TRANSCRIPT
Satellite Symposium during the 18th Annual SHEA Scientific Meeting

Clostridium difficile: *Changing Diagnosis, Epidemiology, and Treatment*
April 7, 2008

Slide 1
Clostridium difficile: *Changing Diagnosis, Epidemiology, and Treatment*

Gerding:
Good evening, everyone. Welcome to the symposium. I'm Dale Gerding. I'm Professor of Medicine at Loyola University in Chicago and I'm Associate Chief of Staff for Research at the Hines VA.

I would like to welcome you to this symposium entitled, Clostridium difficile: *Changing Diagnosis, Epidemiology, and Treatment.* It is jointly sponsored by Robert Michael Educational Institute LLC and the Postgraduate Institute for Medicine. It is CME accredited, as you know. And I would also like to thank ViroPharma Incorporated, who have provided an unrestricted educational grant to conduct this satellite symposium, and to SHEA for their help in setting up the schedule for this activity.

Now you probably have noticed that there's only two of us up here rather than three. Cliff McDonald, unfortunately, has had a family illness. He is in Chicago and he is going to call in and do his presentation on a teleconference call. So we will be able to hear from Cliff. We have all his slides, and he has been very gracious in agreeing to do that while he is in Chicago.

He will speak first on the changing epidemiology of *C. diff*. I will follow with a discussion about something that we've never really talked much about at these kinds of symposia, that is, surveillance definitions and the issues around diagnostic testing that are seeming to become more and more important all the time. Then my colleague Stu Johnson will talk about the current and evolving *C. diff* treatment strategies, which really are changing very, very rapidly. And finally, we'll both be available, and I think Cliff may also be available by phone, to take your questions.

Housekeeping items. If you could please put your cell phones, pagers, beepers on vibrate mode and that will help to eliminate disturbance for other people. That's a reminder for me also to do that. Second, please refer to your workbook for the learning objectives of this program as well as the full disclosure information. And last, in order to receive your CME credit, you must complete the activity evaluation. That form is located in the back of your workbook. Please hand it in to one of the attendants at the conclusion of the program.

So I'd like to begin by introducing Cliff McDonald. He's the medical epidemiologist at the Centers for Disease Control and Prevention in Atlanta. Cliff is a real expert in the epidemiology of this disease and he's going to talk to us about that changing epidemiology. Those of you who read *MMWR* probably know that there was a report just last week on the community *C. diff* rates

coming out of Connecticut. So I'd like to begin the program by having Cliff give his presentation.

So while we're waiting, Stu Johnson has an announcement.

Johnson:
Thank you, Dale. Just while we're waiting for the technical difficulties, I do want to mention that the Anaerobe Society of the Americas is meeting in Long Beach June 24 through 27, this summer [2008]. This is a meeting on anaerobes in general, but it has a special emphasis on *Clostridium difficile*. One whole day will be devoted to various aspects of *Clostridium difficile*. John Bartlett will start the day and Sherry Gorbach will end the day. So if you're a *C.diff*-ophile, very interested in this, it's a great meeting, great location. I've got some flyers up here. You can come up at the end of the meeting.

Slide 2
***The Changing Epidemiology of* Clostridium difficile**
L. Clifford McDonald, MD, FACP, FSHEA

Hello. This is Dr. L. Clifford McDonald. I want to thank you for your willingness to allow me to participate via teleconference.

The first slide I want to discuss, *The Changing Epidemiology of* Clostridium difficile, is my title slide. I want to draw attention to my obligatory disclosure, courtesy of the CDC.

