

# Prospecting for Zoonotic Pathogens by Using Targeted DNA Enrichment

*[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. Elisha Enabulele, a postdoctoral research associate at the Texas Biomedical Research Institute in San Antonio, Texas.

Welcome, Dr. Enabulele.

[Elisha Enabulele] Thank you so much, Sarah. Thank you for the invitation.

[Sarah Gregory] Your study is about looking for zoonotic pathogens using targeted DNA. Remind us first what zoonosis is.

[Elisha Enabulele] Sure. When we talk about zoonosis, we are referring to diseases that can be transmitted between animals and people. Some of the zoonosis can be caused by viruses, bacteria, fungi and, of course, parasites.

[Sarah Gregory] How much of an impact does it have on transmission of pathogens to people?

[Elisha Enabulele] Many pathogens of public health importance result from zoonotic transmission, including about 61% of known pathogens and 75% of emerging pathogens. And if you take Lassa fever, for example, which is a zoonosis transmitted by rats, recently we saw earlier this year there was a report of an outbreak in Nigeria, and about 4,000 cases were reported, and had deaths for about 16 to 25% of deaths. In the case of Ebola virus for example, as well, you could have up to 60% of those infected also die.

[Sarah Gregory] You've just mentioned some. What are some of the other known pathogens that are spread to people by animals?

[Elisha Enabulele] Oh, well it's a long list we have. In addition to the previous two I mentioned (I mentioned Ebola virus and Lassa fever), we also have the rabies virus, the plague, bird flu, of course recently we had monkeypox...the list really goes on. But I noticed on the CDC website that you guys have really done a great job having an A-to-Z list of most zoonotic pathogens, which really helps.

[Sarah Gregory] Glad to hear that. What are small mammals and how many bad pathogens are there from them?

[Elisha Enabulele] Specifically when we talk about small mammals like rats or rodents, they do transmit a lot of pathogens and though they are relatively benign (most of these pathogens), they do transmit leptospirosis. But over 60 pathogens transmitted by small mammals are quite deadly, such as...you have monkeypox, Lassa fever (I had mentioned earlier) and hantavirus. They can really have very high rates (death rates).

[Sarah Gregory] What part can natural history museums play in studying zoonotic pathogens? I've recently done three other podcasts about specific pathogens found in museum specimens, and I really think this is an interesting topic.

[Elisha Enabulele] Yeah, this is really interesting and very important to our study talking about natural history museums. Natural history museums are a critical and underutilized resource for studying pathogens. Scientists collect specimens from different fields and deposit them into

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natural history museum collections. And natural history museums hold over several millions of specimens that have been collected from different countries and different time points. Some even date back to the early 19<sup>th</sup> century (some of the samples that they have in their collection). So each specimen is really a data point that can be used to understand zoonotic pathogens. So they really play a vital role in understanding zoonotic pathogens.

[Sarah Gregory] What kinds of tools exist or are needed to dive into these museum specimens and find these pathogens?

[Elisha Enabulele] Well, for the tools, broadly there are four categories. We have the immunological-based test, and we also have the culturing test, and then you also have visual inspection where you just look at the specimen and you can tell if its infected or not and you can sometimes be able to tell which pathogen you have in the particular sample. And fortunately, we have the nucleic acid that has to do with DNA or RNA. So the nucleic acid method (which is the DNA method) is really more sensitive, specific, and it provides more genetic information for answering important biological questions such as pathogen evolution and epidemiology. So basically, in our own study we actually focused on nucleic acid detection tests.

[Sarah Gregory] Since these museum specimens can carry dangerous pathogens in their DNA, can they also potentially be dangerous to handle or for people to breathe while they are inspecting them?

[Elisha Enabulele] Yeah, that is really an important question you mention, because one has to be really careful. Now at all times, adequate safety protocols should be followed when handling any kind of biological specimens. However, there are a wide range of nucleic acid extraction reagents (so a lot of chemicals) we can use when we extract DNA from those samples. So those reagents actually inactivate the pathogens in those samples before we start handling them. So once we introduce those pathogens to the samples (to our reagents), we actually inactivate any bacteria, virus, or parasite in those samples so we can make them safe and we can work on them.

