

Museum Snakes Preserved in 1945 Found To Contain Fungal Pathogens

[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. Jeffrey Lorch. He's a diagnostic microbiologist and research scientist at the U.S. Geological Survey National Wildlife Health Center in Madison, Wisconsin. We'll be discussing the history of snake fungal disease in wild snake populations through museum specimens.

Welcome, Dr. Lorch.

[Jeffrey Lorch] Thank you for having me.

[Sarah Gregory] Okay. To start with, what is ophidiomycosis?

[Jeffrey Lorch] Ophidiomycosis is a scientific term that we use to refer to a disease in snakes that's caused by this fungus *Ophidiomyces ophidiicola*. So it's more commonly referred to as snake fungal disease, and the fungus that causes—or infects—snakes is primarily limited to the skin, but in severe cases it can cause the animals to look quite disfigured.

[Sarah Gregory] This is really strange stuff that you've done here. You examined snakes in museums dating back to 1945. How did you even think to do that? And why did you?

[Jeffrey Lorch] Yeah. So ophidiomycosis is a disease that has been described fairly recently, and what we wanted to do is figure out if the disease was something new or if it had been around longer and was maybe simply overlooked. And for many other wildlife diseases, people have turned to museum specimens or museum collections to answer these sorts of questions, and so we wanted to do the same.

[Sarah Gregory] And should the average listener be concerned about this disease?

[Jeffrey Lorch] That's a really good question. As you know, snakes are probably some of the most maligned animals in the world but they are actually very beneficial. A lot of snakes eat pest species like rodents, which can damage food or destroy crops and also carry diseases that can infect humans. So I think there was a study conducted where people looked at the effect of timber rattlesnakes and Lyme disease and demonstrated essentially that timber rattlesnakes consume enough mice to potentially have an impact on the transmission of Lyme disease. So like all living things they play an important role in the ecosystem, and any negative aspects that we associate with them are far outweighed by the benefits they provide.

[Sarah Gregory] Yeah, that's an interesting point. I have a couple friends that they just randomly kill every snake they see in their yard, even the little beneficial ones. And I'm like, "Oh no, don't do that." But...yeah.

So what makes a fungal infection different from viruses and pathogens?

[Jeffrey Lorch] On the most basic level, fungi are a very different type of organism than, say, viruses or bacteria, which also cause diseases. But really it's how these pathogens behave that's important when we talk about disease. And fungal pathogens tend to be able to infect a wide variety of different hosts. They're not as specific to a given host species like viruses, for example, often are. And so in the case of snake fungal disease, for instance, it seems that nearly all snake species are capable of being infected with *Ophidiomyces*.

Also, fungal pathogens have a tendency to persist in the environment for long periods of time, even when there's no host around to infect, and sometimes fungi will even grow or replicate in the soil without a host. So this means that animals like snakes can actually become infected just by crawling over contaminated surfaces. They don't need to come into contact with another sick snake to develop disease. And so I kind of like to think of it as telling people to imagine that something like a cold virus could survive for months or years on a surface like a door handle, and then just imagine how much more prevalent colds would be or how much more frequently you would get sick.

And then finally, it seems as though many individuals don't necessarily develop immunity to fungal pathogens in the same way that they do to viruses and bacteria. And this means that you can actually become infected time and again by the same fungus. In fact, there's never really been a successful vaccine developed for a fungal disease. And so all these factors combined can make fungal diseases a lot more difficult to manage in wild animal populations.

[Sarah Gregory] I know I've said this before in other podcasts about fungal diseases, but of all the types of things they scare me the most just because of everything you just said.

[Jeffrey Lorch] So I was about to say it, luckily so far most fungal diseases in humans have been limited to immunocompromised patients. But yeah, being an animal that's more susceptible to fungal diseases even if you're healthy seems like it would be very scary.

[Sarah Gregory] Yeah. So we often hear about diseases in animals such as bats or pigs or chickens. Do snakes have fewer diseases, or do we just know less about them?

[Jeffrey Lorch] Yeah. If anything, I'd say snakes probably have more diseases but we just haven't studied them enough to document what diseases they get. And this is really because humans don't have a long history of living side by side with snakes or having them in captivity like we have with things like pigs and chickens.

So reptiles really only became popular as pets in the 1990s, and most of the diseases in snakes that we know about were discovered after that time. So, a lot less of a history with humans than some of our domestic animals. And then, also mammals and birds are warm-blooded and their high body temperature actually prevents a lot of fungi that might normally be present in the environment from being able to grow in their bodies. But snakes are cold-blooded, so their body temperature is more similar to that of their surroundings. And so what that means is that under the right conditions, many more of these fungi or bacteria that might just be in the environment can opportunistically infect them.

