Are enteroviruses coming back in 2021 after COVID-19 is tapering off?

You can listen to the audio of this video at: https://youtu.be/-rWmw0mGpxQ

Dr. Carlos Pardo: [00:00:00] Good afternoon, everybody. This is the March meeting of the Acute Flaccid Myelitis Working Group. Thank you so much everybody around United States and other countries that have joined the meeting. The Acute Flaccid Myelitis Working Group is a group of clinician scientists, researchers, and healthcare providers and parents that are focused on understanding acute flaccid myelitis and particularly the, the improvement of acute flaccid myelitis, and particularly the, the care and improvement of acute flaccid myelitis and the care and improvement of healthcare for patients affected by this disorder. Today we have virtual forum that is going to be focused on a recent paper that was published in Science Translational Medicine about enterovirus circulation.

[00:01:05] But before we go on the, the forum, I'd like to make some announcements. The first announcement is that Acute Flaccid Myelitis Working Group meets every month. The third, the third week of the month. The next meeting is going to be on April 22. I encourage people to join the virtual meeting because it is going to be focused on what is going on with acute flaccid myelitis around the world. And we are going to have a participation of researchers and scientists from around the world who have experience with acute flaccid myelitis. And we'd like to learn from them what is going on with acute flaccid myelitis in other parts of the world different to United States. The second announcement is Acute Flaccid Myelitis Natural History Study is going. It was launched by NIH and is based at the University of Alabama in Birmingham.

[00:02:00] So, this comprises more than 30 medical centers that have been recruiting patients with acute flaccid myelitis or mimickers for a study of this disorder. So, we encourage all people around the United States and clinicians that have patients or suspected patients to contact us in case that there is interest in referring patients for the study. We are very interested and, and be alert and ready to deal with a potential outbreak of acute flaccid myelitis in 2021 and 2022. So again, the Acute Flaccid Myelitis Natural History Study that is based at UAB, Dr. David Kimberlin is the leader of this study. I mean, all of the groups are basically in a high alertness to start recruiting and evaluating patients with this disorder. With that brief introduction, I'd like to pass the microphone to my colleague, Dr. Kevin Messacar at the University of Colorado, who is going to be the moderator of this symposium. Kevin.

Dr. Kevin Messacar: [00:03:14] Thank you so much, Carlos, and thanks for putting together this session and hopefully it will be an interesting and informational one. Before we get started, I'm super excited to get to introduce Robin Roberts to make a very exciting announcement I think for our Working Group and for AFM in general. Robin and I met I think back in 2014. It's been several years now. And she is one of the most amazing parent advocates for AFM. And the work she has done along with the AFM Association has just been incredible, and I will let her tell you a little bit of her story and make the exciting announcement before we get to the panel. You can go ahead, Robin.

[00:03:59] Yeah.

Robin Roberts: [00:04:00] Thank you Dr. Messacar, and thank you to the Work Group for allowing me to present a little bit for a few moments this afternoon. My son Carter was diagnosed with AFM very suddenly back in 2016, at the age of three and a half and literally overnight was one of the patients that was severely affected, was vent-dependent within a matter of hours. And other than ultimately trying all diagnostics that were ideation probably at the time, really, was only able to regain control of a few toes. And so, unfortunately he passed in August, oh excuse me, September of 2018. And through the Work Group, through the AFM community, always looking for ways to advocate, fundraise, and make a difference. But, I also work in healthcare professionally. And so, one of the things I know ... sorry, one of the things I know is that insurance companies need coding to identify patients, align coverage, and understand what's going on with the patient. And this is one of the few ways we can tell an insurance company, even researchers that look for data on patients, how to find someone.

[00:05:12] So in order to stop labeling pa-, labeling patients with a generic diagnosis or not a specific diagnosis that is befitting to what we are able to now clinically define and identify as acute flaccid myelitis. The year before last, I tried to get a specific code that can go on every claim, whether you're, you're inpatient during the acute phase, you're dealing with a secondary problem maybe with a pulmonologist or a therapist, or even years later when you're still trying to make progress. And so, this year or just last year, in the second year of trying, with the support of the CDC, Dr. Janell Routh, Dr. Kevin Messacar, who's on here, who I've probably tweeted and messaged to way too many times over the past five years, I said I can't do this alone. They said I don't ... I'm not, I'm not clinically relevant enough. And so, they came to my aid to help fight against some of the health information management in the CDC process to get a specific code.

[00:06:11] And collectively, with them and the support of the community, we are so happy to announce that beginning October 1st of this year, a doctor can have specificity in labeling a patient who is clinically diagnosed with AFM by a physician to nail a code that on a claim. And so code G04.82 can be used to stop the disparity in coding, define who these patients are, identify them, quantify them. And this will help guide research, therapies, allied medical coverage for devices and equipment, and ultimately help track and better manage and support this entire population. So, thank you to Dr. Kevin Messacar. Thank you again to the Work Group for your time. Just very excited that we're able to have this now.

Carlos Pardo: [00:07:01] Robin do you mind repeating the code for AFM is G?

