Cryptococcus gattii in the Southeastern U.S.

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

[Sarah Gregory] Hi, I'm Sarah Gregory, and today I'm talking to Dr. Shawn Lockhart, a lead research microbiologist at CDC. We'll be discussing his article about the history of the fungus *Cryptococcus gattii* in the southeastern United States. Welcome, Dr. Lockhart.

[Shawn Lockhart] Thank you. I'm happy to be here.

[Sarah Gregory] So, what is *Cryptococcus gattii* and how does it affect people?

[Shawn Lockhart] So, *Cryptococcus gattii* is a yeast. It's a yeast that's closely related to mushrooms. So it's more…more related to mushrooms than the yeast we're used to—baker's yeast or brewer's yeast, that we put in bread or we put in beer. This yeast lives in the environment, specifically in trees and in tree hollows and the soil around trees. It infects people when people move into the area around those trees, when they dig inside 'em, and it gets into the air and they breathe it into their lungs. In most people, it doesn't do anything at all, and they never noticed that they've ingested the yeast or they've breathed it in. In some people, and we don't know why, it causes a very devastating disease, which can go from either a pneumonia or a meningoencephalitis, which is an infection in the brain and in the spinal fluid, and it can be a devastating infection.

[Sarah Gregory] The *Cryptococcus gattii* has a complicated history in the United States. Would you like to take us through that?

[Shawn Lockhart] Yeah. And I'm actually going to start with a closely related species, called *Cryptococcus neoformans*. That's a sister species. We've known about *Cryptococcus neoformans* for about a hundred years, as an infectious agent. In about the 1940s, we noticed that *Cryptococcus neoformans* actually had four serotypes. And by "serotypes," I mean four different things that were recognized independently by our immune systems. So, they're seen by the body as being four different things, and they were labeled *Cryptococcus neoformans* A, B, C, and D.

[Sarah Gregory] Catchy!

[Shawn Lockhart] Scientists aren't known for their catchy names.

But anyway, so we knew they were different, and over the years, we started to see other biological differences: places they grew, things they grew on, sugars that they liked to eat. And we started to realize that we actually had two different species. And so, serotypes A and D stayed as *Cryptococcus neoformans*, and serotypes B and C became *Cryptococcus gattii*.

Cryptococcus gattii was known to be in tropical and subtropical regions, or so we thought, because the place we'd seen it first in the United States was in California. But we also knew it existed in Australia and in Brazil—all places, you know, tropical, subtropical. Now, what really got us excited in the United States and got all these crazy fungal researchers excited, all of a sudden, we started to see cases in Vancouver Island, British Columbia—not quite a tropical or subtropical region.

[Sarah Gregory] Not hardly!

[Shawn Lockhart] And so, around the late 1990s, some very astute scientists, some of our colleagues in public health in Canada, noticed that they were seeing cases in otherwise healthy people, and also veterinarians were noticing cases in dolphins. They'd never seen *Cryptococcus gattii* there before and this wasn't a tropical region, so everybody became interested. At that time, I was still at the University of Iowa, and I remember reading this thing, going, "What is that doing up there?" So, that's the beginning of the history in the United States.

[Sarah Gregory] What year was that?

[Shawn Lockhart] It emerged approximately the late 1990s; the first papers started coming out in the early 2000s. I got to the CDC in 2008, and about that time, we were seeing cases, not just in British Columbia, but it was moving southward down into Washington state and into Oregon. And our public health colleagues were very interested in tracking this emergence, and so I got the opportunity to join this group that was tracking the emergence from British Columbia down into the Pacific Northwest of the United States. And that's kind of where my story began.

[Sarah Gregory] Why is it interesting to scientists, as you were saying, that *Cryptococcus gattii* has emerged in the Pacific Northwest?

[Shawn Lockhart] So, as I said before, we had always thought it was kind of a tropical-subtropical organism, and this emergence in a temperate climate allowed us to see two things: number one, that it can change environments where it grows, and number two, that we could watch an emergence from the beginning, that we could see this thing entering a new environment, a new niche, establishing itself, and then spreading. And so, we were able to be on the ground floor to watch this happen. So, that was very, very exciting…and a little bit scary for public health.

