C. difficile in Pigs and People, Europe

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

[Sarah Gregory] Hello, I'm Sarah Gregory, and today I'm talking with Dr. Geraldine Moloney, an infectious diseases physician at Cork University Hospital in Ireland. We'll be discussing the transmission of *C. difficile* in pigs and in people across Europe.

Welcome, Dr. Moloney.

[Geraldine Moloney] Thank you so much for the invitation to speak with you today.

[Sarah Gregory] To start with, tell us a little bit about *C. difficile*. What is it?

[Geraldine Moloney] *C. difficile* is a bacteria which thrives in surroundings where there's no oxygen or air. And to get around this and transmit between people, it has adapted by forming these spores which can survive both exposure to oxygen and lots of different cleaning methods, which can make it very troublesome to get rid of once it's established in a place. So a human can ingest one of these spores, the spore passes through the stomach to the intestine, and within the intestine it depends on local conditions or that the spore will be inactive and won't cause any trouble, or the spore starts to grow. The conditions that we think about include what other bacteria are present in the intestine. So we all have these really good bacteria which form the gut microbiome, and when they're working normally, they can actually keep these spores under control and not let the *C. diff* develop into an active bacteria. But when something happens like a person takes antibiotics for a different type of infection, unfortunately the antibiotics can damage the gut microbiome (the other bacteria which are present). The other bacteria can no longer control those spores, and then the spores reactivate which allow the *C. diff* bacteria to grow. When the bacteria is growing, it produces toxins. And it's these toxins which can cause the infection of a human all within the colon, so we call it colitis.

[Sarah Gregory] Originally, it was thought that *C. difficile* was only acquired in hospitals. But according to your study, that is not the case at all. Where and what are other sources of transmission are there?

[Geraldine Moloney] So when we look for it, we can find *C. difficile* in lots of different types of places. So in other studies, it has been found in people's homes, it has been found in other buildings, it has been found in retail outlets, even on retail baskets on the handles of shopping trolleys. It can be found in rivers and river water. It can be found in farms, in meat processing plants. It really could be found in lots of different types of places. It has been identified in lots of different types of food. It has been found in meat, in seafood, and in vegetables. But we don't really know if it could be a foodborne illness or not.

[Sarah Gregory] Okay. So nowhere is safe from it.

Is it very contagious? I mean, I know you said that you had to have the right environment for the spores to turn into infection. But is it contagious?

[Geraldine Moloney] It is a very contagious infection. So in the hospital, whenever we suspect that a person might have *C. difficile* infection, even before that's confirmed by doing certain tests we take extra precautions that any member of hospital staff or any visitor to that person will use lots of extra personal protective equipment, we ask people to wash their hands with soap and water instead of using alcohol hand gel, and that we really recommend that the person with

symptoms should be in a single room with a private toilet facility to reduce the chances of transmitting the bacteria onto any other person.

[Sarah Gregory] And what are some of the symptoms of infection?

[Geraldine Moloney] The most important symptom is diarrhea, and we would expect to see at least three episodes in 24 hours. Because it's an infection of the intestine, the person can experience quite a lot of abdominal pain, and then they can have some systemic symptoms like fever, nausea, malaise, and really feel very unwell in themselves.

[Sarah Gregory] What kinds of treatment are used for it? I guess if antibiotics can cause it or make the environment more amenable to it, then....

[Geraldine Moloney] Yes. So antibiotics are a part of the cause for *C. difficile*, but we also use antibiotics as part of the treatment. So mostly these are antibiotics by mouth, so we use vancomycin or fidaxomicin and much less commonly nowadays, there's another antibiotic called metronidazole. Some of the newer treatments have included a monoclonal antibody called bezlotoxumab—are not intended to reduce the risk of a person falling into a cycle of having recurrent episodes of *C. difficile*. And then if someone has had lots of different treatments but unfortunately get recurrent *C. difficile*, there's even an option of giving fecal microbiota transplantation to try and replace those good bacteria in the intestine, and almost reset their system. And very, very rarely that if somebody has a severe, complicated infection with a lot of damage to their intestine, they may actually need surgery.

