

Evolution of Zika Virus in Puerto Rico, 2016–2017

[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. Jorge Muñoz, team lead of CDC's Dengue Surveillance and Research Laboratory based in Puerto Rico. We'll be discussing the origin, spread, and evolution of Zika virus in Puerto Rico from 2016-2017.

Welcome, Dr. Muñoz.

[Jorge Muñoz] Hi Sarah. Thanks for having me here.

[Sarah Gregory] Let's start off with you reminding us what Zika is.

[Jorge Muñoz] Zika is a virus transmitted by a mosquito (a mosquitoborne virus) that was first found in 1947 and named after the Zika Forest in Uganda. This virus is—it was known for a long time, but it was first introduced in the western hemisphere in 2015 and it caused a large epidemic. The virus is part of a large group of viruses called *Flaviviruses*, and other known *Flaviviruses* are dengue, yellow fever, and West Nile.

[Sarah Gregory] And how is it spread?

[Jorge Muñoz] The Zika virus is spread mainly through the bite of a mosquito, and specifically two species of mosquitoes (the *Aedes aegypti* and *Aedes albopictus*), but mainly by *aegypti*. It's a household mosquito—so a mosquito that is present around the houses in urban areas (the urban mosquito). And it's the same mosquito that spreads dengue and chikungunya viruses. There are other forms of transmission by Zika virus—that's something that we learned from the recent epidemic—which is that pregnant women can pass a virus to the fetus during pregnancy or around the time of birth. And in addition, a person infected with Zika can pass the virus to their sex partners through semen or vaginal fluids. Another possible way in which Zika has been known to be spread through limited numbers is by blood transfusions.

[Sarah Gregory] Blood transmission—would that include, like, organ transplants?

[Jorge Muñoz] Yes, that may include organ transplant. It's a very rare event, but it has been known.

[Sarah Gregory] And what are the symptoms usually? At its least and at its worst?

[Jorge Muñoz] Many people with Zika virus will not have symptoms or will only have mild symptoms, such as mild fever. Some people develop a rash or headaches or joint pain, red eyes and muscle pain. Some people can progress into more severe manifestations such as the swelling of the brain or spinal cord and this other blood disorder that has been shown in Zika, which results in bleeding and bruising or slow blood clotting. Those are rare cases. So mostly Zika can stay asymptomatic. Not many people have a lot of symptoms.

Now, in addition, Zika can produce a symptom called Guillain Barre, which is a syndrome which is uncommon. It's a known sickness of the brain where the immune system attacks and can damage and cause muscle weakness and sometimes paralysis. Now the most feared manifestation of Zika infection is when the virus infects the fetus, and it can cause birth defects—defect of the brain called microcephaly and other severe birth defects. It's also linked to a problem such as miscarriage, stillbirth, and other birth defects.

[Sarah Gregory] And that's what was happening a lot during the epidemic, right? During this time period?

[Jorge Muñoz] Yeah. In some areas, one of 10 pregnancies with Zika resulted in some form of congenital Zika syndrome resulting in poor health outcomes in newborns.

[Sarah Gregory] Are there treatments?

[Jorge Muñoz] No, Sarah, there is no treatments for Zika. There is no medicine or specific medicines of any kind or vaccines yet available. So people, when they have symptoms, are treated with fluids (drinking fluids) to prevent dehydration, resting, and taking acetaminophen to reduce fever or pain.

[Sarah Gregory] So if a pregnant woman gets Zika, there's no intervention to protect the fetus?

[Jorge Muñoz] That's right. There are no interventions, just management of the case.

[Sarah Gregory] And you mentioned there's no vaccines. Is there a vaccine in the works? Like, I know they've been working on a dengue one that hasn't been successful yet. What about for Zika?

[Jorge Muñoz] Yeah. There's several attempts in the direction of having a Zika vaccine, but none yet has surpassed enough in human trials or in the regulatory pathways.

[Sarah Gregory] In your study, you reconstructed the 2016-2017 Zika virus epidemic in Puerto Rico by using complete genomes to uncover origin, spread, and evolutionary dynamics. So we're going to go through all of that, but first of all, what's the difference between an epidemic, like Zika was, and a pandemic, like we're having for COVID-19?

