Act and the Public Health Service Act requirements for adequate and well-controlled studies, and then discuss a couple of regulatory mechanisms to facilitate the development of products for serious conditions. These would be the accelerated approval regulations and the Animal Efficacy Rule. Most of what I will be speaking about today is found in the guidance for industry on providing clinical evidence of effectiveness and in the regulations.

[Slide]

Drugs are approved under the authority of the Federal Food, Drug and Cosmetic Act. In 1962 the FDC Act was amended to add a requirement for demonstration of effectiveness by substantial evidence.

[Slide]

Substantial evidence is defined as evidence consisting of adequate and well-controlled investigations, including clinical investigations by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved.

[Slide]

FDA=s position has been that Congress generally intended to require at least two adequate and well-controlled studies, each convincing on its own, to establish effectiveness. The 1997 Modernization Act amended the FDC Act to state that the agency may consider data from one adequate and well-controlled clinical investigation and confirmatory evidence to constitute substantial evidence, if FDA determines that such data and evidence are sufficient to establish effectiveness.

[Slide]

Turning to biologic products, these are approved under the authority of the Public Health Service Act. Section 351 requires that licenses

for biologics be issued only upon demonstration that products meet standards to ensure the continued safety, purity and potency of the products. Potency has been interpreted to include effectiveness.

[Slide]

In 1972 FDA initiated a review of the safety and effectiveness of all previously licensed biologics. The agency determined then that proof of effectiveness for biological products would consist of controlled clinical investigations, as defined in the provision for adequate and well-controlled studies for new drugs, unless waived as not applicable to the biological product or essential to the validity of the study when an alternate method is adequate to substantiate effectiveness.

[Slide]

This part of the regulations goes on to say that alternate methods, such as serological response evaluation and clinical studies and appropriate animal and other laboratory assay

evaluations may be adequate to substantiate effectiveness where a previously accepted correlation between data generated in this way and clinical effectiveness already exists.

[Slide]

The Modernization Act also amended the Public Health Service Act to make clear that a single license is required for biological products and the establishments in which they are produced. It did not change the evidentiary standard that these products had to be shown to be safe, pure and potent.

In another section FDA was directed to take measures to minimize the differences in the review and approval of products required to have approved biologic licenses under Section 351 of the Public Health Service Act and products required to have approved NDAs under the FDC Act.

[Slide]

Regarding substantial evidence of safety and effectiveness, this section of the regulations defines the characteristics of an investigation

that are needed to consider it adequate and well-controlled for purposes of either drug approval or for demonstration of potency or effectiveness for biologics. This section describes things like the characteristics of trial design, the way that data should be analyzed, the way the trial should be conducted, and Dr. Wang will be presenting some more about this in her presentation.

[Slide]

As I mentioned before, adequate and well-controlled studies FDA has generally interpreted to mean at least two adequate and well-controlled studies. There is a need for independent, substantiation of experimental results. A single experimental finding of efficacy, unsupported by other independent evidence, has not usually been considered adequate support for a conclusion of effectiveness. Reasons for this are that a single study could have unconscious or conscious biases that might lead to flawed conclusions. A single positive trial result

might occur by chance alone. Results of studies performed at single centers or with single large enrollers might be dependent on site- or investigator-specific factors and results that are not generalizable to the intended population. And, rarely there are instances of scientific fraud.

[Slide]

There are, however, situations in which a single adequate and well-controlled study might support approval. One case is in which a single study for a specific new use is supported by information from other related adequate and well-controlled studies. This could include studies of other phases of the disease process; different populations; a closely related disease; different dose or duration of use or a different dosage form.

[Slide]

Another situation in which a single study might support approval is when a single multicenter study of excellent design provides highly reliable and statistically strong evidence of important

clinical benefit, such as an effect on survival, and a confirmatory study would be difficult to conduct on ethical grounds.

[Slide]

It must be kept in mind, however, that in those instances in which a single study supports approval, that a study must clearly meet the requirements for adequate and well-controlled studies as set forth in the regulations. Also, a single favorable study among several similar attempts that failed to support a finding of effectiveness does not constitute persuasive evidence for a finding of effectiveness.

[Slide]

Now I would like to move on to a brief discussion of two regulatory mechanisms for facilitating the approval of drugs or biologics to treat serious conditions. These are the accelerated approval regulations and the Animal Efficacy Rule.

[Slide]

The accelerated approval applies to

certain products for the treatment of serious or life-threatening illnesses and that provide meaningful therapeutic benefit over existing treatments. The applicable regulations occur in Subpart H, Section 414 for drugs and Subpart E of Section 601 for biologics.

[Slide]

There are two situations for accelerated approval, one of which is when FDA has determined that a product is effective but it requires restrictions to assure safe use. In this case, distribution could be restricted to certain facilities or physicians with special training or experience. Another instance will be when distribution is conditioned on performance of specified medical procedures.

[Slide]

The second situation in which accelerated approval may apply is when approval is based on a surrogate endpoint or an effect on a clinical endpoint other than survival or irreversible morbidity. If a surrogate endpoint is used, it

must be reasonably likely based on epidemiologic, therapeutic, pathophysiologic or other evidence be able to predict clinical benefit. When there is uncertainty as to the relation of the surrogate endpoint to clinical benefit or of the observed clinical benefit to an ultimate outcome, the applicant must study the product further to verify the results in additional postmarketing studies that are adequate and well-controlled.

[Slide]

A surrogate endpoint is a biomarker that is used to predict clinical benefit, the clinical benefit being a direct measurement of how a patient feels, functions or survives. Surrogate endpoints are useful in that they are often detected earlier or more readily than a corresponding clinical endpoint. But in order to be considered acceptable one must have confidence that the surrogate marker changes reliably predict the desired clinical endpoints.

[Slide]

The Animal Efficacy Rule applies to

certain products that have been studied for their safety and efficacy in ameliorating or preventing serious or life-threatening conditions caused by exposure to lethal or permanently disabling toxic biological, chemical, radiological or nuclear substances. These regulations are found in Subpart I of Section 314 for drugs and Subpart H of Section 601 for biologics.

[Slide]

The Animal Efficacy Rule applies only to new products for which definitive human efficacy studies cannot be conducted. It is unethical to deliberately expose healthy human volunteers to a lethal or a permanently disabling toxic substance, and field trials to study the product=s efficacy after an accidental or hostile exposure have not been feasible. The Animal Efficacy Rule does not apply to products that can be approved based on efficacy standards described elsewhere in FDA=s regulation, for example under the accelerated approval regulations. The Animal Efficacy Rule also does not address the safety evaluation for the

products to which it does apply.

[Slide]

When the Animal Efficacy Rule is used approval is based on evidence of effectiveness provided from adequate and well-controlled animal studies that establish that the product is reasonably likely to produce clinical benefit in humans.

[Slide]

There are some conditions that have to be met. First, there must be a reasonably well-understood pathophysiological mechanism of the toxicity of the agent and its prevention or substantial reduction by the product. The effect must be demonstrated in more than one animal species expected to react with the response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well characterized animal model for predicting the response in humans.

[Slide]

Third, the animal study endpoint must be

clearly related to the desired benefit in humans, which generally is the enhancement of survival or the prevention of major morbidity.

Finally, the data or information on the kinetics and pharmacodynamics of the product, or other relevant data or information in animals and humans, allows selection of an effective dose for humans.

[Slide]

For products that are approved under this rule there are three additional requirements. One is that a postmarketing study to verify the drug=s clinical benefit must be performed when these studies are feasible and can be done ethically. Second, the distribution of the drug can be restricted to ensure safe use, if necessary. Third, the sponsor must draft and distribute to patients information that explains that the approval is based on studies in animals, as well as other information that will permit the drug to be used safely.

