DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL AND PREVENTION
FOOD AND DRUG ADMINISTRATION
NATIONAL INSTITUTES OF HEALTH

Emerging Clostridial Disease Workshop
May 11, 2006
Atlanta, Georgia

Certified Verbatim Transcript

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Opening Session

[CONVENE 8:31 A.M.]

E. SPEAKMAN: Good morning. Thank you all for coming. Before we get started, we'd like to have our great host, the CDC, have some opening remarks.

C. MCDONALD: Hello. My name is Dr. L. Clifford McDonald, a medical epidemiologist here. And I'd like to introduce the Director of the National Center for Infectious Diseases here at CDC, Dr. Rima Khabbaz.

R. KHABBAZ: Well, good morning. Can you hear me? It's a pleasure to welcome you to CDC and in particular to our new Communications Center, the Tom Harkin Global Communications Center. I am Rima Khabbaz, Director of the National Center for Infectious Diseases at CDC. And I want to start by apologizing for those of

you who are new here or coming in for the inconvenience of our heightened security process.

It's unfortunately a reality of the new world we live in. And for those of us who live here, we've gotten used to it. But I realize that if it's your first time here in recent times, you might think it's special for this workshop, but believe me, it's not. So welcome and thanks for taking the time to participate in this important workshop.

It is our hope that it will help focus our research and prevention work related to both *Clostridium difficile* and *Clostridium sordellii*. As you all know, both are very serious and challenging infections associated with significant morbidity and mortality. And we are committed to get a better understanding of what is causing this apparent increase in disease severity and what the next step ought to be for dealing with these infections. My heart goes out to everyone who has suffered from these infections and to the family of those who have tragically succumbed to them.

I want to thank the planning team for organizing this workshop. On the CDC side, in particular, Dr. Cliff McDonald and also his counterparts at CDC, NIH for a great collaborative effort to assure that all facets of issues and concerns related to these infections are addressed. Also want a special — give a special thanks to our panelists, esteemed colleagues for your participation, and your insights and contributions.

I look forward to hearing the results of your discussions and your feedback regarding needed research and surveillance. So thanks for everyone for being here

and participating in this workshop. Let me assure you that CDC is committed to following up on the feedback. And I believe that Dr. McDonald will touch on specific CDC next steps at the close of the workshop. So best of luck in your discussions. I hope they're fruitful. Thank you.

E. SPEAKMAN: Thank you, Rima. And I'd also reiterate that I feel much safer knowing that these security provisions are in place right here today. Paul, you have some opening comments from FDA? Can everybody hear from the podium — speakers from the podium in the back?

P. SELIGMAN: Can everyone hear all right? It's fine. Good morning and welcome to the Workshop on Emerging Clostridial Diseases. My name is Paul Seligman. I'm the Associate Director for Safety, Policy and Communication at the Center for Drug Evaluation and Research at the FDA.

On behalf of my colleagues at the FDA, NIH and CDC who planned this meeting, I want to welcome you to this meeting, as well as thank the distinguished — our host — thank our hosts at the CDC for allowing us to conduct this meeting here, as well as the distinguished speakers and panelists who have joined us today.

Over the past few years in the United States, there have been emerging reports of serious infections due to two forms of *Clostridium* bacterium: *Clostridium difficile* and *Clostridium sordellii*. Infections associated with *C. difficile*, a toxin-produced anaerobe, occur mostly in association with antibiotic use, especially in the hospital.

It is a presentation that physicians in nearly every type of clinical practice are familiar with, with thousands of cases occurring every year in the United States. It causes illness by way of its toxin, leading to severe diarrhea. This well-known affection — infection is for the most part manageable with established therapy.

By contrast, *C. sordellii* until recently has been an organism known only to a few. Most had never heard of it, probably because it is a bacterium that rarely produces a toxin much less clinical illness. Over the past few years, the picture has changed. In December 2005, the CDC published reports of unusual patterns and characteristics of *C. difficile* beginning with a cluster of cases in Pennsylvania.

These cases are notable in that the patients had no association with hospitals or long-term care facilities and many had not even taken antibiotics. Their illnesses were far more serious than usual with an appearance of toxin-mediated sepsis that was fatal in some cases. Interestingly, several of the cases were among pregnant women. Also in 2005, Marc Fischer from the CDC published a case series of deaths from *C. sordellii*.

These events involved four patients that had been reported to the FDA as deaths in young women who had undergone medical abortion in very early pregnancy just days before their fatal septic illnesses. These cases had been reported to the FDA as deaths associated with medical abortion medications. But it was only when the CDC became involved that the organism associated with the deaths, *C. sordellii* was identified. All four cases occurred in California.

Both of these clusters raise important public health questions. First, why are we seeing fatal illnesses related to these *Clostridium* organisms emerging? What has changed? Is there something about *Clostridium*'s microbiologic ecology that is leading to its emergence in certain areas of the country? And why is this occurring? And finally, can these illnesses be prevented? How can we mitigate the rapidly progressive nature of the illnesses once we suspect them?

The answers to these questions are not readily apparent. Some researchers have suggested that patients with a newly virulent *C. difficile* may have been placed at risk in a different manner than usual. It is clear that the toxin being produced by the organism is different from the organism-producing hospital infections and is increasingly prevalent. The relationship of pregnancy in these cases is not clear.

Some have suggested that patients with *C. sordellii* were placed at risk because of medical abortion, exposure to mifepristone and misoprostol, or because the misoprostol was administered intra-vaginally instead of orally as the labeling recommends. But this combination of drugs is used around the world in many more patients than in the United States. Why then are these cases only here, and why now and only in the Western United States?

Despite our limited knowledge of factors associated with infection, groups like Planned Parenthood have begun to require that medical abortions in their clinics utilize misoprostol orally as labeled. Whether this will alter the incidence of *C. sordellii* illness

is not clear. If answers to these questions were readily available, we would not be holding this workshop.

What we do know is that in this country, we are seeing the simultaneous emergence of two virulent often fatal illnesses affecting otherwise healthy people produced by species of *Clostridium* that either do not usually produce toxin at all or do not produce a toxin that leads to such devastating illness. We must approach these issues with vigor and scientific rigor.

In developing this workshop, some thought it unusual to mix a discussion of these two organisms. While we could have developed separate meetings for each organism, our goal for this workshop is to identify research needs and priorities that will bring us answers to the questions posed by the emergence — emerging presentations of both organisms.

The research community for *C. sordellii* is fairly limited. We feel that this latter group could be stimulated and enriched through the expertise of scientists who are with us today on the panel and in the audience, many of whom have devoted decades to the study of *C. difficile* and other species. FDA, CDC and NIH need you to consider how best to approach such research and surveillance in order to mitigate the public health effects of these two organisms.

The outcome of this workshop will be a draft agenda which we expect will provide recommendations for detecting cases and conducting surveillance of these diseases

and organisms as well as enabling rapid progress in our understanding of the virulence, pathogenesis and treatments for both *Clostridium difficile* and *sordellii*.

Of course the question on everyone's mind is what next after this meeting? After today's workshop, FDA, CDC and NIH will individually and jointly review the discussions and recommendations that this meeting generates and the information submitted to the docket. A transcript and summary of this meeting and all submissions to the document — docket will be publicly available and posted on the web site of the FDA as well as the other agencies. We intend to publish proceedings of this conference in a medical journal to ensure widespread availability and opportunity for peer comment.

In addition to these collective activities, each of the three agencies sponsoring the meeting will be taking various steps. The CDC plans to examine closely the role of additional types of monitoring for healthcare-associated *C. difficile* infections and emerging community-associated — community-associated infections as well as for defining new and evolving risk factors for these disease — these diseases. CDC also expects to develop definitions and strategies to better monitor *C. sordellii* infections.

The NIH and the National Institute for Allergy and Infectious Diseases currently supports a few investigator-initiated basic research and early product development grants in *C. difficile*. The NIAID will continue to be the leader in the field of understanding the pathogenesis and biology of emerging infections such as these. It is their hope and our hope that if clear research needs and strategies can be articulated,

the NIH will be better able to engage the research and product development communities to address these emerging diseases in subsequent research proposals.

And for the FDA, we will be listening carefully to these proceedings and weighing the information presented here and submitted subsequent to today's workshop. We have many of the key FDA scientific leaders with us today. Developing a realistic set of expectations regarding the time it will take to understand the pathophysiology and etiology of these illnesses is an important element in determining whether any regulatory action that affects the appropriate use and availability of these drug products is warranted.

On that note, thank you all for coming. I'm looking forward to learning a lot today. And I'm welcoming the opportunity not only to learn from the panel, but as well as those of us who've joined us, experts here as well as members of the audience. Thank you.

E. SPEAKMAN: Good morning. Again, thank you all for coming. And thank you Paul and Rima for great opening comments. Paul talked about things moving forward. What I wanted to point out to everyone here is that it was a lot of effort done in advance of this workshop to be able to address the concerns, the issues, the passion and the compassion around both of these infections.

And for just a brief second, for those that were on the lead team, the planning team that put this all together, if you could stand up for just one moment, take a quick applause; endless, endless conference calls over the last several — endless, endless

conference calls, e-mails at midnight, 4:00 in the morning, conference calls at 6:00 on Fridays, people being late for dates and families. So a lot, a lot of effort went into being able to ensure a productive workshop.

And you know what we want to accomplish in the workshop and how we're going to accomplish that, our agenda? First, we have a morning panel that will be dealing with the clinical syndromes, and pathophysiology and host factors associated both with *C. diff* and *C. sordellii*. That will take us up to lunch. Lunch will be at 12:15 — excuse me — 12:30, an hour at lunch from 12:30 to 1:30.

And then we have a second panel that will deal with the surveillance for disease and sources of infection. We will take a quick break at 2:30. And at 2:45, we will reconvene to look at identifying a research agenda, providing recommendations for priorities for this research agenda. A lot to do — a lot, lot to do in a short day.

So what we have done is establish some ground rules to be able to make sure that we get as much participation today, but we do it to be able to accomplish the objective to set the stage for those next steps that we discussed. And those ground rules are, is that we want to reiterate, today we're not creating consensus.

We're not making formal decision-making collectively or individually by the agencies represented here; that we are here about open dialogue and discussion; that you heard that the next steps would include a chance for public comment on the transcript and summary of what occurs today at the workshop. Also, this information

will be reviewed by this planning team and the panel chairs and look to publish this in a medical journal as well as the appropriate steps that each one of the agencies have mentioned that they will take moving forward from here.

So we've got a lot of people, not a whole lot of time. So, but we want to make sure that everyone's time is valued and important, so we're going to give everyone an opportunity to speak. For those that are sitting in the front, if you notice there's little microphones up front. All you would do to actually when you want to speak is touch those; it looks like a little triangle kind of thing. You push that if you want to talk; speak into the microphone.

For those that are seated in the back, I would ask that during each panel discussion, we will have allotted time for comments, questions, feedback from everyone. For those in the back, just form a line to the mikes to the left and the right. And we ask you very kindly, if you want to ensure that your comments are recorded accurately to speak into the mike and also to state your full name so we can get that recorded. And that's how we're going to get your input.

Unfortunately, lot of people, lot of time — I mean, with not a lot of time, so we have to limit conversation, questions to two minutes apiece and I will do my best. If you have a question, or comment or feedback, give me the Carol Burnett sign or some way to make sure I know that you want to speak and I will make sure that we get your comments and suggestions. But remember that it's limited to two minutes apiece.

Everyone's input, no matter who you are today, we're all equal today in terms of providing dialogue, discussion and participation. And these are some touchy subjects here and sometimes that we have conflicts that occur between individuals. We're not here to deal with that today. We're here to look at the issues, look at the scientific issues and facts, and then move forward from that. So ground rules on how to be productive.

Now a few logistical ground rules, the important things: Lunch, as I mentioned, 12:30. Box lunches will be available in the corridor where you had breakfast. Restrooms — we do have breaks that are allocated during the time. If you need to leave before or after a break, feel free to. Restrooms — when you step out of the workshop, you can go to your left. Right behind the area where you picked up your name badge are restrooms. Other alternative is when you leave here, take a right. You take your first right and then there's restroom facilities on your left.

I want to reiterate that this is high security in that you are limited to movement within the facility: basically this room, outside the corridor and to the restrooms. Okay. You can't go downstairs; you can't get in the elevators. There'll be security people there to make it obvious to you at that point and time. Also, we apologize for those that are addicted to nicotine. This is a non-smoking facility, so even going outside is not a solution today for you today. I apologize.

So let's dive right into what we want to — the work, the presentations and discussion. This first panel, as I mentioned, will deal with clinical syndromes, pathophysiology and host factors associated with *C. diff* and *C. sordellii*. This panel is chaired by Dr. Dale Gerding.

And the objective we want to accomplish during this morning session is define the current emerging clinical syndrome, knowledge gaps and recommendations for basic, applied and clinical research. There are bios for each one of the speakers and what we want to do is if you want to know more about them, there's a bio in there. But we don't have time; we want to get right into the presentations. But I'd like to very quickly, starting with Dr. Gerding, is just announce your name, your position and what agency you represent.

D. GERDING: I'm Dale Gerding and I represent both the Hines VA Medical Center as well as Loyola University in Chicago.

C. KELLY: I'm Ciaran Kelly. I'm representing Beth Israel Deaconess Hospital and Harvard Medical School in Boston.

A. SONENSHEIN: I'm Linc Sonenshein from the Tufts University School of Medicine in Boston.

J. BALLARD: I'm Jimmy Ballard from the University of Oklahoma Health Sciences Center.

M. FISCHER: I'm Marc Fischer with CDC.

J. McGREGOR: I'm Jaime McGregor from USC.

D. STEVENS: Dennis Stevens from the VA Hospital in Boise, Idaho.

R. MIECH: Ralph Miech from the Department of Molecular Pharmacology, Brown Medical School, Providence, Rhode Island.

E. STERNBERG: Esther Sternberg, National Institute of Mental Health, National Institutes of Health, Intramural Research Program.

E. SPEAKMAN: Thank you very much. Dr. Gerding?

PANEL 1-SESSION 1: CLINICAL SYNDROMES, PATHOPHYSIOLOGY AND HOST FACTORS OF *CLOSTRIDIUM DIFFICILE*

Clinical Aspects of *Clostridium Difficile*-Associated Disease

D. GERDING: Thank you, Erik. First challenge is going to be to see if I can get the presentation up here. I think we're okay. That doesn't fill in; this is it. Okay. Well, good morning and thank you for the organizers for asking me to participate in this conference. I think it's a very important one. For someone who's been working in this field for 25 years to have an increased interest in this very significant problem now I think is very gratifying. And I hope we're going to see an emerging era, a real agenda to move the research in this area forward.

I have some disclosures, the most important one on the bottom: "unapproved use" in an FDA meeting. Metronidazole, which is the drug of choice for this disease, is

not approved by the FDA. So, and if you didn't know that, that's very important. And I may mention a couple of other drugs here: rifaximin, nitazoxanide that also are on the market, but unapproved for use.

The organism that we're really concerned about is pictured here and I probably could put this up as *C. sordellii* or *C. difficile*. But the organism is a gram-positive spore-forming anaerobic *Bacillus*. And you can see the — let me go back here — you can see the spores of the organism are really what are critical to the transmission of this organism in hospitals. The anaerobic organism itself does not survive very well outside of the body or outside of anaerobic conditions at all. And we think that spores are probably critical to the spread of both of these infections that we're currently seeing.

The disease itself has pretty much been associated with prior antibiotic exposure. That seems to be by far and away the largest risk factor. And when this infection is acquired, like catching almost any infection, there was a lot of thought initially that this was sequestered somewhere in the colon, that it was hiding away, that it was uncovered by antimicrobials. But the data we have now on typing, relating organisms within institutions suggests that this really is a problem of acquisition or an acquired infection just as many other infections are.

Following administration of an antibiotic and during that period, there's a variable length window during which the patient remains susceptible to *C. diff* because of the

disruption of the normal bacterial flora that is the primary protective mechanism against this disease.

The most common symptom is diarrhea, though there are other symptoms as well, including abdominal pain. Some patients go into shock. And although we think of this as a diarrheal illness, it actually is much more serious. There is a colitis associated with it in at least half the patients, called "pseudomembranous colitis." Patients become septic and we have had multiple deaths.

The hypothesis of how the disease occurs is shown here in this schematic. I caution you that it's a hypothesis and that we have modified it many times. Whether we've got it right this time, I'm not sure, but Dr. Kelly will be the next speaker. We'll talk about the material that's in the box, which is that down here. We think patients come in the hospital and I put "hospital" here because hospitals are the highest risk area for this disease.

When they come in the hospital, they're at risk of acquiring *C. diff* at any time during that admission, either through contaminated environments or healthcare worker hands. And unless they take an antimicrobial, generally there's no risk to them of acquiring that organism; their normal flora protects them.

However, if they get an antibiotic, and then acquire *C. diff* and it's a toxigenic strain, then we believe one of two things will happen. If they are unable to ramp up

fairly quickly, antibody against those strains — and Dr. Kelly's going to talk about this because this is his work — then they will get *C. diff* diarrhea.

And the antibody that he has noted to be most important here is IgG serum antibody directed against toxin A, one of the two toxin — major toxins of this organism. If they develop that antibody, then they become asymptomatically colonized and are at relatively low risk of ever getting *C. diff* diarrhea.

There is one other possibility here and that is they can acquire a non-toxigenic strain. So there are about 40 percent of strains circulating in hospitals that are non-toxigenic. They're harmless and they colonize patients as well, something that's been the subject of my research for 10 or 15 years in how we can protect patients by actually using that principle.

Now when the patients get the disease and get pseudomembranous colitis, this is how it looks to the gastroenterologist. Dr. Kelly is a gastroenterologist, so he probably has a lot better pictures than I do of this. But this pseudomembrane, when you're looking down a sigmoidoscope or colonoscope, you see these pseudomembranes heaped up on the wall of the colon, *pathonomic for this disease.

There are four major clinical problems that I've been harping about for a number of years. Major one is our inability to prevent this disease in high-risk settings, such as the hospital. Our usual strategies — and I'm not going to talk about it much today — are to, one, keep the bug away from the patient. So that's standard barrier precautions

in which we wear gloves, wash hands, clean the environment, the usual kinds of infection control approaches.

The other approach that we use is to try to work from the side of the antimicrobials that are being administered. And we've been able to control many outbreaks by limiting the use of certain antimicrobials, such as clindamycin in hospitals that have been linked to specific strains that are resistant to clindamycin. The same is true of cephalosporin antibiotics and we may see a similar relationship to fluoroquinolones.

Second major problem we have is lack of a sensitive and rapid diagnostic test. We're probably missing 40 to — 20 to 40 percent of diagnoses because of the insensitivity of the current test systems. We do not have a treatment right now that will prevent the current 20 percent recurrence or relapse of the disease. This has been true now for 25 years. We are still using the same two drugs to treat this disease that we originally discovered in about 1979 or '80. And now we are seeing more of this fulminant or very severe disease which we are also unable to manage very effectively.

So general principles of diagnosis: stool culture for the organism, which almost no hospitals do, is the most sensitive test. It's not the most specific because sometimes non-toxigenic strains are isolated. And the cell cytotoxin assay, the original diagnostic test, remains the most specific test and is generally considered to be the gold standard, also not used very much in hospitals because of the labor intensity of the test itself.

The most frequent tests used are enzyme immunoassays for toxins A and B. Formerly, this was for toxin A alone; many hospitals still doing that. And those tests are not as sensitive as either culture or cell cytotoxin tests. You can do a flexible sigmoidoscopy or colonoscopy, but that's an invasive test. But it is very rapid, but rate sensitivity is only 50 percent even in the most severely ill patients and those with both culture and toxin positivity. Finally, a rising — rapidly rising and often very high white blood cell count is a clear clue to this fulminant disease.

This is a survey that — of what infection control/infection disease physicians are doing in their hospitals with regard to testing. And you can see that most of them are using an enzyme immunoassay for toxin A and B. This was presented at the SHEA meeting in March in Chicago this year. The second most common was "don't know," which is also very common, and 39 percent are using toxin A. And you can see the toxin — or cell cytotoxin assay comes in at fourth and nobody is doing culture. So this is the current state of testing for the disease.

So among the available tests, the culture is the most sensitive. It's better than cell cytotoxin. Then the most frequently used test is in the middle: the toxin A and B enzyme immunoassay, which is better than the three tests below it in terms of sensitivity. So there's one area that we need some improvement in.

Now what about treatment and what are the current and future options? I mentioned already metronidazole, not approved by the FDA, but widely used first choice

drug. And vancomycin, also an approved FDA drug, but because of concerns about resistance among enterococci and staphylococci to vancomycin, CDC has made recommendations that we not use it as first choice in hospitalized patients.

So we have two federal agencies, both of which are sponsoring this meeting now, that are at odds about what the use of this drug should be for treatment of this disease. The — both of them do have the same recurrence problem. Half of those patients are relapsing; that is they have the same organism. Half of them actually are getting new organisms as part of that recurrence process.

Newer treatment possibilities are really starting to come along now. Some of them include non-absorbable, very narrow spectrum antimicrobials targeting *C. diff*, but trying to avoid the collateral damage to the other flora in the gut. A number of products in testing right now at FDA. Toxin-binding agent, better than cholestyramine or cholestipol when that actually is a polymer that specifically binds toxin A, and to some extent toxin B, is under testing as well right now.

Biological agents that we think have an opportunity to — perhaps by a biological interference strategy — to prevent the disease are also under development. That's one of the things that I'm developing in my lab. And then active and passive vaccines are both being developed: both active immunization of patients currently in Phase II testing and passive, including monoclonal antibody strategies to try to attack the toxins.

The recent publication of treating first recurrences suggests that as we've suspected all along, that recurrent disease is not a drug problem or a treatment problem — treatment agent problem, but is a problem of the host in that you can treat the patient again with the same drug and they do get a good response. It doesn't make any difference if you switch over to an alternate drug or not; the response is the same.

But what is very important today is that when someone has a recurrence or a relapse, it may be very severe. So in Dr. Pepin's experience here, shock, colectomy, or a perforation of the colon or death occurred in 11 percent of these patients during a recurrence, something that we previously had really never seen.

Multiple recurrences are a nightmare for patients and for their doctors. Many of these patients are kept on regimens of vancomycin for a long period. We use tapering of doses, pulsing of doses, a combination of vancomycin with rifampin. Metronidazole in this setting is not very useful because of long-term side effects, such as neuropathies.

Biotherapeutic approaches have generally been a failure, including *Lactobacillus* and *Saccharomyces boulardii*, which was effective in just one arm of one study for treating these relapses. Passive treatment with immunoglobulin is used but we do — we have very little controlled experience. Toxin binding agents are under development now, but the ones we have — cholestyramine and cholestipol — are not effective.

So the — it's really going very quickly here. Somebody's telling me something. Okay. All right. So the possible — just wanted to show you this — that the new issues

that are coming up right now, and before I exhaust my time, are ones that are very important. And you do have all these slides in your — in your handbook, so you can take a look at them.

But what is happening right now and that what we think has changed the whole landscape for *C. diff* is this epidemic that's going on in the U.S., and Canada and Europe. This week we were told it was occurring in France — and it's going to get away from me here — so there's more severe disease as well as more frequent disease.

It's possible that metronidazole is not as efficacious as it's been in the past. And the disease may be increasing in the community. We may be seeing more peripartum cases in pregnant women. And there may be an added risk now of proton pump inhibitors. And I put "may" in there on all of these because we're not certain.

Here are data; Gary Roselle is in the audience from the VA who assembled these national statistics. Just shows you that the rate in the VA hospitals, which is probably among the best data that we have in this area, has doubled in the period 2000 to 2004. And he tells me today that that is continuing to go up in 2005, so a doubling rate of disease.

The virulence factors of these new strains — can you get that to stop? All right. So the virulence factors in those strains — if we can go back to that one; go back one more. We're doing well, aren't we, Erik? Right there. Okay. The new strains have

three characteristic virulence factors. These are going to be talked about by additional speakers.

So the virulence factors are a binary toxin, a third toxin that has been found in these strains, variations in a gene that we think is responsible for down regulating toxin production; that is, a defect there or a deletion may be responsible for increased toxin A and B production which has been documented *in vitro*. And then resistance to two fluoroquinolone antibiotics for which strains have previously not had high-level resistance and that's gatifloxacin and moxifloxacin.

Now this fulminant disease shown here in a CT scan of a patient — which increasingly our radiologist will tell us, "See, this patient has *C. diff*" — here you see a segment of the colon. This is actually the thickness of the colon wall, which is markedly increased, and this patient also has ascites. And this fulminant disease clearly seems to be increasing.

Patients go into shock. They have rapidly rising white blood cell counts, which may be a major clue. And we clearly need controlled trials and better means to manage these patients. That is one crying need right now and it is frustrating to try to manage these patients when a life-saving measure may mean taking the colon out of the patient. And even then, mortality rates may be as high as 50 percent.

Treatment with metronidazole is controversial because of three recent publications. They're over here that's shown in this review. They all showed higher

failure of the drug in treating the disease. You can see the recurrence rates for both drugs. Vancomycin, metronidazole are about the same at 20 percent. There does not seem to be increasing failures with vancomycin, but there are in metronidazole-treated patients.

The two studies that have been published that are observational showing this by Musher and Pepin are shown here compared to previous prospective randomized trials. And you can see response rates are down in the 70 percent range compared to 90 percent previously. And recurrence is around 30 percent and 34 percent is higher also than has previously been recorded, so this is possibly related to slower responses with metronidazole.

This paper published in 1995 before we had the epidemic strain shows that vancomycin responses are complete by day 7, whereas you see these trailing responders who are treated with metronidazole is exactly what we are finding in our hospital as well. So response rates at seven or eight days are about 75 percent and then another 15 percent or so respond in the next week or so.

Community-associated *C. diff*, something that has been reported from CDC, and Cliff is — Cliff McDonald, I'm sure, will talk about this. This is voluntary reporting: 23 community cases, 10 peripartum from four states over 28 months. When they calculated what was a community rate, it turned out to be right in the range that had previously been reported from Boston by Richard Platt in a — in a prepaid health plan.

But there were some interesting and unexplained events, such as patients reporting no antibiotic use in the previous three months in 24 percent. And then the isolates recovered, each had the binary toxin gene that I mentioned and one had a *tcdC* gene deletion like the epidemic strain. But neither of them were this new epidemic strain, so it remains to be seen what it is in the community that might be causing these cases.

And the peripartum cases are really unfortunate. These are severely ill women who are pregnant. And the first case here is a 20-year old who developed watery diarrhea at 22 weeks of pregnancy, had no recent antibiotics or hospitalization, high fever, high white count, spontaneously aborted, ended up having to have a colectomy, managed to survive.

The next patient: 14 weeks pregnant with twins — reported in this *MMWR* report — three weeks of watery diarrhea, black stools, had just been exposed to trimethoprim sulfa three months earlier, admitted to an ICU five days later, *C. difficile* toxin A- and B-positive, had a dilated colon, poor response to metronidazole/vancomycin, was discharged, but then readmitted in shock three days later, spontaneously aborted. Then the patient died in addition, a very unfortunate case.

And then the two cases at the bottom are both women who had C-sections, entered the hospital, received antibiotics, developed *C. diff* — or we think *C. diff* at least

or toxic megacolon — resulting in survival of the infant, but death of both mothers. So these are truly unfortunate cases.

In the community, reports of exposures in this U.K. database are very alarming. For example, they show that the rate of *C. diff* in the community went from 1 per 100,000 to 22 per 100,000 in a ten-year period with some very unusual findings: almost no antibiotic exposure compared to what we're used to, only 37 percent compared to the normal 90-plus percent exposure to antibiotics; and then exposure to proton pump inhibitors, other antacid drugs, such as H_2 antagonists, also significant; and then even significance with the use of nonsteroidal anti-inflammatories for which we have no explanation whatsoever.

So this is what we're trying to avoid. This is an autopsy specimen of the cecum from a patient who has died of severe pseudomembranous colitis. This cecum is completely covered with pseudomembrane. Typically it always ends right at the ileocecal valve, but the ileum is spared. And we really desperately need better research to develop ways to prevent and treat this very serious problem. Thank you.

E. SPEAKMAN: Is Ciaran Kelly next?

Pathogenesis and Host Response of *Clostridium Difficile*

C. KELLY: So as Dr. Gerding said, it's a great pleasure to be speaking at this conference and it's wonderful that the conference is being held. For those of us who've

been studying *C. difficile* — for a couple of decades in my case — it's bad to see that it's a bigger problem than it used to be, but good to see that more attention is being paid and also good to see that there's increased interest in terms of new therapies under development.

So I've been asked to talk about — I've put my disclosures up there while I'm giving my — while I'm — to cover pathogenesis and host factors in 15 minutes. So let me go and see if I can do it. So this is the overall schematic. It's not — it's not as well illustrated as Dr. Gerding's slide, but it says pretty much the same things. And what I'd like to do is to sort of step through the steps of pathogenesis and make some comments at each step about what we know and what we don't know.

The first is antibiotic therapy, which for the vast majority of patients with *C. difficile* is the insighting factor. And as Dr. Gerding mentioned, we think — although we're not sure — but we think that what antibiotics do is affect the normal microflora, and hence, remove colonization resistance.

However, that general term of "colonization resistance" is pretty much a black box. And we know very little about what specific elements of the colonic microflora, of what specific changes that antibiotics make that allow *C. difficile* to colonize and proliferate. And that's certainly an area that warrants further study.

This is a study from a couple of decades ago by a skilled young investigator, John Bartlett, which I think is very important in terms of illustrating the complexity of

antibiotic effects in *C. difficile*. And what this looks at is the sensitivity of clinical isolates from patients with *C. difficile*, diarrhea and colitis. And you can see that the *in vitro* sensitivities — this has a mind of its own.

E. SPEAKMAN: It's really, really sensitive.

C. KELLY: I'll put it down. Yes, I'll use the keyboard. The *in vitro* sensitivities you can see for ampicillin, which at the time was one of the most common causes of *C. diff*, and vancomycin, which is still arguably the most effective treatment, are essentially super-imposable. And so that tells us that it's not just the antibiotic susceptibility — the antibiotic susceptibility of *C. difficile* that determines disease, but also other factors.

For example, the pharmacokinetics of the antibiotic, whether or not it has high luminal concentrations and so can be effective. And also, the effect probably that these different antibiotics have upon the colonic microflora. And so I think one area of interest will be to discover or identify antibiotics that are less likely to cause these problems.

And also in terms of therapies, to identify antibiotics that are effective in treating *C. diff*, but do not cause these critical changes to the colonic microflora because ultimately relapse is probably a manifestation of the fact that there's continuing antibiotic therapy. And when that therapy is stopped, the patient is again vulnerable to disease.

So not all antibiotics are the same in this regard and there is a list of frequent offenders on the left side, which include the second- and third-generation

cephalosporins. Clindamycin, of course, is notorious and its friend lincomycin was so bad that it's no longer used.

And the quinolones used to be in the middle column of this table, but recently they've moved over to the left and in recent studies had a similar risk for causing *C. diff* as the cephalosporins, for example. So perhaps one element of this is the fact that some strains of *C. diff*, including of course the epidemic strain, now have fluoroquinolone resistance. And therefore, the quinolone's effect on the microflora and its effect on *C. diff*, the balance has changed.

Not everybody, however, has a history of antibiotic use. There are some circumstances where *C. diff* can occur. Cytotoxic chemotherapy may not — may result in *C. diff* prior to or in the absence of antibiotic therapy *per se*, although it's likely that this is again an effect on the colonic microflora.

Non-antibiotic colon preparation with polyethylene glycol solutions, for example, have rarely been associated with cases of *C. diff*. Very frequently, patients with inflammatory bowel disease will spontaneously develop *C. diff* infection in the absence of antibiotic therapy.

And again, it's probable that the disease, in this case ulcerative colitis, has some effects upon the colonic microflora. We don't know what those effects are, but they allow *C. diff* to colonize. Most patients get disease while on treatment. Occasionally it

occurs some days or even weeks afterwards, but the vast majority will occur within a short duration of antibiotic use.

A few words on non-antibiotic-associated *C. diff*. It's certainly very rare in our experience as hospital physicians, but a number of reports from the community have suggested that perhaps it may be more common in community-acquired disease. So the 1994 study from Boston by Hirschhorn and colleagues has already been mentioned. And in that study, only 65 percent of patients have a documented history of antibiotic use within seven weeks or — of the onset of disease.

In a Swedish study, there was a 98 percent antibiotic use. And then remarkably in the recent study from *JAMA*, there was only a 36 percent of patients had a history of antibiotic use. I actually have some methodological issues regarding the *JAMA* study, which we may come to later in the discussion time.

But if these data do suggest that community-acquired *C. diff* is certainly less common than nosocomial, but does occur and it's not clear to me whether the lower history of antibiotic use has to do with less good data. In other words, that it's more likely that somebody in the community may have an antibiotic exposure that they don't report or that they forget about; whereas, those who receive antibiotics in hospitals, it's much easier to obtain that history.

Moving on then to talk a little bit about the next step, which is exposure and colonization. And certainly, this is much more likely to occur in a healthcare institution

where the environment and the personnel can both act as sources of *C. diff* and the spores are present in high numbers. And in the study done by McFarland and colleagues, she was able to culture *C. diff* from many, many places in the hospital and on the hospital personnel. And so certainly, barrier precautions that we heard about earlier are very important.

This is a study from our hospital that looked at the prevalence and the acquisition of *C. diff*. We looked at about 300 patients in a high-risk ward and these were a medical ward. And these were all patients on antibiotics staying in hospital for more than two days. And we found that 31 percent of our cohort were colonized at some point during their hospital stay.

But as has already been mentioned, not all those who were colonized developed disease. Approximately one-half will be symptomless carriers; the other half will develop *C. difficile*-associated diarrhea. And we'll come back to that point later.

I'm not going to discuss *C. diff* toxins in detail because the later speakers will talk about regulation of toxin production and the cellular effects on the toxins. But I did want to mention the question of the relative importance of toxins A and B in causing disease because this is something where attitudes have changed over the past years. And it's also a very important question in terms, particularly, of non-antibiotic therapies for *C. diff* in terms of, for example, toxin binders, or vaccine generation, whether or not it's necessary to block both toxins, or whether it's only necessary to block one toxin.