[Sarah Gregory] And that doesn't kill what you need to be looking at, though. It just kills the dangerous part...

[Elisha Enabulele] It doesn't damage the DNA; it just inactivates them and makes them so they cannot infect you and you are safe. Of course, we are also working in a biosafety cabinet. All safety protocols are followed such as wearing hand gloves and wearing face shields. But basically, the reagent actually makes them no more pathogenic to humans handling them.

[Sarah Gregory] Glad to hear that. I've been wondering about that for a while. Why did you do this study? What was your goal?

[Elisha Enabulele] Natural history museums may be the single biggest repository of pathogen data, because they have a lot of collections that I earlier mentioned. Unfortunately, methods of identifying samples—those specimens in the museums to identify which of them actually have pathogens—is quite difficult and expensive. Sometimes these kinds of studies that don't, researchers normally focus on single pathogen groups. I listened to your podcast and those studies just focused on certain groups of pathogens, because usually they want to focus on particular groups. In those cases, what happens is that when you actually focus on a particular pathogen from the sample, you will miss a lot of other pathogens in those samples. So as a result, museums are underutilized when it comes to studying zoonotic pathogens. So we wanted to develop a method where you can actually target more than one pathogen group so that from a

single test you can tell different groups of pathogens that may be present in that particular sample. So to do this, we had to think about...the best way to approach this was to do a DNA-kind of study using a method called targeted DNA sequencing, which I will explain later on as we progress.

[Sarah Gregory] On that note, tell us briefly about how you went about it and what methods were used. Obviously, this is a pretty complicated and multistep process.

[Elisha Enabulele] Yeah, it sounds really complicated. But it's quite simple, so I'll just quickly run through what we actually did. So if you were to extract DNA from one of these samples you get from the museum...normally they would send you tissue, maybe about less than a quarter of a centimeter of tissue would be sent to you from the museum, and normally it would have those tissues from an animal, say, from a rat. All you have is mostly the rat's DNA (the host DNA), and that would be...that would form a larger percentage of what you really have. And the pathogen would just be less than 1% in the particular tissue we are being given.

So for our method, we wanted to find a way how you can get a read of the host DNA so that the pathogen's presence in that tissue...I wanted to see how really to maximize our chances of early detecting pathogens in the particular tissue. So our method...what we actually did was to choose about 32 different pathogen groups. We looked at about 32 different pathogen groups that we focused on in this particular study, and for each of these groups we used some computational tools to look at the genome of these various pathogens. We looked for regions of those genomes that are conserved that can actually distinguish between different groups. We designed some DNA-based probes from chemical molecules we designed, which we normally bind to this particular region of interest, that would distinguish each of these groups.

So in the second step, what we did was extract DNA from the tissue using conventional DNA extraction methods, and after we extracted those DNA, we introduced the sample to our probes that we designed and we were able to use it to fish out the pathogens of interest that we have designed for. And after that, we went back into the computational tools trying to identify which DNA belonged to which pathogen group. So that was how we designed our method.

[Sarah Gregory] And after all of that, what did you find? What pathogens were identified?

[Elisha Enabulele] In terms of the pathogens that we found, well, we found quite a lot. As a proof of concept, our experiments showed that you can actually enrich the DNA of the pathogen to be about a two-to-six-thousand-fold increase compared to the host DNA. And remember I mentioned the host DNA-specific problem—you want to get rid of the host DNA. So we were able to prove that yes, with that method you can actually enrich the pathogen of interest up to about six thousand-fold compared to the host DNA. And so, we identified about four different groups of pathogens—*Bartonella* (that's a bacteria), *Plasmodium* (*Plasmodium* is the parasite of malaria), and *Ralstonia* and *Paraburkholderia*. So they are all the pathogens we identified for the samples.

[Sarah Gregory] Do we know why some pathogens apparently lay dormant for years and then finally transfer to people?

