

Fatal Human Eastern Equine Encephalitis Virus Infection, Alabama

[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. Holly Hughes, a research microbiologist at CDC in Fort Collins, Colorado. We'll be discussing a case of eastern equine encephalitis virus infection in Alabama.

Welcome, Dr. Hughes.

[Holly Hughes] Good morning and thank you so much for having me.

[Sarah Gregory] Eastern equine encephalitis virus, or EEEV, is an arbovirus. What are they?

[Holly Hughes] Good question. So arboviruses are viruses that are most commonly spread through the bite of a mosquito or a tick. In the word "arbo," the "A-R" comes from the word "arthropod" and the "B-O" comes from the word "borne." So an arbovirus is any virus that is borne from arthropods, or things like a mosquito or a tick.

[Sarah Gregory] So how is EEEV different from other mosquito-borne viruses, and particularly ones that cause encephalitis, such things as West Nile or Japanese encephalitis?

[Holly Hughes] So all the viruses you just mentioned can cause infection of the brain or an encephalitis. And they all have similar symptoms, such as fever, confusion, or altered mental status. But although West Nile and eastern equine encephalitis virus (or we call sometimes "Triple E") are both endemic to the U.S., Triple E is much rarer of an infection and is also considered one of the deadliest arboviruses found in the U.S. We mostly see cases of Triple E in the Atlantic and Gulf Coast states and Great Lake regions where there are freshwater/hardwood swamps, while West Nile can be found all across the country and is one of our most common mosquito-borne diseases in the United States.

[Sarah Gregory] So Triple E, its name has the word equine, so I imagine it affects horses? What about other animals?

[Holly Hughes] Yes. Eastern equine encephalitis virus can infect horses, and horse owners living in areas where Triple E is common should get their horses vaccinated against the disease each year. Triple E can also infect other domestic vertebrates such as cows or domestic birds like emus. It has also been known to be in wild birds and also some lizards.

[Sarah Gregory] Can we get it directly from these animals, from, say, touching them or eating them?

[Holly Hughes] That's a good question, and no we cannot. Triple E is only spread through the bite of an infected mosquito, and you cannot get it from touching or eating animals that have become infected. Mosquitoes can become infected when they feed on a bird or a lizard that already has the virus. But people and horses, they become infected when that mosquito bites them. However, we consider horses and humans to be dead-end hosts, and this means that the concentration of the virus in their bloodstream is too low and cannot infect other mosquitoes.

[Sarah Gregory] Where has it been mostly found in the past?

[Holly Hughes] The mosquitoes that spread Triple E virus usually live in the freshwater/hardwood swamps. These are mostly seen in the Atlantic and Gulf Coast areas, and

also around the Great Lakes region where these types of environments can be found. Historically speaking, Massachusetts, Michigan, and also Florida have had the highest numbers of cases in a given year.

[Sarah Gregory] So Triple E has been in the news a lot over the last few years. It's gone from 8 annual cases to 38. What's going on?

[Holly Hughes] So it's not really unusual to see increases or decreases in the number of cases from one year to the next. For Triple E in particular, we saw increases in 2004–2006 and in 2005 in particular, there were 21 documented confirmed cases of Triple E. With lots of the mosquito-borne diseases there are several factors that can contribute to years with higher than average case counts. And this could be anything from changes in the mosquito population, changes in immunity levels, weather patterns, and even some human behaviors, including like increased awareness, increased testing, or increased exposure (being outside more often than normal).

[Sarah Gregory] Your article says that in infected humans, “viremia does not develop at sufficient levels to infect additional mosquito vectors.” What's this mean, and how is that different from other mosquito-borne infections?

[Holly Hughes] So viremia relates to the amount of virus found in a person or in their bloodstream. And for mosquito-borne infections, people are considered to be dead-end hosts, and this means that while people can become infected with a virus, they won't be able to spread it to a subsequent mosquito because when they get bit by that mosquito, the amount of virus in their blood is not high enough to be picked up and transmitted by that mosquito. Some examples of other viruses that humans are dead-end hosts would be West Nile virus and also St. Louis encephalitis virus. But alternatively, viruses such as Zika, dengue, and yellow fever can be spread from person to person through biting mosquitoes.

[Sarah Gregory] So a mosquito can bite somebody that has the virus and then go bite somebody else and give them the virus?

[Holly Hughes] Yes, that's correct.

[Sarah Gregory] Your study focuses on a woman who contracted Triple E in Alabama in 2019. Tell us about this case, and did she survive?

