Mycobacterium leprae in Armadillo Tissues from Museum Collections, United States

[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. Daniel Romero-Alvarez, an MD and PhD candidate at the University of Kansas. We'll be discussing *Mycobacterium leprae* found in armadillo tissues in museum collections across the United States.

Welcome, Dr. Romero-Alvarez.

[Daniel Romero-Alvarez] Good morning. Thank you very much for having me. It's an honor to be on your podcast.

[Sarah Gregory] So what is *Mycobacterium leprae* and is it what causes leprosy?

[Daniel Romero-Alvarez] So *Mycobacterium leprae* is a slow-growing intracellular bacilli, and yes, it's the etiological agent of leprosy. It's a traditional known etiological agent of leprosy, although the disease is also caused by another agent that is called *Mycobacterium lepromatosis* that was discovered around 2010.

[Sarah Gregory] To clarify here, is this the same kind of leprosy we associate with the Middle Ages with all the horrific outcomes, such as people losing fingers and noses?

[Daniel Romero-Alvarez] Yes. We are talking about exactly the same disease. It is important to mention that the clinical spectrum of leprosy is large and goes from mild forms to other clinical forms that are very severe, and those are the ones related to the horrific outcomes, as you mentioned. And fortunately, we know now how to treat the disease and avoid those severe outcomes, although they can be seen in some parts of the world.

[Sarah Gregory] So tell us about this disease, generally.

[Daniel Romero-Alvarez] Okay. So leprosy is a chronic disease, and as I mentioned, it has a large clinical spectrum. And depending on the way the immunological response is mounted by the patient that is affected by the disease, you can find the forms that are apparent like...skin problems or you then can this very disseminated form that might compromise your nerves, and then cause these amputations or even blindness. So the disease has been controlled in some sense, but it is still prevalent.

[Sarah Gregory] So if it's prevalent, why don't we hear very much about it anymore? And you mentioned something about a treatment. So there is treatment for it now?

[Daniel Romero-Alvarez] Previously the incidence of leprosy was really dramatic, and of course the disease has a very stigmatic and historical context. However, around the 80's, the World Health Organization started a multidrug treatment strategy coupled with some public health interventions that allowed us to control the disease. The multidrug treatment is based on three antibiotics, and those antibiotics are very effective on treating a particular case, although it's a long-term treatment. So you have to take those antibiotics for around a minimum of six months. So...but they are effective in controlling the disease. So in a sense, we were able to keep leprosy at bay—we were able to control it.

However, something that I wanted to bring to the table was a three-tiered strategy that was also established by the World Health Organization. So in the 2000s, they decided that a threshold to mention that leprosy is no longer a public health problem could be achieved if you have an incidence of 1x10,000 inhabitants. If you have less than that threshold, then you can say that you have controlled the disease. So that is why a lot of countries have started achieving that threshold and then mentioning that it is no longer a problem. So based on that threshold, you can easily say that the three countries that are concentrating the majority of leprosy burden in the world are Brazil, Indonesia, and India. However, at the country level, that makes sense. But if you zoom in on geographical localities that are smaller than countries, then you will see that this control idea has not been achieved in multiple places.

[Sarah Gregory] Just out of curiosity, what are the three antibiotics that are used for treatment?

[Daniel Romero-Alvarez] Interesting question. So the three antibiotics are rifampicin, dapsone, and then you have cephalosporin (third-generation cephalosporin).

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[Sarah Gregory] Do we know how it's transmitted? Is it person to person or zoonotic? How is it done?

[Daniel Romero-Alvarez] That question is the reason why I started to study leprosy disease system, because we might believe that because it's an ancient disease—we know leprosy since very, very old times—then the mechanisms of transmission will be completely clear for us. But that is not the case. So we don't know...although we traditionally understand that leprosy is a person-to-person transmitted disease, we are not sure how that is happening. In the traditional understanding of leprosy transmission, we believe that because *Mycobacterium leprae* (the bacteria) has been found in the nose of infected patients, then by coughing and sneezing they are releasing infected droplets and aerosols that are driving transmission.

