

# Enteroviruses and Acute Flaccid Myelitis

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[Sarah Gregory] Hi, I'm Sarah Gregory, and today I'm on the phone with Dr. Erin Graf, Director of the Infectious Disease Diagnostics Lab at Children's Hospital of Philadelphia. We'll be discussing the potential link between a mysterious condition called acute flaccid myelitis and a virus suspected to be its cause.

Welcome, Dr. Graf.

[Erin Graf] Thank you, thanks so much for having me.

[Sarah Gregory] So there's a lot of fear building in parents about acute flaccid myelitis, AFM. Would you explain what this condition is and why it's making headlines?

[Erin Graf] Sure. So, acute flaccid myelitis, currently the way we define it is the acute onset of limb weakness. So, it could be any limb—upper, lower, multilimb—weakness. And it...and it really is a flaccid weakness where the motor function of the limb is absent. And then we pair that with imaging findings. So, a child presents to the hospital and they receive, like, an MRI, and then we know that there are lesions in the spinal cord, and in particular, these lesions appear in the gray matter of the spinal cord. And so, those two features together—the limb weakness paired with the imaging findings—are kind of what we consider our case definition of acute flaccid myelitis.

But in sort of real life or in the patient setting, what it is is children that were previously healthy, reaching all of their age-appropriate milestones, then develop this acute onset of paralysis. And so it's, you know, incredibly concerning as a parent where your child one day is walking or moving their arm normally and then the next day they've lost function. So, it's, you know, appropriately created a lot of concern for the...for parents in the community.

[Sarah Gregory] I was looking at a polio map the other day and I noticed there was a search option on the map for non-polio acute flaccid paralysis. Is this the same thing? It appears to be it all over the world.

[Erin Graf] Yeah. So, you know, we think of...acute flaccid myelitis is sort of a more focused definition of acute flaccid paralysis, but we think of acute flaccid paralysis being associated with poliovirus. And so, while that's been eradicated in most of the world—I think there's currently three countries that have ongoing transmission—certainly eradicated in the United States, thanks to a really great vaccine, there are now emerging non-polio enteroviruses, so you can think of them like a cousin of poliovirus, that can cause a very similar syndrome. So, for example, enterovirus A71 worldwide is a well-accepted cause of acute flaccid myelitis. And then this emerging enterovirus, called enterovirus D68, that within the last decade has become associated with the potential to cause AFM.

[Sarah Gregory] What do we know about the cause of AFM?

[Erin Graf] So, we don't know the cause of AFM in all cases. So, as I mentioned, you know, poliovirus is a well-known cause of AFM, and in certain parts of the world, it still does cause AFM. But in the United States, with the recent outbreaks that have occurred, in 2012, 2014, 2016, and 2018, we don't exactly know the cause yet. So that a lot of these cases have had

testing that was positive for enterovirus D68, a lot of them have had testing that was positive for an enterovirus that we were unable to type, maybe a smaller subset that has been positive for enterovirus A71, and then there's another chunk where the testing didn't reveal anything. And we've done...I should say *researchers* have done pretty extensive testing in a lot of these patients, and still in a subset of them, unable to define an etiology for the AFM.

[Sarah Gregory] So, as you were kind of just talking about the link between this virus, EV-D68, and AFM has been controversial. But I read a study a few weeks ago saying they are now pretty sure it *is* an enterovirus? What's your feeling about this?

[Erin Graf] Yeah, so I mentioned there's been a lot of work kind of trying to do enhanced diagnostics in some of these samples from children with AFM. And there were actually two studies that came out in 2019 applying new considerate...extrasensitive serologic techniques, so looking for antibodies to many different viruses in the spinal fluid and the serum from cases of AFM.

So, in one study they looked at sequences from all viruses to look for antibodies, and found that the only signal that was significant between cases of AFM compared to controls, was for enterovirus. And so, it really did suggest that enterovirus is the common etiology between all of these cases. Now, they didn't detect this in every patient, but at least in the majority of patients, they had antibodies for enterovirus in their spinal fluid. And then there was a similar study that showed essentially the same result. So, the data definitely is emerging to really strongly link enteroviruses and, in particular, enterovirus D68, with AFM.

[Sarah Gregory] Go ahead and tell us about your study, then. It focused on the Philadelphia area, right? And what were you looking for specifically?