[Holly Hughes] Sadly she did not survive. Triple E is one of the deadliest mosquito-borne diseases in the United States, and it's known that over a third of all people who develop encephalitis will die from the infection. And even for those who are able to recover, many are left with long-term physical and mental impairment. This case was...in particular, this woman had a weakened immune system because of another underlying illness which likely led to her getting very sick and dying from the infection of Triple E virus.

[Sarah Gregory] Why did you do this study? What were you looking for?

[Holly Hughes] So we undertook this study because we wanted to learn more about the Triple E virus infections in humans, and particularly we're looking at the viral genome. This case was very important because this woman was immunocompromised and so the virus was able to replicate more freely. Since her immune system wasn't able to suppress the virus's growth, in her samples we were able to get the full genome from her clinical samples. And as researchers, when we look at the genetics of viruses and other organisms, one of the tools we use to study these sequences is known as GenBank. And it's a public database or library full of genetic

sequences, and researchers use the library for things such as developing diagnostic tests and maybe even vaccines, for example. So this was a very important opportunity to add to that library and expand our understanding of this mosquito-borne disease in a human.

[Sarah Gregory] You took samples from the patient's serum and cerebrospinal fluid. What are these substances, and why did you choose them?

[Holly Hughes] So serum is just a part of your blood, and cerebrospinal fluid—also called CSF sometimes—and that's just the fluid that surrounds your brain and spinal cord. Both of these sample types are the best places to find traces of the virus. So when we need to perform a diagnostic test to determine if a person was infected with Triple E or other arboviruses, we typically use these specimen types.

[Sarah Gregory] Do you usually use both of them or sometimes one or the other?

[Holly Hughes] We can use both. We do prefer cerebrospinal fluid as a more confirmatory sample type to know if the virus itself was in the brain, but we can also use serum for similar tests.

[Sarah Gregory] Okay. And the cerebrospinal fluid, that's what's known as a spinal tap, right? You take the fluid directly from the spinal cord?

[Holly Hughes] Yes. That's correct. The CSF (or cerebrospinal fluid) is taken from a spinal tap.

[Sarah Gregory] So how do you detect such tiny amounts of viral DNA from these samples, especially when it's mixed in with human cells and proteins?

[Holly Hughes] You are correct. It's really hard to find viral DNA or viral RNA from a human sample. So in this case, we were looking for viral RNA, and it's really hard to find it in people after they've been infected because normally people quickly develop an immune response and it clears the virus. But this particular patient, like we said, was immunocompromised, so her body wasn't able to suppress the virus. And there was an unusually large amount of virus in her clinical samples which gave us a unique opportunity to further our understanding of the Triple E genome in humans. So in particular, to find the virus RNA or DNA we'll use a technique called next-generation sequencing. And this technique will generate millions and millions of sequencing reads from the sample, and that gives us a better chance to find the very small amounts of the virus RNA in any sample.

[Sarah Gregory] So DNA (and I guess RNA) is usually found in bits and pieces rather than a single long strand, and each fragment can have thousands of nucleotides, which spell out the genetic code. How do you piece these fragments into a whole genome?

[Holly Hughes] So we were able to use these very complex computer programs, and these programs look for places where the sequences of the RNA or the DNA—where they overlap on two or more sequencing reads. And the computer programs put those pieces together and build on them, kind of like a puzzle, to make the sequence longer and longer until you're able to find the whole genome that you're looking for.

[Sarah Gregory] That is really fascinating.

When you finished mapping the whole genome, you compared it to viral DNA sequences from other Triple E patients (or I guess RNA). What were you looking for particularly?

[Holly Hughes] So when we compared the sequences that we found in this patient in Alabama, we were looking at sequences that have been known to be from mosquitoes, horses, birds, and other humans from many years in the past. So in 2019, there was a larger outbreak of Triple E than we've seen before. So we were comparing these sequences from this case in 2019 to genetic sequences from the past years to make sure the virus itself hadn't gone through any genetic changes that would make it easier to infect a person. And we're also trying to understand more about where the virus in this area of the country may have come from, and also what the virus itself looks like inside a human.

[Sarah Gregory] So you mentioned genetic differences. What do these genetic differences tell us about viral evolution?

[Holly Hughes] So in this case, overall, the virus has stayed very consistent over the years. And we found that the outbreak in 2019 was not because the virus had become more pathogenic, but it was more likely due to other ecological or environmental influences such as birds, mosquitoes, or human behavior. We were however able to find out more about how the virus might behave inside a human, because we saw differences in the genome sequences of the viruses that were able to infect the patient's brain compared to what was able to be in the patient's blood.

[Sarah Gregory] Your article talks about intrahost virus variants and consensus-level majority variation. What do these terms mean?