Slide 3
Pathogenesis of *C. difficile* Infection (CDI)
Turning to the next slide, entitled *Pathogenesis of* C. difficile *Infection*, the usual way that we talk about *C. difficile* infection is we focus first on the organism. This is a little different presentation in the sense that to really understand the epidemiology, we must understand the pathogenesis of the disease. *Clostridium difficile* is an anaerobic spore-forming bacillus and it is the spore form that is ingested, which then transits down past the stomach, because it is relatively stomach acid–resistant, into the proximal duodenum and jejunum, where it germinates and then transits down into the large bowel, the lower intestine, where it takes up residence in the deep crypts, proliferates and there produces toxins, which are the cause of the disease and the pseudomembranes and the other damage to the colonic mucosa.

Slide 4
Prerequisites for CDI
Turning then to the next slide, the *Prerequisites for* Clostridium difficile *infection*. Just as what is the pathogenesis, what are the prerequisites? Well, one of the, at least previously thought to be ubiquitous prerequisite, was antimicrobial therapy. That then disturbs the colonic microflora and gives room for the organism to become established in the lower intestine, to proliferate and then produce toxins that cause the disease. And then of course, also a prerequisite, the actual acquisition of the organism, less than 5% of the general healthy population walk around colonized with this organism. Probably all of us have been exposed to it early in life. There's

evidence at least in 50% or 60% of us as adults, in that we have antibodies to toxin A and B, albeit at low levels. Then as we grow older and we have less immune responsiveness or we have underlying illnesses and we take antibiotics, then we're exposed, especially in the hospital setting where there's a higher risk of exposure–we'll talk about that in a moment. There we acquire the illness. We don't know all there is to know about the incubation period between what is really probably a recent reacquisition of *C. difficile* and disease. One study suggested it might be less than 7 days. Maybe it is out to several weeks. But the main point that we do know is that relatively recent reacquisition of *C. difficile* appears in proximity to the development of disease. It's not people walking around chronically colonized with *C. difficile* who get *C. difficile* infection, but more often the person who is exposed to antibiotics in the hospital usually and then is re-exposed or reacquires. And because of antibiotics, of course, perturb the lower GI [gastrointestinal] flora for a number of weeks after you stop the antibiotic, the effect of antibiotics and setting someone up to get *C. difficile* disease or infection, may last for weeks after those antibiotics are stopped. So the antibiotic exposure and the *C. difficile* exposure, the exact timing of those isn't known, but they appear in proximity and appear to occur in proximity and both are necessary for disease.

Slide 5

National Estimates of US Short-Stay Hospital Discharges with *C. difficile* as First-Listed or Any Diagnosis

Let's then turn to talk about what's happening. Here we have a slide entitled *National Estimates of US Short-Stay Hospital Discharges with* C. difficile *as First-Listed or Any Diagnosis*. This is from hospital discharge data, of course. Actually samples thereof. The blue line showing discharges with *C. difficile*, any listed diagnosis, and the orange line, first or primary listed diagnosis. You can see that there's been a marked increase, really since the year 2000, with a 42% increase from 2000 to 2001. And continued increases since then. From 2003 to '04, a 25% increase. And from '04 to '05, a 10% increase. This is drawn from the National Hospital Discharge Survey from the National Center for Health Statistics.

From this sample of hospital discharges, we can estimate that in 2005, there were around 250,000 acute care hospital discharges, where *C. difficile* was listed as the discharge diagnosis.

I will say, however, the number is actually probably greater than that in 2005. There's another data set called the HCUP [Healthcare Cost and Utilization Project] data or the Nationwide Inpatient Sample, which actually collects data on additional discharge diagnoses. The National Hospital Discharge Survey, from what this is drawn from, only has the first seven hospital discharge diagnoses listed, but that other data set lists 15. In fact, a person by the name of Dr. Mike Young presented some of these data here at this meeting. Because of those additional discharge diagnoses, they have an estimate of more on the order of 300,000 cases in 2005. So 250,000 is probably an underestimate of discharges. It's probably even greater than 300,000. But what's also more important is that it continues to increase.