[Jorge Muñoz] Epidemic refers to an increase in the numbers of cases above what's normal in a particular place, okay? So let's say we didn't have Zika, and when we start seeing Zika in a country or around several countries, that becomes an epidemic. Now a pandemic refers to an epidemic that has spread over several countries or continents, usually affecting many people—it becomes global. And the history of Zika, you know, it illustrates how epidemics may become global. We can go from an outbreak to an epidemic and even, you know, we got close to being a pandemic because before 2000, Zika was known in just a few countries in Africa. It has been known to cause illness in just maybe 12-15 cases in Africa. But in 2007, we found of an outbreak in the Yap Islands in the Pacific—the first known Zika outbreak itself. After that, the virus was found in Easter Islands near the Pacific coast of South America. So that was the last time that we could talk about outbreaks that we moved into an epidemic period. Because in 2015, the virus was found in Brazil. It was spread across bigger cities in Brazil (densely populated areas), and it rapidly spread through the Americas, and the WHO declared Zika a Public Health Emergency of International Concern in 2016. Zika is the closest thing that you can get to a pandemic. It was found in 81 countries where the mosquito that transmits Zika is present, okay? That's the story with Zika. It was a very large epidemic.

[Sarah Gregory] Is using complete genomes (which you mentioned in your study) different than using any other kind of genomes, like whole genome? And what's the difference?

[Jorge Muñoz] When we use the complete genome of a virus, we sequence the entire genome. That gives us a full picture of the genetic composition of the virus. You know, in the past when genome sequencing was less affordable, we used to sequence just a portion of a virus genome that codes for the virus structural proteins, which are the parts of the virus that our body responds

to with antibodies. Now, the Zika pandemic occurred when advanced sequencing was more available and more affordable, and we have been able to sequence whole genomes much more frequently. With this technology, which is called Next Generation Sequencing, we can learn about parts of the virus that we didn't normally learn much about, such as proteins that help the virus survive in the cell environment or that are involved in making copies of the virus. We learned not just about the structural components of the virus, but also about functional aspects of the virus. And because we have more sequenced data to analyze, we can also study the evolution of the virus more accurately.

[Sarah Gregory] As I mentioned before, you were also looking at origin and spread, which are pretty clear terms, but what is evolutionary dynamics?

[Jorge Muñoz] When we sequenced the virus from the first few cases of a specific place—you know such as Puerto Rico, which is the basis for our work—we can only learn of its origin as long as there is sequenced data from viruses from other countries, right? So these genetic sequences from individual cases get stored in electronic databases, and with time as more sequences are added to that database, we can reconstruct how the virus spread through the large regions or even become global like Zika did. We can see how, for example, the virus's spread in one country is more closely related to the virus from another country and not as much to the virus from a third country or another region. And so this is called genetic relatedness, and also we learn how the virus is related across the areas where the virus is being spread.

We have developed bioinformatics or informatic tools to understand biological processes, like the evolution of the virus, and understand that relatedness better. We can add time and location in our analysis and understand not just where the virus came from or where it traveled next, but for how long it's staying in one place, for example. Sometimes a place where a virus spreads for a long time becomes the source of virus for other places. At other times, a place may seem like a magnet for incoming viruses, but the virus doesn't spread for very long in some areas. So that's how viruses evolve, and we call this type of knowledge, "epidemic dynamics".

[Sarah Gregory] So what did you find looking at these things?

[Jorge Muñoz] In 2016, we assisted health authorities in Puerto Rico by providing testing for Zika patients. We confirmed more than 30,000 cases during those years, and we were very busy then. We had a devastating hurricane a year after, so it wasn't until recently that we went back and sequenced the genomes of a collection of viruses from around the island. Our surveillance system picked up useful information, including dates and specific locations. And we were able to connect the dots, if you will—that is, discover how the virus was spread across the island. And we found that in Puerto Rico, the Zika epidemic was driven by multiple introductions of the virus that spread across the island.

[Sarah Gregory] And how did these multiple introductions come about? How were they spread and why were they spread? Were you able to figure this out?

[Jorge Muñoz] Puerto Rico is a popular travel destination, and we found that Zika virus was introduced frequently. But not all of those strains spread, you know, through the island. A fewer number of these strains that were introduced really take on and become established on the island. So once some of these strains are well-established on the island where it is transmitted, the other ones may be coming in but do not necessarily spread as much as the other one that is already on the island. As the local virus spreads, the population of the virus becomes more diverse

genetically as well, and two major introductions took a grasp of the island and caused more than 30,000 cases. It's pretty remarkable.

[Sarah Gregory] That is remarkable. So is that how you call them in your study, these “intricate evolutionary patterns” come about?