[Slide]

In summary, I have talked about the evidentiary standards for approval of drugs and biologics, and described two regulatory mechanisms to facilitate approval of drugs for serious conditions, the accelerated approval regulations and the Animal Efficacy Rule, and the rather limited circumstances under which these apply.

[Slide]

Just to go back to the beginning, we ask you to keep in mind, as you hear these presentations today, and to consider what the role of data from animal models would play in the approval of biologics or drugs for the prevention or treatment of diseases caused by Shiga toxin-producing bacteria; consider what the proper primary endpoint for clinical studies should be; and, finally, once again considering the epidemiology and the fact that there is likely to be a limited window in which to intervene, give consideration to trial enrollment strategies that would help to facilitate studies for this condition. With that, I will take questions.

DR. RELLER: Are there any questions about clarification of Dr. Smith=s presentation?

[No response]

Dr. Smith, thank you very much. Our next speaker will be Dr. Patricia Griffin. She is the Chief of the Enteric Diseases Epidemiologic Branch, Division of Foodborne Bacterial and Mycotic Diseases at the National Center for Zoonotic, Vectorborne and Enteric Diseases at the Centers for Disease Control. Dr. Griffin?

**Epidemiologic of Shiga Toxin-Producing E. Coli Infections, Focusing on North America**

DR. GRIFFIN: Thank you. I realized when I heard the committee members= names and affiliations that for only a few of us is, you know, the ideal social encounter is having dinner and talking about E. coli and that your range or expertise is very broad. So, if I get into an area and haven=t explained something adequately, if committee members want to raise your hand and say just back up a little, let me know. But I think most of this is fairly simple.

[Slide]

This is just to remind us that this is still affecting many people. This is a child who died during the spinach outbreak this past fall.

[Slide]

I will talk about clinical illness, surveillance, HUS outbreaks, transmission and the impact of our work.

[Slide]

This is the sequence of events in 0157 infection. First, the organism is ingested and then there is a three- or four-day incubation period, followed by non-bloody diarrhea and abdominal cramps. After a day or two, in most patients that come to clinical attention there is bloody diarrhea. Then, most of those patients go, down the left side of that chart, to resolution but about eight percent develop hemolytic uremic syndrome.

[Slide]

I want to talk about surveillance for this organism in FoodNet. We count every 0157 organism

isolated by clinical labs in the catchment area and we calculate the annual population-based rates and trends over time.

[Slide]

This slide shows FoodNet catchment area in 2005. It included 44 million people, 15 percent of the U.S. population.

[Slide]

This graph shows the percent of clinical labs that were screening all stools for 0157, starting in 1985 and continuing through 2003. You can see that the screening increased markedly after our big western states outbreak. Then, by 1995 about 60 percent of labs were routinely screening. But still, in 2003 only 69 percent of labsB-if you went to your doctor and you had bloody diarrhea and the doctor got a stool culture and said, AI=m just going to send it off and they=re going to find whatever is there,@ only 69 percent of labs would have even looked for this organism.

[Slide]

This is the relative rate compared with

the 1996-98 baseline of 0157 infections in FoodNet sites through 2005. This slide is not obvious; let me point out to you how it works. We set the baseline to 1 and what we are measuring is a relative rate on the log scale. Then we have years. So, compared with the baseline, you can see that there was a marked decrease by 2003 but then it is increased in 2004 and 2005. Still, by 2005 there was a 29 percent decrease. The numbers for 2006 are coming out tomorrow. This number corresponded to an incidence of 1 illness per 100,000 persons. I want you to again note this 2003 decrease, which was really marked.

[Slide]

Another thing to point out is that the incidence of 0157 infections varies by state. I don=t want you to look at the actual incidence numbers, those vary, or the years. The point here is just the gradient from dark to light. For reasons we do not fully understand, the incidence does truly appear to be higher in the northern areas than in the southern areas, and we also see

this in Europe, and we also see this in an upside down fashion in the southern hemisphere.

[Slide]

This shows the rate of 0157 infections by setting in the United States. The point here is that it is much more a rural disease than an urban disease. Again, the numbers here vary by year. What is important is the difference in these bars.

[Slide]

CDC has made estimates of the annual frequency of 0157 infections in the United States. From our 2000 data that I just showed you, there were 1.06 culture-confirmed infections per 100,000 persons. But many ill persons don=t have a stool cultured. Most of you, if you get a diarrheal illness, will not go to the doctor unless you are really sick and some of those diarrheal illnesses are 0157 and many labs don=t routinely test for 0157. Multipliers that we derived from surveys and from outbreaks help us to estimate the true number of infections. So, we made estimates for the United States in a publication in 1999 that we are

still quoting. We are redoing the estimates but they are not out yet. At that time, we estimated that there were truly 73,000 0157 infections, with 2,000 hospitalized and 60 deaths.

[Slide]

Surveillance for non-0157 STEC I want to talk about now, and the Shiga toxin enzyme immunoassay has been a blessing. Before I get into the guts of this slide, let me just explain that E. coli 0157 has this funny characteristic of not fermenting sorbitol sugar very well. So, we can use that characteristic to make a special culture plate to find the organism pretty easily in clinical laboratories. All these other Shiga toxin-producing E. coli don=t have that characteristic so it is hard to find them. There is a commercial assay that tests for the Shiga toxin, and it is an ELISA assay. It improves detection of non-0157 STEC illnesses and outbreaks. Clinical labs can submit Shiga toxin-positive broths that they make from a broth culture of a stool and they can submit it to state health

department labs so the Shiga toxin-producing E. coli are then isolated. Then those E. coli isolates get sent to CDC and CDC serotypes these STEC for the state health labs. Serotyping is very labor-intensive. The reagents are not available in clinical laboratories.

[Slide]

This graph shows human isolates of non-0157 STEC serotype by CDC from when we started through 2002. The real message here is that 70 percent of the isolates fell into 6 serogroups, 026, 011, 103, 121, 45 and 145. We had 55 other O groups in each form less than or equal to 1 percent of our isolates. So, most of our non-0157 STEC disease is due to 6 serogroups.

[Slide]

Surveillance for non-0157 STEC--the Shiga toxin ELISA has been a curse as well as a blessing because in adopting this ELISA some labs have abandoned use of selective media that I discussed, that media that uses sorbitol to isolate 0157. So, they have just stopped doing that and instead they

have adopted the ELISA, but some of those clinical labs discard the Shiga toxin-positive specimens without obtaining an isolate. They just report back to the clinician that it is positive for Shiga toxin and then they throw it away so the serotype is not determined. We don=t find out whether it was an 0157 or something else and 0157 strains are not subtyped for surveillance and outbreak detection, and an important part of investigating and detecting outbreaks is doing subtyping, for example the spinach outbreak that we all heard about last fall.

Another problem is that clinical laboratories don=t use the ELISA routinely. In 2003 only 3 percent of clinical labs in FoodNet sites had ever used it. I am not talking about routinely using it; I am talking about ever using it. But use is increasing and we are right now finalizing a questionnaire to survey labs about how many are using it routinely on all stools that are submitted for determination of pathogens.

[Slide]

Because of these concerns that I have outlined, we published an MMWR article this fall on the importance of culture confirmation of these organisms. What we said is that clinical laboratories should strongly consider including STEC 0157 in their routine enteric panel. We said the best way to identify all STEC infections, not just 0157, is to screen all stool samples for Shiga toxins; that laboratories that use Shiga toxin ELISA should culture all positive broths, in other words, not throw them away. And, when a Shiga toxin-positive broth does not yield STEC 0157 the broth should be quickly forwarded to the state laboratory for identification of non-0157 STEC and all non-0157 STECs should be sent to CDC for serogrouping.