[Erin Graf] Yeah, so, you know, I was really struck when I moved there...I had started my job around 2015, and so was around when the 2016 AFM outbreak happened. And I was really struck by just how devastating this illness is in these children who, as I mentioned previously, you know, developmentally normal, reaching all of their milestones, and some of them now are wheelchair bound and, in some cases, need respiratory support, potentially for the rest of their lives. So, you know, I really wanted to understand more, being a clinical microbiologist, understand more about the pathogen side of things.

So, we were fortunate enough to have archived specimens going back to 2009, to look and see, you know, is enterovirus D68 something that newly emerged or is that something that we have had present in our local population for some period of time. And then to go a little bit further and ask, you know, if it's present, what can we learn about the genetics of the virus. How has the virus changed or evolved over time and is there something different about the virus in these cases of AFM compared to other children who get the virus and have a perfectly normal course of infection? And so, we were able to go back to our samples from 2009 and study those through 2018, so a 10-year retrospective study, to look at the prevalence of D68. And so, we saw what some other groups had reported, which is peaks of circulation in 2012, 2014, 2016, and 2018, which nicely kind of paired with the peaks in cases of AFM that were reported nationally, and then even locally, in our own institution, as well. So, that data together kind of argued to us that D68 probably really is potentially causing AFM in these children.

[Sarah Gregory] You tested over fourteen hundred samples for the virus. Where did these samples come from?

[Erin Graf] Yeah. So, D68 is a little bit different of an enterovirus compared to its, as I described it, close cousins, like poliovirus, in that it's shed in the respiratory tract. So, most enteroviruses, like polio, are fecal-oral, so they're hardier viruses that are built for surviving your gastrointestinal tract. Enterovirus D68 is more similar to another kind of distantly related cousin, which is rhinovirus. They share a lot of genetic similarity. And so, D68 is shed through respiratory secretions. So, these were archived nasal-pharyngeal swab specimens from children who were tested on our respiratory virus panel as part of their clinical care.

[Sarah Gregory] Okay. So, when were those samples taken and how did you keep the older ones from going bad?

[Erin Graf] Um, that's a...yeah, that's a great question. So, they were taken all the way back to 2009. And I am fortunate...I was fortunate coming into that job, that the prior director, Rick Hodinka, did an amazing job of archiving pretty much every specimen that came into the laboratory—would take a split of that sample and freeze it at minus 80 almost as soon as it hit the door. And his reasons for doing that were exactly this: You know, sometimes you don't know what the next pandemic is going to be or sometimes you don't know what the next important infection is going to be, and so having the ability to go back to historical samples and kind of ask was the virus there, way back then, before we even knew it was important, is really something that...you know, it would be great if all labs across the country had the resources and the space to do that. We were just lucky at CHOP that we did.

And so, to your question about how did we keep them from going bad. You know, we don't know that...that the RNA in these viruses didn't degrade over time. You know, it's possible that the samples from 2009, we could have had a higher positivity rate, for example, that maybe some of them were weakly positive and just...it degraded after being stored at minus 80 for ten years. But we still did detect a good amount of positivity in 2009, so we don't really think that's the case.

[Sarah Gregory] You also retrospectively diagnosed cases of AFM from 2009 to 2014. How did you do that? And, since you can't go back in time to when the patients originally came in, what was done?

[Erin Graf] Yeah. So, we...we have a great partnership with neurology, as well as infectious diseases, in our institution, and so, the neurologists are the ones that did that work. So, they went back and reviewed charts from children that met certain criteria. I don't exactly recall the clinical criteria that they used, because AFM wasn't a case definition back in 2009. So they must have had some search terms that they used, like perhaps "acute flaccid paralysis" or "transverse myelitis" or "Guillain-Barre"—other syndromes that kind of have overlap with AFM. And so they went back and reviewed the imaging results and the clinical characteristics of the patients all the way back to 2009. And they actually went a little bit further back than that, as well. And so then we could link that data together with our...our...virology data to say, okay if there was a case back in 2009, did we have a sample still available from that case, and if so, you know, did it turn out to be D68? And I think we had discovered at least one case linking AFM to D68 back in 2009.