Previously, toxin A was called “the enterotoxin” because it’s enterotoxic in animals, whereas toxin B has minimal enterotoxic effect in animals. Furthermore, animals can be protected by immunization against toxin A alone, but not by immunization against toxin B alone. And so for that reason, it was felt for many years that toxin A was more important.

But there’s some recent data that really draw that into question. And the data comes from human studies performed by my colleague, Harry Pothoulakis, as well as clinical observations. The human studies are in — that in colonic explants and in intestinal xenographs, toxin B has at least if not more injurious effect to the colon than toxin A.

And then the clinical observation is the small number of toxin A-negative/toxin B-positive strains of *C. diff* have been found to cause significant disease, including severe pseudomembranous colitis in humans. So more and more, it seems that both toxins are probably important in causing disease, and therefore, it’s likely that both need to be addressed by new agents.

Finally, these toxins cause symptoms: diarrhea and colitis. And I wanted to finish by just talking a little bit about the host factors that predispose other than antibiotic use. And certainly, we all know that the older we become, the more likely we are to develop *C. diff*. And even in — it’s not just a question of being more likely to be admitted to hospital and receive antibiotics. It’s also clear that when you look at in-

patients in hospital on antibiotics, the rates of CDAD in the elderly are higher than in younger patients.

One other important factor is co-morbidity, so being old and ill are really the main risk factors. There's also an important element played by the immune response. This is a picture which I don't know whether it's better or worse than Dr. Gerding's picture, but it's a picture of pseudomembranous colitis. And this is the histology.

And the reason I wanted to show them was to draw attention to the purulent material that is produced in the colon in patients with *C. diff*. And I think this reflects a very marked stimulatory effect of *C. diff* toxins on the innate immune system. And particularly, phagocytes appear to be particularly affected. As was mentioned, leukocytosis is characteristic, and a leukemoid reaction is not uncommon, and a high white cell count has negative prognostic implications.

In animal studies, we showed some years ago that if we blocked neutrophil recruitment using an antibody against the adhesion molecule CD-18, the — we virtually completely inhibited the effects of *C. diff* toxin in that animal model, suggesting that the toxin effects, the direct toxin effects may not be as important in causing severe colitis as the inflammatory response to those toxins.

And certainly *in vitro*, both toxins activate a number of inflammatory pathways in phagocytes, including NF-kB, MAP kinase and pro-inflammatory cytokine production. More recently, Herb DuPont has shown that a single nucleotide polymorphism in the

interleukin-8 promoter is associated with increased production of interleukin-8 and a greater likelihood of severe and symptomatic disease in those infected with *C. diff*, suggesting that a high IL-8, which is a neutrophil chemokine response, may be indicative of individuals who are more likely to have severe disease.

And there's even been a suggestion that perhaps that corticosteroids may be effective in fulminant disease. And I think that this is an area that really hasn't been explored. Certainly, ulcerative colitis has a lot of similarities to fulminant *C. diff* and we reflexively will prescribe and use high-dose systemic steroids for that condition. And this whole area of addressing the immune — the innate immune responses to *C. diff* in fulminant disease is something worthy of consideration.

And then finally, the adaptive immune response. About two-thirds of the population have detectable antibodies against *C. diff* toxins A and B. And in the study that I showed you, the data from earlier, we were not actually looking at epidemiology so much as at host response. And I'll just show you one data slide from that study.

This looks at serum IgG antibody levels against toxin A in three groups from that study. In the middle are the control individuals who are not colonized by *C. diff*. The lower line are those who were colonized and developed diarrhea. And you can see they have a lower, significantly lower antibody levels both than the non-colonized and the carriers.

And then the top line shows that those who later became symptomless carriers started with slightly higher antibody levels, but very quickly upon colonization, mounted an IgG response which is indicative of a memory response to toxin A. And this was strongly associated with protection against diarrhea.

And then a different study which looked at a different population and that is looking at recurrent disease. This shows serum IgG antitoxin A levels at the end of therapy for the first episode of *C. diff*. And they're divided into quartiles: the lowest on the left and the highest on the right. And then it compares this to subsequent development of recurrence or not.

And you can see that those with the lowest antibody levels had a much higher subsequent risk of recurrent disease, whereas those with very high antibody levels had a much lower risk with an odds ratio of a 48-fold greater risk in those with lower antibody levels.

So this again suggests that an immune response, in this case, there was an IgM followed by an IgG response. So in this case, apparently a primary immune response during an episode of *C. diff* appears to be associated with protection against recurrence.

So I just want to finish by speaking to some of the opportunities that our increased knowledge of the pathogenesis of the disease invites in terms of different points in the disease process where we can intervene. And certainly in terms of

prevention, controlling antibiotic therapy and trying to prevent exposure on colonization are — remain and always will remain important.

The immune response, we believe a memory response accounts for symptomless carriers and a primary immune response helps protect against recurrence. So up until now, we've pretty much exclusively relied upon killing *C. diff* with antibiotics as our therapeutics approach using metronidazole and vancomycin. And certainly, there are a number of other newer antibiotics that are being studied at the moment.

Some of these show promise in terms of having a narrower spectrum of activity, and therefore, potentially being less likely to be associated with recurrence, although that remains to be demonstrated. Probiotics are currently mainly used for secondary prevention. And there I would say that in general, their effects are modest, not dramatic. There have been some reductions in recurrence rates, but unfortunately, they are not a panacea in terms of preventing recurrence.

Some new approaches — including the targeted probiotic of using a non-toxigenic strain of *C. diff* to purposely infect the patient and thereby prevent infection with a toxigenic strain — is certainly, again, worthy of additional study. The older toxin binders were not particularly effective. The new binder, the tolevamer that's being studied in Phase III studies currently by Tolevamer, holds promise as a non-antibiotic approach for treatment of *C. diff*.

And as was already mentioned, a toxoid-based vaccine is currently in Phase II studies with Acambis. And a variety of approaches for passive immunotherapy — including using intravenous immunoglobulins, monoclonal antibodies against toxins A and B, and potentially even the production of a hyper-immune globulin from individuals who are vaccinated and then undergo plasmapheresis — have all been considered as ways of providing protection to individuals at high risk, for example, at the time of admission to hospital. So I'll finish there. Thank you.

E. SPEAKMAN: Thank you, Ciaran. Real quick logistics: you heard that loud, very destructive noise coming from the speakers awhile back and everybody said, "What is that?" That's a cell phone. So that big thing that says "turn off cell phones," unless you like that noise, turn off your cell phones.

Okay, and I know that these great presentations are producing questions in your mind. What I would ask right now is just to record that, your questions, and that we'll do all presentations. And then we'll give you time to ask questions and/or points. Next is Linc Sonenshein. Thank you.

Regulation of *Clostridium Difficile* Toxin Gene Expression

A. SONENSHEIN: Great. Thank you, Erik, and good morning. It's a pleasure to be here. I wanted to spend some time talking to you briefly about two very fundamental

aspects of the biology of *Clostridium difficile*. The first has to do with the relationship between spore formation and spore germination and *C. difficile*-associated disease.

As Dale and Ciaran indicated, the spores of the organism are the population, the form of the organism that's most — that's responsible for the initial infection, and presumably, also for the recurrent infection.

So in fact, if cells, if *C. difficile* couldn't sporulate in the gut, then they would be — first, if it couldn't germinate in the gut, it wouldn't be able to produce toxin. If it couldn't sporulate in the gut, there'd be very little spread of the disease. And yet, these are two processes, two fundamental processes that have received very little attention in the past. The second aspect has to do with the relationship between toxin synthesis and the physiological state of the cells, which I'll get to next.

If we can just think about when cells sporulate, it's when they're limited for nutrients; that time when all bacteria try to adapt to the conditions in which they find themselves. And sporulation in *Bacillus* and *Clostridium*, the two best understood sporulating organisms — I didn't do anything — the two best understood sporulating organisms is very clearly a response to that kind of nutritional limitation.

The stages of sporulation, the morphological changes for *Clostridium difficile*, are very reminiscent of those that have been well characterized in *Bacillus subtilis*, shown here in cartoon form. But if we actually look at electronmicrographs of *C. difficile*, we can see — we can recognize the same morphological changes involving the production

of a cell within a cell: the inside cell becoming the spore which will eventually be released from the mother cell.

In *Bacillus*, it's well understood that two fundamental or two basic transcriptional regulatory systems are responsible for determining both the onset and the timing of spore formation. There's a transcription factor called "Spo0A," which needs to be phosphorylated in order to be active. We know that in *difficile*, there is a homologous Spo0A. But it looks like the mechanism, the actual molecular mechanism by which it becomes phosphorylated must be considerably different in *C. diff*, although it remains mysterious at this time.

Once sporulation is initiated, then there's a series of transcription factors which are actually subunits of the bacterial RNA polymerase that allow the polymerase to recognize different sets of promoter sites of genes as the cell progresses through the sporulation stages. In *Bacillus subtilis*, these are recognized as being both timing-specific and compartment-specific so that there are four spore-specific sigma subunits and there are mother cell-specific subunits.

If we look in *C. difficile*, we can see that all of the sporulation-specific sigma factors are recognizable, although it looks like, again, the molecular mechanisms by which they may be activated are somewhat different in *difficile* than in *Bacillus*. There are also other sigma factors which are used for under stressful conditions, one of which is the *tcdR* factor, which we'll see is very important in controlling toxin synthesis.

In fact, if we look at the pathogenicity locus — that is the 19-kilobase segment of DNA which encodes the toxin proteins — we can see that in that locus, in addition to the *tcdB* and *tcdA* genes which encode the two toxin proteins, there's another gene called "*tcdE*" which is suspected of encoding a hole in like protein, a protein that may be responsible for the release of the toxins into the environment. And then there's an upstream gene called "*tcdR*" which had been suspected to be regulatory protein.

The — in work that was carried out both in my lab and in the lab of Bruno Dupuy in the Pasteur Institute, we were able to show that the *tcdR* protein is actually one of the alternative sigma factors for RNA polymerase. It's a protein that interacts with the core of RNA polymerase to direct the enzyme to the specific promoter sites for the toxin B and toxin A genes as well as the promoter for the *tcdR* gene itself.

Now in order to understand the physiological basis for how these — oh, sorry; glad this slide changed. All right. I've got to go back here. It happens that the *tcdR* gene, which controls toxin synthesis in *difficile*, is closely related to genes that control toxin synthesis in other *Clostridium* species, such as *Clostridium botulinum*, and *Clostridium tetani* as well as the gene that controls bacteriocin production in *Clostridium perfringens*.

These proteins are all related enough to each other so that we can mix and match them both *in vitro* and *in vivo* to different extents. It's as if *Clostridium* learned

how to regulate toxin synthesis once in its evolution and simply adapted that regulatory protein to different toxins.

If we ask when are the toxins actually made — it's a little hard to follow in this slide — but we showed some years ago that the timing of toxin synthesis both at the level of the protein and at the level of the messenger RNA occurs at the end of rapid exponential growth. And in fact, it's greatest in the stationary phase, during which time the cells are also initiating the spore formation process. Although we don't think that spore formation is essential for toxin synthesis, there's clearly some regulatory connection between them.

Also you can see on this slide — although I have to explain it to you — that in the presence of glucose in TYG medium, there is no expression of the toxin genes at any time during the growth cycle. So the question was, what is it that restricts expression of the toxin genes to the stationary phase or post-exponential phase of growth?

And we really framed the question physiologically; that is there must be some signal that indicates that the cells had been limited for some — one or more nutrients. And we presumed that there had to be a regulatory protein that would sense this signal. And then there had to be some mechanism by which this regulatory protein would transduce the signal and relate that to gene expression.

Now we were greatly influenced by our previous work in *Bacillus subtilis* which has served as a model organism. And we had found that in *Bacillus*, and in fact, in

several gram-positive bacteria, there's a protein that we had called "CodY" which is a DNA binding protein which serves as a repressor of hundreds of genes that are normally turned on as cells experience nutrient limitation.

And as I said, there are homologs of this protein CodY found in virtually all low G+C gram-positive bacteria: that is all the *Bacillus*, *Clostridium*, staph, strep, entero, listeria, *et cetera*, all those species. So we asked whether the — sorry — the general mechanism by which CodY works is that it recognizes two different metabolites inside the cell simultaneously.

One is the nucleotide GTP, which presumably indicates to the cell what its energetic capacity is, and the second is the amino acid isoleucine or alternatively valine. And I can't really rationalize why the cell has evolved to recognize those specific amino acids. In either case, both GTP and isoleucine work by stimulating the binding of CodY to its target sites and it's usually acting as a repressor, although there are some genes that are also positively regulated by CodY.

So as I said, these CodY homologs are nearly ubiquitous in the low G+C gram-positive bacteria. And certain motifs are particularly well conserved. For instance, the DNA binding motif is virtually 100 percent conserved in all of the homologs throughout the gram-positive world.

Not knowing — not being able to make a CodY knockout mutation in *Clostridium difficile* — and I anticipate that the difficulty of doing genetics in this organism will be

one of the issues that will be discussed at some point during this workshop — we turn to *Bacillus subtilis* as our surrogate organism and ask is it possible that inactivation of a CodY gene in *Bacillus subtilis* would affect the expression of the *C. difficile* toxin genes?

And if I can take you through this slide, first what we have here is a — is a fusion of the promoter site of the toxin gene, toxin A gene, to a reporter gene which is beta-glucuronidase from *E. coli*. That's been placed in the *Bacillus subtilis* chromosome as long — along with a copy of the *tcdR* gene under its own promoter. And what you see here in the bottom line is that if we don't include the *tcdR* gene in *Bacillus subtilis*, there's no expression of the toxin gene.

However, if we do express the *tcdR* protein, the RNA polymerase sigma factor, then we do get expression and it's at least a little bit higher at the end of exponential growth than at the beginning. If in addition we delete the CodY gene in *Bacillus subtilis*, then we have greatly enhanced synthesis of expression of the toxin gene, suggesting that at least in this organism, the *Clostridium* system is under control of CodY. Unfortunately, we haven't been able to do the parallel experiment in *C. diff* itself.

One of the other aspects of CodY, as I said, is that it's a GTP-binding protein and you can see that the G motifs are fairly well conserved in *difficile*. And this is a 3-D — three-dimensional structure of CodY from *Bacillus* showing the binding of isoleucine in a particular hydrophobic pocket near the end terminal domain. And those amino acids are also reasonably well conserved in *C. difficile*.

But still, we wanted to know does *C. difficile* CodY bind to the toxin gene promoters or the *tcdR* promoter? And if so, does it respond to these signals? This is the gel mobility shift assay which does indicate that the CodY protein of *Clostridium difficile* does depend on both GTP and the branch chain amino acids in order to bind tightly to the DNA. And if we ask where is that binding, we find that the tightest site of binding is right between the two putative promoters that drive expression of this gene.

We don't know yet the mechanism, the precise molecular mechanism, but this allows us to construct a model in which the synthesis of the — of *tcdR*, which is the factor necessary for expression of the toxin gene, is itself repressed by CodY protein when the cells are growing in a rich medium so that they can have high intracellular levels of GTP, and isoleucine or valine.

As cells go into stationary phase, one or the other of these pools becomes decreased. CodY loses its ability to bind to the R promoter. R protein is produced and then it stimulates synthesis, transcription of the B and A genes. Now the — I'm sure that we'll also talk today about the potential role of the C gene; that is the last gene of this opera.

It had been suspected to be a negative regulator of toxin synthesis for many years based on the fact that this gene had the opposite pattern of transcription from the toxin genes; that is, if the toxin genes are silent during rapid growth and turned on

stationary phase, the C gene is expressed during exponential growth and turned off in the stationary phase.

And there's been quite a bit of excitement recently about the highly virulent strains that are now known to have mutations in the C gene. So we wondered whether what we — what we could figure out about the regulation of the C gene and its mechanism of action. So our colleague, Bruno Dupuy at the Pasteur Institute, has purified the C protein. And he's shown in work that has not yet been published that the C protein is a direct inhibitor of the R protein. In other words, it acts as an anti-sigma factor blocking transcription of the B and A genes.

So we then asked, well what regulates the expression of the C gene? And we only had one candidate protein; that was our CodY protein again. And we could show that this protein does in fact bind to the C gene promoter as well.

So now we have a more — a more refined, but at the same time, a more speculative model about how this whole locus is regulated; that is that the CodY protein, while acting as a negative regulator of the R gene during rapid exponential growth, is simultaneously stimulating transcription of the C gene which is the antagonist of R.

So this is sort of a double way of preventing toxin synthesis during rapid exponential growth. When cells go into stationary phase, CodY loses its ability to bind to DNA and no longer stimulates C gene synthesis; instead, allows R gene synthesis and now toxin genes can be turned on. In the absence of a genetic system in

Clostridium difficile, we are unable to tell you that this is, in fact, the way it works. And we're totally dependent on *in vitro* assays at this point, which is clearly not very satisfactory.

To sum up, I just want to acknowledge Bruno Dupuy, who used to be in our lab and now has his own lab at Pasteur. The people who did the work: I mentioned Nagraj Mani and currently Sean Dineen in my lab. And we have an ongoing collaboration with Bruno Dupuy and Julian Rood at the Monash University. And last but not least, I'd like to acknowledge the National Institutes of — National Institute of Allergy and Infectious Disease which has allowed us to do this work. Thank you.

E. SPEAKMAN: Thank you, Linc. Next is Jimmy Ballard.

Toxins of *Clostridium Difficile*

J. BALLARD: First of all, I'd like to thank the organizers of this workshop for giving me the opportunity to tell you a little bit about the toxins of *Clostridium difficile*. And what I plan to do for the next 15 minutes or so is give you just a brief update on the status of the field of the study of these toxins; blend in just a little bit of our research from my own group; and then I'm going to end by touching on some work that we've done on *Clostridium sordellii* and the analysis of at least a couple of toxins that are produced by that organism because that leads into the next panel which will be focusing on *C. sordellii*.

Well, this is just a slide to — this is just a slide to remind you that the study of these — the study of these toxins really has progressed through the normal lineage that we see for the study of many bacterial toxins; whereas, it was recognized very early on in 1938 when this organism was originally discovered that it produced factors that were lethal in animal models.

And then many years later, these factors were recognized as two separate polypeptides produced by *Clostridium difficile*. And in the early 1980s, we termed "toxin A" and "toxin B." And there's a significant amount of impressive work that were done on these toxins in the 1980s. There's probably people in this room that made substantial contribution to the studies of those toxins at that time.

In the 1990s, the genes were sequenced for both of these toxins. And as the study progressed on these toxins in the mid-1990s, the enzymatic mechanism of action was determined for this — these toxins and it was found that these were glucosyltransferases that inactivated small GTPase-binding proteins in the cytosol of the cells. Now we're at the point where domains of these toxins are being resolved in three-dimensional crystal structures.

So we're able to get a glimpse of these toxins now at the atomic level. So we've progressed from initial analysis of these toxins, beginning to understand their cellular activities, to making advances in understanding the enzymatic mechanism of action, to

the point now where we're getting to look at these at the atomic level. But that's not to say by any means we understand everything about those toxins.

Well, this is just a summary of the first two toxins I'm going to touch on: toxin A and toxin B. These are both large clostridial toxins. *TcdA* is about 308 kilodaltons in size and *TcdB* is about 270 kilodaltons in size. This is substantially larger than most known bacterial toxins. However, the biological significance of the size of these toxins is still really not known.

These are both intracellular bacterial toxins; they're glucosyltransferases. And based on recent crystal structure analysis, it's known that these are type — these are members of the type A subfamily of glucosyltransferases. These are major virulence factors. And some of the very good — there's a variety of reasons why I believe this. Dr. Kelly mentioned the fact that immune responses to — efficient immune responses to toxin A, for example, provide protection from the disease.

It's also important to note that these toxins are found in almost all of the clinically relevant isolates. But has been mentioned earlier, there are some recent isolates that are thought to be toxin A-negative. This is just a summary of the structure — or at least not the three-dimensional structure — but the arrangement of these toxins.

They're arranged in a classical A/B toxin type of arrangement where there's a single enzymatic domain, which encodes for the A activity, and there's a separate B — or on the same polypeptide anyhow — there's a B activity that encodes for receptor

binding and translocation which leads to the entry of the enzymatic domain into the target cell.

And this is how we think this happens and this is just a summary of the steps in cellular intoxication by these toxins. Toxin A and toxin B are released from *Clostridium difficile*. They engage a yet undefined cell surface receptor — this — or multiple receptors. This triggers receptor-mediated endocytosis.

The toxins enter the cell in an endocytic vesicle. And following acidification of this vesicle, there's conformational changes in the toxin which results in the toxin inserting into that vesicle membrane and then translocating the enzymatic modality, it's thought now, into the cytosol of the cell.

Once in the cell, the toxin hydrolyzes UDP-glucose. It takes that liberated glucose molecule and transfers it to small GTPase-binding proteins in the cytosol of the cell. This essentially inactivates signaling pathways that are regulated by these small GTPases and this has a variety of downstream effects on the physiology and health of the cell. This includes changes in cellular morphology, as most of you are aware; modulation of the mitochondria; and then, of course, important transcriptional changes.

And the reason why this is so — the toxins are so effective at disrupting cellular physiology is known at the three-dimensional structure. And this is the three-dimensional structure of Rho bound to GTP. It has a — it has a 3Na in here, 3Na37, which has a reactive hydroxyl group that is glucosylated by toxin A or toxin B.

The result of glucosylation reaction is that the protein can no longer — no longer stably complex GTP, so it's essentially locked in the GDP bound form. And the reason why that's so important is the GT — GTP bound form is the on form or the signaling form of this protein. Since the protein's locked in a GDP bound form, it's essentially off. It can't carry out its important functions in regulating signaling inside of the cell. So by this mechanism of action, the toxins are able to shut off signaling through these proteins.

And as you can see as I've summarized in this bottom panel, three of the known targets, small GTP-binding proteins — Rho, Rac and Cdc42 — regulate a variety of events. And these are just some of their downstream targets that have been recognized in a variety of different studies. So you can see how by simply disrupting signaling through Rho, Rac or Cdc42 could have a profound effect on cellular physiology.

However, we believe that this is not the complete story. We do know from work in my group anyhow that if you simply silence the expression of these targets, you don't get the same effect as intoxicating with the toxins. So we believe that there may be other targets are important for intoxication. It's also possible, and it hasn't been formally excluded, that simply that the glucosylation is important for the intoxication event so that glucosylated forms of these proteins are important for the overall detriment to the cell. So there's certainly more work that needs to be — needs to be completed in this area.

So the effects of these toxins have already been mentioned and I'll just go through toxin A again. And this certainly is not a complete list; it just hits again on some of the important areas. I'm going to — I'm going to just touch on what happens at the cellular level and then summarize what — just briefly — some of the things that are known at the host level.

At the cellular level, the toxin — toxin A anyhow — is known to simply induce cell rounding. Caspase activation is known to occur in these cells. There's evidence that the mitochondria is directly modulated by *tcdA*. However, there's been a recent report that recombinant *tcdA* is not able to carry out the same function. As Dr. Kelly mentioned, MAP kinase path — signaling pathways are activated by this toxin. In particular, P38 signaling is triggered by *tcdA*.

Apoptosis can be activated in a variety of cell types exposed to *tcdA* and it's also been shown that there's an increase in permeability in polarized epithelial cell systems. At the host level, again, there's a variety of different things that happen. In animal models, you can inject toxin A IV and it's lethal in rodent models. The toxin also triggers a variety of different inflammatory events. And I won't go through these because Dr. Kelly's already touched on them in his — in his talk.

Toxin B is still somewhat of an enigma, at least to me anyhow. It's a very potent cytotoxin. It's probably one of the most potent cytotoxins known. *In vitro*, it intoxicates just about any cell type that you would like to treat. And for this reason, it's been used

as a wonderful reagent for studying different aspects of cell biology that are related to the small GTP-binding proteins.

It induces cell rounding, pronounced actinomorphic effects. It modulates a variety of intracellular signaling pathways as I've already mentioned. Like toxin A, it triggers caspase activation, promotes apoptosis and it also is able to disrupt tight junctions. It's certainly a result of its ability to inactivate small GTP-binding proteins which are known to stabilize tight junctions.

In vivo, however, the role of *tcdB* — has already been mentioned — is still poorly understood. Again, it's lethal. The toxin — the question becomes whether or not this toxin may also be contributing to systemic effects. Since it's such a broad cytotoxin and it's able to impact a variety of cell types, it's possible that release of this toxin into the blood stream may be contributing to illness. And also now, it's been suggested that the toxin itself is possibly enterotoxic.

So just to take a look at these toxins and what's been recently reported for the separate domains, functional domains of these toxins, let's first take a look at the enzymatic domain. The crystal structure has recently been resolved for the enzymatic domain — the first 546 amino acids of toxin B. The sub — there's a substrate specificity domain which determines which of the small GTB-binding proteins are engaged by this toxin.

There's also important motifs that have been recognized that determines the UDP-glucose recognition and hydrolysis. We've shown from our own work that if you delivered just this enzymatic domain into cells, you get many of the same effects that you get from intoxicating with the complete toxin. So we believe many of the observed effects of the toxin can be directly attributed to the enzymatic — the enzymatic domain.

The translocation domain, I really can't tell you much about. This is very putative. It's called the "translocation domain" really for only reason and that's because it contains a distinct hydrophobic patch that is thought to insert into the target cell membrane. So it's been extrapolated from that; that this domain may be important for translocation of the enzymatic moiety into the cytosol of the cell.

There's been some exciting work recently on the receptor binding domain. It's been known for some time now that the receptor binding domain will — can provide protection against the toxin in animal models. The receptor binding domain for toxin A was recently resolved and it's been co-crystallized now with a synthetic trisaccharide. And what's been found is that this enzymatic — or the receptor binding domain appears to engage multiple trisaccharides, suggesting that this receptor binding domain may be able to engage more than one receptor when it interacts with the target cell.

So progress has been made in understanding these toxins and their sub-domains, but that's not going to be the complete story unfortunately because it's now known that there are at least 24 different toxinotypes of *Clostridium difficile*. And what

that means is that there are particular isolates that have variations in the pathogenicity locus that was just mentioned by Dr. Sononshein. And this pathogenicity locus varies from producing almost no toxin — no detectable toxin A to toxin B.

A vast majority, greater than 80 percent, of these toxinotypes do produce both toxin A — produce both toxin A and toxin B. There are specific toxinotypes that produce only toxin B and then, of course, there's some that have neither toxin. And there are also particular toxinotypes that contain distinct changes in that enzymatic domain that causes those toxins to engage a completely different subset of small GTPases, okay, and these have also been associated with disease.

Now I just want to mention that — before we lose track of the binary toxin — I do want to mention that in addition to toxin A and toxin B, *C. difficile* is also known to produce a binary toxin and this toxin is a bit different from the single polypeptide structure that you see with toxin A and toxin B. This is a toxin that is encoded on two separate polypeptides.

This toxin has an A — is an A polypeptide which is enzymatic and it functions as an ADP-ribosylase that modifies actin in the cell. It's similar to the iota A toxin from *Clostridium perfringens*. There's a separate polypeptide which mediates the entry of A into the cytosol of the cell; this is the entry component. Either polypeptide alone is non-toxic. It takes the combination of these two to cause cytotoxicity.

The binary toxin is found — depending on the reports you wanted to look at — is found in anywhere from 6 to 12 percent of isolates and it has been associated with recent epidemic strains of *Clostridium difficile.* Stu Johnson's group has recently reported that the binary toxin may play an important role in colonization. However, the overall contribution of this binary toxin to disease remains poorly understood.

Now I just want to remind you back to the large clostridial toxins. I want to remind you that these toxins are actually part of a family of large clostridial — of toxins and I'll tell you why that's important. So *tcdA* and *tcdB* are members of this family. *Clostridium novyi* produces one of these toxins.

But *C. sordellii* also produces one of these toxins as — or two of these toxins as well: the hemorrhagic toxin and the lethal toxin. And my group has also been studying *Clostridium sordellii* for some time and we've been very interested in the lethal toxin produced by this organism. So I think it's important to touch on this this morning.

Just to show you some of the differences, you can see between these two highly related toxins, this — these are cells that are intoxicated by *tcdB* and you can see these distinct changes in cell morphology: this actinomorphic effect, this arborization that's characteristic of *tcdB* intoxication. *TcsL*, when cells are intoxicated, you get pronounced cell condensation and rounding without the same changes that you see by *tcdB*.

And the reason for this is that *tcd* — *tcsL* engages a completely different set, almost a completely different set of substrates once it gains entry into the cell, although

these two toxins are highly homologous. In fact, there are isolates of the toxinotypes that I mentioned. There are isolates that actually produce *tcdB* that has gained this substrate recognition activity of *tcsL*. Okay.

Well, *tcsL*, by virtue of its ability of the toxin from *Clostridium sordellii*, by virtue of its ability to engage different substrate targets, it has very different effects on cell physiology. And I'll just show you one example of a signaling pathway that we studied in my lab. This was the Akt signaling pathway which regulates a variety of events involved in cell growth and cell — and regulation of cell growth.

And what we see for, just for example, is if you treat cells with *tcsL*, you see a very rapid decline in phosphorylation of Akt which is indicative of loss of signaling through this pathway. *TcdB* does not have the same effect, okay, and this again is probably a result of it hitting a different subclass of — subclass of substrates.

But there are other differences that we've reported a couple of years ago between *tcsL* and *tcdB*. Any one of the strains of *Clostridium sordellii* that we work with, we know that the toxin is actually maintained in a high molecular weight, multimeric complex and we haven't been able to observe this in any of the other large clostridial toxins. And so it's still a mystery to us as to why this one large clostridial toxin exists in this multimeric complex.

This multimeric complex requires low pH for dissociation and it requires dissociation for intoxication of the cell. So that if you cross-link this multimeric complex,

you completely inactivate the toxin. If you do the same sort of treatment to toxin B, it has absolutely no effect. We can actually dissociate the complex and re-associate by simply switching the pH of these conditions up and down, okay, so another distinct difference between the large clostridial toxin of — a large clostridial toxin of *C. sordellii* and *C. difficile*.

But probably one of the most important questions associated with *Clostridium sordellii* is just how frequently does this organism actually produce the toxin? And has already been mentioned, *Clostridium difficile*, there's a distinct association with toxin production. Almost all of these clinical isolates of *C. difficile* are producing potent toxin A and toxin B.

So we set out a couple of years ago to address this question in *Clostridium sordellii*. And we first asked at what frequency does *Clostridium sordellii* produce this lethal toxin as it exists in a commensal in the host? And it was a bit of a challenge to come up with isolates that were commensals from humans and we actually took advantage of a collaboration with Octavio Martinez at the University of Miami who screens cadaver-derived tissue that's being prepared for transplantation for the presence of *Clostridium sordellii*. So he isolates *Clostridium sordellii* frequently from this tissue.

These are from patients who have not died from *Clostridium sordellii*. So we surmised that this was an organism that it had existed as a commensal in those

individuals. And I'll just summarize for you very quickly what we know; is that of the — of the isolates that we've examined from the cadaver-derived tissue, only one of these produces *tcsL*, any detectable *tcsL*.

They all produce a neuraminidase; a few produce a phospholipase. But also, more interestingly is the fact that these isolates produce a cholesterol-dependent cytolsyin, which is a homolysin that's found — a type of homolysin that's found in many species of *Clostridia*. It's not found in *Clostridium difficile*. It's not known to be produced in *Clostridium difficile*, so we see an increased associated production of this homolysin.

So the next question is, well, how frequently is this toxin produced in clinical isolates? And I note that Cliff McDonald is going to touch on this, so I'll just tell you what we found. We've been examining 25 isolates from — 25 clinical isolates that were provided to us by the CDC. And Cliff is going to touch on this in much more detail, but I'll just summarize for you very quickly that not all of the clinically related isolates of *Clostridium sordellii* produced the lethal toxin.

In fact, many of these isolates that we've screened, we have not been able to detect any cytotoxic factor being produced whatsoever. So it's going, I think, to be a very interesting field of research because this goes against the standard paradigm for everything we know about the *Clostridia*. They produce very strong, very potent toxins to cause very serious disease very rapidly. And now we're starting to examine a

Clostridium that does that, but we can't seem to associate that with any toxin production. So that's going to be a fun field of research for the future.

And so just to summarize some of the important questions that I've tried to highlight for you this morning, again, what is the contribution of *tcdB* and its specific *in vivo* effect? What are the specific *in vivo* effects? Again, an important — perhaps an important field or topic to address is what's the result of increased expression of these large clostridial toxins because that's been associated with some isolates of *C. difficile*.

What role does the binary toxin play in disease? Again, I think we don't have a complete glimpse of the overall — or complete understanding of the overall profile of substrate targets in mammalian cells. And the specific receptors, we have several good hints at what the receptor may be, but we still don't know definitively what the receptors are for these toxins. Whether or not that, in the end, would be informative is not known, but it'd certainly be nice to have a look at that.

Again, what's the contribution — back to *C. sordellii* — what's the contribution of *tcsL* and these other toxins to disease? And then, of course, what's the mechanism of pathogenesis in some of these isolates that don't appear to produce enterotoxins?

And finally just very quickly, I want to acknowledge the two students in my lab that work on the — work on the *Clostridia*: Daniel Voth and Elaine Hamm; and Octavio Martinez at the University of Miami that's provided these cadaver-derived isolates to us;

and then the group, the CDC, who's been generous enough to provide the clinical isolates that allowed us to do some of the preliminary characterizations. Thank you.

Panel 1-Session 1 Discussion

E. SPEAKMAN: Another quick round of applause for all the panelists this morning. The presentations ran a little bit long, but I feel certain that the information that was given was important so we still want to provide time for questions and answers first: about 10, 12 minutes for Dr. Gerding to talk, any clarifying questions, and then we'll turn it over to participants to see if you have any questions or feedback. Dr. Gerding?

D. GERDING: Thank you, Erik. I'm just going to use the moderator's prerogative of asking a couple of questions, and I think Linc alluded to one of the things that has really probably not come out explicitly so far, and that is the issues around trying to do things genetically with *Clostridium difficile*. And Jimmy, you might want to comment also on our capabilities with *sordellii* in terms of genetic manipulation. But I wonder if both of you could comment on that so we get that issue out on the table? That's really a major one I think.