[Sarah Gregory] Tell us about this fecal treatment. I know that it has been in the news, but it's probably not as sensational as it sounds. How is it actually done?

[Geraldine Moloney] So it's probably not the most appetizing kind of treatment, so if people are sensitive they may not want to listen to this particular answer. But there are two ways of arranging a fecal microbiota transplant. That one is by using a donor, either somebody known to the patient or somebody who's working within the hospital that they would provide some stool themselves. There's a lot of screening to really minimize the chances of transmitting any other infection with this. The stool can then be changed into a milkshake-kind of consistency for one form of administration, or it can be formed into these kinds of capsules, as the capsules can be swallowed almost like any other type of medicine.

The other way of giving it is actually an organization that was formed in the United States called OpenBiome. And really what they do is that they have a really elite panel of donors, so they really know lots about their health status, about any potential risks of infections, their exposures. And then their donors keep on donating, but they've created almost a bank of these really good, healthy samples that can be used to be formed in capsules and then can be provided as fecal microbiota transplantations when needed.

[Sarah Gregory] Why does this work?

[Geraldine Moloney] So this is about having the community of good bacteria back in the intestine. So it's not just about which bacteria are there, it's also about what kind of metabolism happens with certain bacteria. So what we want is to have a really healthy community of the normal bacteria which should be present, and we want them to promote certain types of bile after metabolism that depending on whether there's primary or secondary bile acids. One type really allows *C. difficile* to flourish, and the other makes it really difficult for *C. difficile* to grow. So we

want the nice, healthy community of other bacteria, and we want conditions to be very difficult for those *C. difficile* spores to regenerate.

[Sarah Gregory] What happens if *C. difficile* goes untreated?

[Geraldine Moloney] So if it's untreated, it can progress to a very severe infection. And I've already mentioned that in some cases, very rarely, that a person may need surgery. But otherwise, there can be profound systemic consequences in which a person can develop systemic shock, they might need admission to the intensive care unit. And in fact, every year there's approximately 15,000 to 20,000 deaths directly caused by *C. difficile* in the United States.

[Sarah Gregory] And hand sanitizing gel is not a better option, or an option at all? We need to actually wash our hands with soap and water regularly?

[Geraldine Moloney] We certainly acknowledge on that washing hands with soap and water is best. In the laboratory when we're working with *C. difficile*, we actually use the same kind of alcohol that's in the alcohol hand gel to kill off other bacteria and allows us to select for *C. difficile* to work with. So I've certainly seen firsthand in the laboratory that the alcohol gel, even at a 96% concentration, it has absolutely no inhibitory effect on *C. difficile*.

[Sarah Gregory] That's very important for people to know. I had no idea.

Who is most at risk for getting this infection? I know you have to have the right environment, but I mean, you know, how young? Old? Immunocompromised?

[Geraldine Moloney] So increasing age and immunocompromised—so any kind of condition or any kind of medication that would reduce a person's immune response to the infection. Because if a person has good antibodies either to the toxin or to the surface layer of protein of the *C. difficile* bacteria, it can vastly alter the probability of they're having *C. difficile* or having an active infection or a recurrence. In terms of people with an immunocompromised, we do see the people with conditions like ulcerative colitis (which is a kind of inflammatory bowel disease) can be particularly vulnerable. And anybody who's had multiple courses of antibiotics, because of the effect in the gut microbiome, would be at risk of *C. difficile*.

[Sarah Gregory] In your study, you talk about different ribotypes of *C. difficile*. What are ribotypes and why are they important?