[Sarah Gregory] That's a really interesting point. I've actually never thought about that before.

So how and when was this disease actually discovered, then?

[Jeffrey Lorch] Ophidiomycosis was first discovered in wild snakes in 2008 in Illinois, and it was found in a species called the eastern massasauga rattlesnake. And that species is very rare, and so its population was being closely monitored. And researchers that were monitoring that population began to find these snakes that had horribly disfigured faces and would inevitably die as a result of those infections, and they eventually attributed it to this *Ophidiomyces* fungus. Retrospectively, we now know that ophidiomycosis has been found in captive snakes dating back to at least the 1980s as well.

[Sarah Gregory] So since the discovery of this fungus in snakes, where has it been found?

[Jeffrey Lorch] Yeah, so *Ophidiomyces* is now found in wild snakes across much of the eastern U.S. as well as part of Canada. And as the disease gained more attention, people began looking for it in other places. So we now know that *Ophidiomyces* causes disease in wild snakes in Europe as well as in Taiwan, and I'm sure if people looked in more places we would find it probably throughout Eurasia. It would be interesting to look in other continents as well and see just how broadly distributed the fungus is. But there hasn't been a lot of effort put into surveillance for the fungus outside of North America.

[Sarah Gregory] Is there a particular kind of snake it affects more than others? You know, for example, more common among a species or a geographic location, or wild snakes or pet snakes or...?

[Jeffrey Lorch] Yeah. So as I mentioned before with fungi having a broad host range, we do believe that *Ophidiomyces* can infect nearly all species of snakes. That doesn't necessarily mean that all species are equally susceptible, so maybe some species are more likely to become infected or will develop more severe disease if they become infected. Ophidiomycosis has been reported to be more common in aquatic snake species, for example, and there's also anecdotal reports that rattlesnakes may get more severe disease when they're infected. But this is something that we need to sort of look into more.

As far as distribution, we see most cases in the eastern half of the U.S. It's possible that the fungus occurs in the western U.S.—or I should say more widespread in the western U.S.—but people haven't looked there as much as they have in the eastern part of the country. And then as far as wild versus pet snakes, I don't really know the answer to that question because there isn't a good reporting system for captive snakes, and our focus has primarily been on wild animals. But as I mentioned, we do know that captive snakes can get ophidiomycosis and it actually has been found in captive snakes on several continents.

[Sarah Gregory] So you mentioned distortion, like physical distortion. What other signs of infection do snakes show or get?

[Jeffrey Lorch] I'd say the most consistent sign of infection are these crusty areas of discolored skin. People often describe them as sort of looking like scabs, technically that's not what they are, but they look very much like scabs. But the signs can be highly variable sort of depending on what stage of infection the snake is in or how it's responding to that infection. So some snakes will look more like they have blisters on the skin, some will have ulcers on the skin. And then it's really just in these sort of more severe cases that snakes might look disfigured. When they do get infections on the head, that's sort of where it's most conspicuous. Often it will affect the eye, so the eye will be crusted over. And then once they're infected, especially if it's a severe infection, the snakes will sometimes refuse to eat or have a hard time finding food, so they can become very thin. And then in these sort of most extreme cases, the fungus can actually penetrate down through the skin into the underlying muscle and sometimes even into bone or other internal organs.

[Sarah Gregory] So Dr. Lorch, is there some kind of treatment or a way to treat snakes (wild snakes) for this?

[Jeffrey Lorch] Yes. So some people have tried using antifungals to treat snakes and have reported that snakes are able to recover. But I haven't seen really good, I should say, controlled designs that sort of say exactly what the efficacy of those treatments are. Many snakes can

recover just based on supportive care in captivity, and many snakes with mild infections probably recover on their own without any sort of intervention.

[Sarah Gregory] I would imagine administration to wild snakes would be somewhat problematic also, like trying to do oral rabies vaccines for raccoons and such.

[Jeffrey Lorch] Yeah, and so a lot of the time we don't really. Because snakes are so difficult to find—they're so secretive in the wild—and because there's ability to potentially be reinfected by a fungal pathogen, we don't necessarily consider treatment to be good management techniques in wild systems. So for highly endangered species where every individual counts or for captive animals, you know, treatments might come into play. But when we talk about population management, it often isn't a very feasible approach.

[Sarah Gregory] So back to the public health aspect of this. You know, people can get *Salmonella* from all kinds of reptiles, among other things, and I know snakes are one of them. Can people get this from snakes (ophidiomycosis)?