The reason we suggested this, the bullet up here, is that they should include 0157 in their routine panel, you can see that these steps take a while and if you want to identify an outbreak quickly, then the clinical lab is fully capable of finding out that you have 0157 in your stool and

that your neighbor has it too, and their neighbor does too, and that is how we quickly find an outbreak and get the implicated food off the market. Or, that is how we identify that a child has that illness that needs extra clinical day that you will soon hear about from Phil.

[Slide]

A couple of weeks ago John Besser, in Minnesota, said, AI am giving a talk and can you give me your data on non-0157 versus 0157 STEC,@ and I said, AJohn, Minnesota has the best data in the country and, in fact, I was about to ask you for your data.@ So, this is their data on surveillance for STEC in Minnesota. What they do is they have an HMO in Minneapolis-St. Paul and a rural hospital that they have been working with for many years now. Each of those hospitals sees patients with diarrhea and they get the routine stool culture and they do whatever they do. Then they send the plates to the Minnesota lab and the Minnesota lab does the SMAC culture and does PCR for toxin. So, in all these specimens they look

for all STEC.

So, what do they find? From the city lab they have gotten 114 STEC over the years and, of those, a few more, 56 percent, were non-0157 versus 44 percent 0157. In the rural hospital they have gotten 77 STEC over the years and a higher proportion of 0157, 65 percent, 35 percent. But in total it is about 50-50. So, you will see a lot of literature saying the non-0157s are more common than the 0157s. Here 0157 is more common. But it depends on where you look. But basically you see these sorts of numbers. But the numbers are very different for 0157. Here, for the entire state they have had 80 non-HUS cases over these years and 91 percent of those with STEC isolated had 0157 and this is pretty typical as well.

[Slide]

Now I am going to talk about HUS. I want to tell you about our national prospective HUS study. We had a convened a sample of nephrologists and hematologists in our inclusion criteria where the typical diagnosis for HUS, which is a

hematocrit less than 30, microangiopathic changes and an increased creatinine, and we were interested in diarrhea-associated HUS so we required diarrhea before the diagnosis. We requested stool and acute and convalescent sera and a questionnaire.

[Slide]

We identified 83 patients and 73 were children and 10 were adults. Most were less than 5 years old and they came from 16 states.

[Slide]

The clinical features--in addition to diarrhea, most had bloody stools; most were dialyzed; a third had red cell transfusions; 20 percent had a seizure; 6 percent of children and 20 percent of adults died, which is pretty typical.

[Slide]

We had stool cultures on 70 patients. Only 43 percent of patients had stool cultures that yielded STEC. This is a bit lower than we usually see, probably due to freezing, shipping and culture delays. They came from all over the country to CDC. Of the serotypes, 86 percent were 0157 and 14

percent were non-0157.

[Slide]

We also did serology. In fact, we developed our serologic test on this study and 81 percent had antibodies to 0157 LPS.

[Slide]

Overall, of the 83 children, 73 percent had STEC by culture or serology. Of the adults, a similar proportion had STEC infection, including all 3 postpartum women having 0157 infection

[Slide]

So, we looked at the subset of patients, 55 patients that had both serology and stool culture. You will remember that a fairly low proportion had stool culture, and 18 percent of them had no evidence of STEC infection. Compared to those with STEC infection, those with no evidence of STEC infection less likely had bloody diarrhea; less likely had onset in the warm months, which is very typical for STEC--isolation in the warm months; and one had pancreatic cancer. So, the message from those 18 percent is that they

probably had a different illness. You can see the criteria for HUS are not specific. You find them in many other diseases. So, these 18 percent of people probably had some other disease.

So, we can focus on the 82 percent that had evidence of STEC infection and 98 percent of them had evidence of 0157 infection. Three of the four with non-0157 STEC isolated from stool had antibodies to 0157 LPS, suggesting that they truly had disease due to E. coli 0157.

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I just want to mention some other studies with stool cultures. Among HUS cases tested within 6 days of onset of diarrhea, the proportion of 0157 isolated was 96 percent in Dr. Tarr=s study and 87 percent in a study from Canada.

[Slide]

Other studies with serology to 0157 antibodies have also found a high proportion of diarrhea-associated HUS with serologic evidence of 0157 infection.

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So, in summary, among patients with diarrhea 0157 and non-0157 STEC are isolated with similar frequency but among patients with HUS 0157 cases over 90 percent of STEC-associated HUS.

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This lists some of the major predictive factors for STEC-associated HUS, some of which have been better proven than others. They include host factors such as the extremes of age; bloody diarrhea; vomiting; high white blood cell count early in the illness; microbial factors. A strain that produces only Shiga toxin-2 rather than Shiga toxin-1 as well is much more likely to cause HUS, for reasons now unknown. And, treatment of diarrhea with an antimotility agent and with antibiotic has also been linked to an increased risk of HUS.

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I just want to mention that we are doing a cohort study right now of 0157 infections to further explore factors related to the risk of HUS. It is being conducted in 10 FoodNet sites. It

began in 2006 and we are evaluating the risk of HUS by some of those factors that I mentioned before-Bmicrobiologic characteristics, clinical and lab features, antimicrobial therapy and other treatments. It began in 2006 and includes everyone in the site who has an 0157 infection. We are doing medical chart reviews and interviews, and in a sub-study we are getting saliva DNA samples to do some further studies.

[Slide]

I will now talk about outbreaks. 1993 was the genesis of PulseNet, which is our molecular subtyping system. In >93 we investigated the big western states 0157 outbreak in which more than 700 people became ill and 4 children died. We developed a pulse-field gel electrophoresis subtyping method. Tim Barrett, in our lab, developed this. This was followed by an increased demand for subtyping. All the state labs in the next few summers would contact us, saying we are seeing a lot of these illnesses. We don=t know how to link them together. If you could do the

subtyping for us we could figure out which ones are part of the same outbreak because they have the same subtype. We weren=t able to do the subtyping, or we did it months later which doesn=t do any good when you are investigating an outbreak.

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In outbreak investigations time isn=t on your side. The faster you track and control the source of an outbreak, the more cases you prevent. And, in a large country like the United States, sending isolates to a central lab and then subtyping them is time consuming. Just the mailing takes a week. So, determining isolates in the state labs can be very fast and the subtype patterns from many states can be compared quickly by transmitting the patterns over the internet. The patterns are like a bar code that you can just send over the internet to the main PulseNet database at CDC and then we have a team of people who compares the patterns and notices if a pattern is increasing in frequency. Then the epidemiologists in Outbreak Net throughout the

country look for a common source.

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So, PulseNet USA is a national network, and it is actually now international, of public health and food reg. labs dedicated to molecular surveillance of foodborne infections. PulseNet detects and investigates clusters of isolates with the same molecular subtype. It is coordinated by CDC which has the central database, and the current method is PFGE.

I can never give a talk without talking about at least one outbreak so I just want to tell you a little bit about one outbreak in 2002 which really was a turning point. The first cases were in Colorado, and PulseNet posted the outbreak strain and identified 45 persons with the outbreak strain in 11 states. We quickly identified beef as the cause. In fact, the outbreak strain was isolated from ground beef, which is pretty unusual. The beef came from one big meat processing plant and the outbreak stopped after recall of 18 million pounds of ground beef. The entire beef industry

got worried about bad publicity because people don=t really remember which plant was the problem; they just remember ground beef is a problem. So, remember that because I will talk about that later.

[Slide]

But I want to compare the 1993 western states 0157 outbreak before PulseNet with 2002 Colorado outbreak that I just mentioned. You can see that it took us, like, 39 days to find that western states outbreak compared with about half the time for this later outbreak when we had PulseNet. You can see the difference in the number of cases. So, finding them fast makes a difference.