[Geraldine Moloney] So the PCR ribotype analysis is a method of comparing a certain piece of DNA between two or more samples of *C. difficile* bacteria. And for each sample that we test, we produce an image which is quite like a barcode. So whenever we see an identical barcode we call a certain ribotype, and it means that the stretch of DNA between those two samples is similar. So there could be a quite close relationship between those samples. And this has been known as the North American pulsed-field gel electrophoresis, which some of your listeners may have seen in other studies.

So when we think about the *C. difficile* epidemic in the early 2000s, that it was recognized that one particular ribotype which was called 027, and that's also known as NAP1 for anyone who used the North American pulsed-field gel terminology. That particular ribotype was associated with far more severe infection and that was eventually understood because the ribotype 027 was able to produce an entirely new kind of toxin that we hadn't seen before. So the ribotype analysis has been very useful at a population or a regional level, but it only looked at one small piece of DNA. So it doesn't help us fully resolve an outbreak or an epidemic. And that's why we have

other techniques, including genome analysis, where we're comparing almost the full DNA code between the samples. And this gives us much more insight into epidemiology at other levels.

[Sarah Gregory] In your study, you looked at the relationship between *C. difficile* ribotype 78 isolates in people and pigs. Why did you want to focus on this specific one?

[Geraldine Moloney] So over the past 15 years, the ribotype 078 has been associated with severe *C. difficile*, but the epidemiology from the start (or from the 15 years) has been quite different. It has far more often been associated with *C. difficile* in the community where the person hasn't been a hospital inpatient, and it has also been associated with animals. So this has been very, very different to what we understood about ribotype 027, which we do think about as the archetype of the hospital infection *C. difficile*. So because this challenge is so many of the conceptions of ribotype 027 and *C. difficile* of the early 2000s, we felt that it really gives us a lot more understanding into the broader picture of *C. difficile*.

[Sarah Gregory] Ribotype 027 (the hospital one) seems to have been brought under control in the UK. How was this done?

[Geraldine Moloney] This relates to how we as humans use antibiotics. And really it looks like the most important intervention has been the control of how fluoroquinolone antibiotics have been used both in the hospital setting but also in the community setting by family physicians, which would be a much wider or much greater number of antibiotics kind of per day compared to the amount that's used in hospital. So I think there's actually been some similar studies on a smaller scale in the Unites States, which has also shown that the fall in ribotype 027 in the United States has also fallen in line with better practices of how fluoroquinolone antibiotics are prescribed.

[Sarah Gregory] But these same interventions haven’t worked for 078, apparently.

[Geraldine Moloney] No. So there's quite a nice study by Kate Dingle which was published in *The Lancet Infectious Diseases*, and they were able to show that the decline in *C. difficile* incidence in England can be fully accounted for by the fall in *C. difficile* which is resistant to fluoroquinolones, which includes the ribotype 027. But the levels of *C. difficile* ribotypes which are susceptible to fluoroquinolones, which include the ribotype 078, they've really remained unchanged in this time.

[Sarah Gregory] You looked at Ireland and nine other European countries. Why these?

[Geraldine Moloney] So my co-author, Dr. David Eyre, and colleagues at Oxford had formed whole-genome sequence analysis on a large number of *C. difficile* isolates from a European point-prevalent study. So that study had involved samples from almost 500 hospitals in 19 different countries. And when they examined each of the top 10 most common ribotypes, they found one of two different patterns of genetic linkages of which we can then infer patterns of transmission. So a ribotype with either are very closely linked to a hospital transmission. And we saw groups which were very tightly linked to the country of origin of the sample, or there was no such group and there was really very little evidence to support hospital transmission.

So we see that ribotype 027 has very tight groups which related to samples from Hungary; there were different groups in Italy, Germany, Romania, and Poland. But ribotype 078 couldn't be linked to any particular cluster or outbreak in any individual country. There had been another study which was performed in the Netherlands a few years earlier, where those authors had investigated the genomes of ribotype 078 *C. difficile* which was found in pigs and farmers on

certain farms in the Netherlands. And they had compared those genomes both to each other and some clinical cases in the Netherlands, and they had found that on some farms, the farmers and the pigs had identical *C. difficile*. So we thought this was quite an intriguing finding, and it was something we were hoping to build upon.