J. BALLARD: I'll beat Linc to the punch. Yeah, it's a big problem and I think Lincoln may want to maybe tell you — be able to tell you a little bit more about some of the problems. But the issues ranging from selectable markers to simple transformation to recombination just plague this field. And as you can see, almost all of these things

that I mentioned today, especially in regards to the toxins, it would be very nice to validate some of our hypotheses.

And unlike most bacterial systems where that can be done by making specific gene knockouts, it's been very difficult in *Clostridium difficile*. And I think that we really need a concerted effort. I see Bruce McClane's here and he's made some remarkable progress in *Clostridium perfringens*. And I wish that some of that technology could be adapted to *Clostridium difficile*, but it's just been a real big challenge.

We're a little more optimistic about *Clostridium sordellii* simply because it turns out to be much more sensitive to a variety of different selectable markers. And I think that that hopefully will be at least a place that we can start, but I think Linc will probably have more to say about that.

A. SONENSHEIN: Well, Jimmy really summarized the difficulties of the — of working with the *difficile* or *sordellii* systems. The — there have been some glimmers of hope with *difficile*; that is, there have been instances, sort of anecdotal cases where specific mechanisms, specific approaches to gene disruption have been successful, at least in isolated cases. For instance, there are a number of genetic tools that are in fact available.

I — let me tell you what's not available. Electroporation generally doesn't work. The electroporation kills the bugs so devastatingly that they just can't be recovered if they ever were transformed. There's no natural competence. There are no transducing

phases that we can use. However, we can use mechanisms of conjugation from heterologous hosts; that is; we can use either *E. coli* as a donor or *Bacillus subtilis* as a donor.

In the case of *E. coli*, we're using broad-host-range plasmids to — I don't mean "me" specifically — but the field is using broad-host-range plasmids to deliver DNAs to *Clostridium difficile*. They could be replicating DNAs or they could be non-replicating DNAs, so it is possible to introduce DNA.

We've also used the — in *Bacillus subtilis*, we've used the gram-positive transposon, conjugated transposon Tn916, and found that if we integrate *Clostridium* DNA into Tn916, we can then deliver that by conjugation in — back into *difficile*. The question is how to direct any of these DNAs to a specific target site in the *difficile* chromosome.

And with the conjugative transposon, there's only been one case where we've gotten directed integration at the desired site. In all other cases, the transposon simply integrates at random. Now that's a process that can be utilized effectively in certain kinds of experiments nonetheless, which I'll get back to very shortly.

In terms of the plasmid transfer, we can use suicide plasmids. The — however, our sense is that the frequency of homologous recombination is very low. If you add on top of that some problems with restriction enzymes acting on incoming DNA, the likelihood of isolating a specific recombinant goes down to somewhere below the level

of detection. So I think we need to try some novel approaches, things that haven't necessarily tried — been tried before.

For instance, some labs are trying antisense RNA. If you can deliver a gene that makes an antisense RNA for a coding sequence that you're interested in, you can hope to reduce the expression of the — of the relevant gene. That again has only worked partially in a few cases so far.

If you really know the gene of interest and you think that you know something about the function of the gene product, it's possible to introduce what you might hope would be a dominant negative version of that gene product. Again, I don't know of any case where that's actually been shown to work so far, so I think we still have a lot of — a lot of work to do to make this a viable genetic system and answer some fundamental questions about this organism.

D. GERDING: Dr. Sternberg, do you have a comment?

E. STERNBERG: I have a question for Drs. Ballard and Sonenshein. Are there any glucocorticoid response elements, or progesterone or estrogen response elements in the promoter region of the toxin producing genes?

A. SONENSHEIN: I didn't quite hear what you said ...

E. STERNBERG: Oh.

A. SONENSHEIN: ... for me to respond.

E. STERNBERG: Are there glucocorticoid response elements, or estrogen or progesterone response elements in the promoter region of the toxin producing genes?

A. SONENSHEIN: Not that either of us knows.

D. GERDING: I have one additional question. Dr. Kelly mentioned that about two-thirds of adult patients have antibody against *C. difficile*. Ciaran, where do they get that from and how come all of us don't have it?

C. KELLY: It's been known for some time that the antibody response appears to be acquired fairly early in life. In one study that looked at individuals over time, most — the two-thirds of individuals who do have detectable antibody appeared to have acquired it by the — by the age of two or three. So presumably, it relates to exposure to *Clostridium difficile* in the environment.

We know that neonates have a very high rate of symptomless colonization, presumably because they don't have an established microflora. And so there's a period of time after a child is born when they're susceptible. So it may be occurring during that or it may be occurring due to environmental exposure. And then there's another possibility and that is that it's actually a cross-reacting antigen and that it's not really toxin A or B that the antibodies are generated against.

D. GERDING: Now kids have very low rates of *C. diff*, right, of diarrhea?

C. KELLY: Yeah, and ...

D. GERDING: But the rates in the elderly are the maximal. So ...

C. KELLY: That's correct.

D. GERDING: ... how do — how does that all fit together?

C. KELLY: Well, neonates have a very, very high colonization rate and a very, very low disease rate. And it's not exactly understood why that is. One possible explanation comes from studies in animals. Harry Pothoulakis and Tom LaMont, my colleagues, showed that rabbits lack a toxin A receptor when they're born and develop the receptor when they're weaned, so around six or eight weeks. And that until the receptor was — is expressed, they don't bind toxin and they're non-susceptible. So it may well be that neonates and young infants don't express toxin receptors.

D. GERDING: And what happens when you really get old and ...

C. KELLY: Well, then everything falls apart.

D. GERDING: And why do — why do our 70-, and 80- and 90-year old patients have such a devastating time with this? What's going on?

C. KELLY: I don't — I don't know what the specific elements of being old are that make us more susceptible to *C. diff*.

D. GERDING: Yeah. Well, figure it out; I'm getting close. So okay, Erik, I think we'll open it up to questions from the audience.

E. SPEAKMAN: Any questions from the audience? We have about 12, 15 minutes for questions of the panelists, questions and/or comments at this point in time.

J. McGREGOR: Jaime McGregor from Los Angeles. Can we clarify ...

E. SPEAKMAN: Use the mike.

J. McGREGOR: Can we clarify the carriage of *Clostridia difficile* in human populations and is it important: their numbers or their conditions? And we've talked about how many are toxigenic at least among the specimens sent in. And then could we — could you mention *Clostridia sordellii* carriage in the human gut as well as in the reproductive tract?

D. GERDING: The question is about carriage of *Clostridium difficile* in the GI tract. And Dr. Kelly has already alluded to the fact that this is very common in infants, particularly in the first year of life where 50 percent or more of them will have *C. diff* in their stool at any time. Once you get into adult populations in the United States at least and Western Europe, it is much, much lower. It's like 1 to 3 percent might have *C. diff* in their stool and even that may be just a pass-through phenomenon rather than true colonization.

And as far as colonization in other populations, when you get into a hospital population at admission, patients do not have high levels of colonization with *C. diff*. But as they stay in the hospital, they acquire the organism at a rate of about 8 percent per week in our experience and they just keep getting higher and higher levels of colonization the longer they stay in hospitals. And the colonization, as shown by Dr. Kelly, is the first step in the infection process.

But you don't get disease in the majority of patients with colonization. But rather, you have to also lack an antibody response, or an anamnestic or recall response that enables you to apparently be able to get colonized with a toxigenic strain or not become ill. And then our data suggests that anybody who's colonized who didn't get sick really is in a protected state. We thought they would be in a high-risk position; they actually are in a very, very low-risk situation. And I have to tell you, I know nothing about the colonization rate for *C. sordellii*, but maybe Dr. Ballard does, or someone else or ...

J. BALLARD: Yeah. There's very little that's known about that. It's been more done in the veterinary situation than in the human population. We do know that it's the most frequent isolate that's collected from these cadaver tissues, so it's definitely out there. But relative to other *Clostridia*, it's simply not known, not to any extent that's understood about *C. difficile*; that's for sure.

D. GERDING: Dr. Fischer, is there any comment there on colonization?

M. FISCHER: Yeah. I think I'll talk a little, very briefly about that. I agree; when I looked for studies done of stool surveys or vaginal flora surveys, *Clostridium sordellii* very rarely is found or at least in the published literature. There's sometimes *Clostridium* species that are not further speciated that may be, but *sordellii* itself is not. But on the other hand, it is found in contaminated tissues so it must've been in the gut in some percentage of people.

E. SPEAKMAN: A question in the back? And remember to state your name, please.

C. BROOM: Colin Broom. I have two questions if I can be greedy: first of all, one related to the pathophysiology of *C. difficile*-associated disease. We haven't talked much about adherence factors, and their importance and I think that is obviously an important factor. Because when the bugs are doing the most damage, where do they sit? I think most people have the naive view that the *C. difficile* bacterium is floating in the lumen somewhere, you know, producing toxin. I think that's probably naive.

More likely, these are actually within the CRIP of the site of the mucosa where they directly release toxin which causes problems. So why is it important to understand? Well, there may be therapeutic approaches to block adherence. They're important to know. The important factor is it may well not be luminal concentration of a therapeutic that's effective. It's actually — what's more important is concentration at the site of action in the vicinity of the bug that's doing the harm. So are there any comments on the pathophysiology?

C. KELLY: Yeah, a few comments on that, Colin. I agree with you that adherence is important and it's something that hasn't — has not been studied extensively. If you look at the disease, what you see are foci of ulceration associated with a marked inflammatory response. And it certainly is possible that a colony of bacteria could be at the center of that focus resulting in this focal disease.

When we think about colonization resistance of true *C. difficile*, we always think about or assume that the bacteria compete for nutrients. But they may compete for adhesion sites. Furthermore, it's been shown that if you alter the microflora of the gut, you change the levels and type of expression of glycans on the surface of the epithelium.

And so that's another mechanism by which antibiotics could facilitate *C. diff* colonization by changing binding sites. And some work has been done by Neil Fairweather in England. And he has described a putative adhesion factor in the surface layer proteins of *C. difficile* and he has one publication at least suggesting that those surface layer proteins may act as adhesives.

D. GERDING: Just a comment; Ciaran also has data collaborating with Dr. Fairweather that suggests that antibody against some surface component — I don't think — you didn't clarify which is was. Is that correct? But it did have just barely protective effect in his patient population.

C. KELLY: Yeah. Those were not the most robust of our data. I would say it was a pretty meager, but statistically significant effect. If I remember correctly, it was surface layer protein A.

D. GERDING: You said "A?"

C. KELLY: Yeah.

E. SPEAKMAN: One quick follow-up question?

C. BROOM: I have a follow-on question related to achlorhydria, okay, so lack of stomach acid. And it's been quite consistent, this association between anti-secreting agents and an increased risk of developing CDAD. That's been consistently seen in a number of studies. Potentially, of course, that's actionable. You could look at the use of PPIs — inappropriate use of PPIs — which we know is prevalent in the elder population.

But one point that I've never heard really addressed is if you look at the old aging population, there's an association between age and achlorhydria, at least fasting achlorhydria. And indeed, I believe if you look at the 75-year olds, the majority at baseline fasting have achlorhydria.

This may be a very important factor and I wonder if anybody's looked at this risk? Would it be one of those factors you can look at perhaps on admission, individuals with baseline achlorhydria, you know, should be, you know, that's one risk factor that we could look at?

C. KELLY: Yes, that's probably in my bailiwick as a gastroenterologist.

C. BROOM: Sorry to pick on the gastroenterologist. Yeah, but it's not many of ...

C. KELLY: So to answer your second — the second part of your question first, yes, it is something that could be looked at in terms of looking, for example, at gastrin levels or pepsinogen in serum. You would get an indirect indication of the presence or

absence of achlorhydria. But to my knowledge, it's not something that has been looked at in terms of autoimmune achlorhydria.

In terms of pharmaceutical hypochlorhydria caused by proton pump inhibitors, I'm not convinced, in fact, by the data. What I — looking into this, there are some early reports of an association. There are a number of reports of associations between proton pump inhibitors and risk for *C. diff*.

But when we looked at this and when Dr. Pepin looked at this in our populations, we found that although there was an association by univariate analysis, by multivariate analysis, the association was lost. And the recent study in *JAMA* — I have a couple of which — has certainly raised a lot of interest in this question.

I have some methodological issues about — the first methodological issue has already been alluded to; and that is this is a very strange population of patients with *C. diff* where fewer than 40 percent have a history of antibiotic exposure. And it really makes me question the database. In addition, many of these patients were diagnosed clinically and we're not sure what a clinical diagnosis of *C. diff* in somebody who has not received antibiotics would be. And so I'm skeptical as regards to the patient population.

And then my second methodological concern is the fact that they did not control for a variable that we all know is very important in *C. diff* and that is co-morbidity. So there was no controlling for global co-morbidity. They controlled for individual co-morbidities, but not a global co-morbidity index, such as the Charleson Index.

And again, our work and Dr. Pepin's work, we both find that when we controlled for co-morbidity with Charleson index, the PPI association is lost. The bias may be there that individuals who are ill and have multiple medical problems are more likely to be on the PPI and are more likely to acquire *C. diff*, but that it's an indirect and not a direct association.

E. SPEAKMAN: Thank you. One last question over here to the left?

J. RANDAL: Yeah. I — my name's Judith Randal. I wondered (a) what the role of carriers, you know? Does anybody know the impact of carriers in spreading this disease? And (b), how long does it — does the organism survive on environmental surfaces and is that a problem in hospitals, for example?

D. GERDING: I'll take a crack at that. The carrier role in terms of spreading the disease is the issue in hospitals, primarily where you do have large numbers of patients who are carriers. We did one study in which we actually cultured the organism from the stools of patients on a regular basis by having them agree to have a rectal swab done every week while they were in the hospital. And then we did DNA fingerprinting of the organisms to see what they were picking up.

And we did show that each of those patients, as they picked up a new organism, had that organism first introduced to that ward by another patient who was carrying the organism. So that suggests that this is playing a role. However, the data for contamination of the environment overwhelmingly suggests that the rooms of the

patients who have diarrhea are the rooms that are most heavily contaminated. And if you go into those rooms and culture sites — like the floor, the toilet, the bed rail, the window sill, the bed covers — you will find 50 percent of the sites are more — are contaminated with the spores.

The spores in the environment, to answer the second part of your question, lasts a long time, probably on the order of many, many months in that environment if they are not physically removed or killed, which is by the way, very difficult to do. You need to use bleach-type solutions or hydrogen peroxide treatment to get rid of the spores.

So environmental contamination, a big factor. We don't know whether it's the environment or whether it's contamination of the hands of healthcare workers that is the most important in terms of spread of the organism. But the overwhelming speculation is that it's the hands of the healthcare workers that get contaminated and then lead to the spread of the spores to susceptible patients.

J. RANDAL: Thank you.

E. SPEAKMAN: Thank you.

D. GERDING: A lot of work still to be done though. Again, as with all hypotheses on this disease, I'm subject to change at any time.

E. SPEAKMAN: Thank you. Any other questions? Yeah?

D. STEVENS: Yeah. I just wanted to know, you know, in the community-acquired cases of *C. difficile* whether the food source was a potential source for *C.*

difficile? I mean, it's multiple environments; it's virtually everywhere. But Dr. Glenn Songer showed that some veterinary animals actually developed *C. difficile*. I don't remember if they were animal husbandry kinds of beasts or not. But anyway, I was curious if anybody had any information about that?

C. McDONALD: Cliff McDonald. I'll be talking about that this afternoon.

D. GERDING: Okay. Well, let's wait to hear what Cliff has to say about it because Glenn Songer's data are disease in swine, newborn swine. But just for everybody's information, this is a big problem in the horse industry where foals particularly can get *C. diff* and it's fatal, and also in dogs, and it's wiped out the chinchilla industry in the past from time to time. And so, it clearly is an animal pathogen.

And one or the big questions are, are the pathogens that are causing animal disease the same ones that cause human disease? Some data suggests that they're clearly different, but now we're starting to hone in on whether there might be similarities. And Cliff, I'm sure, will have more data on that.

E. SPEAKMAN: Okay. One last question?

J. McGREGOR: This is for Dr. Kelly, Dr. Gerding. Is it possible that environmental sources of antimicrobials, such as in water or phages in water, could perturb the host-microbe relationships and disturb niches within the gut?

C. KELLY: Yes, I think that's entirely possible. We know very little about what the crucial element is that prevents colonization or adhesion, but it's certainly theoretically possible that trace amount of antibiotics could disturb that. We talked about probiotics; has been something that has been used in *C. diff* disease.

In gastroenterology in general, there's now a lot of interest in prebiotics — in other words, manipulating or altering the colonic microflora by what you eat. And it's certainly possible that there are certain diets or food additives, *et cetera*, that could predispose to *C. diff*.

But fortunately what we've seen is that the vast majority of patients with *C. diff* have an identified antibiotic exposure in those instances where we've had close monitoring of the patients. And most of the instances where there's been lack of antibiotic exposure in the main have come from database studies rather than direct clinical contact studies.

E. SPEAKMAN: Okay. One last round of applause for the panelists for *C. diff*. We'll break until 10:45. There are refreshments back in the corridor where we had before. Remember, restrooms to the left and restrooms to the right. Don't go downstairs or try to get on an elevator and we'll be back here at 10:45. Thank you.

[BREAK 10:32 A.M.-10:52 A.M.]

E. SPEAKMAN: If we can take a seat, we're going to get started in few minutes. While we're convening everyone again, real quick on logistics. This next panel will go

until 12:30, lunch from 12:30 to 1:30, prepared box lunches for your pleasure out in the corridor. We've had a lot of requests related to getting copies of the presentations. And the reason why we didn't reproduce it for everyone, two reasons: one is that these esteemed scientists were working until midnight — most of them — last night and altering, even up until two minutes ago, altering or revising their presentations and we wanted to be ecologically friendly.

So, but to be able to address your desires, that we will. There's a web site and link on the bottom of the agenda where both the transcript will be available within 30 days, but we've — we'll — we're going to speed up the time-line related to the presentations. So all the presentations will be available by the end of next week via the link that's on the bottom of your web site.

We might be able to get it to you a little bit quicker, but that's the time commitment we'll guarantee right now and we'll see if we can get it a little faster than that. Okay. We've got everyone for panel 2? Okay. Excuse me, panel 1, session 2 dealing with *C. sordellii*.

The objective, just like it was with *C. difficile*, is defining the current emerging clinical syndrome, basically the current situation, and then looking to determine what are the knowledge gaps and recommendations for basic, applied and clinical research. Again, this panel will be chaired by Dr. Dale Gerding and our first presenter is Marc Fisher.

PANEL 1-SESSION 2: CLINICAL SYNDROMES, PATHOPHYSIOLOGY AND HOST FACTORS OF *CLOSTRIDIUM SORDELLII*

Clostridium Sordellii Toxic Shock Syndrome Following Medical Abortion

M. FISCHER: Thank you. Good morning. I'd like to thank the organizers for inviting me to participate in this workshop today. I'll be summarizing the investigation of four fatal cases of *Clostridium sordellii* toxic shock syndrome that occurred following medically-induced abortions. Like *Clostridium difficile* and other *Clostridium* species, *Clostridium sordellii* is a gram-positive anaerobic *Bacillus* that typically resides in soil. Although it can colonize the gastrointestinal and genital tracts of healthy humans, it is not completely clear how often this occurs.

Although *C. sordellii* is not commonly found in surveys of stool and vaginal flora, a recent study isolated *C. sordellii* from the musculoskeletal tissue of 3 percent of cadaver donors. Like other histotoxic *Clostridia*, the virulence and clinical manifestations of *C. sordellii* are determined by two cytotoxins: lethal toxin and hemorrhagic toxin. However, lethal toxin is variably expressed by different *C. sordellii* strains and its cytopathic effects are altered by environmental conditions, such as pH.

C. sordellii is a relatively infrequent human pathogen, but case reports have described it as a cause of pneumonia, endocarditis, arthritis, peritonitis and corneal ulcer. *C. sordellii* bacteremia is also uncommon and occurs primarily among patients with serious underlying immunoconditions. Like many other *Clostridium* species, *C.*

sordellii has most often been described as a cause of contaminated wound infections, including necrotizing fasciitis, myonecrosis, tissue allograft infections, neonatal omphalitis, and postpartum endometritis and episiotomy infection.

Fulminant toxic shock syndrome among previously healthy persons has been reported in only a small proportion of *C. sordellii* infections. *C. sordellii* toxic shock syndrome was really first described by Dr. McGregor and colleagues in 1989. It is an acute and rapidly progressive disease that is characterized by a lack of or minimal fever; refractory tachycardia and hypotension that does not respond to intravenous fluids; local edema at the infected site with subsequent pleural and peritoneal fusions; marked leukemoid reaction; and an elevated hematocrit due to hemoconcentration. The course is fulminant and the outcome is most often fatal.

Between 1976 and 1993, six cases of *C. sordellii* toxic neonatal omphalitis were reported in the literature among infants 2 to 11 days old. Cases were characterized by severe abdominal wall swelling and periumbilical erythema and discharge with markedly elevated white blood cell counts. Five of these six cases were fatal and *C. sordellii* was isolated from the umbilical wound, peritoneal fluid and blood in one case.

Between 1992 and 2000, four clusters of *C. sordellii* wound infections were described among black tar heroin injecting drug users in California. Many of these infections contained multiple organisms, including *C. sordellii*, *Clostridium perfringens*

and other soil contaminants. However, the presence of *C. sordellii* seemed to be associated with a toxic shock-like syndrome and a high case fatality rate.

In 2001, fatal *C. sordellii* sepsis and toxic shock syndrome was reported in a 23-year old previously healthy man who had received a contaminated thermal condyle tissue allograft. This case prompted a broader investigation that identified 13 other cases of allograft-associated *Clostridium* infections. However, the remainder of these cases were due to *Clostridium septicum* or *C. bifermentans* and none of these were fatal.

C. sordellii has been reported as a rare cause of female genital tract infection and fatal toxic shock syndrome. Of the ten previous cases that we identified in the literature, all occurred in previously healthy women ages 23-40 years of age and all were fatal. Eight of the cases occurred after delivery of live-born new infants; one occurred after a medical abortion; and one was not associated with pregnancy.

Symptom onset occurred a median of three days after childbirth or abortion and time from hospitalization to death was three days or less. Two of the hallmark findings of these cases were, again, leukemoid reaction and hemoconcentration. Between September 2003 and June 2005, the FDA received reports of four deaths among women who had recently undergone medically-induced abortions with mifepristone and misoprostol.

In 2000, FDA had approved mifepristone plus misoprostol for medical termination of pregnancy up to seven weeks gestation. Mifepristone is a synthetic steroid that has anti-progesterone and anti-glucocorticoid effects. Misoprostol is a prostaglandin analog that causes uterine contractions.

The FDA -approved regimen for medically-induced abortion includes 600 milligrams of oral mifepristone followed within two days by 400 micrograms of oral misoprostol. These four patients had all received a common off-label regimen of 200 milligrams of oral mifepristone followed by 800 micrograms of misoprostol inserted vaginally. Initial investigation found that the clinical picture for each case was consistent with a fulminant toxic shock-like syndrome and was similar to a case of *C. sordellii* toxic shock syndrome that had occurred following a medical abortion in Canada in 2001.

The Canadian patient had received the same off-label regimen of mifepristone and misoprostol as the currently recognized cases. Despite these similarities, a specific infectious etiology was not identified in the U.S. cases. So in March 2004, FDA contacted the CDC Unexplained Deaths Project to assist in the investigation.

The four women ranged in age from 18 to 34 years. Two were white, one was black and one was Asian. All four women lived in and obtained their medical abortions in California. All four women were previously healthy. The median time from receipt of mifepristone to the onset of initial symptoms was five days. The time from

hospitalization to death was less than 24 hours for three cases and one of the women collapsed and died before reaching medical care.

Only one patient had a documented fever during her brief illness, but all had refractory tachycardia and hypotension. All of the patients reported vomiting or diarrhea and severe abdominal pain. None of the patients developed a rash. Among the three women for whom clinical laboratory results were available, all had a significant leukemoid reaction with a median maximum white blood cell count of 107,000 cells per microliter.

Two patients had significant hemoconcentration with maximum hematocrits of 58 and 61 percent despite intravenous fluid resuscitation. The third patient had a maximum hematocrit of 45 percent. Two patients had moderate thrombocytopenia with platelet counts of 63,000 and 91,000 cells per microliter. One patient had an elevated serum creatinine. None had elevated hepatic enzymes or bilirubin.

Blood cultures performed on three patients prior to antibiotics were negative for bacteria. One vaginal swab obtained pre-mortem grew *Gardnerella* species. And an endometrial tissue collected postmortem from another patient grew *E. coli* and an anaerobic gram-positive *Bacillus* that was discarded prior to further identification. No other cultures or pre- or postmortem specimens were obtained.

All four patients had autopsies performed and fixed tissues submitted to the CDC Infectious Disease Pathology Activity for evaluation. The most prominent gross autopsy

findings were extensive third spacing of fluid, including large pleural and peritoneal fusion and diffused pulmonary edema. None of the patients had retained fetal or placental tissue.

H&E stains of uterine tissue showed extensive acute inflammation and necrosis of the endometrium and myometrium for all four patients. Three patients had areas of edema and hemorrhage within the uterus and two patients had multiple abscess formation. There was no evidence of gas production in any patient.

Although mixed bacteria were seen on gram stain of uterine tissue for all four patients, abundant gram-positive bacilli was the predominant finding for each case. The histopathologic findings for all tissues other than the uterus were unremarkable and none showed evidence of bacteria on gram stain. Immunohistochemistry, using a polyclonal antibody that cross-reacts with multiple *Clostridium* species, was positive on uterine tissue for all four patients.

Extensive bacilli and granular antigens were seen in the areas of inflammation in the endometrium and myometrium, including within inflammatory cells and blood vessels. *Staph aureus* IHC was positive on uterine tissue for one patient, but these antigens were only seen on the endometrial surface. IHC for group A strep and *Neisseria* species were negative on uterine tissue from all four patients. All of the IHC assays, including *Clostridium* IHC, were negative on all other tissues tested, including heart, lung, liver, spleen and kidneys.

16S rRNA gene sequences amplified from fixed tissue showed at least 97 percent identified with *Clostridium sordellii* for all four patients. The presence of *C. sordellii* in the uterus was further confirmed by three *C. sordellii*-specific PCR assays: for the 16S rRNA, the cytotoxin L and phospholipase C genes. By contrast, PCR for the *C. perfringens* alpha toxin gene was negative on uterine tissue from all four patients.

Based on the findings of the investigation, we concluded that these four deaths were attributed to *C. sordellii* endometritis and toxic shock syndrome. The clinical and pathologic findings in these cases were similar to those in the ten other cases of *C. sordellii* genital tract infections reported in the literature, including the 2001 Canadian case that occurred following medical abortion.

Taken together, these cases demonstrate that serious infection can occur after medically-induced abortion much as it can occur after childbirth, spontaneous abortion and surgical abortion. However, many questions remain regarding the possible association between the use of mifepristone and misoprostol and the risk of *C. sordellii* toxic shock syndrome.

Researchers have speculated about several possible mechanisms by which oral mifepristone or intravaginal misoprostol could potentiate *C. sordellii* infection or toxic shock syndrome. Several of these hypotheses will be the focus of the remaining talks in this session, so I will limit my comments to the first two.

The apparent geographic clustering of four cases in California and the use of intravaginal administration of the misoprostol raised some initial concerns about possible product contamination. However, there were no epidemiologic links identified between the patients. Each woman had received their medications from a different clinic and healthcare provider and the medications received were from different manufacturing lots.

As a further precaution, FDA obtained and tested both mifepristone and misoprostol from the manufacturing lots received by these patients and found no contamination with *Clostridium sordellii*. Another concern was possible reporting or case detection bias that could have altered the apparent association between these medications and *C. sordellii* disease.

For example, local and regional attention around the first two cases in California may have increased the awareness of both the public and healthcare providers to this possible connection and stimulated reports of additional cases that may have not — may have not been detected in other states. In addition, the laboratory confirmation of *C. sordellii* in these cases resulted from extraordinary efforts at a national reference laboratory.

Additional cases of pregnancy-associated *C. sordellii* infection in which the organism was not cultured or speciated may exist, but may not have been pursued because they did not receive the scrutiny afforded these four cases. Finally, these

cases were initially detected through the FDA MedWatch system, which is designed to identify adverse events associated with approved medications.

Since there is no centralized reporting system for pregnancy-associated infections or deaths, *C. sordellii* cases following surgically-induced abortions, spontaneous abortion or even childbirth may not have been identified. To partially address these concerns, CDC and public health partners initiated several supplemental surveillance activities to identify additional cases of severe infection or toxic shock syndrome associated with pregnancy, childbirth or abortion.

First, articles published in the *MMWR* and the *New England Journal of Medicine* requested that healthcare providers report any cases of toxic shock syndrome associated with pregnancy or abortion to their local health department. In addition, specific queries were sent to members of the Infectious Disease Society of Obstetrics and Gynecology, the National Association of Medical Examiners, and the Unexplained Deaths Project sites. Finally, the California Department of Health Services initiated a retrospective death certificate review to identify additional possible cases. Preliminary results from these efforts will be presented by Dr. McDonald later today.

In closing, many questions remain regarding the possible association between medically-induced abortion and *C. sordellii*. Are women who use mifepristone or misoprostol at increased risk of *C. sordellii* infection or toxic shock syndrome compared to other women following surgical abortion, spontaneous abortion or childbirth? And if

so, what is the mechanism of that increased risk? And is it limited to *C. sordellii* compared to other *Clostridium* or anaerobic bacteria? And most importantly, how can we further reduce the risk and improve the treatment of *C. sordellii* infections?

Today's meeting provides an opportunity for us to develop a research agenda to begin to address these questions. And in closing, I would like to end by acknowledging the efforts of many of — some of the many people who contributed to this investigation, in particular, my colleagues in the Infectious Disease Pathology Activity here at CDC. Thank you.

E. SPEAKMAN: Thank you, Marc. Next is James McGregor.

An OB/GYN View of Early Medical Termination and *Clostridium Sordellii* Infection

J. McGREGOR: Thank you very much. My name's Jaime McGregor. I work in the Department of OB/GYN at USC Medical Center in Los Angeles. And I've — along with David Soper and others in the audience — I've had a personal interest in this for a prolonged period of time.

This is, as you can see, some of the face of what Marc was talking about. This is edema in a pregnant patient about to go into refractory shock and die. This is her perineum. This is the results from the autopsy, very similar to Marc's slides. In this, you

can see massive necrosis with dead tissue. And if you look hard, you can actually see gram-positive rods with sporulation, suggesting stationary growth.

This is a dead vagina, dead tissue from the vaginal specimen. If we look closer, we can also see margination of really huge amounts of white blood cells in the area going along with a leukemoid reaction. This is a picture of a portal triad where you can actually see the numbers of white blood cells in the vessel in the liver. I would suggest to you, looking from an OB/GYN point of view, that the epidemiology remains to be validated. This is simply a napkin, a back of a napkin approach, so we're looking very keenly to Cliff's talk this afternoon.

This is all from secondary sources, so I would suggest that this kind of micro — epidemiology is really inadequate that I'm presenting to you. If we suggest that there were over half a million early medical terminations since 2000 and now seven reported deaths, that actually put the risks of this syndrome and its — which is so far lethal in this setting at higher than the risk — higher than the risk of surgical termination, which was quoted by our colleague, Michael Green, in the *New England Journal*, at one in a million. And this approach is several-fold that.

And if we're looking at sort of a new Theobold Smith set of equations where we include innate immunity, where we include acquired immunity and the genome of the host — that is, the patient in these settings, as well as the genome of the microorganism and its conditions — I think we would focus on two factors, one of which would be host

immunity which is both innate and acquired. And in this setting, we would look at the medications to see if they played a role possibly in what's going on in these patients.

These are *Clostridia* up close and personal. And we are now aware that, in fact, the microbiology that we've been focusing on actually for decades may be inadequate to the task. This is work by Fredricks in the *New England Journal* where they used nucleic acid basic techniques to actually look for the microbiology in the vagina. And actually in a series of 78 patients, came up with three, so far, unclassified *Clostridia*. So actually, we have a long way to go in terms of further delineating the microbiology of what we're talking about.

The cultures that we've been using are actually set up to recover certain microorganisms, including *Clostridia*, but in fact, may not tell the whole story. *Clostridia sordellii* has a long past, which has actually been featured mainly in animal husbandry in the veterinary world.

This is the cause of a syndrome called "malignant edema, bloat, sudden death in sheep" — that's the term — and humans, as Marc pointed out, pregnancy-associated infections, wound infections, lethal toxic shock-like edema. And we've heard already from Jimmy that lethal toxin and hemorrhagic toxin are certainly important.

These are large toxins. They affect the Ras super family of genes and signaling within the cells. And I would stress too that that's certainly endothelial cells and

myocardial cells which are attacked and produce not only disorganization of the cell signaling, but cell death through apoptosis and other mechanisms.

Jimmy Ballard also pointed out his research showing that increased acid pH — increased acid pH, in his experiments, disassociated the toxin and increased its potency. Indeed, at the optimal pH of four to five, he found that intoxification was increased five-fold and the intoxicating dose was actually lowered by one log, suggesting that local conditions within the vagina — within the uterus, which may certainly be acid in the presence of blood that's been passed in the dead tissue, being passed out through the cervix is an important aspect to be considered in terms of what the *Clostridia* is producing.

I'd like to next talk about the medications. RU-486, mifepristone, has a long history. As many of you know, it was developed first in 1981 by Rousell-UCLAF in France, over 700 publications in the 1980s, and primarily having to do with anti-progesterone effects as well as anti-glucocorticoid receptor effects. I think that was the origin of Dr. Sternberg's question in the earlier panel.

And it's been applied to numerous clinical situations. And the one where it's been used the most is termination of early pregnancy. It's also been used to inhibit follicular development in terms of contraception. It's been used as a cervical ripening agent, labor induction agent. And in my own experience, it was suggested for breast cancer patients who were progesterone receptor-positive. It was not used in those

clinical settings because it also powerfully binds to glucocorticoid receptors in the patient's cells.