[Slide]

The message from this map of the outbreaks of 0157 infections by state is really that this disease occurs all over the United States, and in the states that have very light outbreaks, that look like they have no or very few outbreaks, the chances are their health department is not very well funded to find and investigate the outbreaks.

[Slide]

This is the same outbreaks by year. You can see that early on there were few outbreaks and we really think that this was due to lack of recognition. Labs weren=t at all looking for 0157, and big, severe outbreaks can just be buried and nobody finds them. You know, each case goes to one or two hospitals and people don=t notice.

Here is the western states outbreak. People were more aware; started looking for more. It became nationally notifiable. This is when the lab created PulseNet and also there was a food safety initiative. There was more funding for foodborne illness and the epidemiologists were able to stimulate better outbreak reporting. So, we started to get a lot more outbreaks. Now there are fewer outbreaks being reported and we think this is a real decline.

[Slide]

The other thing that has changed is the median number of ill persons per 0157 outbreaks. Early on we only found the big ones. I am sure the

little ones were occurring but we only found the big ones. Now we are mostly finding small ones and we think that there are many fewer big outbreaks. So the median size of an outbreak today is 5 ill persons, which is progress.

[Slide]

I also want to say something about the non-0157 STEC infections. These are the outbreaks that we found ever in the United States, 12 outbreaks, with the first one in 1990, the most recent in 2006. They were due to some of the more common serogroups, 0111, 0121, 026, 045, 103 and 104.

[Slide]

The suspect modes of transmission were food, milk, salad, lettuce, apple cider, punch. These are similar vehicles to what we see in 0157. Lake recreational water, kids are swimming in a lake where another kid=s mother brought the kid with diarrhea because wasn=t that a nice thing to do while the kid had diarrhea, to swim in the lake? We also see this for 0157, person to person spread

in childcare centers, and undetermined. So, this is similar to 0157.

[Slide]

I think I am on transmission now, but in talking about transmission the committee asked me to talk a little bit about some disease outside the United States. I am really focusing on the United States. I am not talking about Europe. The epidemiology is somewhat similar in Europe. But I just want to talk about some more far away areas. As some of you know, Argentina, especially the Buenos Aires area, has the highest reported rate of HUS in the world, and Dr. Gianantonio there wrote some of the seminal papers on hemolytic uremic syndrome where it is still a scourge. So, we did a study in Buenos Aires and Mendoza.

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It was a case-control study of risk factors for 0157 and other STEC infections. The cases were children with culture-confirmed STEC diarrhea or diarrhea-associated HUS. We enrolled 150 cases and we compared their exposures with

those of 299 well control children of the same age and living in the same area.

[Slide]

In our study the major risk factors for infection were eating undercooked beef, including teething on pieces of tender beef, which is the typical practice there. They love their beef and they will get a very tender piece of meat and they will give it a two-year old to teethe on, and drinking jugo de carne. They heat up the meat a little bit and then they squeeze the juice out of it and they feed this to the child with a spoon. So, it is the juice of the meat. Another risk factor was residing in or visiting a place with farm animals and contact with a child less than 5 years old with diarrhea.

[Slide]

There were also protective factors, which were the caregiver always washing hands after handling raw beef, and the child eating more than the average variety of fruits and vegetables. So, your grandmother was right.

[Slide]

I also wanted to mention an outbreak in Africa. In some of our work in Africa we have looked for E. coli 0157 in our big surveys. We find it very rarely. It certainly has been reported. People find it in Africa but outbreaks are very rare. But I want to talk to you about one outbreak in Swaziland.

[Slide]

It was a massive outbreak in 1992. Sixty-two percent of Swaziland is cattle pasture and in the early 1990s they had many years of drought and the cattle were aggregated close to water and vegetation.

[Slide]

So, here is Swaziland.

[Slide]

This is a riverbed that is dry and you can see the cattle in the riverbed looking for water.

[Slide]

There is a dead cow. So, this is the setting.

[Slide]

In September of that year there was still inadequate water and pasture for the cattle. There was a 7-fold increase in cattle deaths, and the cattle were defecating and dying in the streambeds. Then, in October very heavy rains came and the following month there was a marked increase in human bloody diarrhea. Everyone assumed that it was shigella dysentery, which can be really epidemic there and it really is most likely the cause of bloody diarrhea in that area, but they looked for this organism, shigella dysentery Type 1, couldn=t find it and a South African microbiologist, Margueritha Isaacson, said, well bloody diarrhea, what should we do? We should look for 0157. So, she looked for it and she isolated it from human stools.

[Slide]

We did the investigation and risk factors for human illness were consumption of beef and drinking untreated water, and that was the water in which the cows were defecating and dying. We

estimate that there were thousands of people sick.

[Slide]

Specimens that yielded the 0157 outbreak strain came from human feces, cattle feces, water of all types, surface, river, boreholes stored in the home, and also from cooked maize. So, there was sort of a veneer of 0157 in the whole region during that time.

[Slide]

Here are some young men with dead cattle, probably trying to harvest the meat.

[Slide]

So, back to the United States, and I want to talk about transmission here. The proportion of illnesses due to each mode of transmission for 350 0157 outbreaks was assessed in a publication recently. We took outbreaks through 2002 and we looked not at outbreaks as the denominator but as all the cases in those outbreaks, and there were about 8,500 cases in those outbreaks. The major mode of transmission was foodborne, followed by drinking water, person-to-person, animal contact,

recreation and lab acquired.

[Slide]

I just want to focus on two of these areas, first animal contact and I will talk about agricultural fairs. They are traditionally held in rural areas and they bring farm animals, food and people into close proximity. There were outbreaks at 10 agricultural fairs between 1999 and 2005. They were in 8 states and more than 1,400 people were ill. Many of the children developed HUS.

[Slide]

This is an aerial view of part of North Carolina that shows their permanent county fairgrounds.

[Slide]

In the fall of 2004 there was an outbreak with 108 ill children, most of them were children, and 14 developed HUS.

[Slide] You

You can see this little girl is picking up the straw, and illness was associated with this petting zoo where she was and touching the animal

environment, as she did, and touching the animals themselves. Cultures of the patients, the animals and their environment yielded the outbreak strain. That little girl, who just had a blast playing with the animals, developed HUS. Her sister was afraid of the animals and clung to the grandfather and she did fine.

[Slide]

Now I will talk about foodborne transmission.

[Slide]

The prime suspects are behind the bars!

[Laughter]

[Slide]

This chart breaks down the foodborne transmission into the major vehicles, and you can see that ground beef, other beef and dairy products are all bovine products and they comprise 50 percent of the illnesses in outbreaks.

[Slide]

This graph shows the percent of foodborne 0157 outbreaks that were due to beef. Initially

all the outbreaks we found were due to beef. We eventually got better at finding other causes but now about a third of outbreaks are due to beef, which is still a substantial proportion due to beef.

[Slide]

The number two food that has been associated with 0157 outbreaks is produce, with 34 percent of the illnesses in outbreaks.

[Slide]

I just want to focus on these cattle feedlots. These occur throughout the United States. There are a lot of them in California, in our growing areas, and some of them are very close to our produce fields. We are very careful about treating human sewage and there is no sewage disposal system for animal feces. We hope that they degrade and that the pathogens that are in them don=t somehow get in contact with our food supply but we know that they do.

[Slide]

This slide shows leafy green vegetables

that were implicated in 0157 outbreaks between 1973 and 2005. We didn=t implicate any until 1995, and I am sure there were outbreaks but the outbreaks that we find are all only the tip of the iceberg of those that occur. But we found 26 between 1995 and 2005. Of them, the vast majority were due to lettuce and lettuce-based salads. Others were due to cabbage, to parsley and to spinach. So, we are very concerned about these leafy greens.

[Slide]

You will remember that we had this outbreak due to spinach this fall.