[Jeffrey Lorch] To date there haven't been any cases of humans developing ophidiomycosis, at least not to my knowledge, and this includes people that frequently handle sick snakes. And it's likely that the fungus isn't capable of infecting people, and that's based on the fact that the fungus isn't able to grow at temperatures as high as the human body. So it seems like that is probably a pretty significant barrier for its ability to serve as a human pathogen.

[Sarah Gregory] In your study (or for your study) you examined snakes from the eastern U.S. that were preserved in museums. What was your initial goal?

[Jeffrey Lorch] We really wanted to get a better idea of whether this disease was something new or if it's been around for a long time and was simply overlooked. And the reason that we wanted to do that is because the way in which you manage a wildlife disease is often pretty dependent on whether that pathogen is new to the area and you have a naïve host population or whether it's been present there for a long time, which might signal that other stressors are responsible and which allows you to sort of focus on maybe some of those other stressors for disease management rather than just trying to control spread.

[Sarah Gregory] So how were these snakes preserved that you looked at?

[Jeffrey Lorch] So the snakes we examined were preserved in either alcohol or a chemical called formalin, some people might know that better as formaldehyde, and these were essentially pickled specimens. So they were stored in liquid within glass jars.

[Sarah Gregory] What museums did you look at?

[Jeffrey Lorch] So we worked at a museum in Kentucky called the Morehead Museum, and then most of the specimens that we got that were suitable for this came from the University of Wisconsin Zoological Museum. And that was simply because the specimens from the University of Wisconsin had been stored in ethanol, which is more conducive for the types of analyses we did whereas the samples from Morehead had been preserved long-term in formalin.

[Sarah Gregory] Were you able to visually identify the snakes with this disease, or did you have other methods? And if you did, what were they?

[Jeffrey Lorch] Yeah. So we initially started by just examining the snakes for skin lesions that are typical of ophidiomycosis. But one issue with that is that these signs that I described are not

specific to this disease. So there's other things that can cause snakes to have these types of skin lesions. So in order to officially diagnose ophidiomycosis, we need to examine the skin microscopically and then we also need to detect the fungus. So to detect the fungus, we use...we look for the fungus's DNA using a laboratory technique called polymerase chain reaction. And to do this and to look at the skin under the microscope, we actually need to remove part of the infected skin from the specimen, and this is called destructive sampling because you're sort of mutilating the specimen in the process of doing that. But luckily the museums we worked with were very excited about this project and they allowed us to do this destructive sampling on a subset of the specimens that we looked at.

[Sarah Gregory] And you mentioned lesions. What exactly do you mean by "lesions"? Are they like cuts or scrapes or scabby bits or...what do they look like?

[Jeffrey Lorch] Lesion is just a medical term that essentially means that there's some sort of damage to the tissue. So cuts and scrapes are types of lesions. The type of lesion we're looking for would be ones that have an infectious cause, not a mechanical cause such as a scrape or a cut. So, again, sort of these thickened areas of skin or crusty skin were the most common lesion that we saw in these preserved snakes.

[Sarah Gregory] Was there still DNA in these specimens? I mean, it seems like to get it out and it only be tiny amounts, it must have been difficult.

[Jeffrey Lorch] Some of the specimens we sampled did actually have suitable DNA, others did not. So as I mentioned, samples that were stored in alcohol were the ones that gave us the amount of DNA that was needed to do our analysis whereas those in formalin did not have any detectable DNA. And the amount of DNA is very small but we're still able to detect it with that technique that I mentioned (the polymerase chain reaction), which essentially helps amplify the amount of DNA in the sample. And we get that DNA out by removing the alcohol from the tissue and then we grind up that lesion tissue both mechanically and then digest it using enzymes, and that helps release the DNA.

[Sarah Gregory] And what did you find from all of this?

[Jeffrey Lorch] Well, we were able to diagnose snake fungal disease 55 years earlier than the previous reports. And this was in snakes collected as far away as Florida and Wisconsin. So it appears that the disease was actually quite widespread in the eastern U.S. decades before the disease was officially described. And in addition to that, many more snakes we examined microscopically looked like they had ophidiomycosis but we weren't able to get enough DNA from those specimens to necessarily confirm that the fungus was present. And beyond that, we found even more snakes that had lesions that we did not destructively sample because we wanted to preserve those specimens for future use at the museum.

So what we found were quite a few specimens that had what we thought was snake fungal disease; we were able to confirm that in a small set of them.