My teacher and coauthor, George Chrousos, used to formerly work at the NIH, pointed out that RU-486 is a prototype glucocorticoid antagonist, which is of course important in innate immune responses with strong anti-glucocorticoid activity, both *in vitro* and *in vivo*. The unusually long half-life — I'll just tell you; it's 30 hours — of this drug also poses problems in terms of titrating its dose within the therapeutic range.

Indeed, it appears that really small concentrations of the molecule are quite efficient at saturating both progesterone receptors and glucocorticoid receptors. This is work from Finland actually looking in five female volunteers, but where they looked at the pharmacokinetics of mifepristone and noted that RU-486 actually — actively binds to progesterone receptor in the endothelium and actually similarly the affinity for glucocorticoid receptors.

Indeed in these studies done in humans, the affinity of the mifepristone was four-fold that of the dexamethasone. There are other agents tested, but to my knowledge today, there's still no more selective progesterone receptor antagonists available.

In terms of the pharmacokinetics, this is a fascinating, very rapid oral absorption within one hour and a long half-life. In one study, the effects of the drug in terms of innate immune responses in terms of cortisol metabolism persisted for a week. It's

excreted — importantly, it's excreted by methylation and hydroxylation, but the metabolites are active.

The metabolites are active and they're active not only in terms of progesterone receptor, but also the glucocorticoid receptor. And as I noted before, it has high binding affinities for both the progesterone receptor and the glucocorticoid receptor.

Because of its pharmacological activities, Couzimet and Beaulieu in France in a famous *New England Journal* article from 1986, used this as a way to terminate early pregnancy within the week of the last — of your missed menstrual period and it was quite effective. One of the complications noted in this really seminal study was that many patients had prolonged, over a month of abnormal bleeding which was very troublesome to them clinically. And they suggested that it be used under close clinical supervision.

I think the studies really came to the full expression in terms of early termination of pregnancy. In a study done at my own institution by John Jain and Dr. Mishell — Dr. Daniel Mishell, a great — two great leaders in reproductive practice and they didn't touch anything. But I'll just tell you what it showed.

And it showed 95 percent satisfactory completion of early termination with both the misoprostol and — the mifepristone and the misoprostol, which is a Cytotec, which is another form of prostaglandin. And they actually work together to get to 95 percent. They found in that study that it was only 88 percent if they used the misoprostol alone.

Mifepristone — or misoprostol is expensive. And so my colleague, Lynne Borgatta — if we could go back one here — did a study out of Boston where — this is a retrospective study and not nearly done with the rigor that Dr. Jain's study — where she used mainly the Cytotec or the misoprostol, the prostaglandin, and achieved a 90 percent success race — rate with just using the prostaglandin.

But some of the patients did receive mifepristone or RU-486. And she concluded that mifepristone alone was given to 34 weeks — 34 patients as well and that misoprostol, the prostaglandin alone, worked 88 to 96 percent in terms of the confidence intervals.

Dr. Sternberg, present in the audience — the panel, others in the audience here led us in terms of the importance of the innate immune system functioning, which is a non-specific, non-memory-based immunity and how important it is in terms of responses to sepsis or other infectious conditions. This is simply a slide from one of her many illustrative articles showing that glucocorticoids actually act on every cell in terms of the innate immune response.

Numerous studies have been done which actually, looking at aspects of the innate immune response, actually show increased lethality in animal models in the presence of RU-486 or mifepristone. This is just a sampling. I think Sternberg — Dr. Sternberg's article in 2004 lists actually many more examples.

Of interest to this panel, RU-486 enhanced *Clostridium difficile* toxin A intestinal secretion/inflammation in a well controlled trial. This is — that what just flashed by you — was from Dr. Miech. And he's putting this all together and he will actually talk about this during his presentation. Dr. Miech is a pharmacologist and is actually looking at the drug effects as well as the host effects. And I won't go through that.

But I will give you part of a letter by Didier Sicard, who's a well known French physician, who's actually has a personal interest in this syndrome as well, and who's written to the *Annals of Pharmacology — Pharmatherapy* pointing out some suggestions from Europe. And he would point out that vaginal application is prohibited in France where misoprostol can only be prescribed in oral form.

I've recently learned that that's not entirely the case, so we have to look to see how the drug is given in different settings. But I can just tell you in countries such as the Netherlands, all of this is very well reported and that would be excellent sources for really good epidemiological information.

And Dr. Sicard actually gives the advice that we, physicians in North American — North America should actually be much more freely giving antibiotics at the time of the initiation of the termination as well as giving steroids if anybody becomes ill, all suggests that I don't agree with that advice. But I would suggest some possible primary prevention reduction strategies.

I think it's more likely — or more likely to be effective with these strategies if we recognize that surgical termination, which appears to be better studied, appears to have a lower risk in terms of patient mortality. I would suggest that we either reduce or eliminate mifepristone or at least consider that within our discussions. And I note that there doesn't appear to be a safe or specific alternative that only gets the progesterone receptor.

And certainly I would agree with practitioners, such as Planned Parenthood, who've already gone to the oral only — oral only administration, which is actually what was approved by the FDA. I do not suggest — as speaking as myself, a clinician — that antimicrobial prophylaxis, either short or long course which has been suggested, would be likely effective. Indeed, it might be just the opposite.

And aspects of vaginal/perineal hygiene, cleanliness and antisepsis, I don't think that's really the issue here. Because as we've learned in the first panel, *Clostridia sordellii* or its spores may certainly exist in the vagina or the gut in anyone. And we've talked a little bit about probiotics, and the power of "pro" as well as prebiotics, but there doesn't seem to be a likelihood physiologically that they would be of help.

Another thing that I think we should do immediately is actually change informed consent so that it acknowledges the risks, which I gave you the envelope — back of the envelope epidemiology. This is the informed consent from a large clinic, where I receive patients from, who get complications. And I'm not asking you to read it. I'm just

pointing out that this is very complex — kind of like a lease — and in fact, should include aspects of serious infection in the information or complications.

In terms of secondary prevention, I as an obstetrician who sees patients everyday, would suggest that we need to do better with treatment and recognition of this syndrome. I think the work by Marc Fisher in the — in the CDC really extends, allows us to know much more about this. And we would, I think — actually, we've have a paper in *Contraception* next month where we propose a set of findings, a case definition and with laboratory factors.

And I would suggest that these patients rapidly be admitted; that they get consultations with infectious disease as well as intensivists; that we make sure that we've started multiple organ support promptly. And I would suggest if this is the diagnosis, *Clostridia sordellii*-associated toxic shock, that we consider immediate extra patient of the surgical source and that's either with a hysterectomy or certainly a D&C.

In terms of antimicrobial coverage, Dr. Dennis Stevens is here and he'll maybe discuss aspects of the legal phenomenon. I would suggest that starting with drugs like clindamycin or imipenen may be of some benefit and then other kinds of techniques, including IV/Ig protein C aspects and certainly steroid support, may be of help.

In terms of the future, I think we have a lot of research to do, much more in terms of *Clostridia sordellii*. I think the folks in *Clostridia difficile* have really led the way in our understanding of clostridial epidemiology, pathophysiology and host responses. But

certainly, we now have the opportunity with modern technology to study the sub-cellular, cellular organ and even organismal effects of mifepristone and misoprostol to understand more about the active epidemiology of *Clostridia sordellii*, not only in pregnant women, but other folks as well. I'm very interested in babies as well.

And we also have obvious models of RU-486, which should be provoked in terms of looking at the pathophysiology of infection/inflammation. I gave you a sample of some of these studies that — which have already been done. And then we need to look at aspects of mifepristone/misoprostol metabolism. These are metabolized by P450 enzymes, which have many polymorphisms, and some people metabolize them much differently. So there may be prolonged doses persisting in some patients.

And then, of course, I think we have to have active surveillance of adverse effects. I have personal knowledge of several of these patients in California. And I can just tell you that some of them were relegated to be unexplained by the local authorities or that there was no connection made between the pregnancy termination and the patient who had died. So thank you very much. It was my pleasure to speak to you.

E. SPEAKMAN: Thank you, Jaime. Next, Dennis Stevens.

Clinical Settings, Diagnostic Clues and Pathogenic Mechanisms of *Clostridium Sordellii*

D. STEVENS: Well, I'd like to thank the organizers for inviting me to put in our perspectives about this particular disease. And I'd like to specifically thank Dr. Amy Bryant and graduate student, Dr. — or not yet doctor — but graduate student Mike Aldape, who have done a lot of this work that I'm going to be talking about today.

Some of you may have the conception that *sordellii* has just emerged as a pathogen in the last year or two, but that's far from the case. And it actually was described first by Alfredo Sordellii and named *C. sordellii* clear back in 1937. I want everybody to be clear that it is an anaerobic organism. It's a gram-positive rod that forms spores and it was thought to be a virulent form of *Clostridium bifermentans* for a long time.

Now I'm going to present some clinical data. We've reviewed the literature fairly extensively, but also want to present a brief case of *Clostridium sordellii* infection. And this was a four-year old male who actually fell out of a car, broke his arm in the fall. His arm was casted. The second day, he noted pain and marked swelling, again, no fever. Blood pressure was normal; pulse was rapid. They gave him intravenous cefazolin and did a volar fasciotomy because the arm was massively swollen.

About a half-day later, there was increased swelling of the — of the arm and pain. His blood pressure had dropped a little bit, pulse increased. His white count was

31,000 with a left shift; went to the emergency room or operating room once again and had necrotic muscle and fascia. Tissue sample grew a culture of *Clostridium sordellii*.

On day three, the white count rose further to 41,000. He had increased hypotension. And I apologize for these symbols; that's supposed to be an up arrow, but there's some kind of a computer gremlin in here. And he had increased tachycardia, metabolic acidosis and ultimately died within three days.

Another case, a 21-year old female with vaginal laceration during childbirth, natural childbirth. On day five, she had increased perineal pain, again, normal temperature. Blood pressure was a little low, pulse was 132. Our white count was 67,000. They started her on IV gentamicin and clindamycin, did a fasciotomy of the vulva, added vancomycin. Post-operatively, the white count rose to 123,000. She developed hypotension, metabolic acidosis and super-ventricular tachycardia with a pulse of 170.

She was given copious amounts of intravenous fluids. And just like Dr. McGregor's case, her whole body swelled up because of the amount of fluid she got in because of a capillary leak. She had a cardiac arrest and died, and again, the cultures grew *Clostridium sordellii*.

Well, we have reviewed all the cases of *Clostridium sordellii* that we could find and the yield — yielded 28 reports describing actually 41 patients. Some of those reports were by Dr. David Soper and Dr. Jaime McGregor in this room; two additional

cases were reported by us as you just heard. They were 49 percent males to 51 percent females. And you can see that the mean was from 17 days of age to 95 years of age with a mean of 36.

Interestingly when Mike Aldape looked at this information, you could see there's really no difference in terms of age, striking difference in terms of among the non-survivors among females. It's a deadly disease for females, primarily because of the OB/GYN and postpartum infections. There was no difference in temperature between survivors and non-survivors. And the temperature was not elevated.

This is very interesting though that the white count had prognostic significance. And for the non-survivors, the mean white count was 75,000; whereas for the survivors, the mean white count was 18,500. Also when you look at the different classifications of infections, here's postpartum patients: nine in the literature; they all died. And medically-induced abortion: five in the literature; they all died. Spontaneous abortion: two in the literature; they all died. So horrendous mortality in females.

Among injection drug abusers: ten cases in the literature; mortality rate was 50 percent. Again, I think primarily because one has a little better clue as to what's going on and where the source of infection is. And then other cases included trauma, such as the case I presented, the young boy; surgical procedures; and then "other," such as omphalitis that were mentioned by Dr. Fischer.

And in order to get a clue about which way to go with pathogenesis, I think it's important to have a clinical perspective. And you can see that certainly hypotension is important. And a lot of these symptoms and signs are really fairly non-specific and don't really help us very much. The thing that's interesting though is this leukemoid reaction that's been mentioned several times. But of the 26 cases where there was adequate information for evaluation, you could see a leukemoid reaction was present in 20 percent.

In addition, hemoconcentration, 16 out of 26. What do we mean by that? We mean that the hematocrit actually rises. And there's case reports in the literature of the hematocrit instead of being in the 35 to 40 range is in the 75 to 80 range. So essentially, all the fluid leaks out of the blood stream through the capillaries and the blood vessels. And all that's really left are circulating red blood cells and elevated white count. In addition, to go along with that, are reduced serum proteins. And albumins can get down to .5 to 1 metabolic acidosis and so on.

Now there's not — in terms of treatment, Dr. McGregor mentioned a number of things that we could do. But I think it's hard to read the published papers and really come away with anything that's very meaningful that would help guide us in the future. The other thing that's interesting is that *sordellii* was found in the 26 cases of which there was adequate information, but other organisms were found at the site in 19 of the 26 cases.

And in six of these cases, there was *sordellii* of the blood. And clostridial antigens were utilized to make the diagnosis in the four cases that Dr. Fischer talked about. And this is just a laundry list of all of the other kinds of bacteria that may be isolated from the site of infection. And you could see that there's really quite a large number of both aerobic and anaerobic bacteria that may be co-pathogens.

On autopsy, this is kind of a summary of what you find: local necrosis and acute inflammation is common; marked tissue and visceral edema; big time accumulations of fluid in the pericardial, peritoneal and pleural spaces; thrombosis of localized blood vessels in nine out of the 26 cases; and neutrophil degeneration at the sites of necrotic tissue.

And this is just some histopathology, again showing muscle bundles that have been destroyed. And here are some inflammatory cells in the tissue. Here are some organisms, some *Clostridium sordellii* organisms in the tissue, again, not with a lot of inflammation around them. This is much different than one would see with a *Staph aureus* abscess where there would be probably more white cells than there are bacteria.

And then in the lung, many of these patients develop capillary leak syndrome and ARDS, *et cetera*. And the striking thing here is that you see some red cells, but the capillaries are not well profused. You see some areas of the septal capillaries where there really is no red cells whatsoever.

So in terms of pathogenesis, clearly potent exotoxins play a role. And I'm not going to go through this information because Dr. Ballard has gone into it in great detail. And again, with *sordellii*, and *difficile* and *novyi*, they share some of these toxins and so on.

Now I just want to close out the last part of my talk by talking about some of the investigations that we've done in our laboratory to look at the mechanisms of capillary leak syndrome and also the mechanism of the leukemoid reaction, which we think is more than just a bizarre finding. We think it plays a very important role, potentially in *sordellii*, but also in *Clostridium difficile*.

So for the leukemoid reaction, we utilized HL-60 cells, which are a promyelocytic cell line that can differentiate either into granulocytic or myelocytic — monocytic cell lines. We then collected *Clostridium sordellii* toxins from just stationary phase cultures. We did an ammonium sulfate precipitation, isoelectric focusing, and created fractions with different isoelectric points from 3 to 10. And then we exposed those cells to these various fractions and identified the cells by flow cytometry.

And in this, what you could see is, this is the HL-60 cell proliferation. Here's no treatment; here's PBS; here's GM-CSF, which is known simulator progenitor cells. This is a fraction 6 from the *Clostridium sordellii*. And this is a combination of GM-CSF plus fraction 6, which seems to be additive in terms of effect. And we're in the process of identifying the proteins that are in fraction 6 that account for this.

In terms of endothelial cell permeability, we've used primary human umbilical vein endothelial cells and tissue culture. We cultured these on membrane-lined insert until they were confluent and then we measured the permeability by measuring the electrical resistance. So if there's holes in the membrane, they — then the resistance goes down very dramatically. And then we added various toxins and measured the resistance over 12 hours.

And just in a nutshell, the no treatment, PBS, sterile media and heat inactivated toxins are all up here. This represents the *Clostridium sordellii* crude toxin preparation and this is purified recombinant lethal toxin that we obtained from Jimmy Ballard. And you could see that there's quite a striking difference, not only in terms of the degree of permeability of the endothelial cells, but also with the rapidity of onset. And we're in the process of identifying what factors are there.

The next thing we looked at was the innate immune recognition and response to *Clostridium sordellii*. And to do this, we used the MDCK cell line that was transfected with the genes for toll receptors: toll receptors 1, 2, 4 and 6, and in combination. And then these cells were also transfected with MD2, CD14 and ELAM-1-dependent luciferase reporter system.

The latter systems allows us to basically measure the light produced by this reaction as a measure of the expression of the surface toll receptors. And then we also

measured cytokines from peripheral blood mononuclear cells stimulated in parallel. And these were largely kits from R&D in Minneapolis.

And this is the innate immune recognition of the *Clostridia*. Now for those that are into innate immunity, if we add LPS in this system, this would be sky high. And I think in a nutshell, you could see that with the exception of *Clostridium septicum*, which is — which is in the — these hatched lines, it seems to use toll 4. But all the rest of the *Clostridia* basically use toll 2 and a combination of toll 2 and toll 6 is the best.

And for some reason, *Clostridium septicum* is the most potent agonist we've ever used for this toll receptor expression. But *sordellii* looked — used toll 2 and toll 6 and the combination seems to be somewhat additive. So there certainly are receptors on immune cells that recognize *Clostridium sordellii* in the absence of any acquired immunity.

And this represents the cytokine response to peripheral blood mononuclear cells. Here's LPS as a whole variety of different *Clostridia*: *bolteae*, *clostridioforme*, *perfringens*, *septicum* and *sordellii*. So *sordellii* is about as potent as LPS in terms of cytokine induction. And in this situation, let's see, we would basically use the protein micro array to look at cytokine production with no toxin, *Clostridium sordellii* toxins and then the lethal toxin. And there certainly are some differences here.

In particular with IL-1β, you could see that the *sordellii* toxin crude preparation was a very potent inducer of IL-1β, whereas, lethal toxin was not. And similarly down

here, GM-CSF was induced by the *sordellii* toxins, as I mentioned previously, but it was not produced by the lethal toxin. And interestingly, IL-10, which is an inhibitory cytokine, was produced to a greater extent by lethal toxin than it was the cruder preparation.

Well, I just want to close and say basically that we think the important pathogenic mechanisms are the diffused capillary leak syndrome that occurs with *sordellii* infections. And that seems to be related to direct toxin effects on endothelial cells and we're in the process of defining what specific toxins are involved with that.

The leukemoid reaction, it looks like there may be some synergistic interaction between GM-CSF, which the toxin induces, and the ability of a toxin within the *Clostridium sordellii* group to actually stimulate proliferation of bone marrow progenitor cells in its own right. Thank you.

E. SPEAKMAN: Thank you, Dennis. Next is Ralph Miech.

The Pathophysiology of Mifepristone-Induced Septic Shock Due to *Clostridium Sordellii*

R. MIECH: My remarks today will focus on a theory to explain how mifepristone may have contributed to the deaths of the four healthy women in California who had medical abortions. These four deaths were the result of septic shock due to *Clostridium sordellii* infection following a medical abortion with mifepristone.

These women were all less than two months pregnant, were given a single dose of 200 milligrams of mifepristone orally, and self-administered 800 micrograms of misoprostol vaginally 24 to 48 hours later. They died within the next five to seven days with clinical signs of shock, absence of fever, leukocytosis and hemoconcentration.

How do we explain that a single dose of mifepristone could result seven to ten days later in a fulminating lethal case of septic shock? Most drugs are eliminated from the body in a matter of a few hours. However, pharmacokinetic studies of mifepristone has shown that this drug generally has a long half-life of 20 to 30 hours. With a half-life in this range, it takes four to five days to remove 95 percent of mifepristone from the body.

However, it has been shown that some humans have an unusually long half-life that can be as long as 90 hours. In these individuals, 18 days would be required to remove 95 percent of mifepristone. Mifepristone is moved from — removed from the body principally by metabolism. It is metabolized by the cytochrome enzymes located in the liver microsomes.

Six different metabolites of mifepristone have been identified. Some of these metabolites have retained biological activity as progesterone antagonists. However, I'm not aware of the studies that may have looked at these metabolites of mifepristone to see if they retained or had increased anti-glucocorticoid properties. Studies of these six metabolites for their effect on the innate immune system is grounds for future research.

In vitro studies with liver microsome enzymes have shown that cytochrome P450-3A4 is the enzyme primarily responsible for metabolizing mifepristone. Furthermore, there is evidence that the enzyme itself can be inactivated during the metabolism of mifepristone. This probably accounts for the relatively long biologically half-life seen in humans. The same enzyme is also responsible for the OD methylation of codeine to morphine.

In mifepristone abortions, women are frequently prescribed codeine for pain. Thus, codeine would compete for the enzyme that metabolizes mifepristone and prolong its biological half-life. Mifepristone binds with high affinity to both progesterone and anti-glucocorticoid receptors — to glucocorticoid receptors as evidenced by a disassociation constant in the picomolar to nanomolar range.

Mifepristone blocks cortisol receptors both in peripheral tissues and in the central nervous system. Blockade of negative feedback receptors in the hypothalamus results in increased serum levels of ACTH and cortisol. It appears that the initial effects of mifepristone is blockade of peripheral glucocorticoid receptors as cortisol levels begin to rise. Thus in some experimental animal protocols, mifepristone is referred to as producing a temporary drug-induced adrenalectomy.

Mifepristone, known as RU-486, was initially called “RU-38486.” Mifepristone originally was developed as an anti-glucocorticoid for the treatment of Cushing’s disease. During the development of this anti-glucocorticoid drug, it was discovered to

possess anti-progesterone activity and acted as an abortive agent. Mifepristone's anti-progesterone activity results in four pharmacological actions on the pregnant uterus: cervical ripening, ischemia of the decidua, necrosis of the products of conception, and sensitization of the myometrium to contraction by prostaglandin.

The first three of these pharmacological actions enable the establishment of a favorable nidus in the ischemic decidua for an infection with the anaerobic bacteria, *Clostridium sordellii*. Different species of *Clostridium*, including *sordellii*, have been found in the normal vaginal flora in 8 to 18 percent of women.

Macrophages, monocytes, neutrophils and endothelial cells are the host's first line of defense to counter bacterial invasion of the interstitial space of uterine tissue. I propose that mifepristone anti-glucocorticoid action initially impaired the proper functioning of the cells of the innate immune system within the pregnant uterus. It ultimately led to a uterine infection with *Clostridium sordellii*.

Specific molecular components, such as lipoteichoic acid and peptidoglycan, are unique to the cell walls of anaerobic bacteria and are known as pathogen-associated molecular pattern molecules. These unique biochemical entities bind to and activate toll-like receptors on tissue macrophages.

Toll-like receptors, when activated, function as the principal sensors of infection in mammals. Activated toll-like receptors of the innate immune system cause an outpouring of pro-inflammatory cytokines at the site of bacterial invasion. Tumor

necrosis factor alpha, interleukin-1 and interleukin-6 are the principal pro-inflammatory cytokines that are synthesized and secreted by phagocytes.

Inflammation, when properly controlled, destroy invading bacteria without tissue damage. If local reaction of pro-inflammatory cytokines are not controlled, two things can occur. One, *Clostridium sordellii* can successfully establish an affection — an infection with the secretion of lethal toxin, and two, excessive pro-inflammatory cytokines gain access to the systemic circulation.

Both of these events contribute to the etiology of septic shock and multiple organ dysfunction. The proper timing and proper amount of cortisol are crucial to maintain control of the inflammatory response and prevent tissue damage. Cortisol binds to its intracellular glucocorticoid receptors and phagocytes to cause increased transcription of DNA, resulting in the synthesis and secretion of the anti-inflammatory cytokine, interleukin-10.

This anti-inflammatory cytokine suppresses the generation of the excessive pro-inflammatory cytokines, tumor necrosis factor alpha, interleukin-1 and interleukin cells — interleukin-6 by the cells of the innate immune system. The hypothesis that mifepristone could facilitate infection and lead to lethal septic shock is supported by animal experimentation.

As an example, a single dose of mifepristone given in an animal model of polymicrobial septic shock dramatically increased the mortality rate almost three-fold in

mifepristone-treated mice. It is my opinion that mifepristone impairs the innate immune system just long enough to delay the proper removal of contaminating *Clostridium sordellii* from the decidua. This delay allows the bacteria to secrete lethal toxin into the uterine interstitial fluid.

Lethal toxin — taken in by phagocytes, endothelial cells and other cells in the uterus — prevents them from properly participating in the defensive inflammatory responses of the innate immune system. When lethal toxin is internalized by uterine phagocytes and internal endothelial cells, it functions as an intracellular enzyme that catalyzes the glucosylization of G-proteins.

G-proteins are the molecular switches that activate or inhibit a multitude of vital biochemical cascades and vital genetic transcription functions that are necessary for cells to function properly. Glucosylization of G-proteins in uterine phagocytes renders them useless in destroying bacteria.

In summary, mifepristone's anti-progesterone effects prepare the aborting uterus as an ideal bacterial culture for *Clostridium sordellii* by causing ischemic decidua that leads to necrotic products of conception. Mifepristone's anti-glucopharmacologic actions disrupt the hypothalamic pituitary adrenal axis and interfere with the functioning of peripheral glucocorticoid receptors at a crucial time.

This results in a lack of control of the pro-inflammatory cytokine response. This allows for the establishment of a nidus of infection with *Clostridium sordellii* and the

localized secretion of lethal toxin. Phagocytes in the decidua are permanently inactivated by lethal toxin. This allows *Clostridium sordellii* to multiply unchecked and secrete excess lethal toxin into the systemic circulation.

In conclusion, the combination of both lethal toxin in excess inflammatory cytokines in the systemic circulation would work together synergistically to produce the clinical findings of rapid fulminating lethal shock syndrome that were the hallmark of the four cases that occurred in California. Thank you for your attention.

E. SPEAKMAN: Thank you, Ralph. The last presenter, Esther Sternberg.

Bacterial Toxin Repression of Nuclear Hormone Receptors: Host-Pathogen Hormone Interactions and Implications for Therapy

E. STERNBERG: So first I'd like to thank the organizers for inviting me to this really very important conference. It's really what public health is all about; getting NIH, CDC and FDA together to try to solve what is an emerging problem. I'm going to present to you our work on host hormone immune system bacterial interactions because this problem, I think, is a very complex one as you've heard up until this point.

We have been studying the effects of hormone on immunity and I'll start with three questions that I will address. First, do hormones affect host inflammatory responses? I'll discuss briefly glucocorticoids, estrogen and progesterone. The second question is do bacterial toxins interact with these host hormone responses?

I'll present to you our published data on *Bacillus anthracis* lethal toxin because it is a model for the other data that I will discuss briefly on *C. difficile* toxic — toxin A and B and *C. sordellii* lethal toxin. And finally, I'll address the question of whether these interactions blocking these interactions can predispose to inflammatory sequelae *in vivo*.

So the answer to the first question — that is, do hormones affect host inflammatory immune responses? — is a resounding yes. We originally showed back in 1989 that blocking the hypothalamic pituitary adrenal axis predisposes, as you've heard, to increased inflammation. And this is because glucocorticoids play a very important role in regulating the immune response. In general, they are immunosuppressive and anti-inflammatory.

In addition, the hypothalamic pituitary gonadal axis plays a very important role in regulating information and the immune response. It's a little more complicated. Estrogens can be stimulatory or inhibitory and progesterone is generally inhibitory.

At low concentrations, estrogens tend to stimulate a TH1-type pattern or a cellular pattern of immune responses. This is generally pro-inflammatory. And at high concentrations, estrogen, and progesterone and glucocorticoids all cause a shift from a TH1 to a TH2 pattern of immunity that is a humoral or antibody pattern and these tend to be anti-inflammatory.

As you know, estrogen and progesterone vary throughout the menstrual cycle, progesterone peaking during the luteal phase. And various studies have shown that there are associated immune function changes at these different points in the cycle: decreased T lymphocyte chemokine receptors, increased antibody production, and increased infection susceptibility to a variety of infectious agents.

These hormones generally also increase throughout pregnancy and peak in the second and third trimester of pregnancy. And there have also been associated immune function changes reported during pregnancy, mostly during the second and third trimester with strong immunosuppression, increased suppressor T cells, decreased cytotoxic T cell function, and increased antibody production, as well as increased susceptibility to toxoplasmosis *gondii* infection.

Many of the studies during pregnancy focus on the effects of these changes in association with autoimmune diseases. We have chosen to look at the effects of progesterone on dendritic cells because dendritic cells are at the interface between innate and adaptive immunity. They're very important in pathogen recognition and response and they produce a lot of pro-inflammatory cytokines.

Using FACS analysis — fluorescent activated cell sorter analysis — we found that, in fact, dendritic cells do express glucocorticoid receptors and progesterone receptors. And you can see on the right-hand slide that using fluorescent microscopy,

you can see the cytoplasmic expression of those progesterone receptors in the fluorescent green.

In additional studies which are not yet published, we found that progesterone is generally inhibitory on dendritic cells. It does not affect immature dendritic cells and it primarily suppresses pro-inflammatory TNFα production in mature dendritic cells. It does not have an effect on anti-inflammatory IL-10 production. It also down-regulates co-stimulatory molecule expression: MHC Class II and CD80.

And this is some of the data that we've found on the effects of progesterone on TNFα secretion in rate bone marrow-derived dendritic cells. And what you can see in the first — in the second bar, you can see that LPS induces TNFα production and increasing concentrations of progesterone inhibit that TNFα production.

These are concentrations commiserate with the luteal phase of — the luteal phase of the menstrual cycle and the highest concentration we use is commiserate with the pregnancy levels. RU-486, or mifepristone, reversed that effect. In other words, it prevented the progesterone inhibition of TNFα production.

So the next question is do bacterial toxins interact with host hormone responses? In our published data, we've looked at *Bacillus anthracis* lethal toxin. And in more recent studies, we asked the question whether our findings in *Bacillus anthracis* lethal toxin extend to other bacteria; that is, *C. difficile* toxin A and B and *C. sordellii* lethal toxin?

So in our first studies looking at *Bacillus anthracis* lethal toxin, we showed in a transient transfection system that the lethal toxin repressed transactivation of a number of nuclear hormone receptors, including the glucocorticoid receptor and the progesterone receptor. And this repression occurred at very small concentrations of lethal toxin — nanogram per milliliter concentrations — and it was both receptor- and promoter-specific.

We also found in looking at the toxins, *C. difficile* and *C. sordellii*, that these toxins also repressed the glucocorticoid receptor. In this slide, you can see that increasing concentrations of *C. sordellii* toxin repressed, partially repressed transactivation of the glucocorticoid receptor. On the right-hand slide — sorry — in the next slide, you can see that toxin A and toxin B of *C. difficile* also repressed this transactivation of the glucocorticoid receptor, toxin B being more potent than toxin A.

In other studies that I am not going to show you the data for at the moment, we have found that *C. sordellii* lethal toxin partially prevents dexamethasone suppression of splenocyte TNFα production. In other words, the effect that we've seen of the lethal toxin in blocking glucocorticoid transactivation also translates to an effect in the physiological system in preventing dexamethasone suppression of TNFα production.

We found that sub-pharmacological concentrations of RU-486 plus the *C. sordellii* toxin completely reversed that dexamethasone suppression of TNFα production *in vitro*. And I emphasize "*in vitro*" because these studies are done *in vitro* and the most

important question at this point is to determine whether these findings *in vitro* translate to an *in vivo* situation. That's going to be my take-home point for future directions, but I'll show you some of our *in vivo* data that we've done to date.

The previous panelists have already referred to my original study published in 1989 showing that RU-486, given together with streptococcal cell walls to otherwise resistant Fisher rats, resulted in 100 percent mortality in these rates. RU-486, I would point out, alone had no effect.

Others have since then used other methods to block the hypothalamic pituitary adrenal axis: either using adrenalectomy, hypophysectomy and using other pro-inflammatory antigens or pathogens, including salmonella, MCMV virus or shiga toxin. And in every case when one blocks the hypothalamic pituitary adrenal axis, together with exposing the animal to these pro-inflammatory triggers or pathogens, there is greatly enhanced mortality. In most cases, glucocorticoids do reverse or prevent this effect.

We also did these studies more recently looking at the effects of lethal toxin in mice. We treated otherwise lethal — this is anthrax lethal toxin — we treated otherwise anthrax lethal toxin-resistant mice, but we adrenalectomized otherwise anthrax-resistant mice. And that adrenalectomy greatly enhanced mortality in these mice. It also enhanced mortality in the mice — mouse strains that were partially sensitive to anthrax lethal toxin.

Unfortunately, dexamethasone did not rescue those mice. And I'm going to emphasize this point because it does address one of the points that was raised in the panel and that is the suggestion that glucocorticoids should be used to treat these sorts of cases. Again, this is another reason that one must test these kinds of questions *in vivo* before you apply them to human treatment.

The reason we think that the dexamethasone did not rescue the mice — the adrenalectomized mice from anthrax lethal toxin mortality — is that these toxins attack the glucocorticoid receptor, not at the glucocorticoid binding region, but they take out a variety of co-factors or accessory proteins that fundamentally inactivates the receptor. And so giving glucocorticoids is, one would predict, would not rescue these animals because the glucocorticoid receptor becomes non-functional.

We do not fully understand why giving dexamethasone actually enhanced mortality in these animals. It is possible that it might've down-regulated the remaining receptors further. But the important take-home point here is that before recommending treatment with glucocorticoids, we need to do these studies with the specific toxins in mind.

In these studies with anthrax lethal toxin, RU-486 had variable effects on *Bacillus anthracis* lethal toxin mortality, which suggests that adrenal factors other than glucocorticoids was playing a role in the mortality related to this particular toxin, another

reason that we really need to do *in vivo* studies with the specific toxins in question and in the context of the different hormones that we are concerned with.

So in summary, glucocorticoids and progesterone generally suppress inflammatory responses. *Clostridia* bacterial toxins partially repress glucocorticoid receptor transactivation. *Clostridia* bacterial toxins partially reversed dexamethasone suppression of TNFα production. And RU-486, at sub-pharmacological concentrations together with *C. sordellii* lethal toxin, completely reversed the effects of dexamethasone suppression of TNFα production *in vitro*.

In future directions, the most important studies to perform are to determine if these *in vitro* findings can translate to *in vivo* situations of shock. It's very important in these conditions to look at the time course after exposure to the bacterial products; the dose response or the concentration of hormones and drugs to which the individual is exposed; and drug interactions, such as the prostaglandins.