[Sarah Gregory] Yes, I am thinking about that, for sure. You used molecular clock analysis with whole genome sequences to conduct your study. Tell us what that means.

[Shawn Lockhart] Okay. So, let me go back just a little bit and keep going with my story. So, as it emerged up in British Columbia, there were a lot of scientists who were very interested in figuring out why. And so, they were looking at sections of the DNA of the cases in both humans and animals, and by that time they'd also found it in the environment, in water, in wood. And they took all these cases, and they looked at sections of the DNA. And what they found is there were three different groups: two in British Columbia, that were also in Washington and Oregon, and one that was just in Oregon. Between these groups, they were fairly different; within a group, they were almost identical.

So, at the CDC, we worked with a set of colleagues in Arizona, at a place called TGen, and we did whole genome sequencing on these isolates. So, what that means is we got the sequence of all 18 million bases pairs in these organisms, and we compared 'em to each other. And what we saw is that between any two individuals, you only had about 15 to 20 nucleotide differences out of 18 million. And if you compared it to a nearest neighbor, say one from Australia, or one from Brazil, you'd have thousands of base pair differences between any two individuals. And so, that told us that it was there very, very recently; and we wanted to know *how* recently. So, that's where the molecular clock analysis comes in.

[Sarah Gregory] It…That told you it was there recently because it hadn't started having variations?

[Shawn Lockhart] You're exactly right! And so, so…the molecular clock…we know, over time, when an organism replicates, when it makes a copy of itself, it makes a copy of all of its DNA, and sometimes it makes mistakes, or mutations. And we know about what rate these mutations occur. So, by counting mutations over time, we can set a molecular clock. Now, that's what we did in the Pacific Northwest. What we did is we collected a population of these isolates, we looked at all their mutations, we used our molecular clock, knowing how many mutations appear over a set period of time, and then we counted backwards to find the nearest common ancestor—the one that gave rise to all these, the Adam and Eve of *Cryptococcus gattii* in the Pacific Northwest, or as we tend to think of it, the Noah, and I can talk about that in just a minute. So, by using that molecular clock, we counted backwards and found that the isolates that were up in British Columbia had been there somewhere between 80 and 100 years. And the isolates that were down in Oregon were a little bit more recent—around 60 or so years.

[Sarah Gregory] That's fascinating, really, fascinating. So, okay, what did you find?

[Shawn Lockhart] So, that's the outcome up in British Columbia. Now, we've also seen *Cryptococcus gattii* down in the Southeastern United States. But we don't see as many cases. They're very rare and they're a little bit different. The people that get it in the Southeastern United States get a very devastating disease and, in most cases, it's fatal. But there just aren't that many cases. So, we said, "Okay, let's go back and look at these isolates."

[Sarah Gregory] Is this the fungus that killed the people in Joplin, Missouri?

[Shawn Lockhart] No. That was *Apophysomyces trapeziformis*. That was a mold—another fascinating story.

[Sarah Gregory] Okay, but molds and fungus are similar, right?

[Shawn Lockhart] Molds are fungi; molds and yeasts are both under the name of fungi, yes.

[Sarah Gregory] Okay, alright.

[Shawn Lockhart] So, down in the Southeastern United States, there were these cases. And when we looked at just a few sections of DNA, they looked very similar to each other, and we thought, "Okay, we have another clone here." So, we did whole genome analysis again, and it was a different outcome this time. Instead of between any two individuals having about 15 or 20 mutations, we were seeing four and five thousand mutations, meaning this population had probably had a much longer time to change itself. So, again, we decided to do molecular clock analysis. So, we counted backwards on the molecular clock using this population and we were able to show that it's actually been down there, in the Southeast United States, somewhere between 10 and 20 thousand years. It's been here a very, very long time as compared to 100 years up in British Columbia.