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I want to tell you just a few things about this outbreak. I am not going to go through the whole story but we identified 206 patients, and these were just the patients who came to medical attention, had a stool culture that yielded 0157 so they are the tip of the iceberg of the number of patients who really became ill from the spinach. Seventy percent were female, 51 percent hospitalized, 15 percent developed HUS and 3 died,

including a toddler and two elderly women. The strain produced only Shiga toxin-2.

[Slide]

This graph shows the number of patients with illness and HUS by age group. Really, the most striking feature here is that most of the ill people were adults. There aren=t that many kids that you can get to eat spinach so that was fortunate. Eight percent of the adults developed HUS. But for those unfortunate kids who ate the spinach, the rate of HUS was markedly high, 32 percent, and still quite high in the older children and this is higher than we usually see in outbreaks.

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In summary, the spinach outbreak was a large international outbreak, it involved Ontario as well, with a high rate of HUS. Rapid action by health authorities was still too late to have much impact, and prevention measures are needed to improve the safety of leafy green vegetables that will be consumed raw.

[Slide]

Now I just want to talk about the impact of our surveillance and outbreak investigations.

[Slide]

As a reminder, this is that graph that I showed you earlier with this marked decline between 2002 and 2003.

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I want to talk about the impact of 0157 surveillance and outbreak investigations of beef safety. In 1993, I mentioned the fast food hamburger outbreak with over 700 illnesses and 4 children died. During the outbreak, the FDA revised the model food code for restaurants to incorporate temperature guidelines for cooking ground beef because at that point the temperature guidelines were not sufficient to kill 0157. So, that happened very quickly.

The following year the Department of Agriculture made a very bold step in making 0157 an adulterant in ground beef so that, if it is found in ground beef, that lot has to be recalled from

the market. So, this required mandatory recalls.

[Slide]

Then, in 1996 the Agriculture Department initiated a new meat inspection system based on hazard analysis and critical control points that focused on cleaner carcasses and included microbiologic testing for salmonella, which was, you know, a step into the 20th century even though it was at the end of the 20th century, to finally not rely on just sight and smell.

Then, in 1996 the Agriculture Department helped FoodNet=s creation by providing money to CDC to enhance our surveillance to track the incidence of 0157 infections, and we issued an annual report card and that is coming out tomorrow. The Agriculture Department helped to create FoodNet because they had to know whether their new meat inspection system and measures were working.

[Slide]

In 2001, I mentioned that there had been a lot of bad publicity due to beef recalls, and the beef industry decided to collaborate not to compete

on food safety and they got clearance from whoever regulates antitrust to do that. That was a great step forward for them, and it is something that the produce industry has not yet been willing to do.

Then, in 2002, I mentioned this outbreak earlier from one plant that caused over 18 million pounds of beef to be recalled. After that the industry began Atest and hold.@ They now test all lots of beef trimmed for 0157 and the positive lots are not distributed.

[Slide]

We also think that ground beef is safer. Fast food hamburgers are safer. Fast food chains are requiring their suppliers to provide cleaner beef and they are cooking their hamburgers better. Eating from a fast food restaurant was a risk factor for illness in the 1990 study but it wasn=t in the later study. Consumers are also aware of the risk and are cooking their ground beef better. And, the ground beef itself is less contaminated.

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Here are some data showing what people are

doing. The percent of people who recently consumed ground beef in 1996 was about 12 percent, but by 2002 it was 6 percent.

Here is data on the beef itself, the percent of ground beef samples that yielded 0157 declined markedly in 2003, around the same time that we saw this marked decline in the incidence of 0157 infections. So, we can=t say that that is the reason for the decline, but it sure would be a great coincidence and it suggests that it could be the reason.

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There have been other impacts. In >96 there was one of many unpasteurized apple juice outbreaks causing HUS. In this outbreak it was due to a major commercial supplier, not just a local cider stand. There was a death. So, as of 2001 apple juice shipped interstate must either be treated to kill pathogens or have a warning label. If you go to your supermarket you will see warning labels on juices now. Since then, there has been a marked decrease in juice outbreaks.

[Slide]

I told you about the outbreaks due to contact with animals and their environment at county fairs. As a result, we now have a compendium of measures to prevent disease associated with animals in public settings.

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We see signs like this at county fairs and petting zoos, and there are hand washing stations.

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In summary, there has been a decline in the incidence of 0157 infections by 2003, but there has been a recent increase. I didn=t focus a lot on that but you saw that big decline in 2003. It was sustained in 2004. It went up in 2005 and I think you need to brace yourself for the data tomorrow. HUS 0157 is a major cause. Pathogen and host risk factors are still being determined. For outbreaks, the median size has decreased to five ill persons.

[Slide]

For transmission, petting zoos can be

hazardous. Beef is still an important cause but produce consumed raw is a major source. Surveillance and outbreak investigations prompt industry changes that decrease illness. Ground beef is safer. Apple cider and juice is safer. Petting zoos now have guidelines but produce needs work.

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This talk was made possible by the efforts of people in many groups, including PulseNet, Outbreak Net, the enteric disease laboratory branches, state health departments and my colleagues in Argentina and Swaziland, South Africa.

[Slide]

This is the current and former members of my branch. Thank you.

DR. RELLER: Thank you, Dr. Griffin. Questions about the epidemiology of Shiga toxin-producing E. coli for Dr. Griffin? Yes, Dr. Edwards?

DR. EDWARDS: In your global estimates of

the incidence of 0157 with the estimated 60 deaths, is it going to be possible for you to give us a rough estimate of what we are going to see tomorrow on the number of deaths? Will deaths actually increase substantially?

Secondly, I wonder if you could tell us what would be the estimates for deaths due to HUS. There would be other causes of death, other than HUS. I realize this is a difficult question.

DR. GRIFFIN: The estimates tomorrow won=t say anything about deaths. The estimates just give the estimates of the incidence of disease. For deaths you need a study. You need a study in which you then call up every patient with 0157 infection or their physician and you wait, you know, and a month later and you find out if they died. So, that is done in special studies and it is not something that we have been able to follow every year. That estimate of 60 deaths was made based on data that was available to us in 1997 when we wrote that paper. So, we don=t have estimates by year of how many deaths there are.

As far as whether deaths are from HUS or from other factors, again, we don=t have that information readily available. The last time that we looked at it, I think we published a short letter to the editor and we found that about-BI can=t remember the numbers exactly, but in the small data set we looked at about half the deaths were due to HUS and about half due to other features of the disease. Phil may talk about that later.

DR. RELLER: Dr. Ward?

DR. WARD: You mentioned the frequency of toxin number 2. Do you have some data about the distribution of types of toxins among these outbreaks?

DR. GRIFFIN: We do not collect that information. I wish we did, and we are hoping to do that sometime in the future. Your question may also be do we have information about the distribution of these infections in general, and in the past our impression in the United States was that about 80 percent of strains produce both

toxins. About 20 percent produce only Shiga toxin-2 and somewhere in there, there were about 3 percent which produce only Shiga toxin-1.

But before you say okay, that was data that we had from our laboratory and our surveillance, in the cohort study that we are now doing we are seeing those numbers sort of turning upside down and we are not sure when that changed. That is something that we are actively looking into because it looks as though, at least in FoodNet, most of the isolates are producing only Shiga toxin-2. So, that is a change and when that changed I don=t know, and whether that will hold up with more information I don=t know.

DR. WARD: That was going to be the follow-up question. Do we have just a point estimate or do we have two or three years showing that shift?

DR. GRIFFIN: We collect E. coli 0157 from state health laboratories that receive them from the clinical laboratories and we test them and salmonella strains for antimicrobial resistance.

We have been doing that for about 10 years. What we are planning to do is to go back and look at that collection and look at the toxin types. Our laboratory has not had the resources to do toxin typing for all those strains in the past, but we are hoping to be able to do that.