However, we can never be able to actually see how that is happening—how the transmission event is occurring. The only clear risk factor for someone to acquire leprosy is to be chronically in touch with the source of leprosy. So that is why household residents of an infected patient are usually at more risk of acquiring the disease. But then what is happening when patients are showing to be positive for leprosy without any other human that...without another patient that transmitted the disease in the first place. So that is why recent studies have shown that potentially there are other reservoirs of leprosy, including other mammals and also the environment. So potentially being exposed to those other mammals or those other sources are also a condition for getting leprosy.

[Sarah Gregory] Your study is about leprosy in armadillo museum specimens. You found these bacilli in armadillo museum specimens, particularly. Why were you looking for them?

[Daniel Romero-Alvarez] As I mentioned, because it is not clear if there is a zoonotic pathway that allows leprosy to move from wildlife to patients. Then around the 70's/80's in the United States, the nine-banded armadillo started to be seen as a reservoir of *Mycobacterium leprae*. And there is this pioneer study that was published in the *New England Journal of Medicine* in 2011 where they found that there was a clear link between the nine-banded armadillo and patients infected with leprosy. They found the same strain of *Mycobacterium leprae* in both populations—armadillo and persons. So there is this understanding that armadillos (the nine-banded armadillo)—which the species name is *Dasypus novemcinctus*—is a well-known

reservoir of the disease. So we decided to study museum specimens to see whether we can find *Mycobacterium leprae* in those armadillos (in those species), and we were able to do so.

[Sarah Gregory] In which museums did you find your armadillos?

[Daniel Romero-Alvarez] At the end, it is important to mention that there is a super interesting...a super good network of information of specimens that are available in the different museums of the US. So by assessing this information, we were able to find multiple specimens scattered around ten museums of the US, and from those ten museums we found positive armadillos in the Yale Peabody Museum of Natural History in Connecticut, the Museum of the North from the University of Alaska, the Museum of Southern Biology from the University of New Mexico, and the Texas Tech University Natural Science Research Laboratory.

[Sarah Gregory] What countries were the specimens from? Just because they were in that museum didn't mean they were from this country. What countries were they from?

[Daniel Romero-Alvarez] The specimens came from multiple countries (at least eight countries). The majority of our samples were collected in the US, of course, but we also obtained samples from Paraguay, Argentina, Bolivia, Peru, Brazil, Panama, and Costa Rica.

[Sarah Gregory] Were you looking at a particular kind of armadillo? And actually, are there different kinds?

[Daniel Romero-Alvarez] Yeah. There are at least 20 species of armadillo, but they are mostly concentrated in South America. From the 20 species, the one species that is broadly distributed is the nine-banded armadillo. So this particular animal lives from Argentina to the mid-US (let's say, up to Canada). So it has a super, super large area of distribution, and it is a very successful species that can adapt to multiple environments and multiple ecosystems. So we were looking at this particular armadillo, although there's published literature showing that at least two species can be considered a reservoir of *Mycobacterium leprae*—the nine-banded armadillo (*Dasypus novemcinctus*) and also the six-banded armadillo, which is *Euphractus sexcinctus* (the species name). And because there was this information about this species being a reservoir, we decided to obtain a specimen from all the Dasypodidae family—so, from all the species available. So ended up studying ten different armadillo species in our research, although we only found positives from the nine-banded armadillo.

[Sarah Gregory] And how exactly were the specimens chosen?

[Daniel Romero-Alvarez] We basically asked for any tissue from the Dasypodidae family that was out there. So after looking into these databases, we found the presence of different armadillos in these ten museums, and then we asked them for a loan, like "Hey, we are doing this research, we would like to see if *Mycobacterium leprae* is present in the tissues of your collections. Would you like to contribute?", so they would say, "Yeah, of course". So then they sent us some tissues to analyze. It was more on availability rather than a particular case.

[Sarah Gregory] And once you had them, how were they tested?