Slide 6

Evidence of Continued Increase in 2006

If we look to the next slide, *Evidence of Continued Increase in 2006*, is impressive. This is from a smaller hospital discharge data set (the Premier Inc. Data). This was presented at the IDSA

[Infectious Diseases Society of America] meeting last year. The main point to take home is that again, from 2005 to 2006 in this smaller discharge data set, there's again evidence of significant increases. So this epidemic appears to be continuing to rise. It has not peaked yet, it has not yet turned the corner. We hope that that will happen very soon.

Slide 7
Yearly *Clostridium difficile*–related Mortality by Listing on Death Certificates, United States, 1999–2004
Especially because when we look at the next slide, we see that this is not just an academic exercise in discharge diagnoses, but there's also real evidence that more people are dying because of this. This is *Yearly* Clostridium difficile–*related Mortality by Listing on Death Certificates*, again, from a national sample of death certificate data. From 1999 to 2004, we can see the steep rise in the number of deaths with *C. difficile* listed on the death certificate. Certainly up to 2003 this doesn't seem like this is just because of increased awareness. Before 2003, actually before 2004, there was not much awareness here in the United States that something was going on with *C. difficile*. Yet you see this steep rise in the number of death certificates listing *C. difficile* as a contributing death. In 2004, if you extrapolate this estimate of deaths per million, 23.7 deaths per million population, that comes to just over 8,000 deaths in the United States in 2004. Of course, we know what's listed on death certificates is not always what people really died of, but the converse is also true. There's probably many more people, and we'll see that in a moment, who died with *C. difficile* contributing to their death, that no one realized that that was a contributing factor.
This has focused so far on acute care hospital discharge, on acute care hospital cases. And here on deaths, that probably occur predominantly in acute care hospitals.

Slide 8
Public Reporting in Ohio, 2006
But as we see on the next slide, from the public reporting that took place in Ohio in 2006, as many of you are aware, the state of Ohio mandated the reporting of *C. difficile* in nursing home patients and in acute care hospitals just for that year. Mainly because they had some very prominent outbreaks in the latter part of 2005. From that year reporting from nursing homes and acute care hospitals yielded 14,100 cases.

Actually when you fill in some of the missing data from that year of reporting, it's more on the order of 18,000 cases.

But what's most interesting here, we won't get into the details of initial versus recurrent cases, but there's a tremendous burden of cases from the long-term care facilities. And these were predominantly nursing homes. On the order of 7,000, actually almost 8,000 cases, from long-term settings, compared to just 6,000 from acute care hospitals.

Now granted, some of these cases that have their onset in long-term care facilities do get admitted to the acute care facilities and they may very well be represented in that hospital discharge data that I showed you a couple of slides ago. However, we have to keep in mind that there may be tremendous burden of cases in long-term care.

Slide 9

Outcomes of CDI in Setting of Endemic Disease

Turning now again to better understand some of the outcomes. We mentioned the death certificates. But this slide, entitled the *Outcomes of* C. difficile *Infections in Settings of Endemic Disease*, brings home something else. And that is that previously a lot of estimates, both in cost and in attributable mortality, have been made in the outbreak setting. These are some studies that have been done at the Washington University in St. Louis, Erik Dubberke and colleagues. First they showed that even in an endemic setting, with endemic disease, *C. difficile* is very costly and they estimate that ranging from $2,000 to $3,000 per case, just for the index hospitalization. But then when you look at inpatient costs over the next 180 days in these people and compare them to other patients, the cost can become quite significant, with a larger range, from just under $4,000 to $7,000 of inpatient cost. That doesn't, of course, account for other things like lost days of work and other things.

Then the other outcomes here listed again, excess hospital days for that index hospitalization, attributable re-admissions on the order of 20% of persons and I guess that probably has a lot to do with these recurrences that we know so much about. And here the attributable mortality measured out to 180 days was just under 6%. Again, significant, because this is an endemic situation. And again, this is much higher than historic attributable mortality rates of 1% or 2% recorded in the '90s.