[Sarah Gregory] Okay, so how does it get from the Southeastern U.S. to the Pacific Northwest?

[Shawn Lockhart] So, that's a great question, and the answer is, it didn't. So, one of the other things that we—and I mean the royal "we," all my fungal colleagues—have noticed over the past

couple years that, 'member I said, you know, first we had *Cryptococcus neoformans* and then we found out we have *Cryptococcus neoformans* and *gattii*? Well, now that we look at *Cryptococcus gattii*, we've actually discovered that it's not one species either, but a set of species called a *species complex*. And there are at least four different species in this *Cryptococcus gattii* species complex. There's one species that lives in California; that's now called *Cryptococcus basilosaurus.* There's the one that's up in British Columbia; that's *Cryptococcus deuterogattii*. And then there's the one that's in the Southeastern United States; that's the original C*ryptococcus gattii*.

So, it didn't actually move—it's been there for a very long time. And what we think happened…if we think back about a hundred years or so, we try to think, "What happened a hundred years or so ago when this thing moved from where we knew it used to live, down in the Amazon basin, to where we find it now, up in British Columbia?" We know it came from the Amazon because that's where all its closest neighbors are. When we do whole genome sequencing, we find that the ones that are most closely related to the Pacific Northwest, are down in the Amazon basin.

Now, the fun thing about science is, because no one was there a hundred years ago, we can speculate and no one can tell us we're wrong. And we know there was a lot of movement back then because that's right around when the Panama Canal opened. And there was a lot of movement of lumber, and we know *Cryptococcus gattii* is associated with trees. And there was a lot of just movements of ships, in general, and ships carry bilge water. They fill up with water where they stop, they go to where they're going, they empty out the bilge water. And we know that *Cryptococcus gattii* survives very well in sea water, and we can isolate it from sea water. So, we speculate that perhaps all this movement between the Amazon basin, a hundred years ago, and the Pacific Northwest, allowed it to travel from down in Brazil up there, very recently, and is unrelated to how it moved into the Southeastern United States, 10 to 20 thousand years ago.

[Sarah Gregory] Okay, so that's all part of the global world and fungus and diseases just moving freely all over the place.

[Shawn Lockhart] Exactly. And, you know, it's a little different from the fungal world. We're not like viruses and…and bacteria, which are inside the body and are spread from person-to-person. You don't see that in fungi; they're not generally spread from person-to-person. They're generally picked up in the environment. So, this is the first opportunity we've had to watch an emergence in the environment, to watch it move into humans from the environment that they're occupying.

[Sarah Gregory] Okay. We need to look into this a little further, just so the listeners can understand, so I can understand.

[Shawn Lockhart] Sure.

[Sarah Gregory] You've got these boats with wood and bilge water, and they go to the Pacific Northwest, they dock in Seattle or something. But then, how does that exactly get introduced into the environment?

[Shawn Lockhart] So, the bilge water could have just spilled out. And once it gets in the sea water, it can…it can go out into the environment. You know, there's spray, and it can be picked

up by waves in the spray, and move out into a tree, find a tree it likes, and start growing there. If they're bringing lumber, you know, it goes to a lumber mill, it's sawed up. We know you can find it in the sawdust around lumber mills, so it could have been spread that way. Now, this is all speculation, but I think that's a pretty good speculation of how it could have spread, either through sawdust or bilge water or even some of the other goods that were shipped back and forth between the Amazon basin and the Pacific Northwest.

[Sarah Gregory] So, it spreads from an item to another item and then people get it from the environment, but you don't get it from another person?

[Shawn Lockhart] That is correct. So, you don't catch it from another person, it doesn't really grow in us. It's deep inside. When it causes an infection, it's either deep in the lungs, and when you cough it up, it's too big to go into someone else's lungs. When we breathe it in, it's the spores that we're breathing in, and they're very small, or a desiccated yeast that's very small. When it's in the body, it tends to get a lot bigger, and so it's very difficult to transfer from person-to-person, even if you're coughing right on them.