DR. RELLER: Dr. Daum?

DR. DAUM: I was intrigued by the system with the pulse-field gel electrophoresis that you have. My guess, and it is a question really, is that there must be a lot of heterogeneity between strains to be able to pick them out like that. So, I have two questions. One, is there a lot of heterogeneity? Secondly, is anyone working on a better system, I guess, that is more simple to do, say PCR-based or short sequence-based to tell strains apart in these epidemics?

DR. GRIFFIN: Yes, before the big outbreak in 1993 people had tried doing plasmid profiles and everyone said that the strain was just too clonal, that you really couldn=t do those sorts of gels and see much difference. But with pulse-field gel

electrophoresis and breaking up the strains usually you need two enzymes so you have two different patterns, one with one enzyme and one with the other. With that combination we find that it really is quite discriminatory. It still requires the human eye, basically looking at bar code patterns so, of course, we are interested in going to DNA-based methods. There are a couple of methods that we are trying out. The advantage of PFGE is that people can afford to buy the equipment and to do the test and it can be standardized so it meets some of those criteria of feasability. But we are planning to go to more molecular type methods.

DR. DAUM: Thank you. Do you have a comment on the degree of heterogeneity? I would think that if you are putting patterns up on a website, unless they are really quite different from each other, there is a lot of room for error in looking at them and interpreting your own patterns compared with what is on the computer.

DR. GRIFFIN: Usually when you are looking

to identify an outbreak, you are looking for a cluster of patterns that looks very similar in a particular period of time. Even in an outbreak setting there are sometimes patterns that are slightly different because the organism can acquire an extra plasmid or break up in a different way. So, some outbreaks have a single set pattern and others have related patterns that are still considered part of the outbreak. So, it is actually not too difficult to initially identify what looks like an outbreak strain.

For an individual isolate that might be submitted from an individual health department over the internet, you have to make sure that the gel is done exactly the same in all places; that it is transmitted well; that it doesn=t blur. People go through training to do that correctly, but it still requires people to sort of line them up visually, and it is difficult and sometimes they need to ship the strain so that it can be done in the same place. But the system works pretty well.

DR. RELLER: Dr. Gorman and then Dr.

Rosenthal.

DR. GORMAN: As you were going through your presentation it struck me once again how effective systems approaches can be, as we were talking about the ground beef success, how effective systems can be in changing outcomes that are health related, especially when the economic incentives of the public health system and the beef industry are so closely aligned. Has the beef industry given you any estimate of the cost to make these improvements into their system? Because we are going to be talking this afternoon about a systems-based approach for taking care of a very specific disease in pediatrics.

DR. GRIFFIN: I don=t know of the cost estimates in general. I think that they are really eager to be partners in this effort and might be willing to share some of that information with you.

One person that you could talk to is David Theno, who is in charge of food safety for the company that took over for the company that was involved in that 1993 big outbreak. After that

outbreak their company began testing all lots for 0157, way before other companies did. And what he said, and I can=t confirm it and I don=t think it is in print but what he said was that because they were looking for 0157 or other organisms in the meat, and that meat I think got converted perhaps to make chili or something like that, that their meat actually had a longer shelf life because it was less contaminated and that pretty much the intervention paid for itself. So, he said it was very cost effective. Of course, you can=t measureB-it is very hard to measure the cost of not having another big outbreak and the consumers not buying beef anymore. The industry has done surveys on consumer perceptions and their impression is that it has been worth it to them.

DR. RELLER: Dr. Rosenthal?

DR. ROSENTHAL: Thank you. I am wondering if you can teach me about whether clinical illness is manifested in animals, cows, that have this infection. If not, in which animals is illness manifested and what are the characteristics of the

animals that manifest illness when they are infected with this strain?

DR. GRIFFIN: Well, you know, that is a whole lecture in itself. I don=t think anyone is giving a talk on animal models here. It really is a big topic. Basically, animals don=t get this illness. You will find exceptions. You know, some baby calves have been reported to have this illness, or you can get gnotobiotic pigs to have this illness, or greyhounds sometimes that are fed raw beef and they develop a similar illness, or baboons in an animal model can get this illness. But basically animals, and basically our food animals don=t get this as an illness. But cattle carry the organism and if you look well enough in any cattle ranch in the United States, and probably in most countries, you will find E. coli 0157. The other Shiga toxin-producing E. coli are actually more common in cattle than is 0157.

The one thing that is different about E. coli 0157 from, say salmonella, is that salmonella is in all of our food animals. E. coli is pretty

much a cattle-associated organism. Does that answer your question? Is that what you were looking for?

DR. ROSENTHAL: Yes, it is exactly what I was looking for. Thank you.

DR. RELLER: Also, after the break this morning there will be an extensive presentation on animal models and components of this disease that may be reflected, you know, in one of the other to some extent, although the cattle that Dr. Griffin has been talking about as the source of beef for consumers are not themselves affected by the organism. Correct?

DR. GRIFFIN: That is right. Another question from Dr. Hilton.

DR. HILTON: Is the problem in produce as a source of infection associated with animal sewage?

DR. GRIFFIN: Well, there are some E. coli infections that are transmitted directly from one person to another. There is a low infectious dose. But, basically, the only major reservoir for E. coli 0157 of which we are aware is cattle. Yes,

you can find it in deer. Yes, you can find it in many other farm animals if you culture them. But cattle are really the reservoir. So, if there is an E. coli 0157 infection we look for how cattle feces got into the food or water that that person consumed. So, for produce, when produce causes an illness we are looking for the cattle connection.

DR. RELLER: Yes, Dr. Wong-Beringer?

DR. WONG-BERINGER: I was wondering if there has been any look at host genetic susceptibility to developing HUS.

DR. GRIFFIN: Yes, a little. I don=t think there is a lot of information. We certainly think that immunity has a role because the most severe illness occurs at the extremes of age. There have been some papers on host susceptibility that have then been refuted. We have a cohort study in which we are going to be looking at some host factors. Phil may be able to answer that question better, but offhand I can=t think of major host factors.

DR. RELLER: Dr. Griffin, thank you very much.

DR. GRIFFIN: You are welcome.

DR. RELLER: That question is a perfect lead-in to our next speaker. Dr. Phillip Tarr, from Washington University in St. Louis, will be reviewing with us the clinical course and consequences of infection with E. coli 0157:H7 and other Shiga toxin-producing bacteria. Dr. Tarr?

**Clinical Course and Consequences of Infections with Escherichia coli and Other Shiga Toxin-Producing Bacteria**

DR. TARR: Thank you, Dr. Reller. Thank you, members of the committee and guests.

[Slide]

In the interests of complete transparency, I feel compelled to mention that in the 1990s I was the guest of Teijin, in Japan and in Seattle at several dinners. I have also been a co-author with several members of the Caprion clinical advisory board in the recent past. Nonetheless, I have no current or pending financial arrangements with these organizations.

[Slide]

This is a very complex topic, as Dr. Griffin set the prelude with her excellent talk as to how this organism behaves in populations. Today I will focus on how this organism behaves when it gets into a human, particularly a child; how the child behaves at that point from a pathophysiologic and care-seeking behavior; and how healthcare providers behave when confronted with a patient with possible or proven E. coli 0157 infection. The whole motif of this talk is an attempt to identify how we might find patients earlier accurately; how we might intervene to prevent regrettable outcomes, most notably the hemolytic uremic syndrome.

How many people in this audience have ever seen a patient with E. coli or HUS?

[Show of hands]

So, there is a reasonable subset.