[Sarah Gregory] Your investigation found that outbreaks occurred at two-year cycles. Why would that be? And do the outbreaks occur at the same time in each country?

[Erin Graf] Wow! Those are all excellent questions that we still don't have the answers to that, you know, researchers in this field really want to understand better, is why are we seeing this biennial pattern, at least in the United States? And so, Colorado has reported similar data; Ohio has reported similar data; Kansas City has reported similar data. So, nobody seems to understand why it's this striking every-other-year cycle since 2012. And so, now with it 2020 coming, you know, we would predict that we might see it again this year. And it does kind of have a sharp peak around the same time every year. So, enterovirus season we always think of as kind of when kids start going back to school—so, end of summer, early fall. So, we usually see the highest peak of circulation in September. And that's what all of these studies have shown.

Across the globe it's a little different. So, I think Japan had reported outbreaks in *odd* years, so 2013, 2015. And there are pockets of circulation in Europe that are kind of also different than the years that we have reported. So, you know, we don't totally understand why the virus seems to go away and come back, although we know that's a common feature with most enteroviruses. It's...logical to assume that it has something to do with immunity, that the population mounts an immunity to the virus, and that slows the circulation, and then the virus mutates, and a more susceptible population is born. And so, you know, we do seem to have association of AFM with younger children and so, that would suggest that they just don't have the immunity to the virus yet.

[Sarah Gregory] Why aren't adults getting this?

[Erin Graf] Again, that's a tough question to answer, because the, you know, we think logically oh, it probably has to do with the fact that they have immunity to the virus. However, there's some confusing data around that. So, I think the first thing I'll say, in talk with colleagues who—at least neurologists in the adult setting—is that maybe the case definition for them is not as clear-cut as it is in children. So, maybe individuals are getting a diagnosis of something like Guillian-Barre. Maybe they truly do have AFM, but it just isn't...is not something that is really thought about, or looked for, or considered, because the focus is so much on pediatrics.

So, it just begs the question: Do more adults maybe have this or have a more milder form of it, than we're realizing? There are adult cases reported, so I think in the CDC's, at least their 2018 AFM data, I believe that the oldest person reported in that data set was in their 20s or 30s. So, at least younger adults—I'll consider someone in their 30s a younger adult—are at least maybe susceptible to it. So, it's not...it's not as though they don't develop it, but for whatever reason, it seems to be more common in children.

So then, again, you would think that if viruses truly are the cause, that it relates to immunity. And so there was a study in Kansas City where they took serum from adults as well as some older children, and they found neutralizing antibodies to the strain of enterovirus D68 two years even before that virus came to circulate in the population. So, meaning that, before that virus emerged in 2014, people already had immunity to it back in 2012, 2013. And so, you know, that kind of strengthens the idea that it's the younger children that don't yet have immunity that are more susceptible to these viral infections and therefore developing AFM, but we still don't know.

[Sarah Gregory] Your study refers to something called “neurotropism.” Explain what that means.

[Erin Graf] Not all enteroviruses can infect all types of cells. So, most viruses have a few or maybe many cell types that they can bind and then enter and then replicate inside of. And so, for

enterovirus D68, there's lots of data emerging to say that it is neurotropic, that it can infect neurons. We don't...we still don't know the exact mechanisms of how it gets into the brain. If it...does it through transport from kind of distal sites and then traffics retrograde along the neuron into the brain tissue or if it maybe crosses the blood-brain barrier. Those are questions that still need to be answered, but we at least know that it can infect the cell types that are present in the brain.

[Sarah Gregory] You used whole genome sequencing to track the virus's ability to invade the brain, as you just said. So does this mean viruses have DNA, even though they are technically not considered living things?

[Erin Graf] So, all viruses have nucleic acid, whether that's RNA or DNA and, you know, we can debate whether or not they're truly living things. Viruses are really good at highjacking their host cells' machineries to make copies of themselves. I mean that's really their end goal is to make a lot of copies of themselves and then spread on to the next thing. And so, they can do that as an RNA virus, in this case, which is what enterovirus D68 is, you know, it can do that, it can highjack the bulk machineries to make more copies of itself and to make proteins and make more virions.