[Holly Hughes] Yes, great question. So often times when people think of a virus infection, they think of the virus as one single thing inside someone's body. But really the virus multiplies many, many times, so you have a whole population of viruses in a host or in the human or animal that has been infected. So each population of viruses can have these little tiny differences, but the whole population likely functions as a cooperative group to infect that person or that animal. So when we talk about intrahost virus variants, we're referring to the small changes in the different viruses that are a part of the larger virus population within a single person. When in contrast we say the consensus-level majority, we're looking at all of the viruses together (not on the individual level) to see which of the genetic sequences are most common or what's most dominant. So for this study, we were looking at intrahost virus variation between the virus population in the serum or the blood compared to what was seen in the cerebrospinal fluid.

[Sarah Gregory] And how do they relate to this case of Triple E?

[Holly Hughes] So in this case, identifying intrahost virus variants was one of the key findings of our study. We were able to identify the variants or the changes within the population of Triple E viruses in one human for the first time. It gave us the unique opportunity to compare these small variants and small changes within the population of viruses found in the serum and compare it to the population of viruses in the CSF (or the cerebrospinal fluid).

[Sarah Gregory] So is there anything more you want to add to what you found out about your case study?

[Holly Hughes] Yes. So in addition to the intrahost variants, we were also able to find that the genetic sequences of the virus from the patient in 2019 were very similar from previous years. And also this person infected in Alabama, the genome was very similar to strains that have been identified from northern Florida.

[Sarah Gregory] Did the serum and the cerebrospinal fluid samples produce the same results?

[Holly Hughes] No. They actually did not produce the same results, and it was one of our key findings and very unexpected. So what we did find was that the virus population in the blood was different from what we found of the virus population in the cerebrospinal fluid. So each sample had different small variant populations and then also a different majority consensus population. So we believe that this suggests that the virus in the blood is a more variable population, and this population variability allows it to infect different areas of the body. But the virus in the cerebrospinal fluid was more limited in its variation, and we believe this limited variation in the cerebrospinal fluid is likely linked to its ability to pass through the blood–brain barrier. And this feature creates what we think is a genetic bottleneck and limits the genetic variation.

[Sarah Gregory] Okay. So what is a genetic bottleneck and what causes this intrahost variation from it?

[Holly Hughes] So a genetic bottleneck is an event that eliminates some of your genetic pool. For example, if we have a population, say, of 10 viruses and there is a situation that causes that population to shrink to two, the genetic variation in your population is inherently reduced. So all subsequent generations or progeny of this virus will carry that surviving population's genetics. So in this case we believe the bottleneck is the blood–brain barrier, which is a very specialized portion of your body meant to prevent outside organisms from entering into the brain. So only the viruses with the unique sequence variation were able to pass through that barrier, and their genetics were then passed on during replication within the brain.

[Sarah Gregory] How did this variation of Triple E get from northern Florida to Alabama? Do we know this?

[Holly Hughes] So that is a great question, and it's believed that in more northern states where the climate is colder in the wintertime, that Triple E is always found in the summer and spring, while warmer climates (such as Florida) there's more year-round circulation and transmission of the virus. So it's believed that likely migrating birds from Florida who winter in Florida carry the virus to more northern states. And that's where the birds will get bit by mosquitoes and become infected after feeding on the birds, and then transmit the virus in the local areas in the northern states.

[Sarah Gregory] Why are these results important for public health?

[Holly Hughes] So first we know that immunocompromised individuals are at a greater risk for mosquito-borne diseases. But this case in particular highlights how quickly and to what high levels the virus can multiply and replicate in a person whose immune system isn't functioning properly. Second, understanding the genetic sequences and where they differ within a human could allow scientists and researchers to target these parts of the genome to develop medicines or vaccines against Triple E. Thirdly, sequencing the virus has really helped us understand the transmission of the virus and how it goes from one state to the next in overwintering. And it also helped us confirm that the 2019 outbreak was not due to any changes in the virus's genetic makeup.

[Sarah Gregory] So after the patient was bitten by the mosquito, how long was it before you actually were able to see an increase in her...virus in her blood?

[Holly Hughes] So we really can't say for when we saw this increase based upon when she was bit by the mosquito, but we do know when we got samples post her illness onset (so days post-onset). And for this case, we know that about at seven days she started feeling ill but was found

unresponsive at home at that time. And so we received samples on day 24 of her illness that we were able to test. And at that point (24 days) we were still able to find virus in her blood and in her cerebrospinal fluid in very high levels, which is extremely unusual for eastern equine encephalitis virus. Usually we're lucky to find it at only three days or less post-symptom onset. But likely since she was immune compromised, we were able to detect it for longer periods of time at higher levels.