When one is considering predisposing host factors to determine whether we would predict whether certain individuals are more susceptible to shock or infection in these cases, it's important to look at hormone levels in the individual: both pregnancy and menstrual cycle hormone levels as well as receptor polymorphisms or mutations that might, in certain individuals, make them more susceptible to the inhibitory effects of other factors that block these receptors.

I didn't talk about this at all in the talk, but there is a mounting literature showing that glucocorticoid receptor morphisms or mutations or mutations in polymorphisms in associated proteins that are necessary for the glucocorticoid receptor to function are prevalent in numerous autoimmune and inflammatory diseases and may play a role in enhanced inflammatory responses in certain individuals.

So if the goal of this conference — and I hope that is the goal — is to identify risk factors to prevent this from happening in other people, we need to think about all of these factors. We need to think about the host factors and the bacterial products interactions with the host. And we need to think about the immune responses and hormonal responses that interact with all of these factors.

And in closing, I'd like to thank the many people in my lab and the collaborators, both at NIH and in other organizations. The beautiful data on the lethal toxin — *Clostridia* lethal toxin was done by Sasha Tait, who is here in the audience, and I would thank her as well. So thank you very much and I'll take questions.

Panel 1-Session 2 Discussion

E. SPEAKMAN: With limited time we have until lunch, what we'd like to do is hold things to questions and not comments. We will have time in the afternoon for comments and dialogue as we try to get things more focused. So for the panelists and

for you all, just limit it to questions only. Dr. Gerding, any questions that you have as the chair?

D. GERDING: The proposed treatments are fascinating for preventing this problem in the patients undergoing abortion and I'm not real excited about it. But I wonder if Dr. McGregor would comment about the antibiotic use? Because in the patients with pregnancy, that's one of the things we've been looking for is antibiotic use as a risk factor for getting a very similar looking illness, which is fulminant *C. difficile*, in pregnant patients.

And antibiotics there are considered to be a provocative agent or an agent that sets those patients up. And I, you know, noticed you weren't enthusiastic about antibiotic use in this situation to prevent *C. sordellii* infection. I wonder if you might comment on that?

J. McGREGOR: Sure, that's a — and Marc will too. I don't think that we should be giving broad spectrum or even focused antimicrobial agents for the problem of *Clostridia sordellii*. As I gave you the back of the envelope epidemiology, this is still a very rare occurrence, although fatal. Certainly, I think we should be treating sexually transmissible infections like chlamydia, gonorrhea and others, but then only selectively.

So I think that the suggestion of giving antimicrobial agents with activity against the *Clostridia sordellii* at the time of initiating the medical termination is a bad idea because it would provoke multiple changes in the microecology of the patient. Probably

10 percent would get immediately yeast and would change the GI flora as we talked about, so I think that's a bad idea.

D. GERDING: Dr. Sternberg, I wonder if you can comment on how you can tie these two clostridial infections together in the pregnant patient? It looks like some of the risk factors that you're identifying may be coming together, putting the patient potentially at risk for both of these infections.

E. STERNBERG: Well, I think in the pregnant — the main point of your question in the pregnant patient is very important because if you think about the first half of my talk, progesterone is elevated. And if progesterone turns out to be *in vivo* as important an anti-inflammatory factor as it looks like in our *in vitro* studies, and it certainly is consistent with what other studies that have been published during pregnancy, that the pregnant woman would tend to be more at risk for infection.

If at that point there is greater bacterial growth — and I'm saying "if" because we certainly don't know this — of certain bacteria that produce these toxins and then the toxins inhibit the host's own inflammatory responses that are meant to protect from the bacteria, then you might go to the other extreme and develop shock. It's a hypothesis, but it's a hypothesis that is testable certainly in animal models.

D. GERDING: Dennis, you want to comment on that?

D. STEVENS: Well, I think it's — I think that a message the audience ought to take home is that *Clostridia sordellii* is a — is a very well equipped microorganism to kill

normal people very quickly. And I spent a fair amount of time talking about that. And certainly, this can affect normal people that have broken arms, legs, postpartum women, certainly therapeutic abortion, but it doesn't really require much in order to be able to cause this infection.

I think the real fascinating thing is that why is infection so uncommon in pregnant women or even women that have therapeutic abortion? I mean, I — you know, over the eons of development of the human condition, it's amazing to me. Given the number of bacteria that might be at the site of child delivery, contaminated with feces, and urine, and stool and everything in the environment, it's amazing that the normal person doesn't develop more infection.

E. STERNBERG: Well actually, part of that has to do with the fact that females of all species have a higher, in some cases two- to ten-fold higher incidence of autoimmune inflammatory disease, probably related to the estrogen component which is pro-inflammatory.

But there was an excellent report by the Institute of Medicine in the year 2000 called "Does Sex Matter?" that summarizes the many, many factors that contribute to females having a much stronger inflammatory and autoimmune response. And that's probably why females survive pregnancy.

D. STEVENS: Great point.

E. STERNBERG: Don't quote that.

E. SPEAKMAN: Any questions from the audience? Yes?

S. KWEDER: Sandy Kweder from FDA. I think it's interesting that in the speakers who talked about pathophysiology and with mifepristone as a potential mechanism never mentioned misoprostol. And that really has been absent from the discussion in two ways.

One is the intravaginal use of the misoprostol. These are oral tablets that are inserted vaginally with a very unpredictable pharmacokinetic profile when you do that. One of the things that's been well reported is a high rate of uterine rupture, in general associated with intravaginal misoprostol that seems to be dose-related, not in this setting, but in general obstetric use.

So I wonder if anybody could comment on potentially the role of the intravaginal misoprostol either in creating greater than in — higher than in ten did uterine contractions with perhaps tissue dioxygenation, one; and secondly, potentially with reflux of the vaginal content into the uterus that might set up the uterus for that infection given the other physiologic circumstances that are occurring?

And I wanted to correct one thing that was said. We have actually spoken with our counterparts in other countries about how often intravaginal misoprostol is used. We've spoken with French regulators and others in Western Europe. And in all countries, it's — that regimen is being used widely, despite recommendations otherwise.

E. SPEAKMAN: James?

J. McGREGOR: I can answer some of your questions. I took out slides about misoprostol and the prostaglandins because they actually play direct primary as well as secondary roles in controlling innate immunity. So there's another story there. In our own work and others, we've shown that when the uterus contracts, that actually substances from the vagina are normally transported up inside the uterus so that would include *Clostridia sordellii* spores or other microorganisms that would probably happen physiologically.

We also talked about the prolonged bleeding, which is sometimes a part of this medical treatment. So I think those are of significance and the products are used together. My — speaking to people from all over the world and my own colleagues at USC, I think countries like the Netherlands, France — as you've already alluded to — have, I think, I would think excellent, I would presume excellent information because you don't die in the Netherlands without somebody figuring it out. I can just tell you ...

S. KWEDER: That's right.

J. McGREGOR: I can just tell you, in my part of the world — which I won't elaborate — some of these patients died mysteriously. And it wasn't until everything was brought together that these cases were brought to you. In some of these cases, you could actually — people would tell you, "Well, the practitioner's office called, but

they didn't get through to do the follow-up because the patient had died." And so in fact, all these facts weren't completely put together in our milieu in my part of the country.

S. KWEDER: Right, but they certainly are in countries with better tracking and nationalized medical systems ...

J. McGREGOR: Yes.

S. KWEDER: ... such as the Sweden, Denmark, the Netherlands.

J. McGREGOR: So I would think that the epidemiology would be much clearer there. We also considered contamination as Marc did in both the misoprostol as well as the RU-486 and came to the conclusion, without as much information that you did, that that wasn't the problem.

In terms of the contamination in terms of the vagina over the — say placing a tablet in the vagina on your own over the perineum, this gets into contentious issues and many anecdotes. But in my own opinion, I think that the patients put the tablet in the vagina without difficulty or without massive contamination.

And I think that in fact, as we talked about in my presentation although briefly, that in fact the microecology and microbiology of the vagina certainly has been studied by Dr. Bartlett and several other folks in the audience. And now we have new techniques to go into the microecology/microbiology of what's actually there. We have new tools to sort of figure this out.

But I would be actually quite humble in all that because I think actually these microorganisms are shared between the reproductive tract and the gastrointestinal tract. And I think that they may exist in very low levels of populations and actually may be difficult or impossible to culture or even to discover, such as the techniques that Marc used in the *New England Journal* paper, which is set to about 105th.

So I would remind all of us, in terms of pediatric studies, that a study was done with *Clostridia sordellii* where they actually looked in the mother for *Clostridia sordellii* — excuse me — *Clostridia difficile* in the GI tract in the vagina and they didn't really find it very often. It was just like we quoted: 5 or 10 percent.

But then they looked in the babies, and in fact, *Clostridia difficile* was actually quite common in the babies — between 30 and 60 percent — suggesting the babies were being either colonized from the mother's microecology or from caretakers in the nursery. And the authors of the paper didn't think it gave the opinion that it wasn't from the caretakers, the doctors, and the nurses and the environment in the nursery, suggesting that actually low inocula of microorganisms can be expanded using the baby as a culture system.

E. SPEAKMAN: Questions in the back right?

B. WINIKOFF: Yes.

E. SPEAKMAN: Your name, please?

B. WINIKOFF: Beverly Winikoff. And this question is directed to Dr. McGregor and to Dr. Miech. I — my background is clinical, and epidemiological, and much less basic science and so I'm trying to understand how your hypotheses actually translates into the facts that we — that we see on the ground.

So if I understand correctly, you're proposing some generalized physiological changes secondary to mifepristone that could predispose to infection? Generalized immune deficiency or glucocorticoid effect would result in the appearance of infections with rare pathogens, no septicemia and toxic death rather than more presentations of more common pathogens with overwhelming infection and septicemia?

J. McGREGOR: That's an excellent question — series of questions. First of all, it appears that in our own experience, and now in the literature, that there are cases of non-*Clostridia sordellii* serious infections. And the author of one paper went further with the observations that have been presented here; that the signs and symptoms of this severe infection, which was occurring clinically, weren't manifest to the clinicians.

So that's the same observation with the non-*Clostridia* infection, but these infections are still quite rare as you were about to say, I think. And yeah, and so that, in fact, it must be a coming together of the predisposition, as well as the agent plus maybe some other thing, which I suggested was the polymorphism for the P450 enzyme. Dr. Miech actually took that to — told us all about that.

So other predispositions all acting together, coming together with these relatively rare catastrophic side effects, but again, I think the onus is on us, all of us, to figure out what the real epidemiology is. It would suggest from, again, the back of the envelope kind of epidemiology that the risk of this syndrome is greater than the risk of lethality and surgical termination done in early pregnancy.

E. SPEAKMAN: There was another question in the back? And that'll have to be the last question before lunch.

G. SONGER: It'd be my pleasure. I'm Glenn Songer, University of Arizona. I'm wondering why — and a couple of the recent answers have sort of brushed up against it — wondering why we don't see *difficile* in these same kinds of scenarios as *sordellii*? There's a potentially emerging condition in late gestation sows, which is characterized mainly by sudden death.

"Sudden death" in the veterinary world means it died since I last looked at it, so it can range from minutes to hours, sometimes days. And these cases have — although we can't tell yet if they're starting in the reproductive tract, that they do have a lot of the earmarks of the *sordellii* problem in humans. They yield *Clostridium difficile*, ribotype 078, toxinotype V with the larger *tcdC* deletion, and binary toxin-positive. So it's the same thing that's coming out of the babies.

Anyway, I — to go back to my question, I wonder why we're not seeing these *sordellii*-like cases caused by *difficile*? Are we — is the prevalence so low that we just haven't run across one yet or are people dying and not being cultured?

E. SPEAKMAN: Anybody? Feel free to speak on that.

J. McGREGOR: From my point of view, I think these are, as we've said, rare circumstances. In the animal husbandry area, these clostridial syndromes have been under discussion and there are actually vaccines for different kinds of clostridial infections in agribusiness.

And as an example of how things could go wrong, there was a vaccine report from Spain where 100,000 animals were actually — roughly 100,000 animals were given the antitoxin, but it was contaminated with *Clostridia sordellii* spores. And so half of the animals became sick and half of those died underscoring the lethal circumstances of *Clostridia sordellii*.

But you're right; there are other kinds of *Clostridia*. Before pregnancy termination was legal, *Clostridia perfringens*, *Clostridia septicus* were common infections in large hospitals, such as my hospital in Los Angeles, and common causes of death and renal failure.

D. GERDING: Glenn, those sows are dying of a *C. diff* enteric infection, you think, late in pregnancy or you don't know where the site of infection is?

G. SONGER: No, it does not appear to be enteric.

D. GERDING: It does not appear to be enteric? But they appear to be dying suddenly as though they may be intoxicated? And you're getting *C. diff* from what site: from the ...

G. SONGER: Bloodstream.

D. GERDING: From the bloodstream? So they actually have bacteremia. Yeah, so I mean, we are seeing fulminant and catastrophic *C. difficile* infections in pregnant women, not to a great extent and, you know, we're — we always look to that antibiotic risk, which I suspect the animals that are getting sub-therapeutic antibiotics could have that added risk factor.

Most pregnant women, we stay away from antibiotics if we can do it. Although in the peripartum period, they frequently receive them as prophylaxis for Group B strep. But I was wondering if there was a difference there in the — you know, we just don't see *Clostridium difficile* bacteremia virtually ever in humans.

And so I'm really kind of intrigued by the fact that this is being found in the blood of these animals and they do not have a colitis or pseudomembranous colitis as, you know, many of these animals do have. So I think something clearly is going on that's different in the animal obviously.

E. SPEAKMAN: Great. All right. One last applause for all the panelists who've been up in the front, all of them at one time. And Dale, thank you. Thank you for chairing such a great — we're going to break an hour for lunch. We want to remind

everyone that lunch is right behind you; come back at 1:30. We'll start panel 2 related to surveillance for disease and sources of infection at 1:30. And then we'll take a small break and we'll begin identifying the research agenda at 2:45. Thank you.

[LUNCH 12:35 P.M.-1:37 P.M.]

E. SPEAKMAN: We'll get started in one more minute, so if everybody could take their seats. Good afternoon. A mentor told me a long time ago that if people come back after lunch, you're putting on a pretty darn good workshop, he told me a long time ago. So I see the same faces that were before, so I guess we're doing a good job of presenting and doing the workshop for you.

The morning was basically saying what's the current situation with these two diseases? Now we'll talk about sort of the surveillance and the sources of infection. And then we'll spend a vast majority of this afternoon looking at from what we've heard, determining and getting thoughts, recommendations for priorities for a research agenda moving forward.

So basically, we know some; we don't know a lot. So what do we need to do to answer all the remaining questions to be able to deal with them, reduce the morbidity and mortality of this? As I've mentioned before, this is panel 2, chaired by Clifford, Cliff — which do you prefer?

C. McDONALD: Cliff.

E. SPEAKMAN: Cliff McDonald, CDC. The objective here is to identify current surveillance and future surveillance needs and barriers. Thank you.

PANEL 2: SURVEILLANCE FOR DISEASE AND SOURCES OF INFECTION

Federal and International Initiatives

C. McDONALD: Thank you. Today I'll share with you initiatives at the federal level to survey and understand emerging disease caused by *Clostridium difficile* first, followed by *Clostridium sordellii*. For *Clostridium difficile*, I will be discussing surveillance in healthcare facilities, community-associated disease, pregnancy-associated disease, and disease in food-producing animals.

As we set priorities for surveillance for *Clostridium difficile*-associated disease, we need to keep in mind that the majority of *C. difficile* infections are acquired in healthcare facilities. However, as you all know, *C. difficile* infections are not nationally reportable so that alternative sources of national disease rates must be sought.

One of these is CDC's National Hospital Discharge Survey, which indicates that CDAD rates in U.S. hospital patients doubled between the year 2000 and 2003. Here are shown the number of patients discharged per 100,000 population with CDAD as either the primary diagnosis in orange or any primary or secondary diagnosis in blue.

Another yearly update of this survey indicates that CDAD rates in patients increased another 25 percent in 2004. The total overall rate increase was from 61 per

100,000 population (or 178,000 total discharges in 2003) to 75 per 100,000 (or 211,000 total discharges in 2004).

This slide shows these two years in blue and red, respectively, and shows the relative rates and increases by major stratification of gender, geographic region and age group. Notice the markedly elevated population-adjusted rates in persons 65 years and older.

What are other countries doing to survey CDAD in hospitals? The Canadian province of Quebec in August 2004 instituted mandatory reporting for acute care hospital cases, including cases with symptom onset up to one month post-discharge. They also tracked the rate of major complications, including death.

England, in January 2004, instituted mandatory reporting for all patients 65 years of age or older and included in this, cases associated with any and all healthcare facilities within individual national health system trusts. This system does not distinguish community- from healthcare-associated cases, but attributes all cases to a particular trust and/or the facility where they are admitted.

Meanwhile, Canada has performed two national sentinel surveys in 1997 and 2005 in which they demonstrated rates. They — excuse me — determined rates, and in 2005, also collected *C. difficile* isolates from sentinel facilities.

Current CDC priorities to promote surveillance of healthcare-associated CDAD include the development of surveillance recommendations currently being reviewed by

professional organization partners. One objective of these recommendations will be to more formally state what we've been communicating through our web site and public presentations. Namely, that in the current era of epidemic CDAD, all healthcare facilities should conduct some type of surveillance.

In addition, we provide definitions and outline methods that should be used to report infections. This document is intended to provide — excuse me — to guide and inform a number of interested parties, but primarily, the healthcare facilities themselves, the personnel there, followed by networks of facilities, state health departments and public reporting initiatives. We are also studying surveillance methods with CDC-funded EpiCenter hospitals.

Finally, the National Healthcare Safety Network is a voluntary electronic surveillance system for healthcare-associated infections that has been deployed and currently measures device-associated infections. A component for CDAD surveillance is under early development. Once this is developed, it could aid individual facility surveillance and networks and possibly be utilized for public reporting purposes.

As you heard this morning, there are recent reports of community-associated CDAD, such as this report of 23 generally healthy persons without recent exposure to healthcare facilities. Several of these cases occurred without recent antimicrobial use. We at CDC have become aware that community-associated CDAD is increasing among patients who seek care at the Atlanta VA Hospital.

Shown here is an increase in the proportion of all CDAD cases that are occurring among outpatients between the year 2003 and 2006. So far this year, 30 percent of all CDAD cases have occurred in outpatients. During this interval, by the way, they were using the same testing methodology throughout this interval.

In addition, many of these patients developed CDAD without recent hospital or antimicrobial exposure. And as shown in yellow, 50 of 61 outpatients with CDAD had not been hospitalized within the previous three months and 19 of these had not received antimicrobials in the prior 30 days.

Proton pump inhibitors, or PPIs, appear to be increasing the risk for CDAD in these patients. Although only case patients and no control group is shown on this slide, the patients highlighted in yellow suggest PPIs compliment the risk posed by antimicrobials. Note that CDAD case patients without antimicrobial exposure were more likely to have been exposed to PPIs and vice versa. The percentages shown here, by the way, are row percentiles.

As you also heard this morning from Dr. Gerding, there has been a recent report of pregnancy-associated *C. diff*. This included ten cases from four states and there was severe disease in some instances with one death reported. This was one of the three deaths Dr. Gerding mentioned this morning in his presentation. We are actively investigating additional cases of pregnancy-associated-CDAD. And to do this, we have

linked with the Emerging Infections Network to survey members of the Infectious Disease of Society of America.

Of a total of 405 infectious disease clinician respondents, 17 reported having personally seen cases and another 23 were aware of cases in their community. In total, 48 cases of pregnancy-associated CDAD were reported through this survey, including 14 that occurred prior to delivery; 20 percent — the usual percentage — were reported to have developed recurrent disease; three developed toxic megacolon; and there was one fetal loss and one maternal death reported. It is not yet known whether any of these severe outcomes are ones we've already heard about through other mechanisms.

CDAD is emerging in food-producing animals. *C. difficile* has been long recognized as a pathogen in horses, rabbits, hamsters and various other animals. Some recent reports have focused on disease or carriage being found among companion animals. However, reports by Glenn Songer — who's in this audience — and others indicate that since the year 2000, outbreaks of CDAD have been occurring in food-producing animals: namely, in neonatal pigs, and more recently, beef and dairy calves.

In pigs, CDAD is associated with high disease rates in affected production facilities. Shown here is a pig necropsy specimen demonstrating edema of the colon in a piglet from an affected litter — excuse me. Next to this is the typical histopathology

similar to pseudomembranous colitis in humans with an eruption of mucin and cellular debris into the lumen.

The strains infecting animals are genetically different from the most common human strains. Shown here are the proportion of isolates that are of a particular genetic type known as PCR ribotypes. Note that the — note that recent outbreaks in both pigs and calves are being caused by the same PCR ribotype and that this has not been a historically common ribotype among human isolates.

Although this slide indicates only 23 human isolates has tested, there are many more isolates in a large database managed by the British researcher, John Braiser, that also indicate PCR ribotype 078 is a historically uncommon strain in humans. However, the epidemic animal strains do share certain characteristics with the recently described human epidemic strain that could indicate increased virulence.

Namely, both the human epidemic strain and the food animal epidemic strain carry the binary toxin in addition to toxins A and B. And both strains possess a deletion in the putative toxin regulatory gene we've heard about — *tcdC*. These characteristics are highlighted by the red blocks. Whereas the human epidemic strain possesses an 18-base pair of *tcdC* deletion, the food animal strain possesses a 39-base pair deletion.

Although as shown on the first row of this data, the human and epidemic strains are of different toxinotypes. Both are virulent toxinotypes, which is different from

historically common or standard human strains. CDC has become aware of cases of human CDAD caused by strains similar to animal epidemic strains.

Shown on this slide are pulsed-field gel electrophoresis genetic typing results of isolates from seven cases of human CDAD that occurred in seven different states, alongside examples of isolates causing outbreaks in animals. Note that all are toxinotype V, all are binary toxin-positive, and all carry the 39-base pair deletion in *tcdC*.

There are several examples where the dendagram on the left of this slide indicate that animal and human isolates are highly related or at least 80 percent related by PFGE. In one instance, the typing patterns are indistinguishable between human and animal. Although our investigations are ongoing, a review of the case histories of five of these seven human cases suggests that human CDAD caused by these animal epidemic strains appears typical. In other words, this is occurring in primarily older patients with significant co-morbid disease and onset often in healthcare facilities.

There was one death attributed to CDAD among these that occurred in a younger patient without co-morbidities and this case may have, in fact, been community-associated. How should we interpret finding similar *C. difficile* strains in food-producing animals and humans? First, we can hypothesize that disease occurring in food animal production facilities is largely a result — is the result primarily of animal-to-animal transmission.

This would make sense based upon what we know in human disease; mainly, that the majority of human cases result from patient-to-patient transmission. Of course, we also know that the strains responsible for disease in these two groups are for the most part distinct. There is now growing evidence suggesting emerging community-associated human disease, but we do not yet have much data on the responsible strains.

Also, we do know — excuse me — also, we do not know whether there exists environmental sources or reservoirs for strains responsible for disease in any or all of these populations. Finally, we have no information at this time on transmission dynamics between food animals in humans, either from animals to humans or from animals — from humans to animals.

To answer these questions, CDC is actively investigating community-associated CDAD. We are doing this primarily by working with an established network or partners, namely FoodNet, of CDC's Emerging Infections Program. We are initiating pilot studies to obtain isolates from community cases and to perform cultures on retail meat samples. We are also working with the state of North Carolina on a field investigation to investigate community-associated disease and risk factors. Dr. Jeff Engel will tell you more about this during his presentation.

Turning now to *Clostridium sordellii*, I will present to you preliminary results of our investigations of additional cases of pregnancy-associated toxic shock-like syndrome;

methods we are using for finding additional cases; and what we have learned from studying isolates that have been submitted to CDC for reference testing over the past 30 years.

CDC is investigating additional cases of pregnancy-associated toxic shock-like syndrome. We have found four additional cases in which the pregnancy outcome was either medical abortion or spontaneous abortion — more commonly known as miscarriage. All four cases were less than 35 years of age and occurred since the year 2000. Three of the four cases died.

This slide shows the cases of toxic shock-like syndrome following medical abortion. There had been five previously reported cases in the United States and Canada, all of which received mifepristone and intravaginal misoprostol between six and ten weeks gestation and developed a *C. sordellii* intrauterine infection. Three additional cases are still under investigation by the CDC. Two occurred in the Western United States and at least one was known to have not taken mifepristone.

Intravaginal misoprostol was administered in two of the three cases. In addition, two of the cases had an infection with a different species of *Clostridium*: *Clostridium perfringens*. We should note for the third additional case — Case C — that although initially reported as an — as associated with a medical abortion, we have been unable to confirm whether the patient indeed ever had a medical abortion or the regimen

utilized. In addition, we have found no signs of intrauterine infection in this case. Instead, the pathologic findings consist of appendicitis, serositis and pneumonia.

This slide shows the cases of toxic shock-like syndrome following spontaneous abortion or miscarriage. The two previously reported cases occurred in the second trimester. Both cases had a *C. sordellii* infection, and in one, *C. perfringens* infection was also noted as a cause of death.

One additional case is under investigation by CDC. This case — Case A — was similar to the previously reported cases, except that this patient did not die. Interestingly, we have found that the organism that infected this patient — a *C. sordellii* that was recovered from the blood of this patient — actually did not possess genes encoding lethal toxin.

How did — how did CDC hear about these cases? The answer is through a variety of passive case finding methods: including FDA adverse events monitoring, reports through state health departments, direct reports from academic partners, and for the cases associated with miscarriage, CDC's Division of Reproductive Health's Pregnancy Mortality Surveillance System.

The purpose of this system is to prevent deaths by monitoring trends and identifying risk factors associated with deaths. Due to unique privacy rules, clinical and pathology samples cannot be requested and no identifiable information can be

published. In addition, CDC has been working with our partners in California searching for additional cases of pregnancy-associated toxic shock-like syndrome.

By searching death certificates of women aged 15 to 44 years for indication — years of age for indications of a pregnancy-associated death — for example, anaerobic septicemia, toxic shock syndrome or inflammatory disease of the female pelvic organs — by looking for these causes of death, 321 possible cases from the years 2000 to 2003 were identified. Various numbers of cases have been excluded based upon reasons listed here on this slide so that we are currently looking into 18 possible cases with their status listed.

By testing the bank of isolates received at CDC for reference testing over the past — these past 30 to 40 years, we have confirmed that only a minority of clinical *C. sordellii* possess lethal toxin. This is shown highlighted in yellow at the bottom of this slide. Also in yellow is the number and proportion of toxin-positive isolates received at CDC in each of fast — past four decades. There appears to be no increase in the number or proportion of isolates that are toxin-positive according to this data.

Finally, based upon genetic typing of these isolates sent for reference testing, we have now — we have found no evidence of any epidemic *C. sordellii* strains. Very few of these isolates appear highly related to one another and there appears to be no clustering of toxin-positive versus toxin-negative strains.

There are two pairs of indistinguishable isolates. One pair of these appear temporally- and geographically-related — the bottom pair. The upper pair are remarkable in that they are neither temporally- nor geographically-related. Certainly, further studies into the epidemiology sources and transmission dynamics of *Clostridium sordellii* would appear warranted.

I want to thank you for your attention and also take this opportunity to thank all the people at CDC and our collaborators who have been doing this work. Most, but not all by any means, have been acknowledged at the bottom of these slides where they contributed data, so we'll go on. Thank you.

E. SPEAKMAN: Next, Jeff Engel.

State Initiatives and Performance Characteristics of Optimal Surveillance Systems

J. ENGEL: Didn't want to, you know, upset this machine. Good afternoon, everyone. I'd like to thank the organizers for inviting me to present. It's rather surreptitiously that I'm here because of the timing of the EpiAid to North Carolina that was just before this workshop was being planned. And it was in our state that this investigation took place, so I'm very happy to be here nevertheless.

My goals today — perhaps you're going to think this is more of a high school civics lecture than a *C. difficile/C. sordellii* lecture. But I thought it was important, and so

did the organizers, that it's understood what happens at the state level when a new surveillance program is put into place, or discussed or proposed. So I thought a bit of a background in terms of what we do at the state level would be worthwhile.

So I'm going to talk about the passive surveillance system in the United States from a state epidemiologist's perspective, our legal authority under which that resides. Then, this very strange dichotomy exists in the United States between hospital health and public health. I'm a former hospital epidemiologist and, you know, crossing the rubicon — I guess, if you will — to public health, I'm just repeatedly struck by the difference that exists between the reporting mechanisms of infections in the two environments; that is, whether or not you're in the hospital or in the community.

I'm going to talk mainly about state level *Clostridium difficile* surveillance. I'm only going to mention *C. sordellii* briefly because I think we're at such an early stage of discovery and with the rarity of cases that it's been mentioned earlier, there's really not a lot to discuss with that today. And then finally, I'm going to conclude with what are some optimal performance characteristics of the ideal public health surveillance system so you can get an idea of what I think we should all aim for in studying these diseases.

Our current reporting system for communicable diseases in the United States is a passive one. We rely on reports from our providers through what is known as the Nationally Notifiable Disease System in the United States. The states adopt reporting statutes, which are laws. And these become person-based reports, and therefore,

become a confidential medical record because of those personal identifiers on that record.

In most states, the physician is mandated to report by law if they suspect a person has one of the diseases to which the list of diseases — of reportable diseases exist. And the other big groups that report are laboratory directors. Note that in North Carolina anyway, it is a person who must report. Therefore, you have a physician; you have a laboratory director. You don't have a clinic; you don't have a facility; you don't have a laboratory. It is an individual who is responsible for that reporting.

And indeed, failure to do so is a misdemeanor in North Carolina. It's punishable by as much as two years in prison and a fine. Have we ever done that to a physician in North Carolina? No, but you know how laws exist with the threat of the stick.

States then develop rules that flesh-out these statutes. So if we have mandatory physician and laboratory reporting, well, the question is, "Well, what do I need to do?" So our rules list — have the list of reportable diseases and the mechanisms for reporting. And that's all fleshed-out in the rule.

Further complicating state level surveillance in North Carolina and many other states in the Union, we are a "home rule" state, meaning that every county — all 100 of them — are autonomous. Some of them have consolidated and we have basically now 86 autonomous health departments in the state. And that's where the rubber meets the

road because the physician or lab really must report to their local jurisdiction and then that local jurisdiction reports to the state.

Now some of those reports come directly to the state. Indeed, some go directly to the CDC because the physician sees an issue, is worried about it, and calls the CDC instead of going through their local health department. When that happens, the CDC will call the state epidemiologist up and say, "We got this report from your physician." And then I go to the local level, but you can see how this local/state/federal relationship exists.

Not all states are home rules; some have what are more regional districts. And in fact, the states around me — Tennessee, Virginia and South Carolina — all have regional reporting in their — in their health districts. North Carolina is unique in the mid-Atlantic anyway.

How do these disease reports look? We do it by event code. So if you have gonorrhea, you're reported by "GC." If you have tuberculosis, you're reported by "tuberculosis." Again, these are confidential medical records. And public health is what we call a "HIPAA non-covered entity" in that we have the right to obtain all confidential records on any of the mandatory reportable diseases in the state.

These reports are discoverable in that — in that area lies some of the rub that you're going to hear on hospital issues. Because of the Freedom of Information Act, we must give our records to a request or usually a lawyer. We de-identify them for the

purposes of the lawyer's investigation unless it's specific to a person who has signed consent to release their public health record to their attorney.

I will add that a disease does not have to be reportable to be investigatable by public health. So any disease that we view as a potential threat, we actually have the authority to investigate with all of the laws and rules of public health. And examples of that are what we're talking about today: *C. difficile*-associated disease with our investigation in 2005 and active case finding for *C. sordellii*. Neither of these infections are reportable in North Carolina; they fall into the realm of emerging infections.

So how do diseases then make it to this list — this nationally notifiable list? Well certainly, the cases of emergencies in 2003 was a heck of a year in public health and we added SARS and monkeypox infections to the list of nationally notifiable diseases. There are other public health threats though that tend to sneak up on you and I'm just mentioning two here.

Deaths in children 18 years or younger from influenza became reportable in 2004. That's the first time that a named report related to influenza disease became a nationally notifiable entity in the United States. And that's understandable since influenza seasonally is so prevalent. How on earth could any state, you know, keep up with named reporting of all their flu cases?

And then just this year in North Carolina, we added novel influenza virus to the mandatory reportable diseases. So what does that mean? That's the threat, obviously,

of a pandemic when you might have a type A non-H1, non-H3 virus in a human. We want to make that reportable now in North Carolina. Those are, again, examples of public health threats that aren't actually existing emergencies.

Case definitions, when the disease makes it to the nationally notifiable list, is a consensus statement between the Centers for Disease Control and the Council of State and Territorial Epidemiologists, which is our group. It's the 50 or 55 of us. We have consensus statements. We meet with CDC and we agree upon how we define these cases for surveillance purposes.

Now to the issue of hospital health versus public health. Hospitals or institutions generally do not report any of these problems. In fact, the statute in North Carolina is interesting. It's written that hospitals "may" report. Yeah, if you want to, you may. And this was an extremely political hot potato with a very powerful hospital association in our statement. Whereas, physicians "shall" report, laboratory directors "shall" report. Hospitals "may" report. And that's because information cannot be protected and this is a long debate in healthcare safety. And I'm not going to get into that any more than just by saying what it is.

So healthcare-associated infections never make it to the National Notifiable Disease Surveillance System and that would be for most cases of *C. difficile*-associated disease. However, in North Carolina and many other states, outbreaks are reportable to the local health department, so we would indeed hear about tuberculosis in a long-term

care facility. But I don't think we would hear about an *acinetobacter baumannii* ventilator-associated pneumonia outbreak in a SICU. In fact, I've never heard of such a report anywhere. So this is the — what exists now between that interface between public health and hospital health reporting.

Now I want to update you on what two states are doing now because these are, as far as I know, are the only two states that are doing anything at the state level for *C. difficile*-associated disease. And it's a — definitely a tale of two states here, very different programs, and I'm going to talk you through both of them, starting with Connecticut.