[Elisha Enabulele] Well, pathogens will naturally remain in their host animals until when anthropogenic factors bring humans or people get in contact with such infected animals. Pathogens normally...they just lie low in the animal (the host). But when people get in contact with them, then evolutionary factors can come into play that can make them zoonotic pathogens

to make the jump from an animal and subsequently they adapt in people. So basically, that is how this works. Normally they just stay dormant in the animal. As long as we keep animals away, we don't interfere with them, everything is fine. But once you interfere with the animals, then there's a possibility of the pathogen jumping from the animal into humans to cause infection in people, as well.

[Sarah Gregory] So it's more about proximity than it is about mutation?

[Elisha Enabulele] Yes, it's more about proximity, as long as we don't interfere and people don't go close to the wild, without being protected. Of course, scientists...we do a lot with animals in the wild, with protection. But once you don't have this protection, without necessary equipment, there is potential if an animal is infected that it could get transmitted, and it takes a while for the animal...for the pathogen to adapt.

[Sarah Gregory] Were there any surprises in what you found? This is a pretty new area of study, looking at museum specimens, I think.

[Elisha Enabulele] Yeah. We were quite surprised to see how many different species of small mammals are hosts for *Bartonella* bacteria (I mentioned *Bartonella* earlier). *Bartonella* is actually a genus of bacteria that is responsible for bartonellosis in patients such as cat-scratch disease. During the World War, a lot of soldiers died as a result of trench fever, and that is also caused by *Bartonella*. So we were quite surprised to see that a number of groups of *Bartonella* were found in the samples. There were quite different groups of *Bartonella* that were found. And one was quite unique to us, which was quite interesting.

We identified a species of *Bartonella* called *Bartonella mastomydis*. It was in the samples that was collected sometime in 2009 in Botswana (southern African region). But what is interesting about this particular pathogen is that in 2018, it was described as a new species of *Bartonella*. So that really...it gave us proof we need for using museum samples because this particular species was already in museum collections back in 2009. But science only discovered and described it in 2018. So that really was quite interesting to us that, yeah, what really could be hiding in museum samples that we don't know about, what pathogens are there. So that is why it's quite an important question on using museum samples to understand the history of pathogens.

[Sarah Gregory] Are there inherent challenges in looking at museum specimens?

[Elisha Enabulele] Well, not so much of a challenge because, you know, the challenge of samples you normally find with museum samples. Now, most of the studies designed for samples in museum collections, historically organized for systematic and taxonomic study of the host organism. So different scientists collect different animals from the wild for different reasons, and when the scientists are not aware of particular pathogens of interest, they wouldn't know what samples to collect from those animals that are collected in the wild. So that is a major challenge because at times we request from museum samples, for example if you take a...let's say, for example *Trypanosoma* (a parasite)—and this parasite most of the time you find it in the brain—so you may get samples from museum collections from different animals, and you wouldn't have sample specimens from the brain. So there is not much we can really do. That is a major challenge. So I think what can really help is when pathobiologists and organismal biologists can work together—the pathobiologists actually inform organismal biologists what kind of samples or specimens to collect from the animals that they are investigating. So that would really help

when studying pathogens to have the right specimen for study or investigating pathogens of interest.

[Sarah Gregory] Which museums did you get your samples from—the ones that you used in your study?

[Elisha Enabulele] We collected samples from the Natural Science Research Laboratory. It's an arm of the Museum of Texas Tech University in Lubbock, Texas. So they provided those museum samples that were collected about 30 years ago from different parts of Southern Africa and from part of Texas and from some other parts from South America. So they provided the samples for us.

[Sarah Gregory] Are all natural history museums receptive to allowing this kind of study or do some resist it?

[Elisha Enabulele] As a general principle, natural history museums are willing to share samples and specimens collected with different investigators, but the criteria for receiving specimen loan requests can vary from museums. So they have different protocols. You have to fill out forms several forms....you have to fill out. Voucher specimens or tissue samples can be embargoed for study, for example, the depositor may say, "Wait, I don't want anybody to look at this sample over a time period". So that can also be one of the factors that can restrict access at some point. But our experience has been that museums are generally excited, and they really want scientists to make use of what they have, because they have lots of collections.