[Slide]

This slide demonstrates the time line of infected patients. This is a variation of what Dr. Griffin just described. There is about a 33-day

incubation period between the ingestion of an E. coli-containing substance and the first loose stool. These data are derived from outbreak studies where the vehicle was known and the time of ingestion was known. Then, in about 80 percent of cases there is a one- to about three and a half-day interval where there is non-bloody, usually quite painful, diarrhea. Patients usually do not seek medical attention in this interval, though about a quarter of such patients do make a telephone call to their healthcare provider asking for advice. But in the absence of sustained high fever in this interval, the absence of blood in a child who has a median age of about 4 years old and a child who is still urinating there are very few sentinel symptoms, cardinal symptoms that would bring a patient in for evaluation prior to the time that the stool tuns bloody, as it does in about 80-85 percent of all diagnosed cases in North America. That occurs between about day 2 and day 4.

When we have looked at this rigorously between the onset of the first stool that is

bloody, and obtaining that stool for culture, there is a delay of about 12 hours. That is usually at the point of presentation. Soon thereafter the stool is submitted. So, this is the first opportunity anyone could realistically hope to have for diagnosis and intervention.

Hemolytic uremic syndrome, if it is going to occur, occurs with a median of about day 7.5, with day 1 being the first day of diarrhea in recent North American studies. It occurs in about 15 percent of children under the age of 10 with microbiologically diagnosed E. coli 0157 prior to the onset of HUS. There is a subset of children who come in into the system where the first stool culture is obtained for E. coli 0157 at the time they report with hemolytic uremic syndrome, and about 85 percent of children resolve this infection spontaneously without going on to develop renal injury.

For the purposes of this talk, we have chosen to define hemolytic uremic syndrome fairly stringently with a hematocrit less than 30 percent,

a platelet count less than 150,000 and a functional definition of renal injury, namely the creatinine above the upper limit of normal for age.

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So, there are great opportunities. About 90 percent of patients are seen by a physician prior to the onset of hemolytic uremic syndrome. This is a toxemic, non-bacteremic disorder. Certainly, there are two notable examples, the tetanus and botulism, where toxemic disorders can be treated with an intervention, namely antibodies, and one would think that there might be an opportunity here to do the same.

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However, there are also considerable challenges in assessing such strategies and then implementing such strategies in the general population once a product is available. The first problem comes in the ability to identify quickly and accurately infected patients. Dr. Griffin described the haphazard approach to microbiologic diagnosis. You are highly dependent upon getting

the right sample to the lab, having the lab do the right test, interpreting it quickly and confidently in any sort of a time frame where the provider is then provided with information so that they can implement an intervention.

This is a low incidence disorder. Dr. Griffin=s incidence estimates of 1/100,000 patients with a positive culture translates to about 3,100 positive cultures in the United States per annum. It is a sporadic epidemiology. The vast majority of cases are not part of large outbreaks. Perhaps they are small inter-household or, even rarer, inter-daycare clusters, and it is a rural disease because these people do not present to large medical centers in large numbers, the situation that would be ripe for studying and implementing a therapeutic trial.

There is a narrow window of time to prevent sequelae. Patients generally seek care around day 4 or 5 of illness. Hemolytic uremic syndrome is going to begin 71-96 hours later. Big question, how much vascular injury is already under

way? How much more antibacterial or antibacterial product interventions could reverse cascade is an open question. How much one can attenuate the host response is also an open question.

In a forum like this, it is important to identify what the outcome of interest is. Certainly, meeting an objective case definition of HUS is going to be one outcome of interest, but even among children with HUS there are categorically two different kinds of HUS. These can be roughly grouped into anuric, dialysis-requiring hemolytic uremic syndrome and non-anuric HUS, and I will get into that at the end.

Finally, as we begin to look at implementing such a trial, there are going to be important considerations regarding informed consent. How can a family member be appropriately consented in real time in the time frame needed to give an intervention in the hopes of doing some good? These are all very complex issues.

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Once a therapeutic is approved, and it would be terrific if one could be approved and given to children who present around day 4 or 5, in addition to the standard concerns that a practitioner is going to have about safety and efficacy, cost must be addressed. If this is a massively expensive intervention it will not be used well. In addition, for a very rare event like this, there are important supply carrying costs to an institution. If an antibody is administered once or twice a year in a rural hospital and the antibody has an expiration date 6 or 12 months after distribution or manufacture, there is going to be a lot of institutional pressure against using such a product.

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Dr. Griffin set the stage for the big debate, how does one diagnose microbiologically an infected child? A practitioner taking care of a patient with a diarrheal infection, that practitioner is only as good as the microbiologist to whom he or she sends that patient=s stool. Once

in the microbiology laboratory, today the laboratorian has two opportunities. They can choose to plate it on sorbitol-McConkey agar or they can choose to seek a toxin-based study. Most laboratories, if they plate it on sorbitol-McConkey agar, will look at the colonies 24 hours to 48 hours later, depending on growth; take a candidate sorbitol non-fermenting colony and then, fairly quickly, make a presumptive diagnosis of E. coli 0157. Confirmation takes an extra day or two, by which time the patient is better or in the ICU with hemolytic uremic syndrome.

With a toxin-based assay, this is not generally a toxin assay as applied to the stool; it is a broth culture of the stool after overnight incubation. With a fairly rapid enzyme immunoassay toxin can be discerned in the broth so at least a presumptive preliminary signal can be gotten out. If there is an organism in this broth that produces Shiga toxin that is important information for a practitioner to know. In many hands that may be a more robust, durable readout than the

sorbitol-McConkey agar plate.

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However, the situation is somewhat more complex. In much of Europe, in most of North America, Canada, Japan and South America one single serotype, E. coli 0157:H7, which is best detected with the sorbitol-McConkey agar screening is the predominant cause, causing at least 90 percent, probably closer to 95 percent of all post-diarrheal childhood cases of hemolytic uremic syndrome. Here is a sample of references that support this statement.

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When we decided to look at this intensively at a single point of care in a study, performed largely by Dr. Eileen Klein at the Seattle Children=s Hospital and regional medical center emergency department, we began to extrapolate some of the microbiologic findings to a real-world, real-time human population. In this population there were 1,626 stools collected during a 3-year period. In fact, there were nearly 5,000

children who came in with diarrhea but sample obtaining in this situation is remarkably difficult and many of these stools were only swab specimens. It is very difficult to get this analyte for study.

In this 3-year study, 39 times did the Meridian EIA broth, which was performed on a daily basis, give a signal that there was a toxin-producing organism in that broth. So, 39 times through 3 years, almost once a month.

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Of those 39 signalsB-now, this is looking entirely hierarchically at the toxin, not the sorbitol-McConkey; this is done in parallel but looking at toxin as the nodal test here for those 1,600 stoolsB-of those 39 signals, 11 yielded a non-0157 Shiga toxin-producing E. coli. These are the serogroups. I think all of these are the ones that Dr. Griffin just mentioned. Three of them produced no Shiga toxin-producing E. coli despite our best attempts to dig through that broth and find the offending organism. Twenty-five of those

signals were caused by E. coli 0157:H7. This epidemiology is very similar to the Minnesota HMO ratio. Two E. coli were 0157's in an urban pediatric emergency room to one non-0517 Shiga toxin-producing E. coli.

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There were 3 additional children whose EIA was absolutely negative and, yet, grew toxin-producing E. coli 0157 on the sorbitol-McConkey agar. So, even though this assay has considerable intuitive and theoretic appeal, it is not 100 percent sensitive for the detection of E. coli 0157:H7 which is the leading cause of hemolytic uremic syndrome in most of the world. So, our overall recommendation is to do both tests in parallel.