[Jorge Muñoz] That's right. As the virus continues to spread around the island, it finds favorable conditions. It finds mosquitos that haven't been infected before with Zika virus and people have ever been infected. You know, Puerto Rico's rainfall is a factor in this, and the mosquito eggs need water to hatch, so that's the reason why a virus like Zika can take on over here. You know, there's susceptible people that are more prone to being affected by Zika, and the geography of that place and the population density and everything plays a role. So it becomes an intricate form of transmission, and you can see that in the evolutionary patterns that we find.

[Sarah Gregory] You also mention about 10 months of “cryptic transmission.” This is not a term we hear much, if ever. What does cryptic transmission mean?

[Jorge Muñoz] Yes, about 10 to three months of cryptic transmission. So what that means is that the analysis shows that there could have been undetected transmission for some time before we found it, and we call that cryptic transmission. This happens often with epidemics. It takes some time for the virus to cause disease or to be noticed by the system and for the health system to react and find more cases. The analysis of all the data that we have and location analysis in Puerto Rico from all over the region helps us understand that, because we can look at that relatedness that I was telling you about and see that "hey, this virus is more related to this possible origin, even though it wasn't seen here before". And so, with those kinds of tools, we were able to reconstruct a timeline of the virus coming in Puerto Rico and identify what we call cryptic transmission.

Now, cryptic transmission has been found for Zika in other places and for other viruses transmitted by mosquitos. Because you could imagine that for the virus to be transmitted, it has to salvage infection in the mosquitoes. And it's not until there is a number of mosquitoes infected with the virus that can transmit a lot of the virus to people that we start seeing more cases.

[Sarah Gregory] There were some different kinds of surveillance implemented to find this, is what I'm understanding from your study. So it wasn't able to be recognized by whatever regular surveillance tools you had?

[Jorge Muñoz] You know, mosquitoes with Zika spread the virus to their eggs. Maybe that the virus is present in the mosquitoes before some threshold is reached and we can start seeing human cases. Also, many people don't have symptoms or have very mild disease, making cases more difficult to detect. And so our study shows that this cryptic transmission could have been actually short—as short as three months but as long as 10 months. In terms of the difficulty, I think the epidemic originated in Brazil and it went to Colombia and other places, and it took a little bit of time for the epidemic to hit our area. We were able to prepare a little bit better to face this epidemic.

[Sarah Gregory] I see. So Brazil and Colombia had similar problems initially identifying the virus. Is that right?

[Jorge Muñoz] Possibly. Some of the studies that have been published show cryptic transmission as well in some of those countries.

[Sarah Gregory] You created a Zika genome dataset. How will that be useful in the future?

[Jorge Muñoz] Well, genome databases have become essential parts of disease surveillance. The technology has gotten more affordable and efficient. And it gives us a lot of information about the virus—the genetic composition of the virus, how the virus evolves, how it is transmitted in places and becomes an epidemic. So we assessed genome databases to look at relatedness of the virus in the Caribbean and to understand the epidemic in Puerto Rico better, and our own datasets in turn can be useful for other people to understand their outbreaks as well. We use it constantly now, and the full potential of those global databases is not always reached immediately. For example, if Zika reemerges, it could be interesting to know if the population once exposed to the virus will be protected from new strains. And these databases may be able to help determine, you know, some of these answers once the virus reemerges.

[Sarah Gregory] And who will have access to this dataset?

[Jorge Muñoz] Health officials and scientists from all over the world have direct access to genome databases. Data is available for more than Zika, you know, it's available for a lot of viruses that we see now with COVID. Very large databases with genome sequencing have been built and are accessible to people. COVID databases have been extremely helpful in documenting the spread of COVID recently, and that's how databases are used constantly for disease surveillance, particularly infectious diseases.

[Sarah Gregory] What do you think this study contributes to public health and why is it important?

[Jorge Muñoz] These studies are, I think, most helpful when combined with other datasets such as numbers, cases associated with geographic data and time. They help us understand transmission through time and space and even identify patterns of transmission. We have learned of hotspots of transmission that is, you know, in areas where transmission was more intense or lasted longer here in Puerto Rico or in other countries, and scientists have found that the genetic diversity of the virus increases in areas of more transmission. So whenever we see strong transmission in someplace, it's usually correlated with more genetic diversity. The virus is more diverse in its genetic composition in places where the transmission is intense.

So with all of this acknowledged, we can better assess the epidemiology of the virus. We look at human population density and socioeconomic status as well. So this study can help make public health decisions and inform the implementation of prevention or control methods to reduce the burden of disease in these areas.