So, our study was interested in sort of looking at the mutations that other folks had identified that may or may not be relevant for this "neurotropism." And we did not find the same common mutations across all of the viruses that we studied, particularly not the ones in 2016 that were associated with our cases of AFM. So, we really felt like those...some of those early mutations that folks had pointed out, that may or may not be related to neurotropism, we really felt like that wasn't the case in our study.

And there is a paper that actually just came out, which is the first paper to show that some of the earlier strains that were first identified of enterovirus D68, so these were the strains that were identified in the 1960s, way back when, when we thought this was just, you know, a respiratory virus that on occasion caused pneumonia. But those early viruses possessed the ability to infect neurons in a couple of different test systems. So, really the data now's suggesting that it probably isn't specific changes that happened over time to allow the virus to be neurotropic, it probably has the ability to be neurotropic, it's just more the question of how does it get into the brain and, you know, how is it circulating in a population—I think those are the areas that need further pursuit.

[Sarah Gregory] How can you use the virus's genome to track these changes?

[Erin Graf] Our initial question with the genome sequences, at least that we generated, was to look at the virus in the cases of AFM compared to all the cases that had normal responses or normal outcomes. And just ask the question of is there anything different about those cases, or the virus in those cases, from the normal presentation? And the short answer is there was nothing we could see, there were no specific mutations that we could identify that suggested there was anything different about the virus. So that then kind of suggested that there might be something different about the host.

But you can look at things like either the evolution over time of the virus in total or the evolution of specific proteins. So, you know, this virus tends to mutate rapidly in capsid genes, which are the genes that are expressed on the surface of the virus and therefore the ones that the immune response is targeted against. So, you'd imagine that those would kind of mutate more rapidly.

And then you can look at other changes in the virus. So, there's a five-prime untranslated region that's really important in...as I mentioned, with the virus making copies of itself. And so, if that mutates and drives now the virus becoming potentially more infectious, then maybe you just get higher titers of virus, higher levels of virus, in an individual. But those things could sort of drive higher levels of virus, more likely to get into the brain, and then, potentially more likely to get AFM. So, none of those sort of changes fell out as being obvious, you know, we didn't see anything more of as a slam dunk, "okay, this is, you know, clearly what's changed in the virus over time to drive AFM." So, I think it really does kind of point to maybe more of a host-specific issue.

[Sarah Gregory] Are these results pretty specific to Philadelphia or are they probably similar to AFM studies across the rest of the world?

[Erin Graf] I would say they're similar to studies across the rest of the world. Other groups have characterized some of the different strains, at least the ones that were circulating in the community during the different outbreaks, and showed that the B1 clade and then the B3 clade more recently have been what have been predominantly in circulation. And then, in terms of association with AFM, I think maybe the...one of the early papers on the 2014 outbreak showed that, in their handbook cases of AFM, it was the B1 clade associated. So, I think it would be similar to what's been described in the US, certainly, similar papers have been published in parts of Asia, as well, showing the B1 and B3 clades having a role.

[Sarah Gregory] You mentioned briefly, earlier that, possibly, children could be on respiratory support for the rest of their lives. So, is this something like it used to be with polio, that once you got it, you don't recover? Or are children recovering or partially recovering?

[Erin Graf] Yeah, that's...that's such a great question. So, we're not far enough out yet to really know how it compares to polio. So, the first thing I'll say is that children with D68-associated AFM seem to not recover as well as children with other enteroviruses or other viruses that are associated with AFM, outside of polio. So, the group in Colorado, at the children's hospital there, has published a study where they had a cohort of children with enterovirus A71-associated AFM, and those children seemed to do better. They seemed to regain limb function better, get closer back to baseline than children with D68. And other viral causes of AFM, like West Nile virus, that, you know, we won't talk about, people with AFM associated with those viruses tend to do better. So, there's something about D68 that maybe is slightly more similar to polio. Some children have done okay. Again, there has been some published work where children have been fortunate enough to regain limb function, but there's a large number of children who haven't.

And so, one of the sort of experimental approaches is nerve transfer surgery. So there have been children who have had a nerve transferred who then regain some limb function in their arm or their legs, with some success, although some children are not candidates for that. But I think, you know, the concerning thing is we don't know the long-term progression because it's so new. You know, years from now...with polio you can have kind of like a polio relapse, it's called a post-polio syndrome. We don't know if we'll see a similar phenomenon with children with D68, where years later the virus can kind of come back again.