[Sarah Gregory] Do these results tell us anything about the origins of this disease?

[Jeffrey Lorch] Yeah. So as I mentioned before, you know, the way we approach management of diseases often sort of varies based on whether something is introduced into a naïve population or whether it's been present for a long time. And these results tell us that *Ophidiomyces* has at least been present in North America for several decades. That doesn't mean that it could not have been introduced to North America, it just means that it happened before we began noticing

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problems associated with the infection. So it could mean that the fungus has always been here but like I said, we really need to sort of look at more specimens to get an idea of whether it has been present for longer than the 55 years that we were able to go back and confirm.

[Sarah Gregory] So in your opinion, are there any environmental implications for ophidiomycosis being around, either new or always?

[Jeffrey Lorch] Yeah, that's a really good question. And a major issue we face in trying to look at the impacts of disease on snakes is that we really just have no long-term data for most snake populations. We do know that snakes have been declining worldwide for a variety of different reasons, but in North America we don't really necessarily know if those declines are disease-related. We do know that some snake populations are experiencing negative impacts from ophidiomycosis, but that really hasn't been tested over sort of...on a broader scale among snakes as a whole. And then we also don't know what sort of domino effect that will have. So if you start removing snakes from the ecosystem where they're—not just provide benefits to humans—but are also served as an important food source for many other animals like birds and mammals. We just don't know what the impact of that could be.

[Sarah Gregory] How will these results you found be used going forward?

[Jeffrey Lorch] Well, I think our work is really just a first step in trying to figure out what the origins of *Ophidiomyces* are. So we're hoping that other research groups might examine museum specimens from outside of eastern North America to give us a better idea of where the fungus occurs and when it started showing up in a given region. And that's what's really nice about these museum specimens is that not only are we able to get sort of a geographical coverage, but we're also able to look temporally to get an idea of maybe when something began to show up in a particular place. And we sampled a relatively small number of snakes in our study. So by looking at larger museum collections and more specimens it might actually be possible to determine things like how the disease has varied over time; whether it has become more prevalent, more severe, that sort of thing; which snake species might...are more likely to be infected; and then as I mentioned, if the pathogen was introduced to North America, when that introduction may have occurred—so when do we start seeing it show up in museum specimens and then in what regions does it show up first and you know, from where in those regions might it spread.

[Sarah Gregory] Why did you pick specimens from 1945, why that date?

[Jeffrey Lorch] So we did actually go back further, but we weren't able to sort of find the combination of factors we needed to confirm a diagnosis prior to 1945. Either DNA was too degraded in some of the older specimens or the lesions were just so small that we couldn't do multiple types of sampling from those snakes. So it's possible that it had occurred prior to 1945. Also we're sort of limited in what was present in the collections that we looked at. If we went to larger museums that maybe have snake specimens going back further, maybe we would have found cases that predated 1945.

[Sarah Gregory] What were the challenges of working with museum specimens rather than, say, live animals?

[Jeffrey Lorch] Yeah. So working with museum specimens, first of all, is great because you don't need to worry about causing harm or disturbing live animals. You can examine specimens from various time periods. But museum specimens do pose some challenges, mainly many

specimens are very precious and so with the type of destructive sampling that I mentioned earlier, we weren't able to get confirmed diagnoses for all of the specimens we saw that we believed may have had ophidiomycosis because you do want to preserve those specimens for other people to use for future use. You can't go back and get more specimens from 1945. What you sort of have in collections is you know, all you'll ever have.

Also it can be a lot difficult—a lot more difficult to get enough suitable DNA from preserved specimens versus if I were to get a fresh snake, for example, and that does limit a lot of the analyses we can do. So we can detect *Ophidiomyces* in these samples, but we often can't, you know, look at what particular strains of *Ophidiomyces* they may be or that sort of thing because there just isn't enough DNA that we can recover from those samples with our current methods to do that.

And then finally, there's often a lot of bias in museum collections. So a particular collection may be dominated with a particular snake species that was collected from a certain location where a particular study was done. So it can often be hard to get a good balanced study design when you're using these opportunistically collected museum specimens.

[Sarah Gregory] Have museum specimens been used to retroactively identify any other diseases that you know of?

[Jeffrey Lorch] Yeah, absolutely. So museum specimens are really valuable resources and they're used in these types of studies quite frequently. And that includes both, first studies on human diseases, as well as diseases in animals. So for example, scientists have used museum specimens to sequence the entire genome of the bacteria that caused the Black Death in Europe and that helped them sort of compare it with what that bacteria looks like now and whether they thought it might be more pathogenic during that period than it is now. I guess more related to the topic at hand, scientists have also used museum specimens to study other fungal diseases of wildlife such as chytrid fungus in amphibians and white-nose syndrome in bats. And in both of those instances museum specimens helped to identify the likely origin of those fungal pathogens.