Connecticut's program began with a concern. Their state epidemiologist — a smart guy who's been at it a long time — Jim Hadler, said, "Are toxic strains now emerging in the community?" And I'd like to thank Pat Mshar for an interview here to understand what Connecticut is doing. So the process to add a new communicable disease to the — to the list in Connecticut consists of a committee consensus of hospitals, labs, community input, and then the committee approves the new disease.

So they started this pilot program, which is basically descriptive epidemiology looking at trends. And they're now what they call their "evaluation phase." What they are interested in is community onset *C. difficile*-associated disease. It was made reportable as of January 1st, 2006, so obviously I don't have a lot of data to show you right now.

Their definition was “illness onset while living in the community and no contact with healthcare in the previous three months.” Surveillance in that state for this program is done by infection control practitioners in 31 acute care hospitals. So in other words, to get to the attention of the — of the system, the person must obviously present at a hospital. It’s an intensive questionnaire. I do not know all the questions on that, but I’m sure they’re looking deeply into the risk factors that you’ve been hearing about all day today.

It includes chart review and follow-up at physician offices to see how people do with this disease. What Pat was able to tell me as of May 1, they’ve had 86 reported; 39 have been ruled out; 17 now have been shown to be true community-onset *C. difficile*-associated diarrheal disease; and 30 are still under review.

There is a laboratory component as well. They are collaborating with the CDC FoodNet program. There are 11 sites across the country that are submitting samples. They’re seeking ten isolates of *C. difficile* organisms from Connecticut in FoodNet. The challenges of this surveillance are resources. It initially took three-quarters of an epidemiologist to start this. They’ve now got it pretty well tuned and it’s now down to about .5 FTE.

Their lab challenges are notable because the diagnosis is usually made by an ELISA of toxin A or B. So they want to gather stools to see what’s going on in terms of that epidemic strain. You need the organism, so they’re finding it very difficult to get

labs to store stool samples while cases are under investigation. So that's been a challenge in their surveillance.

Now let's look at Ohio, a very different system. Here, citizen and media concern regarding healthcare facility outbreaks of *C. difficile* began their program. The governor directs the department of health to "act." I hope I never get that call from my governor. I've been at it for four years and it hasn't happened yet, but I would certainly dread that.

Thus, mandatory hospital and long-term care facility surveillance was established on January 1st, 2006: 200 — at least 200 acute care hospitals in the state, at least 1,000 nursing homes now report numerator data only by week. So it's just count data by facility and it's an amazing report. And I'd like to thank Bob Campbell, the epidemiologist at the Ohio Department of Health, for sharing this with me.

So if you were to log on to the Ohio State, this is a screen shot of their cover site — cover page. You can see all the programs under their features, but I highlighted their *Clostridium difficile* initial case reports. You click on that and they give you a very nice description of *C. difficile*-associated disease.

And then if you click on the bottom link, you get a 17-page pdf file that has the county, the institution by name, and the case count by week. So I guess if you're a consumer looking for, you know, a nursing home for your father or mother, you might want to use that. But of course, it's limited and we'll go through why it might be.

So this — the — is a new trend coming now on this mandatory public reporting of healthcare-associated infections. It is now true for community — for *C. difficile*-associated disease. Their report is that the onset must be after — two days after admission. Their new version, however, is beginning to look at rates. So they're going to try to collect denominator data, which is much harder in the hospital, and they're going to do it by bed day or patient day.

So as of April 1, the report is going to now reflect, in their acute care hospitals, true rates by patient day; as of July 1 in the long-term care facilities, rates by bed day or occupied bed day. No risk adjustment, you know, no co-morbidities, no age, nothing. It's just, again, it'll be gross rates by facilities.

The early benefits of this program in Ohio have been that they've been able to establish a secure web-based reporting tool that's statewide, and indeed, that is a big accomplishment. And we're trying to do something similar to that now in North Carolina and we're under development. The educational opportunities are that they've used this as an appropriate tool to bring out that important message of appropriate antibiotic usage and infection control in healthcare facilities.

So a tale of two states. In summary, Connecticut and Ohio have developed surveillance systems: one based on true scientific concern regarding an emerging community health threat; whereas, the other, a public concern regarding a common nosocomial infection. The question is, what health policy is going to emerge out of

these two systems? And time will tell. It'll be very interesting to watch what happens in these two states.

Finally, I'd like to tell you what is going on in North Carolina. Cliff already mentioned the EpiAid. We asked for an EpiAid when Chris Woods, who's in the audience, I think — he's jumping between two meetings: one across the street at the Emory Inn and Conference Center. Chris is a physician and ID doc, former EIS officer at Duke and the Veteran's Administration Medical Center who has a very nice registry of *C. difficile*-associated disease in all the four Veteran's Administration Medical Centers in North Carolina.

And what his registry noted was a marked increase of suspected community-onset disease in veterans. Because of that, we asked for an EpiAid. Cliff sent a crew of folks to North Carolina to spend a week in Durham County to investigate this in a retrospective fashion from January 1st through December 31st, 2005 — the entire calendar year. They looked at our four VA facilities on their very nice electronic medical record: one tertiary care center and one regional hospital as a sentinel surveillance, not that they were targeting those for any other reason.

Preliminary results that classified 625 cases this — so far this year, the community onset is defined as really more of a — it's more of a database definition; that is, the person was in the community when they got sick and the culture of the stool was obtained before hospital admission. But I want you to focus on the — what we're calling

"community-associated;" that is that 24 percent of the 149 cases there because that's — those are the ones that we're going to look at a little bit more carefully.

The definition for North Carolina was "no healthcare contact within two months." And if you were looking now at just the Veteran's Hospital population of 58 patients that made that — met that case definition, you can see these descriptive epidemiologic parameters here: median age, whether or not they were on proton pump inhibitors, on H2 blocker, non-steroidal anti-inflammatory drugs, and antibiotics.

What Dr. McDonald's team did next was a similar analysis as to what you've heard in other studies looking at the known risk of antimicrobial exposure and crossing that with a proton pump inhibitor exposure. They found actually no significant additional risk when controlling for antimicrobial exposure.

But if you look at these 58 patients, what is striking is all those patients with no antimicrobial exposure. And that's what needs more investigation and that's what's underway. For a smaller subset of 33 patients, again, no association with proton pump inhibition when controlled for antimicrobial exposure.

To conclude, I wanted to let you know what an ideal public health surveillance system is and you can think about the Connecticut and Ohio experience as I go through these parameters. I'm reminding you of this definition of a "public health surveillance system." You can read for yourself, but this is from an *MMWR* in July of 2001 that gave very good guidelines on how to set up the ideal system for a population.

The tasks of a public health system are to engage the stakeholder in the evaluation; describe the surveillance system to be evaluated; focus the evaluation design; gather credible evidence regarding performance of the surveillance system; justify and state conclusions and make recommendations; ensure use of evaluation findings; and share lessons learned — all from that *MMWR*.

I want to focus on gathering credible evidence regarding the performance of the surveillance system. To optimize performance of any surveillance system, you really need to focus on these functionalities or parameters: simplicity, flexibility, data quality and acceptability. And you'll — I'll have you go through the definitions and you can think about the Ohio and the Connecticut system as we look at these parameters in a surveillance system.

And concluding with sensitivity, predictive value positive, representativeness, timeliness and stability of the system. I can give you some examples of setting up what you think is going to be a great system and then you fall down because of the timeliness issue.

And it has to do with that: the delay in the — from the onset date of symptoms from the patient, to the time the report makes it to the local jurisdiction, to the time it gets into the state's hands for analysis, and feedback to the people who need to know. Timeliness is one of the things that is — it's just — it's one of the easiest things that can go haywire in any new surveillance system.

However, the ultimate guideline for states — and I would argue too for federal people — are the resources to do the work. The Ohio Department of Health received no additional resources to begin their healthcare-associated CDAD surveillance; that's 200 hospitals, 1,000 nursing homes — no additional resources. I — we'd have — if that happened in our state, I'd have to give something else up to do that — just to do that amount of work.

Connecticut, however, is part of what is known as the Emerging Infections Program, which is 11 states in the United States. They participate in the Active Bacterial Core Surveillance and the FoodNet programs. And they, because of that, they've been funded like the other ten states with extra federal dollars to do these emerging-type problems in the United States.

And in North Carolina, we're not an EIP state, but EpiAids are free. I love them. The CDC comes in with a core of really good scientists. We learn a lot. I wish they could stay. So here are the EIP states. Most of these were funded in the early '90s. There was a second wave of funding in the mid-'90s.

Unfortunately, North Carolina is not one, but you can see that — nor is Ohio — but you can see that Connecticut is an EIP state. And I think as we move forward with learning more about state level surveillance for new infectious diseases like *C. difficile*, we're going to look to these EIP sites for getting those programs up and running. Thank you for your attention.

Panel 2 Discussion

E. SPEAKMAN: A few questions to the panelists for clarification and then we'll turn it over to the participants. The first question — either one of you — is what do you feel the priorities for *Clostridium* disease surveillance in terms of populations, first part of the question, and the second one, form of disease? Any thoughts on what the priorities are?

C. McDONALD: Yes. For the federal level, it's — and probably also the state level — is healthcare-associated disease in terms of that's where the major burden is. But the federal level also, I think, has a responsibility to understand any emerging forms of disease as well.

So healthcare-associated disease, and as I tried to indicate, what the federal government can do is probably try to bring order, and guidance and some parameters to try to get things done the same way. In emerging disease forms, as we discussed, community-associated disease in special populations, those, I think, are the priorities for the — for federal side of things.

J. ENGEL: Yeah. I think in terms of the state or the public health getting into the business of hospitals is going to be an ongoing continuing challenge. However, we're looking at this mandatory public reporting of hospital-associated infections that is now coming into being. And many states have passed such legislation as beginning the opening of those doors.

For example, if we had a simple intervention — like for most hospital-approved disinfectants or quots that don't inactivate *C. diff* spores, if we could put a simple intervention that would replace it with a dilute bleach solution for routine terminal cleaning of rooms, if that could make a big impact and we could monitor that at a state level for healthcare-associated disease — that the simple infection control intervention can reduce disease, that's the type of thing that I think we can look toward doing.

But we will only do that when hospitals begin to share data in a standardized fashion across the board. That's a challenge though. And then secondly as Cliff said, we need to understand what this community-associated disease is all about. We may be dealing with a very separate issue there.

C. McDONALD: In terms of *Clostridium sordellii*, I think the issues right now are case finding, and case finding, and case finding, and just leave it there and then whatever creative mechanisms can be used to do that. The trickier issues maybe come down to is how broad to cast the net? What exactly is the entity we're most interested with *Clostridium sordellii* disease? Is it only pregnancy-associated? Should we be going beyond that — those substantive questions?

E. SPEAKMAN: That leads into the — this — the next question of, you know, what's the most promising methods to conduct human disease surveillance?

C. McDONALD: For healthcare-associated CDAD, again, electronic reporting holds promise. This is predominantly diagnosed through laboratory tests. There are all

the caveats of laboratory testing and this assumes, of course, that only liquid or semi-form stools are being tested for *C. difficile*. There's all the limitations of diagnostic testing which go beyond surveillance certainly.

But once those have been worked out or hashed out, it does hold promise at least that it could be quickly channeled and conducted in that fashion in most cases. A positive laboratory test on a liquid stool would be a surrogate at least for *C. difficile* disease. The — right now where we're at also though is just working out the — what defines the period of risk of exposure to a hospital?

Is it just while you're in the hospital or does it extend out for some period afterwards? And certainly, the movement right now in many of these other places is to go out four weeks or so. I'm talking about like in England — well, in Canada specifically. And I know the European Union is talking about the same type of idea of conducting post-discharge surveillance for *C. difficile* out to one month.

E. SPEAKMAN: And so electronic reporting for healthcare-associated or community-based?

C. McDONALD: Yeah. It would be electronic reporting using the hospital as the — hospital laboratory as the source of reports. That, of course, assumes that people come back to their same facility where they were admitted before, which is problematic we know in other post- — when we do post-discharge surveillance for, say surgical site infections.

We know that it's problems in terms of actually capturing those cases that leave the hospital and you've had a lot of experience with that kind of thing as a hospital epidemiologist. At least with *C. difficile*, we're only looking at a month. If we're going to do post-discharge surveillance, it's only going to be a month. And the hope is that most of those go back to the same hospital for testing where they left before.

E. SPEAKMAN: Anything to add on that, Jeff?

J. ENGEL: Well, I'll just reiterate that if we were to bring on *C. difficile* at a state level, it would absolutely depend on electronic reporting. And to that end, as we're developing our system to replace our current paper-based system — it's called NEDSS, the National Electronic Disease Surveillance System — electronic laboratory reporting is a key component of our requirements for our electronic disease system.

So if we have true electronic lab reporting — not just from hospital labs, but from big community labs like Lab Core which is a big presence in North Carolina — we could set up a pretty efficient surveillance system. It would be flawed, but at least it would be consistently flawed if you know what I mean. You could definitely know when you have outbreaks, I think. And you could also look at interventions and see if they're making an impact.

E. SPEAKMAN: Good. Thank you. A last question: are there any animal or environmental sources of human exposure to *C. diff* or *C. sordellii*?

C. McDONALD: Well of course with *C. difficile*, that's why we did present that data. We want to be transparent about that; that there are or we're reporting today is that we have found strains of *C. difficile* that have been epidemic in food-producing animals. Those same or similar strains are also being seen at a very low rate. I mean, we have hundreds of *C. difficile* isolates — and I showed you seven cases — but at a low rate in humans.

That, of course, does not mean that we have any evidence that there's transmission through the food supply or any other means. There's no evidence of that at this time, but it does mean that it's something that we want to actively look at; that animal health can affect human health we know in other instances. But also, there's very much the possibility that there's something going on in the environment or sort of subterranean; that a bigger picture view that there's something perhaps going on in *Clostridium difficile* and maybe *Clostridia* in general.

And we've heard that there is some shared regulatory genes and whatnot that could be going on in the environment at large and is affecting both human health and animal health in somewhat a similar time frame because the — as Glenn Songer can attest — the outbreaks in animals that he's been seeing really started occurring around the year 2000, which is right where we saw that — the epidemiology take off in humans also. So it may just be something that's happening in both populations simultaneously …

E. SPEAKMAN: Great.

C. McDONALD: ... and it's really in the environment.

E. SPEAKMAN: Any questions from the audience? Okay.

M. CRAWFORD: Can you hear me? Mark Crawford. I just wanted to know for mifepristone-induced medical abortions, what efforts are being made to evaluate adverse event reports or data from other countries, like China or in Europe?

C. McDONALD: We are evaluating reports that we hear about through — if we — when we hear about possible reports from other countries, we are inquiring of them, even would be interested to work with them to try to get tissue in the case of the Canadian case, evaluate tissue here at the CDC. And so we would do that as those cases became available to us or we were notified of them.

We've spoken with other countries directly who are interested in trying to case find. We all have the same issue of case finding. And again, getting back to that issue as should necessary — should case finding only be focused on mifepristone medical abortion or should it be broader? Our feeling is that it should be broader, but inclusive of that. So the quick answer is just as we hear about those cases, we do investigate them and discuss those with other countries.

E. SPEAKMAN: One more question?

B. WINIKOFF: Yes. Would we know for *C. diff* ...

E. SPEAKMAN: Sorry, your name?

C. McDONALD: Oh, and the microphone.

E. SPEAKMAN: Microphone and name?

B. WINIKOFF: Right. Sorry. Just clearly, do we know about how many estimated deaths and how many estimated cases there are of healthcare facility-related *C. diff* in the United States?

C. McDONALD: Okay. Well, "healthcare-associated" is a very difficult term. That means, of course ...

B. WINIKOFF: Well, use your own term.

C. McDONALD: Let's just say in hospital discharge release, I showed you that data.

B. WINIKOFF: Yes, you did.

C. McDONALD: The hospital discharges ...

B. WINIKOFF: Yeah.

C. McDONALD: ... from acute care hospitals, that survey suggests in 2004, 211,000 hospital discharges had indication that those patients had *C. difficile*. Now the question comes as how sensitive is coding data because that's what that was based upon for all *C. difficile* infections.

One look you can get at is actually from Ohio. They do have their cases now on the web and you can actually go to that site and look at the total number of cases. I

don't have that number in front of me, but it's several thousand. They also are looking at long-term care facilities, by the way, because that's another major ...

B. WINIKOFF: Yes, I know.

C. McDONALD: ... proportion of cases which the survey, the data I showed you did not include long-term care facilities. Based upon them being about 125th of the U.S. population, if you take the total number of cases that are reported over the four months — their first four months of surveillance, both in acute care and long-term care — we're looking more at around 400,000 cases.

A number that we've thrown out before is maybe it's really more like 500,000 when you get the community cases too. You get the fact that no surveillance is 100 percent sensitive and that has been a number we've thrown out just because it's a round number: half a million cases a year.

But I've heard other — that's something we've said; CDC has said publicly before — but I've heard other outside experts try to suspect that it might be maybe a fold higher than that, but that's at least where we have data; 211,000 in 2004 maybe the true rate. When you add long-term care facilities, you add community, add the insensitivity of coding, maybe it's more like 500,000.

B. WINIKOFF: And does discharges include deaths?

C. McDONALD: I'm sorry. Now deaths is — there actually is data in the — in the discharge — in the National Hospital Discharge Data Set, there is numbers of people with discharge who actually died rather than discharged ...

B. WINIKOFF: Yeah.

C. McDONALD: ... and the number who had *C. difficile* listed as one of the diagnosis. Now realize that's one of eight diagnoses. We have no idea whether that's attributable, and in most cases, it's not. Okay. Historically, around only 1 percent to 1½ percent of people with *C. difficile* infection died of attributable *C. difficile* — a death that was attributable to that infection.

Now we know with the new strain in Canada where these studies have been done, that 30-day mortality is more on the order of almost 7 percent, upper 6 — 6.8 percent. So it'll depend a little bit on the strains, how severely they're affected. But I think you could make some estimates of the number of attributable deaths based upon these figures for yourself.

E. SPEAKMAN: In the back left?

A. ALLINA: Thank you. Amy Allina from the National Women's Health Network and I'm here today on behalf of a number of reproductive health organizations. Women's health experts and advocates are, you know, across the country are concerned about the deaths — while rare — of women from *C. sordellii* following

medical abortions. So we're very glad that you're having a meeting today to address it in the context, you know, the broader context of the emerging infections.

But, and we have a few questions that we want to put out there. We're really glad to be able to be here learning from your discussion. Some of my questions do relate to surveillance and others don't, but I'm going to take the chance to just put them all out here so you don't have to hear from me again.

E. SPEAKMAN: We don't have much time so ...

A. ALLINA: I'll be quick. I'll be quick. The first is whether there are examples of *C. sordellii* infection among the obstetric and gynecologic patients who've survived the infection? And if I understood Dr. McDonald correctly, I think the answer is at least possibly yes. And if that's true, then we would like to ask you to look into what sets these cases apart and whether the cases provide any insight for treatment options or guidelines?

Then are there — are the confirmed deaths from *C. sordellii* following medical abortion related to an alternate, unusual or a mutated form of the organism? A pressing question that came up earlier today with the panel was in the context of the abortion care. Is the role of prophylactic treatment with antibiotics — and as some speakers noted and also the FDA has cautioned — there are questions about that approach?

And with limited data, it's not really clear that the benefits outweigh the risks. So we would really like to ask for prioritization of a research agenda that will help

healthcare providers better evaluate treatment options for patients, and then just finally, if there's a potential antitoxin that could be developed as a reasonable approach to saving lives from this infection.

So I'll just finish by saying that we really want to express our commitment to work with everyone here on determining prevention and treatment options, particularly as it relates to obstetric and gynecologic care.

E. SPEAKMAN: We'll look at — we'll look at priorities coming up.

A. ALLINA: Thank you.

E. SPEAKMAN: Do you have any follow-up with that?

C. McDONALD: Yeah, just in response, I think some — they're not the priorities, but you asked the question about obstetric infections with *C. sordellii* that were (a) not induced abortion-related. And yes, I did mention one. There were the two that were recently reported. That was, again, from the pregnancy mortality surveillance system. Those were reported as a letter in the *New England Journal*; they were on my slide.

And then case A, which was a Midwest — case from the Midwest. It was a spontaneous abortion or miscarriage and that woman did survive. Interestingly, the *C. sordellii* was recovered from the blood and that — but also interestingly on that isolate, we have — we've looked for the lethal toxin gene and it is not present interestingly enough. So we don't know exactly what to make of that right now, but just another thing that I think we want to continue to follow and look at.

A question about confirmed deaths — if there's any evidence of a mutated form, remember that all these cases that you've heard about have been diagnosed postmortem through non-culture means. This is through DNA amplification from tissue. All the *Clostridium sordellii* that we've talked about today in women were diagnosed in that way. And so all we're able to do is say that *Clostridium sordellii* is present; that the — it is actually also looking at the toxin.

So we know it's not only a *Clostridium sordellii*, but it's a *Clostridium sordellii* with the gene for lethal toxins. So that's what we know about these cases. We can't say anything further about mutations in regulators or anything of that nature. And then I think the prophylactic antibiotic issue has been discussed already.

The only thing that I think we brought to the table also is that there is *C. difficile* disease occurring in pregnant women, albeit that that is also relatively infrequent when we look at the — if we'd done the same survey of ID physicians for *C. difficile* disease in general, they'd all be reporting to us hundreds of cases — well, not hundreds, but you know, tens of cases, tens and twenties of cases, not a couple of cases here and there. So it's still relatively infrequent, maybe not rare, but uncommon: *C. difficile* disease in pregnant women.

A. ALLINA: Thank you very much.

E. SPEAKMAN: Thank you very much. One last question?

D. HAWALESHKA: Thank you. My name is Dan Hawaleshka. I'm a writer for ...

RECORDER: Please turn on your microphone, sir.

D. HAWALESHKA: I did.

RECORDER: It's not on.

D. HAWALESHKA: How's that? Better?

C. McDONALD: No.

D. HAWALESHKA: How's this microphone? If you want me to just speak up ...

RECORDER: Sorry, you have to be on the mike.

D. HAWALESHKA: I wasn't clear on how you felt about the role of PPIs in community-acquired CDAD. I've been getting different messages. Some people think it's involved; some don't. Could you recap how you characterize the link if any?

C. McDONALD: Well, we gave you two sides of the issue actually here also because two studies that CDC is involved in. And there's previous studies I've been involved in too: some were positive associations; some were negative. Of course, what we do is look for epidemiologic associations and so I presented some data from the VA Medical Center. And actually, maybe Bob Gaines may be in the room if he wants to comment also. He might have some more information to share.

But basically looking at the — just for those cases, it did seem that there was this interesting overlap, such that those who didn't have an antibiotic were more likely to

have the proton pump inhibitor. So that's interesting. It's not the be-all and end-all because there are other studies. Like Jacques Pepin's study in hospitalized patients where he carefully controlled for other confounders, did not find it associated.

Could it be that the PPIs are somehow associated more with age or something like that in this group that we've not looked at yet? We don't know yet. We're not there yet. I think from the epidemiologic data, it's going to be hard alone. It would be very nice if we could look beyond the epidemiologic data and to look at if there could be a model developed that could try to show how it might be doing it.

We've been focusing a lot on the acid suppression activities and there's good reason to. Because oftentimes you'll see H2 blockers, the — those are the histamine-2 receptor blockers that are also acid-secreting suppressors — often, you'll see in epidemiologic studies, you'll see the PPI is associated with usually an odds ratio of 3 and the H2 blocker with an odds ratio of 2, suggesting that it is something to do with the acid secretory ability of those drugs.

But the other thing is that the PPI agents, the proton pump inhibitors, some of them do have or maybe all of them have some antibacterial effects that we need to look at more closely. And perhaps Bob can comment further or you can talk to him also — Bob Gaines in the back of the room, right? So I don't — my title was — my slide was still titled the way I want it.

It's that we are finding evidence that from that — from this VA population here at least evidence to suggest the PPIs are playing a role. But is it the final study? No, but it's a reason to keep pushing forward and keep studying this issue until we understand it. It has tremendous repercussions as you can imagine. PPIs are used widely and now with over-the-counter use, ever widening use.

E. SPEAKMAN: Sounds like a potential priority for a research agenda moving forward, could be? All right. Did you have a question in the far right?

B. KRUSE: Yeah.

E. SPEAKMAN: Yeah.

B. KRUSE: Beth Kruse. I think it's one of the things that I've really been enjoying about learning today is the ways in which *C. difficile* and *C. sordellii* have some similarities and some differences. And with the deaths from *C. sordellii* that are so disturbing, but also, there's been more deaths from fulminant *C. difficile* and how that relates to pregnancy.

And it reminds me of particularly, you know, when Amy was talking about from a women's health standpoint of the time when toxic shock syndrome deaths sort of hit the airwaves. And I remember seeing a reference to an article, a recent article in the *Journal of Clinical Microbiology* about an increase in toxic shock syndrome between the years of 2000 and 2003.

And so I'm wondering if you could speak to that at all? If there's any way that looking at that might tie into this or how, you know, how there might be other features that, you know, from yet another branch to look at?

C. McDONALD: We were aware that reported in the toxic shock syndrome. I think there was some reasons in terms of actually confirmed isolates and some other issues. We're not able to confirm that basically, that toxic shock syndrome has been increasing. We're talking now. We're just sort of — understand, we're using the term "toxic shock syndrome" several-fold over.

That article you're talking about is staphylococcal — *Staphylococcus* toxic shock syndrome, right. Right. And we have not been, as far as I understand, we have not been able to confirm that. And if anyone else in the room wants to get up and speak to that, they can, but — from CDC. But no, we've not been able to confirm that, no. In terms of — speaking for CDC, if someone knows from CDC and some — knows something more, but we've not been able to confirm that just from the — some data.

That is not something we've been able to confirm and so I don't really know what to say about that. It's a different organism altogether. We do know there are issues with staphylococci and with the — especially with the emergence of methicillin-resistant *Staphylococcus aureus* in the community, more virulent strains of that. But in terms of that — and maybe you can speak to the toxic shock syndrome also?

D. STEVENS: Well ...

E. SPEAKMAN: Turn your mike on.

C. McDONALD: Yeah, it's not — it's not on.

D. STEVENS: Dennis Stevens. Yeah, I think that the — clearly, there's some data to suggest that some of the methicillin-resistant strains, the community-acquired strains have some of the staphylococcal enterotoxin genes in greater proportion than hospital-associated and methicillin-sensitive strains. So there are strains out there that are endowed with the genes which can cause toxic shock syndrome.

And the only potential way to connect all this stuff is maybe through fluoroquinolones, where fluoroquinolones clearly have been associated with an increase in *C. diff*. I think you and others have pointed that out. And clearly, fluoroquinolones are also a big risk factor for methicillin-resistant staph, so maybe that's the common denominator.

C. McDONALD: Yeah, I was speaking directly to the toxic shock syndrome toxin. You're talking about other toxins as well, right?

D. STEVENS: Well certainly, toxic shock syndrome can be caused by either enterotoxins or TSST-1.

C. McDONALD: I was just speaking to TSS.

D. STEVENS: And the USA-300s are more endowed with the enterotoxins.

E. SPEAKMAN: All right. A round of applause for this — we are — we are running about 15 minutes late right now. My guess is that we need two hours

discussion on priority to push adjournment closer to 5:00, just letting those aware that need to leave at 4:45 that there — things may run a few minutes over.

I want to give enough time obviously for the priorities. We'll also allow a few minutes during the discussion for any more clarifying questions to help provide the priorities. So 15 minutes, come back and we'll get started looking at priorities for a research agenda.

[BREAK 2:48 P.M.-3:10 P.M.]

PANEL 3: IDENTIFYING A RESEARCH AGENDA

E. SPEAKMAN: All right. If you could go ahead and take your seat, let's get started. All right. Could somebody please shut that back door? I thank you all for coming back and here is — we've heard so far today basically what the current situation, some of the needs. And now we want to begin focusing those efforts down into determining recommendations for a research agenda.

Again, I want to reiterate that in today's workshop, the goal is not to come up with sort of consensus-based decision-making. This is for a discussion, and dialogue, and that we have many processes in terms of next steps collectively and then from all the agencies moving forward to get into decision-making and then into action.

Okay, so the reality is with another — an hour and 45 minutes, we can't get consensus-based decisions as saying "definitely this," "definitely that" over the time. So I just want to let people know that reality before we get started with this session.

Our goal in panel 4 on identifying a research agenda is basically to provide recommendations for research agenda priorities under three main categories: surveillance and epi; basic research is the second one; and the third: diagnosis, treatment and prevention. This panel will be chaired by John Bartlett and the first presenter will actually be Dale Gerding to talk about his initial thoughts on priorities for *C. difficile.*

D. GERDING: Actually, I'll be very brief; I don't have any prepared issues or a shopping list. But I will say that I think that taking off from the previous panel that surveillance of some kind is desperately needed; that we have to have systems similar to what's going on right now in Quebec where they actually are getting reports of good epidemiologic data on *C. diff* rates, collecting isolates.

And that point actually is a second research point that's exceedingly important. We're not getting enough isolates of this organism in particular to really figure out what's going on, so we need better access to isolates. That's a problem of hospitals not doing cultures in isolation and we need to improve that. And then we need to, in addition, improve our ability to characterize those isolates once we get them by typing systems,

by analyzing them for toxin, and at that point, by starting to look more carefully for virulence factors in these strains.

We clearly do not know what all the virulence factors are in this new epidemic strain. We have identified a number of new or different characteristics of those strains: that is, their fluoroquinolone resistance, their binary toxin, their increased production *in vitro* of toxin A and B. But clearly, there may be other factors involved with those strains that will better characterize them.

So we need much better virulence characterization of those isolates. We need to be able to take those isolates into animal models where we get some comparative data of just exactly what's going on. We are doing some of that. The animal model is very time-consuming, tedious.

A hamster model, in order to keep out wild strains from contaminating the animals, you need to use extremely careful, much better than hospital infection control measures, believe me. Everybody in gowns, and coveralls, and booties and, you know, sterilizing the room with bleach and sterilizing all the food in the cages. So it is a major undertaking. It's one that we really clearly need to do more of though in order to advance the field.

The prevention and control in hospitals is another big area of research that continues to demand our attention. We're starting to move now to increasing

environmental disinfection using bleaches. There's hydrogen peroxide methods that are also being adapted. I think those are excellent.

We need to resolve the question of the alcohol gels and their lack of sporacidal activity. No one seems to have found increasing rates of disease associated with alcohol gels, but I think we're all concerned about that. And we need to determine when it's appropriate to use hand hygiene using hand washing versus alcohol gels.

And the issue in hospitals of whether we can affect or control these rates by the choice of our antimicrobial use is also one that has huge opportunities. We've been able to do this very effectively with clindamycin and I think we have an opportunity here to potentially do it with other antimicrobials in terms of how we use them in the healthcare setting.

The diagnostics, I didn't mention, but I did earlier this morning; that we do have really a crying need for fast and a sensitive test for *Clostridium difficile*. We are doing way too much empiric treatment because we know that the test is not very sensitive. And we know that failure to treat a patient who has the disease because you have a negative test can result in fatal illness.

So we need better testing, and it has to be rapid and it has to be sensitive. And right now, we have not got those kind of operating characteristics in our current diagnostic tests. And I said I would be brief and I will stop there. Thank you.

E. SPEAKMAN: Thank you, Dale. With *C. sordellii*, David Soper.

D. SOPER: Okay. I'll try to go per instruction through the different categories. Epidemiologically, I think where are the cases and in whom do they occur? Is this really a problem and how widespread is it? And how do we go about ascertaining that? And is the pregnancy mortality system adequate for us to be able to determine abortion deaths?

What proportion of these are due to infection? What proportion of these due to toxic shock syndromes? And what's the deal with all of these cases being in California? I think that CDC is doing some more active surveillance in that state, which would be a recommendation, and it will be interesting to try to further investigate that.

Can the institute place a call-out to infectious disease experts in the country and through the organizations of IDSA and IDSOG to let them know that they can send specimens to a central repository, if you will, for investigation to these unknown causes of death that might fit at least a case definition of *sordellii*-associated toxic shock, and therefore, identify the microorganisms? So that ID clinicians that are exposed to these unknown cases that are pregnancy-related and are wondering what the etiology is might have a resource by which they can figure out that this indeed is *Clostridium sordellii*-associated toxic shock.

Diagnosis, treatment and prevention — is there a continuum of infection? In other words, if you — if you look at all of these cases, what you find out is that some patients have a run-of-the-mill bacterial infection that in my view is not associated with

toxin production; that leads to cellulitis or severe soft tissue infection; that when treated in the usual fashion with surgery and antimicrobials results in response to therapy as opposed to the patient; that has a very rapid downhill course associated with toxin production and death.

Essentially, such a rapid downhill course that there really is little opportunity for any kind of therapeutic intervention at all. Therefore, the issue of prevention. Again, respiratory infections in nine gynecologic non-obstetrical soft tissue infections tend to respond to therapy, although there are some mortalities. But pregnancy-related infections uniformly result in death. Why is that?

And if there is a continuum, are there biomarkers that can alert the clinician to the need for aggressive intervention: for example, intravenous immune globulin, which might have an antitoxin in it and surgery? Is there — are these biomarkers identifiable for early disease? Because it appears that early antimicrobial therapy, and if the patient can get to the operating room and get surgery, it's still not enough. Because in pregnancy-related *sordellii* infections, the patient still succumbs.

What is the — can be the impact of antitoxin? We know that both lethal and hemorrhagic toxins associated with *sordellii* or similar to *C. difficile* A and B and that there have been some work, I think by Dr. Bartlett, that looked at *sordellii* antitoxin that could neutralize *difficile* A and B toxins. So is there an opportunity to possibly

manufacture? We get this IgG, make it universally available through IV/Ig or some other potential, more uniform available.

It has to be uniformly available and on the shelf; otherwise, you don't have enough time to respond to this kind of problem. The good thing is the fact that patients do take a couple of days after delivery, after miscarriage, after therapeutic abortion to actually develop the syndrome, suggests that there might be a window of opportunity for intervention. But I'm not very optimistic about that.

And lastly, Dr. McGregor covered this a bit. But should antimicrobial prophylaxis be recommended for medical abortion given that all patients that undergo surgical abortion are prophylaxed with either doxycycline or metronidazole? Since the risk of fatal anaphylaxis with penicillin is estimated to be about 1 in 50,000 in this country, that risk is greater than the risk of *Clostridium sordellii* toxic shock.