[Sarah Gregory] So, through your study and all this information you've put together, will this help eventually to treat AFM, to create a vaccine, something hopeful?

[Erin Graf] Yeah, I mean as a...I'm the mother of three young children, and so this virus definitely keeps me up at night and concerns me because it...you know, we know it's probably coming again this fall and there's nothing we can really do to prevent it. You know, we say hand washing and staying away from sick contacts, but I mean it's...these viruses are everywhere, they get shed everywhere, so your likelihood of getting it is so high.

You know, our hope is that the more data that we accumulate as a community, the more we can really show a definitive link between this virus and AFM, because it is still controversial. And that then can potentially help drive, as you said, development of vaccines or development of targeted antivirals, because we currently don't have any specific treatment for this condition or for this virus.

[Sarah Gregory] In light of the recent media coverage, is there anything else you'd like to add that I haven't asked?

[Erin Graf] I think, you know, just increasing awareness of this and increasing partnership with public health to submit samples so that, you know, the earlier these kids can get diagnosed with AFM, then the earlier that it can be coordinated to collect the right sample types. So, you know, getting a respiratory sample early in the presentation is gonna help us be more likely to detect the etiology, to detect the virus—if it is D68, if it is, you know, another virus. Getting those samples early on is the key to better understanding the causal relationship.

[Sarah Gregory] So, I guess, because almost everybody is vaccinated from polio nowadays, there's no sense in thinking that maybe the polio vaccine would help prevent this?

[Erin Graf] I got the same question in a journal club I presented around this topic and someone asked me “Well, does the polio vaccine work?” And, you know, the answer is...is “no,” because everybody gets it...

[Sarah Gregory] Right.

[Erin Graf] ...and we don't see that these cases of D68 disproportionately happen in children who haven't received vaccines. So, it's clearly not cross-protective, and again, back to that study I mentioned where there were neutralizing antibodies in the population, but yet people still got AFM. So, I think it's...you know, we don't know yet what immunity we need to this virus.

[Sarah Gregory] I think you touched a little bit on this, but tell me how you became interested in this subject.

[Erin Graf] Yeah, so I mentioned I'm a mom of three young children, and I think when I...when I started my...you know, the job at Children's Hospital of Philadelphia, I had one young child at the time. And so, as I said, in 2016, when the outbreak occurred, you know, I was just really struck by how devastating this illness was for these children and these families. And thinking of my own child, who, you know, was reaching all of her developmental milestones—learning to walk, you know, using her hands and arms—you know, thinking about what it would be like for my husband and I to have to kind of sit there by her bedside and watch her kind of go through this process. You know, a lot of these children needed to be mechanically ventilated while they...while they had AFM. And while many of them were then weaned from the ventilation, I think just, you know, having experienced that as a parent and then kind of looking at your child and knowing that the trajectory of their life is forever changed, if they're wheelchair-bound or lost the use of the hand that they would write with, for example. So, I think that, just that picture of what that is like for those families and for those children is what motivated me to want to

learn more about this, and do as much as we could do with the resources that we had and the samples that we had to investigate.

[Sarah Gregory] Tell us about your job. What do you do besides investigate this?

[Erin Graf] I love my job—it's the best job ever! I get to kind of bridge the sides of the clinical practice with the research and the laboratory piece of it. So day-to-day, you know, I'm sort of involved in the real-time operations of the laboratory—interacting with clinicians on complex cases or challenging results or, you know, sort of finding the right tests for their patient or for their clinical question. And then, you know, on the other side of it, in the lab, we have this wealth of clinical specimens that come through our doors every single day, and so, you know, that patient...that baby's spinal fluid, that baby's blood sample, that child's respiratory sample, you know, those are all precious samples to us that we can then use for the greater good, to do these kind of research projects, to answer these kind of questions.

[Sarah Gregory] Thank you so much for talking with me today, Dr. Graf.

[Erin Graf] Well, thank you so much. I appreciate it.

[Sarah Gregory] And thank you for joining us out there. You can read the full September 2019 article, “Association of Enterovirus D68 with Acute Flaccid Myelitis, Philadelphia, Pennsylvania, USA, 2009–2018,” online at [cdc.gov/eid](http://cdc.gov/eid).

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

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