[Sarah Gregory] You mentioned that it went quickly. So how quick was it from when this person (this woman) was bitten to when she died?

[Holly Hughes] We can't say from when she was bitten by the mosquito, but we do know that after about 20 days post-onset she became ill, and that's when she was put into hospital around that time. And we received samples even later than that—about 42 days post-onset of her symptoms—and she still had a lot of virus in her blood. So she had a lot of virus in her blood for a long time.

[Sarah Gregory] How can people protect themselves from this incredibly scary virus, and their horses of course, from Triple E?

[Holly Hughes] So the best way to protect people from mosquito-borne diseases like Triple E is to use insect repellent, wear long sleeves and long pants when you're outdoors to prevent the bites of the mosquitoes. You can also help reduce the number of mosquitoes in and around your house by emptying standing water around your house where mosquitoes can lay eggs. Also using things like air conditioners or just screens on windows and doors prevent mosquitoes from getting inside the house and it'll keep them outside.

For horse owners living in areas where Triple E can be found should really speak with their veterinarian about vaccinating their horses against Triple E. However, there are no approved vaccines for use in people.

[Sarah Gregory] So a vaccine that works for a horse wouldn't really be the same vaccine that would work for a person?

[Holly Hughes] For Triple E, that's correct. There are not any vaccines that are used for humans yet. There are some under development and under research, but nothing approved.

[Sarah Gregory] So every time you go outside in your backyard, you should put on socks, long pants, and a long shirt. I know this, and I go outside, and I forget to do it and I try to keep my yard pretty water-free, and I still get bit and they follow me inside. It's very distressing.

[Holly Hughes] Yes. Sometimes, best efforts, I still get bit myself.

[Sarah Gregory] And I hate to use pesticides on myself, but I know I should.

Tell us about your job at CDC, how you came to be here—or I guess in this case I'm in Atlanta and you're in Fort Collins, so, there—and what you like most about it.

[Holly Hughes] So in my current job I focus mostly on next-generation sequencing of viruses that are held in our reference lab. So by sequencing these viruses, I center my work around developing diagnostic tests for orphaned or rare viruses that don't currently have existing, commercially available tests. And I also use this technology to ensure that existing diagnostic tests will work because over time, the virus's genetics can slightly change just because of normal

drift. And we can just make sure our tests remain sensitive enough to keep identifying infections over the years.

I got my start at CDC actually while I was studying microbiology as an undergraduate at Colorado State University, and I took a work-study position in the cell culture lab and that's where I got my first taste of public health and I've never gone back. I just...I love my job. And especially here, what I like most is the opportunity to work with these orphaned and rare diseases like Triple E. They're so rare and uncommon that there are not commercially developed tests and there's not much incentive to put lots of research effort into them. So that's where CDC can help fill that gap and make sure that we can diagnose these rare diseases just as well as the more common ones.

[Sarah Gregory] You really do have a fascinating job.

[Holly Hughes] I do love it.

[Sarah Gregory] So as a lab expert, I imagine you had to keep going into the lab while the rest of us stayed home. Was that just very strange?

[Holly Hughes] Well, I actually was not able to come in as often as normal. But some staff who work on clinical diagnostics on a routine basis were able to come in to continue to perform the essential diagnostic needs. So I'm able to come in sometimes, but not much. But since I wasn't going into the lab to do much routine work, I actually did a couple deployments to assist in CDC's COVID-19 response. And I was able to serve as a liaison at the Laboratory Task Force, and I helped field teams who were out responding to outbreaks or doing special field investigations make sure they had what they needed to perform the clinical diagnostics for COVID-19. And it was an amazing experience and I had a chance to work with some amazing talented and dedicated people that I normally wouldn't get the chance to in my routine day-to-day work. So I really enjoyed that time.

[Sarah Gregory] Oh, yes. Well good for you, it does sound fascinating. Did you work with Dr. Stephan Munroe? He just retired.

[Holly Hughes] No, not directly. I worked mostly under the Laboratory Task Force chain of command. So I worked with Brandi Limbago a lot and Wendi Kuhnert, they were the heads of the Task Force when I was working there.

[Sarah Gregory] I know Wendi.

Well, thank you for taking the time to talk with me today, Dr. Hughes.

[Holly Hughes] Oh, thank you so much for having me. This was absolutely my pleasure.

[Sarah Gregory] And thanks for joining me out there. You can read the July 2021 article, Fatal Human Infection with Evidence of Intra-host Variation of Eastern Equine Encephalitis Virus, Alabama, USA, 2019, online at [cdc.gov/eid](https://www.cdc.gov/eid).

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

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