[Sarah Gregory] Are there certain countries or continents where it occurs most often?

[Geraldine Moloney] I think it's reasonable to consider *C. difficile* as a global infection. So while it can be hard to compare even within a country because of different testing practices and different diagnostic methods in the laboratory, it is well recognized in North America, in Europe, Australia, Asia, Middle East, South America. And in the last couple of years, I've actually seen some papers published on *C. difficile* in parts of Africa. So I think it's fair to consider it as a global infection.

[Sarah Gregory] Let's talk about pigs now. We just did a podcast on *Taenia solium* in pigs in Peru. What makes them such good hosts for infectious diseases?

[Geraldine Moloney] So I think that's a really interesting question, and my focus really is about zoonotic infections, which are infections which can be shared between animals and people. So when we think about the anatomy and the physiology of the pig, we can actually see that there's enough similarity between the structure and function of their digestive system, their cardiovascular system, and their renal systems. That means that infection which is able to take place in a pig, it actually isn't that difficult for a bacteria, a virus, or a parasite to cross the species boundary and infect a human. And so then we need to consider the conditions in which pigs are being raised and how do people come in contact with them, either directly or indirectly, for how a zoonotic infection can occur.

[Sarah Gregory] And how do pigs get infected with *C. difficile*? They don't mostly do any of the things you mentioned earlier on, like go and grocery shopping.

[Geraldine Moloney] Not to my knowledge. So pigs also get exposed to *C. difficile* spores in the environment. Most of the reports of *C. diff* that we see in pigs have been in very young piglets which are less than 10 days old. And for pigs, it's not quite as closely linked to antibiotics as what we see in humans.

[Sarah Gregory] And what about other livestock? Cows, sheep? Chickens?

[Geraldine Moloney] There are many other animals which can also get infected with *C. difficile*, and they would include cattle, sheep, goats, buffalos, bears, raccoons, horses, rabbits, dogs, cats, and mice. And when we look at animal infections, we can see that it's more often the young of the species—like piglets, calves, or foals—rather than the adults. Which is actually the opposite of what we see in humans where we see it as much more commonly for older adults than in young children.

[Sarah Gregory] And are the symptoms and treatment the same in pigs as it would be in a person?

[Geraldine Moloney] The main symptom is diarrhea. But from the, I suppose, the industrial aspect, pigs can suffer weight loss or failure to thrive which affects their market value and what happens after that. They can also develop symptoms relating to altered fluid distribution—so dyspnea, ascites, edema. There isn't a strong evidence-base for how to treat pigs, so there really is much greater emphasis on how to prevent the infection occurring in the first place.

[Sarah Gregory] So can pigs transmit *C. difficile* to people? I mean, I think you just said it's the other way around, but we can give it to pigs?

[Geraldine Moloney] So it's a really important question, and we think yes, that pigs can transmit to people but we don't know exactly how it happens. So it could be direct contact or the shared environment, which was certainly a possibility in the study in the Netherlands where the pigs and the farmers had identical *C. difficile*. Or it could be indirectly, so something like pig manure which is then used to fertilize crops in a different location, possibly through water, possibly through the food chain. There was another study in Australia which found identical samples of *C. difficile* ribotype 014 between pigs and humans which were either 1,000 kilometers (or over 600 miles) apart from each other. That certainly seemed to be more indirect contact rather than a close environmental link.

[Sarah Gregory] Tell us briefly about your study and how you went about it.