[Daniel Romero-Alvarez] So once we received the portion of tissue, then we used molecular techniques to diagnose *Mycobacterium leprae*...it's presence in that tissue. We first extracted DNA and then used at least two approaches based on polymerase chain reaction (PCR), which is means that it amplifies DNA largely and then uses a specific target to detect the presence of

both *Mycobacterium leprae*, and we were also looking for *Mycobacterium lepromatosis* in the samples, although we never found that other species.

[Sarah Gregory] What kinds of samples were the bacilli found in? I mean, was it in body parts or was it in types of preservatives? Where exactly did you find it?

[Daniel Romero-Alvarez] Because we were only looking for...we were very broad in our inclusion criteria on how to test. Our aim was to address any armadillo species...rather, any particular tissue. There were some reports mentioning that the places where *Mycobacterium leprae* would be in higher concentrations in armadillos would be the liver and the spleen, but these organs are not necessarily collected in the specimens that are held in museums. So we were super broad (just asked for tissue). So at the end, we received a lot of liver, muscle, spleen, heart, kidney, and we used our molecular detection techniques across all those tissues. Something that was surprising is that we found a lot of positives in the muscle tissue. That was unexpected, because we were aiming to find a lot of *Mycobacterium leprae* in either the liver or the spleen, but muscle resulted to be a good tissue to find the bacteria (at least in our sample).

[Sarah Gregory] What percentage of the samples had the bacilli?

[Daniel Romero-Alvarez] We ended up analyzing 159 individual armadillos from ten different species. From those, 122 belonged to the nine-banded armadillo, and we only found positives there. So we found 18 positives out of 122, which gave us a prevalence of 14.8% of *Mycobacterium leprae* in our sample.

[Sarah Gregory] Are there different subtypes of this bacilli?

[Daniel Romero-Alvarez] Yes. So regarding subtypes, it is important to mention some background. So around 2010, there was a very interesting study published that compared genomes of *Mycobacterium leprae* that were collected across the entire world. Through this study, the scientific world concluded that *Mycobacterium leprae* is one of the pathogens with the lowest patient rate ever. So they had...these 200 samples (around 200 samples) were super similar—they were not different. And because they are so similar, any mutation is able to characterize a particular *Mycobacterium leprae* with a particular geographic location. These mutations are called single-nucleotide polymorphisms or SNPs. So based on these SNPs, in 2010, the researchers developed this categorization system that allows you to classify *Mycobacterium leprae* in four types—one, two, three, four—and also 16 subtypes labeled according to the letters in the alphabet—so you have subtypes 1A, 1B, 1D, 2A, 2B, etcetera. Using this subtypification method since 2010 until now, there have been other subtypes that have been detected, so now we access more than 16 subtypes, but traditionally we only mentioned 16 subtypes. So yeah, there are a lot of subtypes.

[Sarah Gregory] Was the one found in the United States different than the ones found in other countries or samples from other countries?

[Daniel Romero-Alvarez] So to answer this question, it's important to note something. Because this subtypification system is based on very specific mutations that are found in the genome, whenever you are trying to characterize a *Mycobacterium leprae* strain at the subtypification level, you need a good quantity of DNA. And that was usually not the case when we were extracting DNA from these museum specimens. So from the 18 positives that we found in our study, we were able to properly characterize subtypes (so, *Mycobacterium leprae* at the subtype level) on only five specimens. And from those five, we were able to actually see exactly what

was the strain in only two...sorry, only four, and from those four, all belonged to the subtype 3I, which was the subtype expected for the US and for North America.

[Sarah Gregory] You mentioned the area that these infected armadillos roam around in is pretty extensive in the US. Where exactly can we find them?

[Daniel Romero-Alvarez] Armadillos with *Mycobacterium leprae* have been detected across the majority of the southern states of the United States, so they have been found in Texas, Mississippi, Louisiana, Florida, Alabama, Georgia, Kansas, and there is an interesting report in Oklahoma as well.

[Sarah Gregory] What are the public health considerations of what you found?