Again, something else that we haven't really put our hands around fully, but may make *C. difficile* very, very costly to the entire society, is the fact that a person who comes from home, is admitted to an acute care hospital and there develops *C. difficile* infection, is more likely to be discharged to a long-term care facility.

Slide 10

Current Epidemic Strain of *C. difficile*

What are the reasons—let's turn to the next slide—and talk a little bit about what is one of the main reasons we think that *C. difficile* may have changed so dramatically. And that we think is due to the current epidemic strain of *C. difficile*. This strain has been known as the BI strain, the letters BI, by restriction enzyme analysis performed at the Hines VA there in Chicago. The North American Pulsed-Field type 1, which is the typing system where we're using pulsed-field gel electrophoresis at the CDC. And the PCR [polymerase chain reaction] ribotype 027. PCR ribotyping commonly done in Canada and Europe. Also this strain has got a particular variant toxinotype known as toxinotype III. Toxinotyping involves genetic typing of the toxin genes themselves and surrounding regulatory genes.

This strain, though, is unique by all these markers, it is a strain that has been around. In fact we know from Dale Gerding's database, going back to the early 1980s, this strain has been around. But it's been historically uncommon and now has become epidemic throughout the United States. Its change in behavior is coincident in the terms of timing, with this strain becoming more resistant to the fluoroquinolones, more highly resistant to all the fluoroquinolones. This strain always was peculiar in that it carried an extra toxin, known as binary toxin. It's not totally unique in that, there are other strains that carry this extra toxin as well. And this extra toxin, we really don't know if it in any way contributes to increased virulence. But there also are some polymorphisms in the toxin A and B regulatory gene known as *tcdC* and these polymorphisms

may be, we don't know, but may be linked to the fact that at least *in vitro* this strain does appear to produce more toxins A and B in broth culture systems.

Slide 11
Increased Toxin B Production *In Vitro*
And that's shown on this next slide entitled *Increased Toxin B Production In Vitro*. This was a broth culture method that Michel Warny performed in his laboratory in Kansas when he was there, where they cultured the NAP1/027/BI strain and compared its toxin production, shown in the orange line here, with the squares. Compared it to other strains. You can see that at least here in toxin B production, there's much more toxin production earlier in log-3s of growth, and overall increase on the order of 23-fold more toxin B and 16-fold more toxin A.

Slide 12
States with BI/NAP1/027 Strain of *C. difficile* (N=38), November, 2007
Let's look now at the next slide and see that this strain has in fact become rather ubiquitous. Here's the strains, the 38 states where this strain has been recovered, usually from outbreak situation. And this was as of November—at least since then we at the CDC are not aware of any additional states. But basically if you look here on the map and there's a state that's not colored pink, it's probably because no one has cultured for *C. difficile*. Because very few people are culturing for *C. difficile*. It's very likely that if any outbreak occurred tomorrow in any of the states, it would probably have some association at least with this strain. At least that's what we've been seeing thus far.

Slide 13
Countries in Europe with BI/NAP1/207, November 2007
And this strain also, if we go to the next slide, see the countries in Europe with this strain, as of again, November '07. You can see there's over 10 countries in Europe that have seen this strain, usually associated with outbreaks.

Slide 14
CDI at an Atlanta VA Long-Term Care Facility
Some of the early outbreaks, in fact this next slide entitled, C. difficile *Infection at an Atlanta VA Long-Term Care Facility*, from the early outbreaks, the early part of this decade, we notice that fluoroquinolones were often associated. And this outbreak occurred temporally related to a formulary switch from levofloxacin to gatifloxacin. They showed through an outbreak, through a case-controlled study during the outbreak, that gatifloxacin was an important risk factor for cases. They switched back to levofloxacin and they saw a decrease. It made a lot of people think that maybe these fluoroquinolones are not equal in terms of their ability to cause disease.