By doing both tests in parallel we found 28 children infected with E. coli 0157, 25 of whom would have been picked up by that EIA. Eighteen percent of those children in an emergency room population-based prospective study developed hemolytic uremic syndrome and 92 percent of the

children with E. coli 0157 had bloody diarrhea as noted by the parent on a questionnaire we asked them to complete. Only in 70 percent did the laboratorian say there is blood in this. We also asked the laboratory technicians to write down was there blood.

This discrepancy demonstrates don=t rely on your laboratory to decide what test to use in your patient. Don=t rely on them to say, oh, there is blood and I will, therefore, look for E. coli 0157. It should be a broad-based approach without selectivity at the laboratory end. Of the children with non-0517 E. coli infection, none developed hemolytic uremic syndrome. About half had bloody diarrhea. That suggests that if you are looking for non-0157's don=t use bloody diarrhea as your culture criteria. Half of them will have non-bloody diarrhea and should be included in the screen.

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Why not test the stool for the toxin? We think this is a toxemic disorder. There is $10^7$ to

$10^8$ E. coli 0157's per gram of stool on or before day 4 of illness. One would think that there should be a lot of toxin there. Well, it is a difficult analyte to get. You often fail to get it. Even though a patient is coming to you for diarrhea, children often will not produce that substance for testing immediately. Interestingly, in a study we published with Dr. Nancy Cornick at Iowa State University several years ago, only about 40 percent of children overall who subsequently developed hemolytic uremic syndrome had free fecal cytotoxin, free fecal Shiga toxin in their stools so even though they were $10^7$, $10^8$ viable 0157's pre gram of stool, when we took that stool, filtered it at the bedside and subsequently tested for toxin, it was not detectable in a Vero-cell assay over half the time even though those children went on to develop hemolytic uremic syndrome.

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One then has to ask if you get a signal in a non-culture test, such as a toxin assay, is it for real? How much credibility to do you need to

put in it? I think you need to put a lot of credibility in it, to the point that you have to really ask what kind of patient it came from. It is not something that just appears in a physician=s mailbox two or three days later. This really does obligate a call, much like a positive blood culture should obligate a call from a microbiology laboratory. But the setting in which the specimen was obtained, the clinical setting, is often quite critical and this relates to how seriously one should take a positive signal.

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For example, if a patient emanates from an emergency room, if the patient has bloody diarrhea, if the patient has painful diarrhea or the patient is hospitalized and a toxin enzyme immunoassay is reported as being positive, whether an E. coli or 0157 is also simultaneously detected, that would be a highly credible result and would make me suspicious that this patient is infected with one of the serogroups highly likely to cause a serious gastrointestinal disease, probably much less likely

to cause HUS though the risk is zero.

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However, if the stool originated in a patient with chronic diarrhea, an infant with diarrhea, at least in the United States, with non-painful, non-bloody diarrhea and one hears about a toxin signal being positiveB-and this is something that comes up, for example, in these large industrial laboratories where doctors= offices send stools from children with illnesses that are less acute than that seen in emergency rooms and you start to bring in less specific signals-BI would say that that is considerably less likely to be related to the patient=s illness and is probably not going to have very much in the way of medical consequences for that patient.

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So, to summarize the diagnostic challenges for a randomized, controlled study and also for the implementation of a therapeutic in years to come, right now and for the foreseeable future any testing for toxin must be coupled simultaneously

with a test for E. coli 0157. Right now, the best test in my opinion is the sorbitol-McConkey agar screen plated simultaneously, as Dr. Griffin also recommends.

Direct stool tests appear, for reasons that are unclear, to be insensitive. Choose your population well if you want to find children infected with E. coli 0157. Doctors= offices are probably not the right target. Also, as I will start to demonstrate, the time to a positive result for any test is critical. Every minute counts with this illness. This is not just a dehydrating diarrhea. The model for an E. coli infection that precedes the hemolytic uremic syndrome is a myocardial infarction.

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So, let=s get back to this time line. We have intensively looked at what happens at the point of presentation, at the point of microbiologic diagnosis, and tried to discern what is going on in the human host as early in illness as we can possibly identify them.

We do this by a variety of notification systems using a network of laboratories in the Pacific northwest and more recently in Missouri. When a culture is positive for E. coli 0157 we are notified; the same for a toxin assay being positive. Children who fulfill a clinical profile and present to selected emergency rooms are also eligible for enrollment.

What we are trying to do is study intensively the pathophysiology of this infection obviously in the most relevant human host, an infected child. Unfortunately, animal models have been problematic and have not completely recapitulated the series of events between oral infection with Shiga toxin-producing E. coli, gastrointestinal symptoms and subsequent microangiopathic hemolytic changes and renal failure. So, we have to rely on looking at the infected child, ideally, at the colitis stage in advance of developing the thrombi characteristic of hemolytic uremic syndrome.

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When one sees a patient like this in the conduct of a trial or the administration of a therapeutic one has to decide should I diagnose and then treat if positive, in other words, can I get the test now, send the patient home and, if positive, will call them back? Or, should I start to syndromically profile this patient, rule out MI and rule out sepsis, possibly E. coli infection and then withdraw therapy if it turns out to be another infection?

In my opinion, for a patient with acute bloody diarrhea in North America, and most particularly a child, this is a medical emergency. This is an outlying symptom. This is a fairly unusual problem. It has to be respected. This is not rotavirus diarrhea. So, my preference is to profile such patients and to start an intervention in advance of microbiologic diagnosis. At the same time, we hope microbiologic diagnosis will start to improve, enabling us to make a bedside or soon thereafter diagnosis of an infected child.

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Now, what would I propose for syndromic profiling? This is based on several published studies, as well as having taken care of many of these children in the past 23 years since I first saw a patient with E. coli 0157 in Seattle. Briefly, a child who has diarrhea for one day that then turns bloody is a candidate for an E. coli infection. A child who has bloody diarrhea as the first loose stool is fairly unlikely to be infected with E. coli 0157. It can happen. It is somewhat unusual. Bloody diarrhea that goes for more than 5 to 7 days without turning into HUS or resolving spontaneously is very unlikely to be E. coli 0157. Children infected with E. coli 0157 have almost always at least 3 bowel movements in the previous 24 hours. A child coming in with a single bloody stool, it is very unlikely to be an infectious colitis.

At least in the United States though, perhaps in South America it is somewhat different, hemolytic uremic syndrome is extremely rare under the age of 9 months. So, while we have seen

children infected with E. coli under the age of 9 months, we rarely see kidney failure in this interval. If I had to exclude a population because of low likelihood of developing this consequence, I would probably set the cut point at 9 months.

Another hint that it might be E. coli 0157 infection is that these patients rarely have fevers over 38 degrees centigrade in healthcare settings. About half of such patients will report having had a fever prior to coming to the hospital or to the clinic, at home, but very, very few are febrile at the point of presentation.

Finally, the abdominal pain is well out of proportion, especially when having a bowel movement. This is another clue that it might be an E. coli infection. And, I have found this profiling relatively helpful. It may or may not be possible to put this into a strict algorithm.

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Syndromic profiling is best when applied to high acuity venues, namely emergency rooms. It will miss about 20 percent of cases because to get

into the profile you have to have bloody diarrhea. Twenty percent of patients will not have bloody diarrhea. You are going to admit an awful lot of kids with salmonella and shigella. Even with the best attempts to try to focus on children with E. coli 0157, you get 2 or 3 children with salmonella, shigella or campylobacter who don=t have 0157 who will be admitted. It must be coupled with expeditious testing so that you can lend clarity to the situation. It is unreasonable to expect a patient to sit in a hospital for several days, not knowing what they have, and if it is in a research setting there will need to be appropriately funded support for the patients who fit a profile yet might not be infected.

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We have given some thought to how to handle the pre-symptomatic patient or the contact of a patient with a bona fide or highly likely E. coli 0157 infection. Epidemics, household contacts, daycare centers are all such opportunities potentially to intervene with larger