[Sarah Gregory] And you said that there was no viable DNA before 1945, but that's a really long time ago as it is. Why do you think the fungus survived so long in the specimens?

[Jeffrey Lorch] I guess the first thing to point out is the fungus is not actually alive in these museum specimens. It's the DNA that we're finding, and DNA is actually a very stable molecule under the right conditions. And one of the things that helps preserve DNA quite well is alcohol. And so these specimens that had been preserved and stored long-term in alcohol actually have a much better chance of yielding DNA than specimens that might be of similar age but stored by other methods.

[Sarah Gregory] Tell us about your job, what you do, where you work, how you got there, and what you like most about it?

[Jeffrey Lorch] Sure. So I'm a microbiologist at the U.S. Geological Survey National Wildlife Health Center, and our center is a federal institution that's dedicated to wildlife disease detection, control, and prevention in the U.S. So here I do both disease investigation work, which involves helping to identify the causes of wildlife die-off events, and then I also do research into various aspects of wildlife diseases. So I've always been passionate about nature and I combined my undergraduate degrees, which were in microbiology and wildlife ecology, so that I could focus

on disease issues. And that sort of came about having taken some courses where we talked about wildlife conservation, and it became clear that disease was quickly becoming a much bigger factor in species conservation than it had been even a few decades ago, primarily because humans are moving disease agents around the world much more quickly than we ever have. And so that really sort of solidified my desire to move forward and focus on wildlife conservation from the standpoint of trying to manage disease. And as far as what I enjoy most about my job, I guess I would say I really enjoy the part we're trying to figure out how the pieces of the puzzle fit together when we're conducting those challenging investigations into new wildlife diseases. And then also helping management agencies come up with ways of protecting wildlife from disease threats.

[Sarah Gregory] Well, on a personal level, do you have a pet snake?

[Jeffrey Lorch] I currently do not have a pet snake.

[Sarah Gregory] You currently do not, does that mean you did at one point, or you're going to get one?

[Jeffrey Lorch] I've had snakes in the past, I've always been interested in reptiles. So as a kid I've had some pet snakes. But no, currently I don't have any pet snakes.

[Sarah Gregory] I actually wouldn't mind having a pet snake, but I cannot feed a pet snake a live mouse or even a frozen mouse, so sort of a nonstarter. But I think they're very appealing.

[Jeffrey Lorch] There's snakes that eat all kinds of different things.

[Sarah Gregory] Really?

[Jeffrey Lorch] Well, I mean there's none that eat vegetables, but...

[Sarah Gregory] Well, yeah.

[Jeffrey Lorch] There's some snakes that eat insects, most of the captive species I think are mouse eaters. It's a little bit easier to feed them. But garter snakes will eat worms and fish and all kinds of stuff.

[Sarah Gregory] I used to have some lizards, I guess they were, that ate crickets. But, oh my goodness, those things would hop all over the place and then they'd get out and I'd have little crickets hopping all over my house.

[Jeffrey Lorch] Yeah, then it gets under your refrigerator and keeps chirping at night and you can't get it but it keeps you up.

[Sarah Gregory] Exactly, it's just startling when they hop up at you unexpectedly.

[Jeffrey Lorch] I was about to say when you were talking about snakes and what they eat, you know snakes are a pretty diverse group of animals and some of them are really specialized. And one of the species that we work with feeds exclusively on crayfish, and in fact some people say they eat almost exclusively freshly molted crayfish because the ones that haven't freshly molted have too hard of a shell.

[Sarah Gregory] Oh geez.

[Jeffrey Lorch] So they've definitely evolved into some pretty specialized niches.

[Sarah Gregory] That's really interesting. Can you imagine the enormous aggravation of trying to keep freshly molted crayfish for your snake?

[Jeffrey Lorch] Yeah, that species hasn't made it into the pet trade.

[Sarah Gregory] Okay. Well, thank you for taking the time to talk to me today, Dr. Lorch. This has been a very interesting conversation.

[Jeffrey Lorch] Yeah, thanks so much for having me.

[Sarah Gregory] And thanks for joining me out there. You can read the July 2021 article, Confirmed Cases of Ophidiomycosis in Museum Specimens from as Early as 1945, United States, online at [cdc.gov/eid](https://www.cdc.gov/eid).

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

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