Slide 15
The Problem with Fluoroquinolones is a Class Effect
However, we show on this next slide, the problem with fluoroquinolones is really a class effect. This was another outbreak in Pennsylvania where we tried doing the same thing. They had a formulary switch from levo- to moxifloxacin, shown in the first arrow to the left. You can see the orange line of cases. The other lines on this slide are total antibiotics in blue on the top, total fluoroquinolone use in purple in the middle, and the red line is days of moxifloxacin, and defined

daily doses of levofloxacin in green. You can see soon after that formulary switch, there was this big increase in *C. difficile* cases and, in fact, during the outbreak, moxifloxacin was a risk factor for being a case. So they tried switching moxi back to levo and you can see that that did nothing to their *C. difficile* rate.

Unfortunately, the medical staff started using more levo than they had been using levo and moxi combined during the outbreak. After they switched back to levo, they actually used more levo than they had been using in terms of moxi during the outbreak.

So without controlling overall fluoroquinolone use, this study suggested that simple formulary switches is not going to achieve anything.

Slide 16
Interventions to End an Outbreak, Hospital A, Baltimore 2004–2007
This next slide shows some data from a poster that was presented yesterday here at SHEA. And this was just a severe outbreak in the Baltimore area and tried several different interventions. You can see here the number of cases on the Y axis and the month and year on the X axis. Tried several different infection control interventions, while the outbreak, number of cases, continued to climb. Finally, because the cases were so high in number, the medical staff came together and agreed to restrict all fluoroquinolone use.

Soon after that, unfortunately these things always happen, that several things happen at once, and in fact the environmental services company was fired and a new one was hired, and this was all happening coincident with a decline in cases. So it's hard to draw a firm conclusion of exactly what drove the decline.

Slide 17
Defined Daily Doses of Most Commonly Used Antibiotics, Hospital A, Baltimore
But it is interesting that, if we look at the next slide, the defined daily doses of the most commonly used antibiotics at this hospital, you can see that probably overall antibiotic use did decline. Actually I don't have it on this slide, but on another slide we do show that, that fluoroquinolone restriction you can see in yellow, the bright yellow line, how all fluoroquinolones went away. That actually there was a decline in use even before the restriction was actually enacted. With that there was an overall decline in overall antibiotic prescribing.

So just through restricting all fluoroquinolones, they were able to reduce total antibiotic use by about 20%. That was interesting in its own right, just in terms of how much movement we probably can have in our formulary in terms of total antibiotic usage reduction, just by making people think a little bit more about antibiotic prescribing. So that's one important point from this. And maybe that total antibiotic prescribing is the most important thing that may have happened in controlling this outbreak. We don't know.

Slide 18
Novel Risk Factors, Washington University Prevention Epicenter (n=36,086)
But let's look on to other novel risk factors. And these aren't really so novel, but they are kind of interesting, the way they were presented. Again, this is work from Erik Dubberke's group at

Wash U. And one thing he was able to show from this large cohort of patients that he had detailed data on, are over 300 *C. difficile* cases in this endemic study period. They were able to show that there was this significant independent risk just from being in proximity to other *C. difficile* cases, even when controlling for other variables. This is known as the *C. difficile*-associated disease pressure or CDAD pressure. You can see the increasing odds ratio going up with increased *C. difficile* pressure.

Also some other interesting, somewhat unique risk factors, in that they were able to show that IV [intravenous] vancomycin, 7 days, was an independent risk factor. Again, as others have shown, but not uniformly, but others have shown that acid-suppressing medications, stomach acid–suppressing medications, may be an independent risk factor for disease.

Slide 19
***C. difficile*-Associated Disease Pressure**
Just to say more about, in the next slide, with *C. difficile*-associated pressure, this is a schematic showing what we mean by *C. difficile*-associated pressure. It just goes back to one of those earlier slides I showed you, that the two things that are necessary to get *C. difficile* is to newly acquire the organism and to be exposed to antibiotics. At least in the hospital, those two are required. This just shows how you're more likely to be exposed to *C. difficile* if there are more people who are symptomatic with *C. difficile* on that same ward. Even though they're not in the same room, they're on the same ward, therefore within proximity to the patient through contamination of healthcare workers' hands and other shared medical equipment.