[Daniel Romero-Alvarez] I can say at least three public health implications of our study. The first one is that one of our positive armadillo detections came from a sample collected in 1996. This sample is *Mycobacterium leprae* of subtype 3I, and that is interesting because the subtypification strategy was developed in 2010 (around 2010). So we only knew about the circulation of the subtypes 3I starting this date. However, our study showed that the subtype 3I has been circulating in the area (in the Southern United States) around...since the 90's, which is super interesting, and suggests that monitoring should be encouraged.

The second public health implication is that from our 18 positive samples, 16 came from the US, but two positive samples were found in armadillos collected in Bolivia and in Paraguay, and these are the first identifications of *Mycobacterium leprae* on those South American countries. So by confirming the presence of the bacterium in these armadillos, we are suggesting that these armadillos might function as zoonotic sources of leprosy in both countries. And of course, both countries have shown people infected with leprosy. Traditionally, we believe that those persons get the disease from other persons, but the zoonotic source must be included in surveillance research to see if those are acting as zoonotic sources of the disease.

And the third public health implication of our work is that museums should be seen as biorepositories for infectious disease surveillance, because they are hosting a lot of biological information that should be leveraged in order to understand infectious disease dynamics. Here we are showing the case...this interesting case with the 1996 identification of *Mycobacterium leprae* in the United States and also the positive identifications in Paraguay and Bolivia. All of this information came from museum specimens, and then we should actually start watching these institutions on these early detection alarms that will help us to anticipate outbreaks.

[Sarah Gregory] Going back to transmission, can a person, do you think, get leprosy from contact with an armadillo? I see all these Reels on Facebook and Instagram of people having these pet armadillos and it worries me.

[Daniel Romero-Alvarez] Yeah. And that is that most direct question and the most direct implication considering leprosy is a zoonotic disease. And the answer is that we still don't know that. As I mentioned, the person-to-person transmission is not completely understood, and of course, the armadillo-to-person transmission—and also the person-to-armadillo transmission—is completely hindered. There is the possibility that contact with the armadillo is the starting point of the bacteria moving from one species to the other. However, we don't know is handling the armadillo is appropriate; we don't know if being around the fluids of armadillos is the transmission route; we don't know if eating the armadillo is the way to get infected. So more research is needed to understand that connection.

[Sarah Gregory] Well, on that note, what kinds of future studies do you think are needed? What needs to be investigated particularly?

[Daniel Romero-Alvarez] I can mention three particular topics that need further research in this area. The first one, as you correctly mentioned...as I mentioned is that shed more light on how the pathogen is actually transmitted. Now it is accepted that leprosy can be a zoonosis in the United States and also in Brazil. However, if you go and review the recent report of the World Health Organization, they are barely considering the zoonotic route as a clear transmission pathway to acquire leprosy. And in this matter, it is also important to mention that *Mycobacterium leprae* has also been found in the environment—in soil samples and also in water samples both in India and in Brazil.

So there is a source of leprosy contamination that goes beyond humans, and this is not well-known and this is not well...and people are not aware about this route of infection. So it is super important to be aware of that, and also to understand how disease happens. The only clear condition that allows the transmission event involving leprosy is the chronic exposure to the source. So we need more information regarding how that chronic exposure transforms exposure into a clinical case. That is one of the most important research to be completed.

Another area of study that should be encouraged is to actually understand how much of leprosy is caused by this new pathogen that was found in 2010 (*Mycobacterium lepromatosis*). So in our study, we were aware that our samples should be tested for both (*Mycobacterium leprae* and *Mycobacterium lepromatosis*). However, almost nobody is aware of the presence of *Mycobacterium lepromatosis* as an agent causing leprosy. So to actually understand how this bacteria...what is the role of this bacteria in the global incidence of human leprosy or wildlife leprosy in the world is also another very important piece of research that should be completed. And more awareness is granted because if you are not aware that there is another pathogen causing leprosy, then of course you are not going to test for it. And the current methods of leprosy detection are based on only detecting if leprosy is present or not. So they are not concerned or trying to understand, "Yeah of course, this patient has leprosy". Which one? *Mycobacterium leprae* or *Mycobacterium lepromatosis*? If they have *Mycobacterium leprae*, current methods are also not interested in understanding which *Mycobacterium leprae*.