So if you use that beta-lactam antibiotic, you may get more deaths than you really bargained for. But if you use non-beta-lactam antimicrobial prophylaxis, what is the more serious adverse effect? And given the fact that death by infection has increased a bit in at least with the case ascertainment that's gone on now with medical abortion isn't indicated to give antimicrobial prophylaxis.

I think we have to be somewhat tentative in looking at that in that all the data related to *Clostridium difficile* infection suggests that antimicrobials increase the risk of infection. So if all of a sudden we starting prophylaxing all patients with medical

abortion, we actually could potentially see an increase in *Clostridium sordellii* infection and death.

And basic science issues — as many as 43 different strains of *sordellii* exists, but not all are toxigenic. What indeed holds the key for toxin production? Is it interaction with local conditions? Is there some sort of interaction with medicines that play a role? Is there some epidemiologic reason that these are always seem to be associated with pregnancy-related infections? And does pregnancy hold the key to toxin production?

If you look at the presentation of these illnesses, they always come after delivery, after miscarriage, after passive of a therapeutic abortion. And at that point in time, there's tremendous changes that are going on in the body, both hormonally in the micro-floor of the vagina. And none of these patients with *sordellii*-associated toxic shock associated with medical abortion had any retained products of conception, which would put them at low risk or no risk for infection.

So what's the deal with the changes, I think, more important than any absolute levels that are occurring any time that occurs with pregnancy-related-associated toxic shock syndromes? I mean, what stimulates toxic production in the first place? I mean, we know that there's something that stimulates *botulinum* toxin. We know that there's something that stimulates *tetani* production. What's the deal with *sordellii*?

And although we learned a bit about this, about the mechanisms of toxin production, is — and that there is an animal model or maybe that could be a

development of an animal model to determine if antitoxin's effective in actually preventing the overall syndrome in general.

And of course, there's tremendous interest these days in individual patient's ability to respond to different infectious insults, so all their genetic polymorphisms that actually explain patient's response to either the toxin or to the microorganism. I think that's it. Thanks.

E. SPEAKMAN: Okay. Thank you, David. And Cliff, you want to talk about surveillance related to both *C. diff* and *C. sordellii*?

C. McDONALD: Actually, I'd like to talk first about surveillance and then public health research also. In terms of surveillance, some of this I've said already; I'll say it again. The priority for *C. difficile* for surveillance should be healthcare-associated *C. difficile* disease at this point in time. And this would include both acute care hospitals, but also long-term care facilities, which we also mentioned. For *C. sordellii*, it is in the area of case finding.

In terms of public health research, which is — which goes much broader, one area — the first area probably — well, not necessarily the first; this is not necessarily in priority — list of priority, but public health research in the area of better defining the epidemiology in a way that could help assist us with making proper surveillance definitions. This would include determining just how long after leaving the hospital are

you likely to get — to manifest disease that was acquired in the hospital and this will make a big difference in the — in the burden of surveillance reporting for hospitals.

I mentioned already that we'll probably go forward, depending on input from partners, with the idea of one month post-discharge surveillance as, at least as a — as an optional form of surveillance. But is that even necessary? Would surveillance be adequate just to look at all cases that occur while someone's still in the healthcare facility? We don't know those things.

And we also need to know just what is the incubation period of *C. difficile*? I know Dale presented some data that suggests it's very short; that you are not colonized with *C. difficile*, then you get exposed, and then you develop disease and it may be within seven days. But if we're now talking about stretching that out to one month, has something changed, or is that just something to do with the way people report their disease or things like that?

And so these types of issues can be answered with better kinds of difficult-to-do studies. And they probably need to be redone where you culture people over time, and look at when they acquire a new strain and when did they develop disease. And then what — how are they related? Where was that likely acquired?

Also, I've already mentioned that we do need to go forward with public health research. I won't call it "surveillance" in these special populations like pregnancy; continue to monitor that. Is that on the upward trend or is it — is it just something that

we've just gotten aware of just because the overall disease rate has increased a little bit to — gotten to a point that we're all looking at it and we're just — so I think to check that periodically over the next several years will be important.

And of course, the community-associated disease, a lot of questions there. Again, although Connecticut has started to perform surveillance, that's going to be a short-term period. It's really almost a public health research, again, and we are doing those. I've mentioned FoodNet. The priority there, of course, is to get isolates. And this sort of dovetails with what Dr. Gerding said; is that maybe the approach should be more.

And I think he was intimating this; that we should be looking at our neighbors to the North and across the Atlantic and getting some type of large surveillance system where we get isolates on a periodic basis so that the next time *C. difficile* shifts its virulence, we won't be caught. Really now, what may have started happening in the early 2000, right?

This probably all started in the year 2000 when the — when the discharge rates started going up so that we know those things a little sooner. Certainly, England knew the introduction of the new epidemic strain to their hospitals very early in the whole process. So a large sentinel surveillance system where isolates are collected at different points throughout the country would be a priority as well.

And then I've mentioned already, of course, this issue of sources of infection. And I do think we need to look in the environment further. We talked about the hospital environment and that's another big area. And it opens up the whole area of prevention research. Let me turn to that in a moment, but also environment in the home, in the community and including the food supply.

In the hospital environment, we talked a little bit about some of the innovations. We have not talked a lot — much about prevention here today at all. We know that, okay. Prevention of *C. difficile* is, of course, largely the prudent use of antimicrobials across the board. And then in healthcare facilities, it's both the prudent use of antimicrobials and interventions to prevent cross-transmission from patient-to-patient.

There's a lot of different research that could be done, that needs to be done, and some of it is being done and will continue to be a priority. The reason why we didn't focus so much on that today is because we felt like the audience and the group that we're bringing together here is not as heavily represented by infection control. We know there are some here.

And if you're here and you're wondering why aren't we talking prevention in infection control, that's not because it's not important. It's very, very important. But it's just that we couldn't do everything for everyone and so that is a whole other area that we've not talked probably enough about.

Dale mentioned hand hygiene and this issue of alcohol gel. I think this goes across healthcare-acquired infections though beyond just what agent used to clean your hands. How can we improve hand hygiene to prevent cross-transmission? And then the issue of the environment: what are some innovations that could really make a difference?

And I think you might've touched on it; that probably only a minority of cross-transmission is really — cross-transmission in hospital occurs through the environment, but that may be different when you go into outbreak settings. In other words, hospitals that have very high rates, the environment may be playing a bigger role. So optimizing strategies to reduce environmental contamination are still important, but they probably fit in a little bit below hand hygiene and stopping the transmission through the hands of healthcare workers.

Public health research in *C. sordellii*, again, really comes back to case finding. It also does include risk factors and I should go back to *C. difficile* also. The risk factor issue with PPIs, we've already mentioned that. That has to be a priority too for *C. difficile*. We need to get that solved. Is it or is it not?

And if it — if it is, we probably need to start quickly coming to grips with what is the model? How has it happened? Is it the acid suppression? Is it — is it other things? I think that about encompasses through the public health research and surveillance. And I kind of mixed them in there together. Did you get all that?

E. SPEAKMAN: Cliff, the needing more isolates and, you know, better definition, the epi, you know, so it's consistent across. I assume that applied to both *C. diff* and *C. sordellii*?

C. McDONALD: No. No, that was for *C. difficile*. Of course, we didn't actually — I'm glad you brought that up. Yeah, I had that on the bottom here. You know, what has happened to anaerobic bacteriology in the United States in the last 30 years? And some of you know exactly what's happened and laboratories are increasingly being compressed, their budgets. These are clinical laboratories we're talking about.

The budget crunch is bearing down hard upon them and they're looking constantly at what is the clinical benefit of each culture. And a lot of the sense in clinical infectious diseases has shifted toward, well, anaerobic infections are somewhat predictable in terms of their coverage of antibiotics. I'm not saying this is all right; I'm just saying that this is sort of what happened. And therefore, determining the actual cause of an anaerobic infection is not as important as just saying it's an anaerobic infection or not.

This is clear areas where there — we're kind of shot in the foot. Because I don't know if you remember that slide of the number of isolates sent to CDC over the last 40 years of *C. sordellii*, but the biggest number was in the 1970s. We had 30 isolates. And the last several — I think in the 1980s, we had one isolate or something.

And a lot of that — and I don't — I didn't present that, by the way, to think that's really scientific surveillance of virulence in *sordellii*. I'm just trying to get what can we learn from that. That's the only reason I presented it, but it does say something about anaerobic bacteriology. And we need to find some — if we can't — we're not going to be able to overnight increase the budgets of clinical laboratories.

But what can we do in public health to stand in the gap — to sand in the gap of that sentinel surveillance that was always there? There were clinical labs always there doing that: getting the isolates, they could send them on up. What can we do? Even the area of anaerobic susceptibility testing, you know, what do we have there that's really a safety net, if you will, if suddenly *Clostridium difficile* tomorrow would develop resistance to metronidazole?

Now certainly Dale Gerding, your laboratory would pick it up eventually. But is "eventually" good enough? But certainly would find clinical failures and everything, but what if it's a slow creep? Should someone be minding the — minding that switch, if you will, or minding that issue? Because there, just so you're aware, almost no clinical laboratories perform susceptibility testing on anaerobes.

That's not to say there aren't standards; it's not to say that some don't. Because I mean some do, but very few do because of the expertise that's required, because the sense that it's just not worth the trouble. Some labs may do a yearly survey of all of the anaerobic isolates they have, but many more don't do anything in that sense. So that

brings out a larger issue and maybe Dennis Stevens would have more comments about that too from being the president of the Anaerobe Society.

E. SPEAKMAN: Yeah. David, I want to ask you a clarifying question. There's a lot of talk here, and obviously, probably a lot of concern from those present around, you know, sort of what's the role of misoprostol and mifepristone? But, and your thoughts on priorities are to really get an accurate assessment of that, we have to broaden that and look at — look at an assessment of all abortion-related deaths? Is that correct?

D. SOPER: Yes.

E. SPEAKMAN: That produces a better research agenda by broadening it a little bit?

D. SOPER: Well, a couple of things. First of all, it can't just be a simple issue of mifepristone because we have all of the postpartum deaths that are associated with no mifepristone. I mean, it seems to be more logical to think that it's related to some sort of post-pregnancy or peripartum sort of change that's going on.

But I think when you have such few cases, if you have four cases in the country and you're trying to determine if you really have a problem with infectious complications of abortion, you need an accurate determination of exactly what kind of infectious complications do you see with abortion, both surgical abortion and medical abortion. Are we underestimating surgical abortion and overestimating medical abortion or, I mean, what's the deal here?

And so I think the passive pregnancy mortality system does take us down the road to some accurate information about a relative risk and that the studies that are in the literature would suggest that the risk is infinitesimally small. But it may be that we're not good enough with respect to the surveillance that we're doing.

And that's why I recommended maybe a little bit more of an active sort of role or at least an assessment of the current pregnancy mortality surveillance system. Is it doing what we want it to do so that we know what the risk factors for significant morbidity and mortality are in pregnant women and related to live birth, related to termination of pregnancy and the like?

Panel 3 Discussion

E. SPEAKMAN: Great. Thank you. What I'd like to do right now is broaden out the feedback, get some feedback from the panelists themselves, if they have any particular thoughts on what was mentioned up here and what they feel is their top priority for a research agenda moving forward. And hate to put you on the spot by giving just "a" top priority.

And then we'll — and then we'll do a — ask a few more — if anybody has any clarifying questions from the crowd and then rather want to get your comments and feedback related to sort of the priority agendas. And then we are going to actually, what's been mentioned, kind of throw it up on the screen. And then Dr. Bartlett will

have some concluding remarks based on what we're hearing from all the panelists and the crowd. So starting with the panelists, did you have a question, Esther, or did you want to go first?

S. KWEDER: You want to go first?

E. STERNBERG: I want to go first.

E. SPEAKMAN: Okay, so we'll ...

E. STERNBERG: I went last on the panel.

E. SPEAKMAN: So we'll look at — we'll look at, right now, *C. sordellii* right now, okay, looking at what do you think are the priorities in terms of surveillance, basic research, diagnosis, prevention and treatment? Okay.

E. STERNBERG: So I actually wanted to follow up on David Soper's last comment; that it's almost certainly not going to be a single factor. If we take what we know from other multi-factorial diseases — and I'm thinking of the autoimmune disease where the immune system is out of bounds, where there's too much inflammation — it's multi-factorial.

If you think about inflammatory arthritis, there are over 20 different genes on 15 different chromosomes that determines susceptibility to such inflammatory syndromes. Whether or not a host will get an inflammatory syndrome depends upon the load of genes, the environmental exposure and that includes the — whatever pathogen might be involved as well as potential drug exposures and so on.

So I think it's a complex issue and I think that we need to examine all of these aspects: including the hormonal balance, including the timing, whether pregnancy is involved, whether the end of pregnancy, as you pointed out, is involved. That's another thing.

In inflammatory syndromes, it's usually the change in hormones that's critical, not so much the hormone level itself. And then of course as I pointed out, there's the receptor end of it, whether these — there are mutations or polymorphisms in the receptor that might — and receptors that might make certain individuals more or less susceptible to these — to these situations.

And the other thing that I also wanted to emphasize that I've been hearing through the whole conference is — and it's something else that you pointed out — that the reasons for infectious death. Because we're kind of blurring the line between toxin inflammatory death versus sepsis and those are two very, very different things.

And actually, Cliff, when you mentioned the one case where there was *sordellii* in the blood, that's really sepsis. Would you agree? I'm not an infectious diseasologist, but that's sepsis. And the other end and that's where there's too little — if you will, surveillance by the immune system — too little of an inflammatory response to shut — to get rid of the bug.

And at the other end, you've got too much inflammatory response where you get death from inflammatory, possibly death from inflammatory excess. And we don't even

know that, so that's another thing that we have to work out. What is the cause of death in the animal models first to determine is it too much inflammation? Is there some other mechanism?

In the anthrax field, there's a lot of debate as to whether TNF or — and excess inflammation of proinflammatory cytokines are actually causing death. You see it *in vitro*, but you don't necessarily see it *in vivo*. Or you see it at certain time points, but you don't see it through the whole time course.

So I think we need to really — if we want to get at the truth of this, which is what I think we're here for, we've got to look at every single one of these aspects and not focus on only one because it's almost certainly not going to be only one cause.

D. SOPER: And just to amplify real quickly about the difference between toxic shock syndromes and sepsis, a different cause of mortality, I think this last case was appendicitis that was associated with *sordellii* bacteremia and not even really abortion-related. So we do have to be careful to make sure that we don't meld those.

C. McDONALD: Let me — let me just clarify on those cases. The one where the *sordellii* was in the bloodstream was a miscarriage-associated case from the Midwest. That was chorioamnionitis intrauterine infection, okay. The Western region case where we have *sordellii* in a tissue, but we don't even know if that person ever had a medical abortion. Okay, that was the appendicitis and pneumonia.

We don't even know for sure that she's pregnant. I mean, we know now for sure because, you know, this is the problem. Just as said to us that this was a case, but then we could never go back and we couldn't confirm this. So it's up there because it's *sordellii* and it was reported to us. And it's up there in case you ever hear about it again. But at this point, it's not really for sure a pregnancy-associated case.

E. SPEAKMAN: All right. So Esther, you think your — the top priority you feel is the — really determining the cause of death and then looking all the multi-factors, including the receptors and inflammatory? Okay. Great.

E. STERNBERG: And *in vivo* studies in animal models.

E. SPEAKMAN: *In vivo* in the animal models, yeah. Great. Thank you. Anybody on *sordellii* that would like to kind of add on to it?

J. McGREGOR: Yeah, McGregor from Los Angeles. I would like to reaffirm Esther's remarks. I think that the cases are so rare that there'll be limits to what we can discover. And the animal models really can allow investigation, not only the pathophysiology of these events, but also the limited number of interventions: including antitoxins, including early surgery, later surgery. Certainly the work of Dr. Bartlett in the earlier decades would lead us to that understanding.

And then we also can use those cell — cellular experiments to see what's happening in terms of these toxins. Dennis Stevens emphasized the importance of the toxin in terms of endothelial function. I emphasized without knowing anything that, in

fact, these toxins may perturb cardiac function and maybe the manifestation of death. And then I think Dr. Miech, and myself and others emphasized the importance of polymorphisms that may have to do with the different, not only drug metabolism, but also the polymorphisms which control inflammation.

Indeed, just the genetic control of inflammation, what determines too much or too little? And certainly, Esther has amplified that in many of her studies. So I think all of these are actually available with targeted funding to really come up with some — a series of critical experiments which may be of benefit to all of us.

E. SPEAKMAN: And that's the animal models while we're looking for the case finding that we need?

J. McGREGOR: Yes.

E. SPEAKMAN: Okay. Okay. Great. Ralph, do you want to ...

R. MIECH: Ralph Miech. As a pharmacologist, I would like to see the development of an animal model to evaluate drugs for their ability to inhibit the innate immune system. With hospitals daily dispensing ten times more drugs than they have patients, such an animal model would help shed light on which drugs possibly might contribute to *C. diff* re-infection.

Plus with regard to women's health, such an animal model could be used to evaluate mifepristone, misoprostol and other drugs that may contribute to post-abortion infections. By post-abortion infections, I would also include — along with the medical

abortion — those that are surgical and those that are spontaneous. So from a laboratory research point of view, looking at the relationship of drugs in the innate immune system may be an area that could provide us with vital information.

E. SPEAKMAN: And I'm going to get to your point real quick; is that you see, we talked before about with surveillance, an accurate assessment of sort of the abortion-related deaths. But you're expanding that and saying not just only the deaths, but also any post-abortion infection should be part of the surveillance ...

R. MIECH: Yeah.

E. SPEAKMAN: ... a part of the study? Is that correct? Okay.

R. MIECH: Yes.

E. SPEAKMAN: Okay. And you, again, you're another proponent of an animal model more specifically related to pharmacology, right? Okay. Jimmy, do you have something to add with *sordellii*?

J. BALLARD: So my group grows *Clostridium sordellii* almost on a daily basis. And anyone who's had an opportunity to do that can appreciate how rapidly this organism grows, and how rapidly it produces and the amount of toxin that it produces. And you begin to understand how an organism like this can cause very severe disease.

And you also begin to understand how as disease progresses, the host-immune response may not be as relevant later in disease simply because this organism is going to outgrow anything that the host can throw at it. And for that reason, I really think that

a priority has to be understanding more about the microbiology of this organism. Germination — we know nothing, do we? We know nothing about how this organism germinates and initiates disease — as a spore, we think.

We really don't know anything about sporulation. We don't know what nutrients this organism grows on, so we don't know what to extrapolate into the host. We don't know why it may grow in certain privileged sites in the host. And the reason for that is because we simply have no idea about the growth physiology of this organism.

So for us to make, I think, any important advances in the study of this disease, we have to understand more about the basic bacteriology of *Clostridium sordellii*. The same thing's going to be true for *Clostridium difficile*. We have no idea how these toxins are being regulated. And we've heard today that there are certainly strains out there that simply don't produce the toxin.

Why do they not produce the toxin? Are there genes or other deletions? Are the genes there? Are they not being expressed? Are we not detecting them? Are there other toxins there? Are there other virulence factors present in *Clostridium sordellii*? We don't have the genome sequence. We have no way of finding out.

We have — we have Dr. Stevens fractionating culture supernates, and testing those on cells and trying to identify those factors. That's the best that we have right now. We need the sequence of this organism if we're going to make any progress. So I really want to make a strong plea for us to make an effort to focus on the basic

bacteriology of this organism and then it'll blend wonderfully with all of these other priorities as well.

E. SPEAKMAN: Okay. Thank you. Dennis and Marc, either one of you have any comments? Dennis?

D. STEVENS: Well, I agree with Jimmy Ballard. I think that having mortality rates of 100 percent is just absolutely unacceptable in 2006. And so I think in the short-term, we need to find out some better ways to neutralize the toxin, kill the organism, and so on and so forth, and suppress toxins.

But I — but I think — I think the women's issue is bigger and I really think that there needs to be some very, very careful epidemiologic studies done in pregnant females. I mean, we're all aware that many different organisms that have been described that have caused real problems in postpartum infections in the last ten years, *Clostridium sordellii* being one of them.

And I think we need to figure out why that is and what's the epidemiology of *sordellii* and the other organisms that are associated with postpartum sepsis. And then I think we could — we could devise better strategies to intervene to know how many women are colonized with some of these organisms.

We've gone to a lot of trouble to know about Group B strep in the third trimester, but what about some of these other organisms? And I'm not saying that should be done

on hundreds of thousands of women, but I think some really careful studies that are done in the right setting would provide a lot of useful information.

E. SPEAKMAN: So you see not only an assessment of abortion-related deaths and post-abortion infections, you also want to go in pregnancy when people are still well?

D. STEVENS: Oh yeah. I think ...

E. SPEAKMAN: Okay.

D. STEVENS: I think, you know, in terms of sheer numbers, I think that the biggest problem is in pregnant females.

E. SPEAKMAN: Marc, do you have any thoughts on what you think priorities are related to *C. sordellii*?

M. FISCHER: I think I would just reiterate two points: the one that Dr. Soper and Dr. McDonald made regarding active case finding and making sure that that case finding is representative of pregnancy-associated deaths or severe illness regardless of whether it's associated with abortion or not.

And that those be followed up with, you know, intensive epidemiologic review to look for factors that may be uncommon as well as laboratory diagnoses using other things than just cultures so that we're putting things on an even playing field and not just talking about culture-confirmed cases versus non-culture-confirmed cases. And I think that's being done to a large extent.

And then I was actually going to say what Dr. Stevens mentioned. From my looking at the literature, I don't think we even know what proportion of women nowadays are colonized with *C. sordellii* or *difficile*. Many of the studies that were done were done decades ago maybe using better or worse bacteriologic techniques. Maybe there have been shifts epidemiologically in women who are carrying the organism.

So I think some sort of survey or study that looks at women, both early and late in pregnancy because of changes that can occur throughout time; and maybe even comparing women when they first present for obstetrical visits to potentially women who are presenting for abortion in case there are epidemiological differences between those groups to compare how many are colonized with these organisms, how many of those organisms are toxigenic versus not would be useful potentially in differentiating what are some of the issues that separate these groups who have disease and do not.

E. SPEAKMAN: Great. All right. Thank you. Each one of the panelists had sort — oh, I'm sorry; go ahead.

J. ENGEL: I'd like to raise the question and ask what the role of the media is in case finding of rare diseases? I think they have an important role to play as long as we're smart about our risk communication, both from the clinical provider point of view and the public health point of view. And I think we could be a lot better at being a little bit more transparent about some of these issues as they're emerging in our society. And I think that's actually a research agenda.

E. SPEAKMAN: What the role is associated with case finding and the transparency of the work that you do?

J. ENGEL: And the role of the media in assisting with case finding.

E. SPEAKMAN: Okay. Okay. Each one of the panelists with *C. sordellii* had expanded upon a slight twist. Is there anything that any of the panel — I don't want to get any fights here right now; I'm enjoying the close consensus. But is there anything that the panelists said that somebody — another panelist says, "No. That's — I just — I just don't see that as a research priority." Was there anything that was said? All the panelists?

I'm just speaking to the panelists right now. I'll get to the — everyone else after that. But just the panelists, that you would say, "No, that doesn't seem like a — I don't — that doesn't feel like a priority to me." Hurt anybody's feelings. Okay.

J. McGREGOR: In terms of the organization, if we could go on to another step, I suggest that the CDC, FDA — cooperating so well — actually set up a consult service for emergency room doctors, obstetricians, gynecologists, primary care providers who might have a question about this.

And being a physician like David, who gets closer to the care of these patients, and I'm aware of the chaos that occurs when we're presented with patients like this. I think it would be a really good idea for — to have a resource person that practitioners could call in terms of when there's a possible case because ...

E. SPEAKMAN: Going across both or just *sordellii*?

J. McGREGOR: I think both.

E. SPEAKMAN: Okay.

J. McGREGOR: But in my talk, I gave the suggestions of when the case is occurring. But I suggested some radical steps, including hysterectomy. So in fact, I think there should be some way to get to informed consultation about what might be the best circumstance in the — this nearly 100 percent lethal situation so far.

And I was talking with David and others and it turns out in veterinary practices, there are antitoxins for *Clostridia sordellii*. An old issue for us where I — when I used to work at Colorado was what was the potency of IV/Ig in terms of anti-clostridial toxins of different kinds? And I think actually that's a — David suggested he wanted something on the shelf.

E. SPEAKMAN: Antitoxins.

J. McGREGOR: And in fact, that would be on the shelf of many hospitals, albeit very expensive, but available. And once again, I think this is most amenable to animal model, either large or small modeling, to see if things like this would work.

E. SPEAKMAN: Going back to sort of consulting or consultant, almost seems like a 1-800 line: "Give us a call if you any questions on ..."

J. McGREGOR: Yeah.

E. SPEAKMAN: It'd also be for reporting: "We found this case. We ..."

J. McGREGOR: Well, it'd certainly — it would ...

E. SPEAKMAN: "... want to provide both."

J. McGREGOR: ... facilitate support ...

E. SPEAKMAN: Okay.

J. McGREGOR: ... of reporting. For many odd infections, I would call the CDC immediately and ask just to speak to the EIS officer who was on that section. Certainly, we did this for staphylococcal/streptococcal toxic shock because we need the very best advice and we would talk to the EIS officer, the manager who had the most information. And I think actually that might be a bureaucratic function that would — which would — which would be a service to us all.

E. SPEAKMAN: And that'd be a dedicated person, dedicated number or person to contact related to that?

J. McGREGOR: Yes.

E. SPEAKMAN: Okay. Great. Great. Any other comments, feedback related — from the panelists — related to their comments? Okay. I know that we had some clarifying questions from a participant that we didn't get a chance to get before.

And then I want to turn it over to the rest of the participants for about 15 minutes to kind of get — 15, 20 minutes to get your thoughts, feedback related to what was some of the recommendations for the priorities. David, you have a few questions? Is that correct?

M. PATTERSON: No, I ...

E. SPEAKMAN: Actually, turn that on.

M. PATTERSON: My name's Monty Patterson.

E. SPEAKMAN: I'm sorry.

M. PATTERSON: I would like to thank the CDC, the FDA and the NIH for having this conference. I do have a vested interest being here. I've listened to your talks about *Clostridium sordellii*. I lost my daughter who — Holly Patterson — to the infection as a result of a medical abortion termination. I have — I would like to contribute to this workshop by presenting to the CDC, and the FDA and the NIH.

I have a compilation of nearly 400 medical and scientific journals on RU-486 *C. sordellii*, the innate immunity and septic shock. This is a result of over 2½ years that I've spent since my daughter's death and thousands of hours of research. And there have been other people involved too. And I personally have read each and every one of these articles.

I am familiar with most everyone here: Jimmy Ballard; I've read Dr. Bartlett's, Soper, Jaime McGregor — Dr. McGregor, Dr. Miech. I've read most everyone here and you too, Dr. Stevens, on necrotizing tissue. So your articles are in here — many, many more. I would like to say to you that I hope this research that you're doing and any of this research here that I have helped to compile will facilitate the understanding. And

maybe we can explore the possible causal relationship of RU-486 and the medical abortion *Clostridia* infections. And I will thank you very much.

E. SPEAKMAN: All right. Did you have any clarifying questions that you have that would be germane to sort of establishing these priorities?

M. PATTERSON: Well, one of the things that I did want to ask is that, you know, my daughter really never had a chance once she developed the *Clostridia sordellii* infection. And short of having, you know, of having a hysterectomy or for early intervention and early recognition of the disease, I don't know at this time what measures are being taken at emergency room facilities to help diagnose what could be, you know — I think she'd be here today if somehow, you know, she had been — maybe had been diagnosed earlier.

But I would say what is being done? What tests are being done through — at hospitals by doctors who can recognize the early signs? And I know listening to the panel members that they're — once a toxic shock-like syndrome sets in of *C. sordellii*, that really it results in death. And I know Holly didn't have a chance. Particularly after I watched what she went through, I knew that there was no hope for her. So my question is what can doctors do early on at this time?

E. SPEAKMAN: Yeah. Sorry for your loss. Any — anybody? I know that Dale, you talked a lot about the need for a fast, a rapid, sensitive diagnostic and that's obviously a priority moving forward. Can anybody else add?

D. SOPER: Well, I, again, share your sense of loss. I can't imagine how horrible that would've been for you. And I have actually seen one of these cases up front and personal myself, so they're absolutely horrible. Because as a physician, what you see is a patient that actually looks relatively well, but is in extreme septic shock. And really, there's nothing you can do about it.

You pull out all the stops. You do everything as a clinician you know what to do to treat these kinds of serious infections and there is nothing to be done. So I would say that as I kind of — or just re-amplify what I said in my presentation; and that is there may be a window that we do not know today that we might be able to identify an intervention that could happen that might save lives, but that I'm not optimistic.

Because the patients that have — as you know having reviewed all this literature — the patients that were aggressively treated, including surgically, all of them still succumbed despite that surgical intervention. And what I am concerned about is that some of these symptoms that are associated with *sordellii*-associated toxic shock are symptoms that are non-specific.

And therefore, I worry about the patient who have undergone the termination, who comes into a clinician, who can be treated with antibiotics and not aggressive surgery, and go home and do well. All of a sudden, ends up with a hysterectomy because of a concern for a more serious infection that indeed is very, very rare.

So I think we're on a very, you know, tight edge there about when to recommend to be aggressive and when we can be conservative. And that takes a lot of clinical judgment, and frankly, it takes more information than we really have available now because of the rarity of these infections.

And I hope, as you said, that this will spark some basic science research in learning about the microorganism that will help us do less invasive sorts of therapies that will be more effective in treatment. And I think those are going to be molecular interventions, antitoxin interventions and that sort of thing.

E. SPEAKMAN: All right. Sandy?

S. KWEDER: Yeah, I think I'm not sure if this is an area of research or if it's an area of simply communication. I have the sense from talking to clinicians who are not part of the FDA, or the NIH or the CDC that despite the *New England Journal* article publications and the existence of a workshop like this, that very loud changes to product labeling for mifepristone and misoprostol because the original cases were identified there.

That there are a lot of clinicians who would not even consider this, you know, a *Clostridium* infection as a cause of their death in a — of a death, an unexplained death in a patient. In fact in many of these cases, an infectious disease physician might not even be involved.

In a woman who is — who's had a spontaneous abortion, or a surgical abortion or a medical abortion, even an OB/GYN might not be involved who might otherwise have been informed about this risk. It's going to be emergency room doctors, primary care physicians. So one question that I had is whether we need to do some research to identify ways of communicating information in order to find cases as well as once we understand better the disease, communicate to clinicians who are going to see these patients at the moment what to do?

Because I do believe that it is not going to be the ID doc who sees them. It — that's — there's not time for that, so I think we don't really understand at FDA. We're always struggling with communicating information about risk. And this falls into that same kettle of — that same kettle; is how do you communicate information about — concerns about the infection first to find cases and study them better? And secondly, I — ultimately, communicate how best to intervene to prevent catastrophic results if that appears to be possible at some point?

C. McDONALD: Just one comment and not to — in total agreement basically, but because that was posed as a — as a research question as something that could be further understood. I think certainly one limitation is we know that there's a lot of overlap between the presentation and many other conditions. One thing that has come up over and over again is this white blood cell count that we've talked about.

White blood — white blood cell count in excess of — which sort of almost puts a hallmark on these infections as being large clostridial toxin-related and it's both *C. difficile* and *C. sordellii*. Granted, maybe once they reach that point, there is nothing that we have in our armamentarium to reverse the situation.

But I don't think there's a general knowledge outside the ID community that white counts of 30,000 or more are very, very likely large clostridial toxin-related. I don't think the general practitioner knows that. That's not something that they're being taught. I mean, it just sort of came out in *C. diff* — I think, Dale? — maybe in the last ten years; that if you see someone with a very high white count as an ID consultant, and you're on the ward, and you don't find a source, and they had this high white count over 30,000 and they may not even be having diarrhea, it's generally now we teach fellows to think about *C. difficile*.

But beyond that — I think maybe in the education of obstetricians, OB/GYN — maybe this is not on the radar screen and needs to get on the radar screen that large clostridial toxins are part of this. And I want to just take it so that — but I don't think that's an answer yet, but that's something; I agree. We should put that on the priority and work on.

But let me just add a little something to that; is, you know, a lot of this is sort of we have an orphan bug. You've heard about orphan drugs for a condition. This is an

orphan bug right now because it is a very small number of cases and it's unlikely we're going to see antitoxins.

I mean, ideally what we have for women who come in there is a drug that de-couples the toxin from the GDP, right? I guess perhaps they get in the cell and get in there somehow. That's not going to be developed, of course, with an N of four or in whatever the total is. You know, even — what?

S. KWEDER: Even 100.

C. McDONALD: Yeah, even 100, right. Right. But I think one thing we need to start thinking about is maybe the *Clostridia* have been ignored for too long and too big a way. And is there even possibly a role for large clostridial toxins in abdominal sepsis? And I'm talking here a clinical entity of people who have a gunshot wound or ruptured diverticulitis and these people go downhill.

They don't usually have white counts over 50,000, but they — probably under 30,000, 40,000 range oftentimes. And you see them in OB/GYN too, but it's usually from spilled intestinal contents in the — in the perineum. And we always think about, "Oh, that's gram-negative sepsis." But if you saw those same people with, you know, urosepsis — of course, with *E. coli* even several gram-negatives — they don't — they don't just go on in this spiraling.

And of course, it's different host factors, certainly. And it's the issue, you can't drain the infection. But we don't know what is the role of large clostridial toxins in

abdominal sepsis and maybe that's something we should be looking into. And that one thing would be is if you could measure toxin levels in the peripheral blood of some of these, that might be useful.

E. SPEAKMAN: All right. It seems like there's a general theme of not just science, but of communication that's needed moving forward on this. Yeah, Dennis?

D. STEVENS: Well, I certainly think the education of physicians is important in postpartum infections and certainly of women. I mean, they need to know what the potential consequences of pregnancy and/or medically-induced abortion are. But, you know, we've got antitoxins for rare diseases like botulism and tetanus. It's only a handful of those cases around a year. Got rabies vaccine for a handful of cases around the country.