Slide 20
CDI in Previously Low-Risk Populations
Let's turn to the next slide. Want to draw some attention to the fact that we're aware that *C. difficile* infection can occur in what we previously thought were low-risk populations. Two of these were mentioned in the *MMWR* back in December '05, and these were pregnant women and the generally healthy persons in the community, some of whom had no precedent antibiotic use.

Slide 21
Cases of Severe Pregnancy-Associated CDI
Looking at the next slide, I just want to update you on cases of severe pregnancy-associated *C. difficile* infection. There are now 10 cases that we are aware of, of very severe infection, that we have recently reported in the journal *Obstetrics and Gynecology*. Two of these were reported previously. But only three of these had prior hospitalization. One had no previous antibiotic exposure. Several were pre-term. Others were just postpartum, within 1 week of delivery. But what was notable in all 10 of these and why we report on these was the severity of disease. Six required intensive care unit care for toxic megacolon. Two of those in fact did have this epidemic strain. Five of these 10 required colectomy. And there were three maternal deaths and three fetal losses. So it's something certainly we should be keeping our eye on.

Slide 22
Survey for Pregnancy-Associated CDI Emerging Infections Network of the Infectious Diseases Society of America

We did try to look into this with the Emerging Infections Network of IDSA. A survey was performed back in '06, of 419 ID clinicians, who reported seeing or being aware of approximately 40 of these cases—I'm sorry, 55 cases of pregnancy-associated *C. difficile* infection. Most of these occurred prior to delivery. There was a high relapse rate. Well, actually the usual relapse rate of around 20%. No real alarming increase in overall—this is a low number of cases—but something we need to certainly keep aware of. So the take-home message on this is that there can be very severe disease in pregnancy or peripartum period, but fortunately it remains fairly uncommon, albeit not rare.

Slide 23

***Clostridium difficile* Surveillance Definitions**

Dr. Gerding will be talking about surveillance definitions, but I want to just use some of those definitions he'll be talking about here in this slide. Change slides to a flowchart, showing *C. difficile* infections, the total number . . . This is some work that we did in North Carolina as an investigation of community-associated disease. But it gives you a sense of where community-associated disease really fits in the overall epidemiology. You can see here it's about 20% of all *C. difficile* infections in these six hospitals in North Carolina. Over half had healthcare facility–onset disease, but around 40% had community-onset disease. And then about half of those, or just under half of those, had what we call community-associated disease, meaning that they had not been overnight in a healthcare facility for at least 12 weeks.

So community-associated disease is an important portion of all *C. difficile disease*; about one-fifth of all cases appear to be community-associated.

Slide 24

Estimated Incidence of Community-Associated CDI in North Carolina, 2005

Now when we looked at this community-associated disease in North Carolina and we calculate, on the next slide, this is the estimated incidence of community-associated infection in North Carolina. This is a population-based estimate, the best we could do at least. You can see we estimate around 25 cases per 100,000 population. Now this is about 2½ times or 3 times what we reported in that *MMWR* back in December '05. And also what was just reported this week from Connecticut, if you're aware of that, *MMWR*, Connecticut Emerging Infections Program, reported their experience with passive reporting of community-associated *C. difficile* infections. AWe think that this difference of 25 per 100,000 may be just because we were actively accounting for all cases and so that the estimates that we've given before of 8 to 12 per 100,000 are probably about half or one-third, one-third to one-half of the true incidence. We think this is probably closer to it.

What's also interesting is that I think in Connecticut they also showed what we're showing here on this slide, and that is that females, women, appear to be at higher risk of this community-associated *C. difficile* infection. We're not quite sure why that is, but now that seems to be confirmed.