[Geraldine Moloney] So we had set up a study to analyze the DNA of all the *C. difficile* infections in our hospital (which is St. James's Hospital) in Dublin in a 3-year time period. And in that time, we found that ribotype 078 happened to be the most common *C. difficile* ribotype that we found in all of our cases. And this was quite unusual because most other reports, most other hospitals, most other countries, we usually see that ribotypes 027, 014, or 020 are the most prevalent. We then checked our own archives and we were able to access a few more clinical samples from cases that had been in our hospital, and we were also able to access some samples from a different Irish study which was looking at community-acquired *C. difficile*. So that gave us 53 samples of clinical cases of *C. difficile* in Ireland.

Then we developed a collaboration with Dr. McElroy and colleagues at the National Veterinary Research Laboratory of Ireland. They had found evidence of *C. difficile* infection in some pig autopsies, both histology and by testing for the *C. difficile* toxin. So they were interested in further molecular typing of the isolates, which we did. And we identified these isolates as ribotype 078. And in total, we had 20 samples from pig cases of *C. difficile* in Ireland. As I mentioned, our coworkers in Oxford had access to the *C. difficile* samples found in the European point-prevalence study, and there were also 67 genomes which were available from the studies in the Netherlands which I described where the authors had studied the *C. difficile*-associated farmers, pigs, and clinical cases. So this gave us 171 *C. difficile* ribotype 078 samples.

And it's important to say that all of these samples had been obtained by comparable laboratory methods and analysis. So we thought it would be really interesting to compare these genomes and look at the overall population structure for possible relationships between these samples. It's also important to stress that there's quite a strict criteria to make the call about whether two genomes are related for *C. diff* or not, that we can only allow up to two differences between the thousands of DNA ladders in each genome to call them as related.

[Sarah Gregory] And beyond that, were you looking for anything in particular in your study?

[Geraldine Moloney] Our hospital study had first been set up to investigate how many of the *C. difficile* infections had been transmitted in the hospital according to the similarity of these genomes. But then when we got access to the 078 *C. difficile* samples from the Irish pigs and the other European studies, then our focus shifted to a much more complex question of the broader epidemiology and the transmission that patterns well beyond the hospital.

[Sarah Gregory] What did you find beyond anything that you've already mentioned?

[Geraldine Moloney] We found that there were two different clusters which both had potentially very significant findings. So one cluster had a hospital case, a community case, and also had some of the samples from pigs. And the pig isolates we could see were the genetic ancestors of the hospital-associated case. So that tells us that somehow, our hospital case was actually derived from a pig case either directly or indirectly. We don't have that particular piece of information. We found another cluster of five cases which had a hospital case in Ireland, a community case in Ireland, a pig isolate from Ireland, but also had some isolates from one of the pigs and one of the farmers in the Netherlands. And this was something we really hadn't expected, that we had thought that the isolates in the Netherlands could be a comparison group (could set a wider timeframe). We really wouldn't have expected to find direct genetic links with samples that we were working with. I will that we also did find some occurrences of hospital transmission and also of patients who had had recurrent *C. difficile* infections who had identical isolates, and these were very much the anticipated findings from the study.

[Sarah Gregory] Was there anything that surprised you?

[Geraldine Moloney] I really didn't expect that we could find such a close relationship between the Irish porcine and the clinical isolates, or that we would find those genetic links so strong to the study from the Netherlands. We also found that there was some identical *C. difficile* isolates between Ireland and Italy and Ireland and the United Kingdom. And again, we can't explain how that happened.

[Sarah Gregory] Were there any challenges and limitations to your study?

[Geraldine Moloney] So one of the major challenges is how do we use genome sequences from different studies from different centers which can have different laboratory methods or the bioinformatic tools—how can we use these to build and integrate the information? So because the criterion is so strict with allowing only two different DNA bases to consider the genomes would be identical. There was a possibility that we've actually underestimated how similar the isolates from Ireland and the Netherlands, in particular, were, given that these isolates come from slightly different sampling timeframes.

[Sarah Gregory] What do you think are the most significant public health implications of what you found?