There's not any reason why we couldn't develop antitoxins against some of these things that are useful, especially young healthy people that could be salvaged. So, you know, I think that ought to — I'm not deterred by the fact that there's not hundreds of thousands of patients to treat this way. I think sometimes you have to make exceptions for highly-virulent organisms that potentially an antitoxin could be life-saving.

C. McDONALD: I was thinking of a boutique drug being developed, a designer drug, which probably would be a different issue. But yeah, antitoxins would be something that I would think would be a shortcut if we could show they're protective.

D. STEVENS: Yeah, I'd also like to just reiterate what Dr. McDonald said about the cultures and I'd put a plug for the Anaerobe Society of America's meeting in Boise in July of this year. And we're going to have a large panel of clinical microbiologists are going to discuss where we should go in terms of anaerobic clinical microbiology because I think it really has deteriorated in this country since its heyday in the days of John Bartlett was doing some definitive studies in anaerobic lung infections.

E. SPEAKMAN: For those on the planning team that have to, you know, go from these priorities, and move forward and do action in their day-to-day jobs, do you have any questions, points of clarity? Any from NIH? Any — yeah, Leslye?

L. JOHNSON: One thing that I ...

RECORDER: What is your name?

L. JOHNSON: Leslye Johnson.

E. SPEAKMAN: Leslye.

L. JOHNSON: I'm sorry. One thing that I found very interesting was with people — from people getting way down deep into the molecular mechanisms and interactions, you know, inducing this pathway, inactivating that.

I think it would be helpful to the government to ask if there are other diseases with similar pathology or pathogenesis that are higher priority targets perhaps that would be able to be translated, if there were an animal model, into something that would

be useful in these two — in — with these two infectious agents. So that's something that I think we need to have further thought on or else we're open to suggestions.

E. SPEAKMAN: Including now? That anybody has suggestions at this point? Oh, in the back?

D. SICARD: I am Didier Sicard from France, Professor, Infectious Disease in Paris. And I am the second father of the — my daughter died. It was the case number 4 presented by the excellent paper of Dr. Fischer. So since June 5, I am a distressed father. But that's not the problem. The problem is that I am impressed by the excellent workshop. I come especially from Paris yesterday evening and I leave tomorrow.

And I ask one question and one commentary. One question is how reporting wide in California and never in France? But perhaps in France, the epidemiologic data are not good. And always I say, CDC is a model for all the world. So if France says there is no case, perhaps there are.

But when I ask to the treating gynecologist, he says, "Well, it's not, for us, a problem." The question is there is difference between for the contraception before pregnancy in France and United States. And it seems that in California, most of the women take contraceptive anti-progesterone device before pregnancy. And if pregnancy occurs, perhaps the device with anti-progesterone intravaginal enhanced level of *Clostridium sordellii* — perhaps — and hello, infection of carriage of *Clostridium sordellii* before pregnancy and when you have abortion. Perhaps, but I don't know.

And the second, it's not a question, but commentary. All the case arrived with intravaginal misoprostol and it's absolutely forbidden in France. So I ask is it possible to stop to give for economic reasons — misoprostol by vaginal and perhaps to take a decision? Because I'm surprised that FDA said it's not allowed; to give by vaginal in France, it's not allowed.

And it seems that several gynecologists said, "Oh, it's no problem to give by vaginal." And I was surprised by a paper in *Lancet* or *New England* says, "Well, it seems there's no problem. It's too expensive to make a survey to evaluate in the next year what is the best." So the question is, is it possible to say, "Well, by prudence, we stopped give misoprostol by a vaginal way?"

E. SPEAKMAN: Anybody to talk on that? Anybody, if you're qualified, to talk on that?

D. SOPER: Somebody from FDA should be able to talk. I mean, it's my understanding that you can ...

S. KWEDER: Yeah, I — so I — what I would take from that is a better, a more specific understanding of drug use patterns in other countries where — as well as case finding in other countries. That might be something that we can work together on.

E. SPEAKMAN: And then the implications for it here, but ...

S. KWEDER: Right.

E. SPEAKMAN: ... in America. Yeah. Great. Thank you. Any other questions? Why don't we just open it up then to all the way back? If you could please step up to the mike, state your name and then, you know, any questions and/or comments?

F. UZAL: Francisco Uzal, the University of California-Davis. I'm a veterinarian and I'm a pathologist. And I have a comment and I think a silly question after that. Over the years, I've seen a few cases of gas gangrene in sheep and a few in cattle in which we cultivated only *Clostridium sordellii*.

However, when we tested by *in situ* techniques, like immunohistochemistry, or FATS or PCR, we detected a cocktail of other *Clostridia* and got to the conclusion that we were dealing with mixed infections. And then I was told by our microbiologist that *Clostridium sordellii* grows so fast it overgrows all the other ones. And now the silly question. This is not the case here, is it? Has it been tested?

E. SPEAKMAN: Anybody?

C. McDONALD: Actually, just so we know, the four cases that Marc Fisher presented, none of them were actually detected through culture. And actually, that's getting back to the fact that it would be nice if we had better culturing going on and it was not done in these cases.

Instead, they were detected through PCR on the tissues for *Clostridium sordellii*-specific genetic sequences. At the same time, some other sequences were looked at, I believe, for a few other organisms.

F. UZAL: That's a specific question.

C. McDONALD: Okay.

F. UZAL: Were other organisms looked for?

C. McDONALD: Were looked for with primers. Actually, one is a 16-S non-specific primer to try to amplify ribosomal DNA from any bacterial species and only the *sordellii* was found. If you find a — if you have a mix of organisms, you won't get a specific signal in that situation. So this is a case, actually where the molecular methods obviate that problem of overgrowth of one species over another.

E. SPEAKMAN: All right. Do you have a question in the back?

C. BROOM: Yeah. Colin Broom; I have a question. And I'm surprised that one of the priority areas of research that really wasn't mentioned was can we better identify and categorize *C. difficile* sources of disease patients in terms of their severity? Can — could — this is quite a thorny issue, but not straightforward. What is "severe disease?"

And what I'm driving at is can we better identify patients at high risk of complications who should be more aggressively managed early on and communicate this? This is something that can be done. It's not straightforward, but needs to be validated. And I think there's an opportunity to impact the disease if we can address this.

C. McDONALD: Did you mean that for *sordellii* or *difficile*?

C. BROOM: I mean this for *difficile*.

C. McDONALD: Oh, you mean for *difficile*? Okay.

E. SPEAKMAN: Yeah, we were just wanting to ...

C. McDONALD: Keep that.

E. SPEAKMAN: ... push up *sordellii*. We'll get that. We'll ...

C. McDONALD: Keep that one.

E. SPEAKMAN: ... hold *diff* for you.

C. BROOM: Okay.

E. SPEAKMAN: Hold that for you. Anybody on *C. sordellii*? Now to *difficile*. Oh, I'm sorry. You had one more? I apologize.

J. COUZIN: I'm Jennifer Couzin, a reporter with *Science*. This is actually about two of the four new miscarriage and medical abortion cases that Dr. McDonald presented earlier and that were linked to neither *Clostridium difficile* or *sordellii*. And I was just wondering, you know, what those might mean; whether they came as a surprise; whether they should have any role in research priorities or surveillance?

C. McDONALD: Yes, that's *Clostridium perfringens* in both instances and we know that *Clostridium perfringens* is a common *Clostridium* also. These cases, one of them — actually both of them being medical abortion-associated: only one of them associated with mifepristone, the other one with misoprostol and laminaria. They were both intrauterine infections: one was necrotizing endometritis, the other one was just stated as an intrauterine infection right now.

One of them actually, interestingly again, these are detected through actually the — one of these was detected through culture and PCR. And I don't think we've yet characterized that *Clostridium perfringens*. I'm looking back at George Kilgore; he does that. And if he — if we have, that's fine; he can come to the microphone.

But what was interesting in that one case — this was a Western case that had been reported, I think, over a month ago now — is that it had a maximum hematocrit of over 60 and a white count of over 50, and yet, it's a *perfringens*. So it does — it kind of raised the questions of, you know, did we just miss — is it — did we just miss the *sordellii*? Did the *perfringens* in this case maybe overgrow the *sordellii*? Because it was obtained by culture as well as PCR, so these are still some questions.

I think the other one with *perfringens*, a Midwestern case associated with intravaginal misoprostol and laminaria, had — did not have an elevated hematocrit and we don't have a white blood cell count. Can't really say any more about that, but it does raise some questions. The organism *perfringens* is very ubiquitous and I don't know. Maybe Dennis Stevens, you can comment on have you ever seen *perfringens* cause a leukemoid reaction like this and elevated hematocrit?

D. STEVENS: No. Did this woman have a necrotizing infection or what sort of ...

C. McDONALD: Right now, I think we just know it's an intrauterine infection and the final report is pending on that, so ...

D. STEVENS: Well, I think it would be exceedingly unusual to have hemoconcentration with *perfringens*. I mean, *Clostridium perfringens* is a common cause of postpartum — I won't say "common," but it is certainly a cause of postpartum sepsis and it has been for decades, and decades and decades. But I've never seen that happen. I certainly have seen hematocrit go from 40 down to 4 or 5 in four or five hours, so you usually get the opposite.

E. SPEAKMAN: Glenn, did you have a comment in the back for *sordellii*?

G. SONGER: Yeah, if I may. The cases ...

E. SPEAKMAN: Name one more time. Sorry.

G. SONGER: Glenn Songer; I'm sorry. The cases that we've heard about, the specific manifestation of *C. sordellii* infection are, I mean, they're absolutely horrific. There's no question about that. But I think we'd probably be short-sighted to go away thinking that's the only manifestation of *sordellii* infection in humans.

My sense, after having seen a few dozen cases of *sordellii* infection in domestic animals, is that the lack of diagnostic testing in many cases is probably leading to misdiagnosis and we would find a lot more *sordellii* infections in humans. I mean, there's just no reason why we shouldn't.

And it probably would help if we were to, you know, somehow start a movement to enlighten pathologists as to the existence of the discipline of microbiology. It's — it really is a lot easier to sort these things out from fresh tissue.

E. SPEAKMAN: I think we get a nod, all the — all the heads around here with that too. But actually, two more questions and we'll move on to *difficile*. Sir?

R. STEINBROOK: Oh, right. Robert — excuse me — Robert Steinbrook from the *New England Journal of Medicine*; basically a follow-up to both of those questions. Dr. McDonald, do you have a system through your active surveillance to get high white blood cell counts in an infectious disease context from clinical laboratories and follow them up above a certain threshold?

C. McDONALD: No, that would be difficult. Our system, again, is retrospective looking at deaths. This is what I've described to you out in California. You could imagine a — or it wouldn't have to be prospective, I think, in hospitals. Of course, you know, it'd be going through a lot of noise still. It'd be really, I think you're saying deaths with a very, very high white count in this range. Right now what we're looking at is death certificates, so we're not there basically.

R. STEINBROOK: Or even patients who are still alive?

C. McDONALD: Yeah, or even patients that are — who are still alive. And it'd be interesting when you go — I think the specificity of a white count over — whatever, 30,000 or 40,000 — is someone, of course, who does not have leukemia.

And so being sure that you're not dealing with chronic lymphocytic leukemia or these other conditions would be one issue and it's a — it's a thought. It's not something we — our focus thus far has been on deaths and death certificates retrospective.

E. SPEAKMAN: And then we've got one last question and then concluding remarks from Dennis. And then we have to move on to the next because we do have people that have to catch airplanes.

D. DAVIS: Dan Davis with the FDA. This is just a point of clarification. In the recent case from the West that was positive for *perfringens*, I'm quite sure that was considerable bowel damage, and I think 11 liters of fluid in the abdominal cavity as well as changes in the uterus. So it would look like a mixed picture in that case, I believe. But it wasn't the classic just uterus only, and in the lung, and pleural occlusions plus abdominal fluid. I think there was considerable bowel necrotic changes.

C. McDONALD: Okay.

E. SPEAKMAN: Go ahead.

C. McDONALD: I'm sorry. The final report is still pending from our ...

D. DAVIS: Okay.

C. McDONALD: ... pathology.

E. SPEAKMAN: Thank you for the clarification. *C. difficile*: briefly kind of some of the highlights that Dr. Gerding talked about was — and everyone else — was more isolates, more isolates, more isolates, more isolates — a reoccurring theme that seems to be right there.

Looking at better determining the risk factors of PPI; getting those isolates both from healthcare-associated acute and long-term; looking at human and animal models.

Within basic research, really, really looking much deeper into the virulence factors associated with *difficile*. With diagnosis, prevention and treatment, key — it looks like here — is really effective diagnosis; that maybe a lot of misdiagnosis going on right now.

Much faster, more sensitive methodology — I mean, excuse me — testing. Exploring better environmental disinfection and exploring, you know, what is the right choice for antimicrobial treatment. Okay. For those that were on the panel related to *difficile*, any additions, comments, questions? Yeah, Linc?

A. SONENSHEIN: I'd like to reiterate the comments that Jimmy Ballard made in the context of *sordellii* for the case of *difficile*. I think it's really important to study the basic physiology of this organism, particularly with respect to the processes of spore formation and spore germination.

Dale Gerding mentioned the many virulence factors that we surely don't know about yet. And in fact, all — in fact, all the special proteins required for sporulation and germination must be among those virulence factors. And if we could target them, I think we'd be able to potentially greatly reduce the severity as well as the spread of the disease.

E. SPEAKMAN: Okay. Anybody else? Dale?

D. GERDING: Just a comment, Linc, that the — it's really just this huge critical need to get better genetic tools to do all those things that — you know, we really need basic fundamental breakthroughs in being able to manipulate this organism better.

We're just, you know, decades behind *E. coli* and a lot of other pathogens in trying to get at what's going on, including doing more sequencing of the genome for which we have just one right now — two actually, I think, because I think the Canadians have done another one. And, but I think that's going to very important. We're getting a lot of clues already from the genome about what's going on. I think we just need to do a lot more additional really basic work in there.

E. SPEAKMAN: Okay. Anybody else that would like to add upon what they feel priorities associated with *difficile*? Yeah, Jeff?

J. ENGEL: Yeah. Jeff Engel; two questions I guess, is what is the importance of zoonosis in *C. difficile* disease? And number two, is the question of whether or not the spores in the human food chain are not a valid question or how aggressively do we need to go after the fact that this disease might be food-borne? To that end, I'm wondering if the USDA has been involved or maybe even the National Association of Public Health Veterinarians? They assemble their task forces and so forth.

C. McDONALD: Well, I think the priority question is just how highly to put this issue in the overall ranking of importance? And right now, we are — we have it high. It's not the highest in some ways. I mean, it's sort of simultaneous, you know. One

arm's doing this and the other arm's doing that. Healthcare-associated disease is over here and it's actually working with the Food-Borne Branch and their networks, FoodNet, to look at the food chain issues.

And USDA is aware of — in fact, I — they might even be present in this meeting, but they are aware of this information that I shared today in terms of similar strains. At the same time, what I shared also is that there's mostly dissimilar strains. I mean, mostly the disease that's being caused is different over here and over there.

The just question is are there — are — could there be occasional passages through the food chain? And I think that culturing — the key things will be culturing the food supply, culturing meats, and in determining how often we find *C. difficile* spores, and then if there are spores that are — can be associated with human disease.

I'll say even before that though, you know, the priority before that will be getting isolates from community-associated cases. If this is important, if there's any importance to the food chain in any of this, it would make sense that it would declare more in community-associated cases. So we can't separate all those things; I'm sorry.

I guess the first priority is to get isolates from community-associated human cases and then start comparing them. But I agree with you; I think these are priorities to look at and maybe do them in a stair-step fashion. I don't think we should go out now and commit to five years of surveillance in food-producing animals, and in meats and all this because there's just no evidence for that kind of concern.

E. SPEAKMAN: Any questions from the participants? Colin, do you want to ask your question one more time? Did we answer that for you? I can't remember. Did we — did we get you a satisfactory answer? Should we get it one more time?

C. BROOM: The question was related to, you know, we're having difficulty in struggling with defining what is "severe disease" in *C. difficile*-associated disease. And the idea here is can we identify patients as early as possible who are more likely to have complicated or even fatal outcome so we can more aggressively treat those patients? I think that's one priority that we should be looking at.

E. SPEAKMAN: Okay. I want to see if everyone's got a — that feel that that's a priority as well? And anyone want to add on that?

D. GERDING: Yeah, I — go ahead, Dennis.

D. STEVENS: Yeah, I'd just like to make one comment to the editor to the *New England Journal* as well as the other gentleman about that. There was a study done by Dan Musher at the VA Hospital in Houston where he actually looked at the white blood count in people that were hospitalized there and then went around and saw those patients. And it was a high correlation with a white count over 30,000 in *Clostridium difficile* and which allowed them to make an earlier diagnosis.

C. McDONALD: Yeah, I think you were talking — you were talking about in the sense of *sordellii*, that'll be another important thing you have to screen out all those with *difficile*.

D. GERDING: Actually, I think a number of groups are looking at clinical scoring systems that you can use early in the disease course to try to predict who is going to do badly or who's going to — in the case what we're doing — that's who's going to fail metronidazole therapy. And we actually have come up with a scoring system that we're just reporting now that looks like it is predictive.

But we're now trying to dissect through the scoring system to see what the elements are that are predictive. Is it more than just white blood cell counts? And can we identify the other elements of that scoring system? And then can we validate that prospectively? That's really the key, I think, to a good scoring system. But I think a lot of us are very interested in that, but nobody has come up with a real good system as yet.

C. McDONALD: I agree with it as a priority too. And I just want to say it's probably one of the priorities which will be met earlier than some of the others, so I don't know where you put that. But ...

E. SPEAKMAN: Low hanging fruit?

C. McDONALD: Well yeah, it might be low hanging fruit. It might be, but also along with that will be surveillance systems that also capture complications too because it'll be important over time not to have to go back five years and say, "Oh yeah, people were dying at a higher rate of *C. diff* way back in the 2002." But to know, you know, in real time if there's a change in virulence again.

E. SPEAKMAN: Unfortunately, we're starting to get into a time constraint. So we've got a question in the far left, far right, then Esther, and then we're going to have to go to concluding unfortunately; that these panelists have to get on planes. So we'd love to be able to stay until late into the night, but they all have commitments they have to get to. So ma'am, you have a question in the far left?

E. HAMM: Yes. Elaine Hamm. I was curious how much the extra colonic activities of *C. difficile* contributes to the morbidity, and mortality, and perhaps the severity of the disease? I don't know if any of the clinicians here would like to comment on that? Because there are patients that have had eradication of the organism, and removal of the colon, and yet, still succumbed to the disease so ...

E. SPEAKMAN: Can anybody — that one? Dale?

D. GERDING: What was the — specifically your factor that you were wondering about?

E. HAMM: It was something more, like perhaps like as Dr. McDonald pointed out: the role of the toxins in a systemic sense of the disease and if outside the colon. Everyone focuses on the colon, but are there other factors? Are there other multi-organ issues that we need to be looking at?

D. GERDING: Yeah, just a superb question and one that we always toss about saying, "Gee, it's probably systemic toxicity in severe *C. diff* disease." Everybody believes that's happening. Nobody, to my knowledge, has measured toxin or really has

good data on the effects on other organs in the body beyond the colon. So it's an area that really needs to be looked at much more carefully.

We need a good sensitive toxin assay in blood or serum right now. We certainly expect that that is what happens in the hamster model where animals die just precipitously. I mean, they have no symptoms whatsoever and six hours later, they're found dead. And we expect that this is systemic toxicity and potentially what could be happening now in these very severe cases in people which previously we had not seen to any great extent. So there's probably something different going on, perhaps with the new epidemic strain.

E. SPEAKMAN: Glenn, then Esther?

G. SONGER: Yeah. Glenn Songer; two quick comments. First of all, I think it's — it may be difficult to overestimate the potential benefits of taking a very careful look at these organisms in what they do in domestic animals. Of necessity, a lot of those things become what we call "anecdotal" because they're diagnostic reports. They're things that are discovered that aren't — really don't lend themselves to publication. There's a huge amount of knowledge out there that could contribute greatly to understanding, especially on *sordellii*.

Second, while I wouldn't be so bold as to disagree with Cliff about the importance of *difficile* in the food chain, for what it's worth, we initiated a study of retail meats: beef, pork and turkey. And at this stage, more than 25 percent of those are culture-positive

— of the samples — are culture-positive for fully toxigenic *C. difficile*, for what that's worth.

E. SPEAKMAN: Esther? Yeah, I'm beginning to think I should just live in a bubble now, you know, right? Let's all move in one big bubble. All right. Esther?

E. STERNBERG: It's a — that's a hard point to follow ...

E. SPEAKMAN: Just don't eat anymore, right?

E. STERNBERG: ... having just had a turkey sandwich. Yeah. I just wanted to get back to the low hanging fruit issue, and using my other hat and at the National Institute of Mental Health, and looking at human behavior, and since hand-to-hand hand washing is so critical in preventing transmission.

I'm working with the American Institute of Architects and the American Institute of Architects of Healthcare Design, who are very, very interested in designing hospital spaces so that healthcare providers will be more likely to wash their hands. And if you analyze this, if the sinks are only at the nurse's station, they're unlikely to wash their hands. And so it's a very simple modification, but it could make a huge difference. And so I would encourage you to work with them, collaborate with them. They're very anxious to do this kind of thing.

E. SPEAKMAN: Hand washing and scoring for severity of *difficile* could be some low hanging fruit. All right. I want to thank everyone for the questions. Dr. Bartlett, you're ready to provide your concluding remarks on the priorities?

Summary of Key Findings

J. BARTLETT: Yeah, thank you. I'll really have to confine most of my remarks to *C. diff* because that's the only one of the two that I really have much experience with. But I'd like to start out by saying something about the fact that these two organisms were mixed and this was kind of a serious contentious issue for the people that participated because you've got *C. diff* that attacks only the colon. I mean, that's the only organ it knows. And you've got *sordellii* that know — that knows other things, but doesn't know the colon. It never goes there, except that this one case that we heard about.

But I did want to recount something that I thought was interesting in the course of these discussions; and that is, *C. diff* was discovered in 1977. And at that time, there was a tissue culture assay that was positive. It was a strongly cytopathic toxin — toxin B — and it was shown that gas gangrene antitoxin neutralized it.

So the question was what in that five-member gas gangrene antitoxin was doing this? Because that was the cause of antibiotic-associated colitis and it was *Clostridium sordellii*. And the way we got it was to go to the military because they had these huge vats of *Clostridium sordellii* antitoxin for gas gangrene in World War II. And then it turned out that it was an antigenic cross-reaction.

The only reason I mention that is that these two organisms may have more in common than we are thinking about, at least on the surface. So I would certainly

subscribe to the fact that clinically, they're probably completely different in terms of who, what, and when and where.

However, in terms of the mechanisms of the toxin, I would certainly endorse the overlap in the body of knowledge that would be gained by studying the toxin regulation, the role of sporulation, the virulence factors, the role of the flora in controlling these organisms, and the mechanism of the toxin. So that's probably an area where the two organisms mix rather well.

In terms of *Clostridium difficile*, I think what we could say is that this was an organism that everybody kind of knew about, and knew how to diagnose and treat, and didn't have many serious questions or problems until this more recent NAP1 strain came. And then all of a sudden, there was a flurry of activity. And I think what we're trying to understand is why the now flurry of activity, but also asking some questions that could've been asked before.

But what we seem to have now is more disease, more serious disease. And we have disease that is more refractory to therapy. And I guess one of the reasons why that's a topical issue right now is because that has forced us to think about it and worry about it. In terms of the risk, I would certainly endorse this — the suggestion that there be sentinel studies in order to collect information because I think now this is a new area which we have a paucity of information. And we've talked all day about it, and therefore, I'm not going to reiterate it now.

But certainly, the known risks are antibiotic exposure, age and being in a — either a chronic or acute care facility, but now we've got these new cases that seem to be unrelated to antibiotic use. And having seen, I think, probably thousands of cases for the last 30 years, it strikes me as complete — you know, 20 years ago, *Clostridium difficile*-associated colitis not due to an antibiotic was a case report.

And there were — our group produced seven of them and I always thought they were probably closet antibiotic takers, but could never prove that of course. But that's how rare it was. We — and we and others certainly looked for PPIs and other things that sort of got rid of the gastric barrier. And it never panned out, so these are some new questions that may be coming up because of this new strain.

The test is not — the one that Dale showed that most hospitals use, the EIA, is not a very good test. And I think we knew that, but we didn't really pay a lot of attention to it. At our hospital, it's 40 percent sensitive. At Hopkins, we have not done the EIA test in three years. We can't do it; it's just not good enough. And I think what we need is — we use the tissue culture assay and the common antigen as a screening test, but I'm not saying that's the right one because it still takes a long time.

I expect there'll be PCR technology that will permit us to identify toxin B and that will be — emerge as the rapid, cheap, sensitive test of the year 2007, 2008 — some time in the near future. What we'd also like is a test that would tell us about the new strain — the NAP1. Now as we've repeatedly said in this conference, people don't

culture stool or almost anything else anaerobically. And therefore, stool culture is, for *C. diff*, is common in England and common in Australia, but it's not very uncommon in the United States.

And I think your poll showed like 2 percent of hospitals are doing cultures. So if we're going to get the strains, we're going to have to do the culture or else we're going to have to — have to find a way to have a non-culture-based way to recognize, either by binary toxin or by the deletion. And that would perhaps do it, but that necessarily wouldn't give us a lot of other information about the other strains of *C. diff*.

In terms of treatment, you know, here's the ultimate paradox, isn't it? You got — you got two drugs everybody uses: vancomycin and metronidazole. They both cause and cure *Clostridium difficile* and that's a real paradox of course. And so I think it would probably be good for us to decide what we need in the way of therapeutics.

I think we clearly have to distinguish between what we need for acute disease and what we need for intermittent disease. And that's pretty patently obvious, but they're two completely different challenges. I think it's going to be very hard to beat vancomycin. On paper, that's the perfect drug. It's going to be hard to do better than vancomycin for acute disease.

The problem we have, that all of us have, is the treatment of the patient that can't take oral therapy. Because if you can't take oral therapy, it's very hard to treat *C. diff*. For the patient with intermittent or with recurrent disease that we've talked about, that's

a big problem because of the frequency of it and the morbidity of these people that have recurrent, after recurrent, after recurrent.

My record is 26 recurrences and I have three patients now that have taken vancomycin for three years. So I mean, those cases are really morbid when we see them. And we would love to be able to — and everybody that does this sees them and we all have our different ways to deal with that.

They're completely different. They're not terribly well studied. And all of those 11 treatments work some of the time and none of them work all of the time. So I expect that's the area of new drug development where we are likely to get a better control in the future, at least I hope so.

For prevention, it's interesting. We got through the whole day on *C. difficile* without ever mentioning antibiotic control until Cliff mentioned it a little while ago. But of course, this is an iatrogenic disease except for these new things, these people that have *C. diff* without antibiotics.

But by and large, this is an iatrogenic disease. And in the process of doing the community-acquired cases, it would be interesting to review the antibiotic exposure to see how often that was a justified use of an antibiotic for the hospital. It'd be nice to know.

You know, you can't treat — if you say the big three are the broad-spectrum cephalosporins, fluoroquinolones and clindamycin, if you say those are the big three —

which is what we said today — then I think what we would say is the guidelines for community-acquired pneumonia — which are marching orders for every hospital that gets Medicaid, which is virtually all non-federal hospitals — is to use one of those three classes. Well actually, two of — you got to do — you got to use those drugs.

So in order to find alternative ways for drugs that don't necessarily drive that disease, I mean, we saw the data and I agree with the — with Dale's list, you know: these are the bad guys; these are the middle guys; and these are the almost never guys. It'd be nice for us to, when we write guidelines, to try to figure out how to use drugs that don't drive *C. difficile* because there's big differences in those classes.

We talked about healthcare-associated disease as a high priority in public safety and public reporting. I would personally like to see *C. diff* be a reportable disease. In addition to that, I think the sentinel reports is something that we've talked about.

The VA is a great resource to do that because they have, of course, the best electronic medical record system that we've got in the United States — a 126-bed system. It's the biggest hospital system in the world, so they've got enormous data stored someplace. And it would be lovely to tap that if we could to get rapid answers to data that are already collected, you know, and to a large extent.

In terms of *Clostridium sordellii*, I think it would be presumptuous for me to try to capture the — summarize the beef of the reports that have been made here today because they've been made with such scholarship by people that are in the field. I think

we do get the feel from both diseases that we need better surveillance systems. We got — we, certainly for *sordellii*, we have to have better ways to detect the infection. I think we all know that.

The business of anaerobic cultures is certainly *deja vu* for me because I went through the anaerobic bandwagon as part of that Mafia. And, you know, that was viewed in retrospect as one of the most successful campaigns in the history of medicine. I mean, you took a period of 1960 when nobody had ever did anything for anaerobes and all lung abscesses were non-specific lung abscesses. Appendicitis was treated with pen with strep.

Nobody knew anything about anaerobes; went through that period where there was that intense effort. And everybody had an anaerobic chamber, anaerobic gas-packed jars, EPI, put the nomenclature in order and whatnot. And it was a heyday and then it just went away. So that now I think laboratories, by and large, have abandoned the issue of anaerobic cultures in large part because just what Cliff said; they don't think they need to do it and they can't afford it.

So I think if we're going to be able to culture *C. diff*, or culture *C. sordellii* or any other histotoxic *Clostridia*, I think we're probably going to have to regenerate some interest in knowing when and how to do that, and especially how to do it fast and how to do it cheaply. I'll finish there and thank you.

Closing Session

E. SPEAKMAN: Thank you. And thank you for everybody on the last panel that decided to kind of stick their neck out and with priorities; appreciate that wonderful, great, great job. I want to thank all the panelists that spent a lot of time preparing for this and your presentations; and also, thank the planning team that spent countless, countless conference calls getting to this point; and the participants for your active listening and your questions.

We'll let you all know that this is not an end; this is essentially really a beginning at the agenda, then the research moving forward. So thank you for your time. I know that Cliff, you have a few concluding — oh, we've got Cliff and then Leslye ...

C. McDONALD: Yeah.

E. SPEAKMAN: ... with just a few concluding remarks.

C. McDONALD: Yeah, I just to say again, thank you for all coming on behalf of CDC. And want you to know that there is still the process open docket that still goes on for most of — the end of June. And to June 15th, you still can get comments. And from this meeting, the priorities that are set, the research — public health research, and basic research and surveillance priorities that are set forth, CDC wants to respond to those and will respond to those.

We are dedicated to promoting surveillance, promoting public health research in the ways you've heard, but want to take queues from this meeting, and from what has

been put on the docket, and what has been put forth in terms of steering us and guiding us mid-course. So want to know that there are processes in place.

We will actually take this report, these recommendations, bring them to our director in our center — coordinating center director, Dr. Mitch Cohen; center director, Dr. Rima Khabbaz, who introduced today — and respond to the recommendations in terms of how to her — being accountable to her and to Dr. Cohen in terms of how we are going to modify our course based upon these recommendations.

E. SPEAKMAN: Thank you, Cliff. Leslye?

L. JOHNSON: I'm Leslye Johnson. I work at the National Institute of Allergy and Infectious Diseases and I'm Chief of the Enteric and Hepatic Diseases Branch. And I want to say on behalf of the panel, special thanks to everybody because I was — I'm not a bacteriologist; I'm a virologist by training. But I was very invigorated by the discussion, by the findings, by everything being so up-to-date. And I really want to thank the speakers for bringing us that up-to-date picture to help us flesh-out where we — where we might want to go.

I want to thank the panelists for bringing — and the speakers for bringing forward recommendations. This is what we need. We need to have direction. You just can't go out and pick something off this tree, and this tree and this tree. You really have to end up with a plan. And I think what's happened here will help develop that working plan, which will always be a work-in-progress because it will be modified.

I also want to thank the people who brought forward some interesting hypotheses because that's going to make everybody think a little more and a little harder about what's possibly going on. This is a very complex area and I hope that everybody in the audience got that as part of the message. It is a set of complex interactions between the host and the pathogen.

It's complicated because not everybody — not every — not everyone has the same reaction to the pathogen. It's complex because the disease progression that's different. And every time I hear this, and you hear it a lot if you're looking at clinical research and clinical sphere, this complexity is really a call for some multi-disciplinary research for a group of people putting their heads together and coming up with incisive research.

And I want to emphasize that I think, to the group here, that that's something that the NIH will take home — take home with us. I want to remind anyone who is an investigator that you can always apply for an R01. We don't see very many of these, but you can bet that it's going to be a high priority for us coming out of here. And not only the regular R01, but some of the smaller more developmental work.

I think one thing that we can do is encourage, coming out of this, use of existing government resources where they do — where they do exist. I think CDC is partnering with a lot of people outside the CDC and I think that's very, you know, that's going to be really important, that type of collaboration. I think we need to keep meeting as a federal

group to try to keep our hands on this, and our arms around it to pull in the things that do change and make it part of who we are and what we do.

And the other thing that I was heartened by, I must say, is to hear that there are several clinical trials out there in the *C. diff* field that are with things that might work therapeutically or preventively. And that's heartening because I think then there is progress being made and there's a place here for the public/private partnership between industry, and the NIH and the other groups in order to have things move as quickly as possible.

So I think I'll close there. You've been a great group to work with, and a great audience keeping all of us on our toes and bringing up important other areas to consider. So have a good trip home.

E. SPEAKMAN: Thank you very much. Remember, transcripts within 30 days on the link down here and also to give presentations within the next week. Thank you for your time. Have a great day.

[ADJOURN 5:00 P.M.]

CERTIFICATION

State of Georgia)
)
County of DeKalb)

I, Nadine Rivera, do hereby certify that the foregoing transcript, consisting of pages 1 – 239 in total, was personally typewritten by me and is a true, complete and accurate transcript of the proceedings recorded by me.

I further certify that I am not related to, employed by, or attorney of record for any parties or attorneys involved herein. I further certify that I have no financial interest in this matter.

WITNESS MY HAND AND OFFICIAL SEAL BELOW.

This 5th day of June, 2006.

_____
Nadine Rivera
My Commission Expires: August 1, 2006

[Seal]