And we in North Carolina in this study by Dr. Kutty, and these data were presented previously in Toronto at IDSA in '06.

Slide 25
CA-CDI in North Carolina, 2005
On the next slide, just shows some of the predisposing factors in the subset of these community-associated cases from North Carolina. And what's most interesting again, and also I think borne out by the data from Connecticut, is that in the community, in community-associated disease, seems to happen maybe even half the time without recent exposure to antibiotics. I think it is interesting to see that borne out from the Connecticut experience also.

This is, of course, throwing quite a wrinkle in the earlier slide I showed you with the important prerequisite of antibiotic exposure disrupting the GI flora. Now we don't know, maybe there's something else now that people are using that disrupts their GI flora, and certainly some of the acid-suppressing medications, namely the proton pump inhibitors, were one important factor. We didn't find it necessarily as a risk factor here, but—I'm not showing that data—but we find prevalent use. Everyone and their brother seem to be on a proton pump inhibitor.

It also appears from some of that data that outpatient visits could be playing a role.

Slide 26
Enhanced surveillance for Strains Causing CDI in Community
So let's turn to just what are the strains that may be cause for some of this, causing this community-associated disease. This is the next slide entitled, *Enhanced Surveillance for Strains Causing* C. difficile *Infection* – not CDAD – *in the Community*. This was from 10 FoodNet sites across the United States, where they collected putative isolates – well, they're not putative isolates, they're isolates from putative cases of community-associated *C. difficile*. So at these ten FoodNet sites – FoodNet is one of the Emerging Infections Programs Surveillance Systems – just based upon chart review, medical record review, no interviews, but based upon available medical record reviews, they looked at cases that had no previous hospitalization for 3 months. And they collected the stool on these *C. difficile*-positive patients, who met this criteria. And they had to be not in the hospital for 3months and not in the hospital for more than 32 hours if they'd just been admitted. And from these, tried to get 100 isolates, and achieved in getting 92 isolates from nine states. And these were submitted to CDC for testing. These data were presented, both in Slovenia at the *C. difficile* conference, and I think also at ASM [American Society for Microbiology].

Slide 27
Recognized PFGE Patterns Among CA-CDI Isolates
If we go to the next slide, we can see the recognized pulsed-field gel electrophoresis patterns among these community-associated isolates. What's most interesting is that there is the NAP1 epidemic strain as a cause of community-associated disease. But then the other thing that's interesting is there's a lot of heterogeneity. So what we see in hospitals is, unfortunately, a lot of epidemic disease, a lot of clonal spread. Out in the community it appears more heterogeneous and a variety of different pulsed-field types. These are all different North American Pulsed-Field types.

Notice North American Pulsed-Field type 7 and North American Pulsed-Field type 8 in light blue and navy blue, respectively, accounting for about 10% of these isolates.

Slide 28
Toxinotypes Represented Among CA-CDI Isolates
If we look at the next slide, we see the same data broken down by toxinotypes rather than pulsed-field types. And the two pulsed-field types I just mentioned, NAP7 and NAP8, are represented on this slide by toxinotype 5. And you'll notice it's about 10% of all the isolates are this toxinotype 5.

Slide 29
CDI in Piglets
Well, why is that important? On the next slide we can turn our attention, we'll come back to this point of toxinotype 5, and just mention the fact that there is *C. difficile* infection in food-producing animals, and this has been the work of Glenn Songer in the United States and Scott Weiss and colleagues in Canada. And it's both in pigs and in cattle. In piglets, especially in Songer's work, has shown that it's causing a disease, actual disease, and actual morbidity and mortality in these pigs, causing scouring from birth, which is diarrhea and edema of the colon. And the usual type of pathologic findings of pseudomembranous colitis.

It appeared that this kind of emerged or really increased just around the year 2000, just when *C. difficile* epidemiology was changing in humans also. And interestingly, a lot of these pigs seemed to get disease without previous exposure to antibiotics.