[Sarah Gregory] In your opinion, is another Zika outbreak of this magnitude likely to happen again?

[Jorge Muñoz] There is current suspicion of Zika virus in India, a country that has previously experienced Zika transmission before. So it shows that it can reemerge. It is likely, though, that the virus will not have a large epidemic or pandemic very soon because there's herd immunity that has been built in populations that were exposed during the Zika epidemic in 2015 and 2016. But there's no reason to think that it will not come back, and it would cause some level of concern.

[Sarah Gregory] And so around that, how can people protect themselves from the Zika virus or any mosquito-borne virus? What are the best ways?

[Jorge Muñoz] The best way to prevent Zika is to protect yourself from mosquito bites. Use Environmental Protection Agency (EPA)-registered insect repellent, always follow product label instructions to use those repellents, you wear long-sleeved shirts and long pants and protect your skin from being exposed to the bite of all the mosquitoes. Remember, those mosquitoes can transmit dengue as well, which is a disease that continues to cause concern in many countries. Use screened windows and doors and remove items that hold water in the periphery of your house. And in the case of Zika, if Zika is transmitted and it's a concern in travelers or in people in endemic areas, the use of condoms is recommended for people who have been exposed to Zika, preferably to avoid sexual transmission of Zika.

[Sarah Gregory] The wearing of long clothes in spring and all of that, that's difficult though, isn't it? I mean, even here in Atlanta, Georgia, just going out in my own backyard in the summer even though I try to do everything I'm supposed to do, I still get bit. They seem to even bite me through my clothes sometimes.

[Jorge Muñoz] Yeah, I mean some mosquito repellents aren't very effective, you know, in avoiding mosquito bites. But remember that this mosquito (*Aedes aegypti*) particularly, it's a mosquito that is not present in all of the United States. It's present in some states (southern states) and it's more frequent that you will be infected in communities where the mosquitoes are present.

[Sarah Gregory] Is this a different mosquito than West Nile virus?

[Jorge Muñoz] Yes, it's a different mosquito.

[Sarah Gregory] Okay. And where can people find reliable information about Zika?

[Jorge Muñoz] You can visit the CDC webpage cdc.gov/zika and find a lot of interesting information about Zika disease, ways to prevent Zika, Zika diagnosis, and other important information about Zika.

[Sarah Gregory] Okay. And Dr. Muñoz, tell us about your job at CDC and how you became involved in this study.

[Jorge Muñoz] I am the team lead of the CDC Dengue Branch Laboratory here in San Juan, Puerto Rico. And our mission is to help control and prevent dengue and other vectorborne diseases in Puerto Rico and the Caribbean, and we assist dengue programs in more than 100 countries around the world. We have worked with dengue epidemics for decades and other mosquito-borne diseases, such as the chikungunya epidemic in 2014 and the 2015 Zika epidemic that we have been talking about.

During the Zika pandemic, we developed a molecular diagnostic test for Zika that also tested for dengue and chikungunya since those were diseases of concern in the same areas where Zika was being transmitted. Our lab in Puerto Rico has been here for almost three decades studying how dengue spreads, and we were uniquely positioned to help during the Zika epidemic and conduct the genetic study to understand how the virus transmits and spreads through the island a little bit better.

[Sarah Gregory] What do you enjoy most about living in Puerto Rico?

[Jorge Muñoz] The people of Puerto Rico and being able to apply research directly into public health programs is what really makes me happy. I love working with people in Puerto Rico and there's nothing more valuable to me than to help strengthen public health programs locally,

increase laboratory capacity, and improve surveillance practices in areas where dengue is a problem. This knowledge that we obtained from directly assisting and conducting research during the Zika epidemic and improving diagnostic systems was very useful for Puerto Rico and for the Americas. I'm lucky to be here after living in New York City for 10 years. I was ready to enjoy the warm weather and relive my diving days, I used to be a diver and I have been able to do that in Puerto Rico and enjoy these beautiful coral reefs around the island.

[Sarah Gregory] Ah, yes. Not much of that in New York City.

Thank you for taking the time to talk with me today, Dr. Muñoz.

[Jorge Muñoz] Thank you so much.

[Sarah Gregory] And thanks for joining me out there. You can read the November 2021 article, Tracing the Origin, Spread, and Molecular Evolution of Zika Virus in Puerto Rico, 2016–2017, online at [cdc.gov/eid](https://www.cdc.gov/eid).

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

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