[Sarah Gregory] Why is it important to public health to develop and do these kinds of studies?

[Elisha Enabulele] Yeah, for public health, we want to use museum collection and ensure provide information and biological collections that can be used to document the presence and the prevalence of zoonotic pathogens in both known and unknown host animals. So they have a huge collection, so if you really want to study pathogens (you want to study the evolution of pathogens, if it has been circulating for a long time or a particular location, then a natural history museum would really be the best place to visit to collect samples for study. Samples in natural history museums may have been collected from a same location across several decades. If you go through the samples, you can actually tell when a particular pathogen came into that particular location and able to track it. So they really provide a vital resource for us. Generally, reservoir studies—animals that have pathogens—are a vital component of any integrated public health response to established or emerging zoonotic diseases. So that is where they are really of use to us.

[Sarah Gregory] Going back to the study for a second here, where did you do the initial research before you started looking actually at specimens?

[Elisha Enabulele] This research was done at Texas Biomedical Research Institute here in San Antonio. It's a non-profit, independent research institute based here in San Antonio. We are dedicated to studying infectious diseases. We and our colleagues study a wide range of pathogens such as Ebola, malaria, tuberculosis, HIV, COVID-19, just to mention a few. And we work to develop therapies and vaccines to help keep us safe from these diseases. that is what Texas Biomed is all about.

[Sarah Gregory] What do you think should be the next step in furthering your research?

[Elisha Enabulele] Well, for our study, you know we actually just looked at a few samples (just less than 50 samples we got from the museum). So ideally, we really want to look at a large-scale sampling. There are a lot of museums all over the world with different collections, and to do this...to make this possible, of course, we need funding to assess more specimens from different museums and across different groups of hosts and different taxa from different geographical locations and, of course, with different museums to get different samples. And if we do this, we will be able to tell which reservoir host transmit pathogens and we get a good picture of different pathogen groups that may be available in different specimens. So scaling up is really what's really...is our focus at this particular point because our study actually was just to prove a concept that we actually can do this particular study and actually detect multiple pathogens from a single test. So we really want to expand it.

[Sarah Gregory] Where do you work? What areas of disease are you most interested in and how do they fit in with this study?

[Elisha Enabulele] We work at Texas Biomedical Research Institute with the Timothy Anderson Genomics Laboratory at Texas Biomedical Institute. So generally, in our lab, we work on malaria, and we work on a parasitic disease called *Schistosoma*. So we do more genomic work on those particular pathogens, but again, we are also interested in developing diagnostic tools (genomic diagnostic tools) for identifying pathogens. So that is what we do in our particular lab.

[Sarah Gregory] Of all the deadly pathogens out there and the new ones you're discovering (like these ones in little mice), is there one that worries you the most?

[Elisha Enabulele] Well, there is ongoing research, drug development, and vaccines for various pathogens that we know about. So what worries me the most is what we don't know (pathogens we don't know about). There's talk about pathogen 'X' or disease 'X', so which is...nobody knows what the next pathogen will be that will be of major concern to us. So I'm really concerned about what is next. So that is why, again, it is quite important to really emphasize why pathogen surveillance is very critical and, of course, we need funding to be sustained, you know, for us to keep working on this particular area of disease surveillance so that whatever shows up...nature throws at us, we'll be ready for it.

[Sarah Gregory] Sort of like COVID-19 or SARS-CoV-2 popping up out of nowhere.

[Elisha Enabulele] Yeah, we just have to be ready. Surveillance...keeping on pandemic preparedness is quite important here. Surveillance will go a long way. Doing genomic surveillance is a verified aspect of this particular interest at this particular time.

[Sarah Gregory] Well, thank you so much for taking the time to talk with me today, Dr. Enabulele.

[Elisha Enabulele] Thank you so much for the time.

[Sarah Gregory] And thanks for joining me out there. You can read the August 2023 article, *Prospecting for Zoonotic Pathogens by Using Targeted DNA Enrichment*, online at [cdc.gov/eid](https://cdc.gov/eid).

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

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