Slide 30
Epidemic Animal Strains Share Characteristics with the Human Epidemic Strain
And if we look at the major strains on the next slide, the epidemic animal strains, causing disease in pigs and also found very prevalent in cattle, although not necessarily causing disease, we see that the strain, the epidemic animal strain is different from the current human epidemic strain. But it does share some important characteristics. It's a different PCR ribotype, of course, on the second row here. First of all, it's a different toxinotype. The current epidemic human strain is toxinotype 3. These strains are toxinotype 5. PCR ribotype is different, 027 versus 078. And the PFGE patterns, as I mentioned previously, NAP1 versus NAP7 and NAP8. They're all binary toxin–positive, they all have polymorphisms in *tcdC*, and they all therefore have a very truncated tcdC protein.

Interestingly, we do, although I'm not showing the data, know that these toxinotype 5 strains do produce a little more toxin than wild-type toxinotype 0 strains. Albeit nowhere near the amount of toxin as the human epidemic strain.

Slide 31
Tox V (BK/NAP7-8/078) Strains
But if we look at what's going on in humans with these toxinotype 5 strains, we look at the next slide, toxinotype 5 strains are also known as REA type BK. Again, NAP1 or PCR ribotype 078 strains. These in humans have been historically rare and recently are becoming more common. Again, thanks to the great database that the Hines VA lab has been keeping track of over these

last several decades, we can tell that in that database of over 6,000 isolates, there were only about 10 of these isolates. From before the year 2000, only 10 of 6,000 isolates in that historic database were the toxinotype 5 type. And then the second row is from 2000 to 2005, around 600 isolates were received at CDC for typing. Only 10 of those were this toxinotype 5 strain. And now since 2005, we've gotten 125 isolates in at CDC and six of those are toxinotype 5.

So there appears to be some increasing incidence, again, just through convenience samples of isolates coming for testing from humans. Mostly from hospitals. In fact, if we looked in the community, like I showed you, these isolates I'm mentioning on this slide are actually mostly hospital outbreaks, but when we look specifically in the community, then we find that these are accounting for about 10%.

So two major points to take home is that these animal strains appear to be increasing in prevalence, being more common in the community than in the hospital.

Slide 32
Human CDAD Caused by Strains Similar to Animal Epidemic Strains, 2001–2006
And on this next slide shows human infection caused by strains similar to animal epidemic strains. Again, these are all the toxinotype 5 isolates. And you can see by pulsed-field gel electrophoresis that they can look even identical. There are some identical matches between human and pig strains, for example, on this slide.

Slide 33
***Clostridium difficile* in Retail Ground Meat, Canada**
And this is all important, I guess, mainly because if we look at the next slide, that *Clostridium difficile* has been found in retail ground meat in Canada and also found in the United States. And in fact, here in the United States, although it's not yet published, has been presented that this toxinotype 5 strain and even the NAP1 strain, the BI/NAP1 strain, has been found in retail meats. So we'll stay tuned there.

It appears, however, that if the food supply is important for *C. difficile* in any way, it may just be in terms of migration of strains between animals and humans. Certainly most human—the vast majority of human cases of *C. difficile* infection are person-to-person transmission in healthcare facilities.

Slide 34
Summary
So just to summarize, the last slide. Rates, mortality and costs associated with *C. difficile* infection continue to increase. Much of this increase may be due to the emergence and spread of this epidemic strain, the BI/NAP1/027 strain. The disease is becoming more notable in previously low-risk populations such as pregnancy and in the community. And the community-associated disease appears associated with variant toxinotypes, somewhat different from the major toxinotypes causing disease in hospitals, where it's more clonal. And more often toxinotype 0. And finally there is now I think some emerging circumstantial evidence for animal-to-human transmission of some strains, namely these toxinotype 5 strains, which so far are not

that important in human disease, but we need to keep our eyes on that. So thank you very much for your attention.