[Sarah Gregory] Alright, along those exact same lines, so what does it mean for the public health? Is this study more about etiology and history, more than impact on the public health?

[Shawn Lockhart] You know, so that's a really good question. And the fun thing about public health is we don't have to just think about public health; we can answer other questions at the same time. And usually those two things fit together and they help. So, really, you have both. You have a public health question, and then you have just a regular scientific, "Wow, this is really cool" kinda question.

And so, let's start with the public health. First of all, this is the first time it allows us to see an emergence, it allows us to track an emergence, and it sets us and our public health partners up for what happens if this happens again. We set up a system of tracking, we set up a system of looking for isolates, we set up a system of alerts. That's all the type of thing that we did. We did a lot of messaging. That's very important for us fungal people, because these are new events. They haven't happened really in fungi before. It's a lot different from the virus and the bacteria people. So, it helped us public health, it helped us set up these…these systems, get 'em in place, for looking for how these emergences take place, and so, the next one, we'll be ready.

But it also answers some other questions. And now we go down to the Southeastern United States, and we say "It's down there. Why aren't we seeing more cases? Are we just not going deep enough into the woods where it hangs out? Are we becoming immune for some degree?" And we don't know. But if we start seeing *more* cases, we can rule out a recent emergence, we know that's not it. So, we can start looking at other things. Are we moving into the environment? Are we building houses around trees that, you know, we didn't used to build houses around before? And we can ask those different epidemiological questions, and put us closer to the answer. We don't have to go backwards and think, "Well, how did that get here, and I wonder if it's new?" We have those answers.

[Sarah Gregory] I think you've touched on it a little bit, just fascinating, but why were you interested in doing this study?

[Shawn Lockhart] So, as I said before, I've been following this since it started, before I got to the CDC. And it was really fascinating because there's never been an emergence like this of a fungus—at least in humans. We've seen something similar in bananas. There was a fusarium, a mold, that wiped out the entire world population of bananas. But we haven't seen anything like that in humans. And so it was a really fascinating story to try to figure out 1) how it got here, and 2) why it was spreading.

Now it's become really pertinent for us, because what we're seeing now are two other fungi that seem to be spreading. There's another yeast, called *Candida auris*, that seems to be spreading worldwide right now. And then there is a mold, *Aspergillus fumigatus,* that's resistant to the most important drug we use against it, that also seems to be spreading worldwide.

And so, we can use the public health lessons learned in our surveillance for *Cryptococcus gattii* to do surveillance for these other emerging molds. And so, that's what I find so fascinating. And that's what allowing our group to use the data that we got from this study and apply it to the new organisms that we see emerging.

[Sarah Gregory] What's your job at CDC and what's your area of expertise? Are you a mold guy? A fungus guy or…?

[Shawn Lockhart] We call ourselves "fun-guys," although, you know, that's a terrible joke. But I've been doing infectious…infectious molds and yeasts for about 25 years. And I came to the CDC to be a team lead and be the director of the Fungal Reference Laboratory. Up until a few weeks ago, that was my title. So, what I did was help our public health partners identify difficult-to-identify molds and yeasts, that's the first part. And the second part of my job was to work on antimicrobial resistance. So, antifungal, the drugs we use against fungi, to work on the molds and yeasts that were resistant to drugs.

Most recently, I've become the senior clinical advisor for Mycotic Diseases Laboratory. And so, my new role will be capacity building for both antifungal resistance, so helping people in the United States and worldwide to develop their laboratories to be able to detect antifungal resistance, and to be able to identify difficult and hard to identify molds and yeasts. And that's the part I really like. I love this capacity building. I love helping other laboratories learn how to identify these things, to recognize them, and then to treat them.

[Sarah Gregory] Thank you so much for taking the time to talk with me today. This was very interesting, Dr. Lockhart.

[Shawn Lockhart] It was a pleasure.

[Sarah Gregory] Listeners can read the November 2018 article, Timing the Origin of *Cryptococcus gattii sensu stricto,* Southeastern United States, online at cdc.gov/eid.

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

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