[Geraldine Moloney] We've got more evidence that the transmission of ribotype 078 *C. difficile*, it occurs mostly beyond the hospital environment and we found these related isolates in pigs and in clinical human cases. These have crossed international boarders, so we really need to investigate both modes of transmission well outside the hospital.

[Sarah Gregory] You mention in your article that a One Health approach to contain *C. difficile* ribotype 078 is needed. Explain that, but first explain what One Health is.

[Geraldine Moloney] So the One Health approach is recognized as the connection between the health of people, the health of animals, and the environment. So One Health is really about all of the professionals who work in public health, animal health, and other related communities really to try and look at the animal/human/environmental interface, and how do we promote the health and wellbeing of all. And part of that means that we will help control, detect, or prevent zoonotic infectious diseases.

[Sarah Gregory] And what do you think needs to be included in this approach to stop this spread?

[Geraldine Moloney] My personal take on this is that we need to have lots more action, resources, and targets for antimicrobial stewardship, which is how well we use antibiotics. And that antimicrobial stewardship really is applicable to those who are working with human health, those who are working in animal health, and those who sometimes use antibiotics in terms of certain crops or in an environmental context also.

[Sarah Gregory] What can individuals do to protect themselves besides washing their hands with soap and water?

[Geraldine Moloney] So everybody can play a role in antibiotic stewardship to make sure that as humans, we are using antibiotics as wisely as we possibly can. Antibiotics are a really important type of medicine, and we should always try to have the very best possible match between an antibiotic, an infection, and the patient. So what I would say to patients is that really don't seek or use an antibacterial medicine if it's a viral infection. Don't alter a prescribed dose or its prescription interval, because they could end up with either an ineffective dose or suffer dose-related side effects. Don't save antibiotics for a possible future episode of infection. And when antibiotics are being given to one person, they should be used by that one person and not given to a family member or somebody else to take in a different circumstance.

[Sarah Gregory] On a personal note, tell us a little bit about your job, where you work, and what you like most about it.

[Geraldine Moloney] So I'm currently on maternity leave, so my daily pattern is a little bit different. But I love infectious diseases because no two days are ever the same. We see an amazing diversity of patients, we see an amazing range of infections, we work in the hospital—both in the wards where we have inpatients, we see consults, and we also do a lot of work in outpatient clinics. And one of the wonderful parts about infectious diseases is that we have some great medical fellows, we have lots of medical residents who work with us, and we also get a lot of opportunities with medical education for medical students.

[Sarah Gregory] So if you weren’t working in infectious diseases, what other careers do you think you’d pursue?

[Geraldine Moloney] I probably would give different answers at different stages of my life. But throughout it all, I've always had quite a strong interest in psychology and behavioral economics. I think there's been some fascinating work done in recent years. So I think that could be an interesting alternative. But I'm not looking for an alternative right now.

[Sarah Gregory] Well, speaking of psychology, we live in extremely difficult times right now. What are some activities that keep you grounded in the midst of this ongoing COVID pandemic?

[Geraldine Moloney] So at times, it has been so hard to switch off from COVID because as a topic, it has been so pervasive and it really has altered so many aspects of daily life for so many people. There's nothing like time with my husband or my sons for getting to unwind, but we're big podcast fans so I was really quite happy to get the invitation to speak on one. And I also—I mentioned that some of the COVID restrictions have been removed recently in Ireland, and one thing I'm quite happy about is that the parkrun community events are restarting in Ireland in September. So they would certainly be two things that I quite enjoy.

[Sarah Gregory] Well, I'm glad to hear it.

And thank you for taking the time to talk with me today out of your maternity leave, Dr. Moloney.

[Geraldine Moloney] It has been a delight, thank you.

[Sarah Gregory] And thanks for joining me out there. You can read the September 2021 article, Human and Porcine Transmission of *C. difficile* Ribotype 078, Europe, online at cdc.gov/eid.

I'm Sarah Gregory for *Emerging Infectious Diseases*.

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