As I mentioned, there are at least 16 or more subtypes of *Mycobacterium leprae*, and it is important to understand which ones are causing the disease. And deciding on this line of thought, the third recommendation on future studies that should be done is that by understanding which subtypes are circulating around, we might be able to actually obtain/recover genomes of *Mycobacterium leprae*, and we need those genomes for areas that have not been studied yet. Specifically in South America, there are very few genomes contributing to the worldwide picture of *Mycobacterium leprae*—the majority of them are coming from Brazil. But there is no information from Colombia, from Peru, from Ecuador. We also need more *Mycobacterium leprae* genomes from West Africa and also from Eastern Asia (from China and Japan). That will allow us to complete the understanding of the worldwide distribution of *Mycobacterium leprae*.

[Sarah Gregory] Going back to museum specimens, this is the third podcast I've done on pathogens in museum specimens based on EID articles. Is this a growing trend in research?

[Daniel Romero-Alvarez] So I had the opportunity to listen to both of those episodes. I liked them a lot. But something interesting about those two episodes is that they are concerned on the fungi and the snakes. And it is curious because the researchers on those episodes were

mentioning that it is a common practice to do this. And yes, potentially it is a common practice from a veterinary perspective, from a biological perspective. But from a clinical perspective, it is not as common. And actually, during the COVID-19 pandemic, there was a super interesting report that was published in *Science*. It mentioned that we should develop an infrastructure to monitor wildlife diseases before they jump into humans, being aware that zoonotic...I mean, the COVID-19 pandemic had taken discourse about zoonoses to the public understanding, because the pandemic affected us all.

And it's curious because this *Science* piece, they mentioned that we need this infrastructure, and then there was a response to the scientists mentioning that "Hey, we have that infrastructure. We don't need to create a new one". That infrastructure is based on biorepositories represented by natural history museums. That response was done by Jocelyn Colella, who is a professor in Kansas University that has been promoting infectious disease research for quite a time now. So I believe the trend is going to continue. More studies are going to be developed, addressing the presence of pathogens in museum specimens with the intention of anticipating outbreaks, and also with the intention of understanding infectious disease dynamics on wildlife, which is super important. But I wanted to mention that for...from the clinical world, from the world of people, it's not common to actually look for pathogens of museum specimens.

[Sarah Gregory] Well, tell us about your job, what you do, and your particular areas of interest and how you became involved in this particular study.

[Daniel Romero-Alvarez] So I'm a medical doctor. I obtained my degree in Ecuador, and after working as a clinician I realized that the clinical world is sometimes limited in the understanding of how actually infectious diseases enter into a population. And that is because clinicians are focused on treating or on curing a particular infectious disease, not on understanding these dynamics. So that is why I changed fields, and I started a PhD on disease ecology—so being able to understand the origin of pathogens, to try to answer the question, "Where are they coming from?". And specifically, although this work involves the diagnosis of leprosy in armadillo populations, I am also concerned on the distribution of ecology of other pathogens. So I have been able to work with the presence of anthrax in Africa, and also with the presence of melioidosis in the US, and I am super interested on understanding patterns of infectious disease distribution and which are the conditions (the environmental conditions) that determine the jump of a pathogen from one species to another, and how that event can become an epidemic.

[Sarah Gregory] Well, thank you so much for taking the time out of your important work to talk with me today, Dr. Romero-Alvarez.

[Daniel Romero-Alvarez] Thank you very much for the interview, and I had a great time in this podcast.

[Sarah Gregory] And thanks for joining me out there. You can read the March 2023 article, *Mycobacterium leprae* in Armadillo Tissues from Museum Collections, United States, online at cdc.gov/eid.

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

[Announcer] For the most accurate health information, visit cdc.gov or call 1-800-CDC-INFO.