Robin Roberts: [00:07:06] G0482. So, G04.82, officially becomes part of this whole encyclopedia of codes. It can be combined with other symptoms and other things going on with your patients as a primary diagnosis in patient and then at all subsequent visits as a secondary diagnosis for any problem related to AFM to help us track these patients.

Dr. Kevin Messacar: [00:07:33] Thank you so much, Robin. This was all due to your effort, but also a wonderful story of, of just how the Working Group works bringing together parents, clinicians, public health, and the CDC came to the backing of this. It just is a wonderful story of, of practical progress being made in this area. And like you said, I think it's going to help quantify cases that are clinically diagnosed that may not meet epidemiologic case criteria. It's going to help families with insurance issues and it's going to overall just help us defining this disease and understanding more about it. So, thank you again, for all of your efforts. And, and really can't say enough, how I'm just in awe of what you're, you've been able to do and, and it's such an honor and a tribute to Carter. So thanks.

Robin Roberts: [00:08:20] Thank you.

Dr. Kevin Messacar: [00:08:23] So, next I'd like to introduce Dr.... soon-to-be-Dr. Daniel Park, PhD student at Princeton under the mentorship of Bryan Grenfell, who works in mathematical modeling and epidemiology. And another congratulations to pass on, just published a wonderful modeling study in Science Translational Medicine getting at a little bit more granular level of the geographic and temporal circulation of enterovirus 68 in cases of AFM that have been seen throughout the country since 2014. So, we'll start with a short presentation from Daniel, and then we will open it up and I'll introduce our other panelists. And feel free to put questions in the chat as we go along, and we will pose them to Daniel and the rest of our panel afterwards. So Daniel, you can take it away.

Sang (Daniel) Woo Park: [00:09:18] Thank you very much for the introduction, Kevin, and thank you for setting this up and coming. I'm very glad to be able to share our work on epidemiological dynamics. So enterovirus D68 in the United States and implications for acute flaccid myelitis. Prior to 2020, we were seeing AFM cases every two years, and enterovirus D68 cases were also reported every two years, suggesting some correlation between D68 and AFM. But, there were still several remaining questions that needed to be answered. So, in this study, we wanted to, we wanted to ask whether we can explain geographical differences in the timing of enterovirus D68 outbreak that is why do some states get later or earlier outbreaks and can we relate the seasonalities of D68 outbreaks and AFM outbreaks across different states. And what, what, what is driving such biennial patterns? Why do we get outbreaks every two years and every year? And then will this pattern, biennial pattern continue? Is this, is this kind of state persist? And does that mean we can get an outbreak in 2020? Then we will ... we started working on these questions in September of 2019 and when 2020 came there was COVID and non-pharmaceutical interventions, social distancing, etc. and wanted to understand what those meant in terms of D68 outbreaks for 2020 and potentially for future.

[00:10:53] So this work is based on ... this work builds on work from our collaborator, Margarita Pons-Salort, and people at CDC, which looked at seasonality of other enterovirus outbreaks in the US, which demonstrated that southern states get earlier outbreaks than northern states which revealed some role of climate in shaping the transmission of enteroviruses. And also Margarita's work on enterovirus D68, and, and enterovirus endemics in Japan, where she was able to demonstrate that these complex outbreak dynamics in particular here is you see that Coxsackievirus A2 and A4 exhibit biennial patterns just like enterovirus D68. She showed that these patterns can be explained by SIR dynamics, by that, which we, we, we, what we mean is that people with lifelong serotype specific immunity can explain these biennial or even more complex patterns. So, in order to a- address these questions, we use data from BioFire which creates, a multi pathogen multiplex PCR, the test for multiple lots of raspberry patterns at once, and in particular, they're able to predict the presence of D68 from these panel data from the, the tested from rhinovirus, enterovirus.

[00:12:31] Previously, enterovirus surveillance in the US have relied on passive surveillance but, with this hospital-based surveillance, we're able to get a more granular details how D68 is circulating across different states at a finer timescale. So here, I want to show what the data looks like in panel A, we see data from US, and so the states that where a lot of data come from here as you, you'll notice that some of the states have been clumped together, for example, you find Colorado and Florida, Georgia and South Carolina, that's because of confidentiality requirements that we have to group these data together. But overall, as we see in the US, New York, and in some other places, we see clear patterns of outbreaks happening every two years. But in some places like Ohio, we see a more intricate pattern where it seems like outbreak is happening every four years.

[00:13:28] So, we, we, we first like to understand when these out- outbreaks happen which we try to apply in figure B, this is when essentially when outbreaks happen, but we can show that these, the timing of the

outbreak which refer to the center of gravity here and figure C and D are correlated with mean latitude and mean longitude. This, this is consistent with earlier work on other enteroviruses, highlighting that earlier states essentially get southern states get earlier outbreaks, northern states get later outbreak hinting at the role of climate in shaping the spread of enterovirus D68.

[00:14:07] Then we wanted to correlate these patterns with AFM dynamics. In figure A, we're showing the D68 outbreaks shown as bar charts and AFM number, AFM cases shown as red lines. We, as we see there's a very clear correlation between what the AFM outbreaks and D68 outbreaks look like although there are some differences that it seems like AFM outbreak has been increasing since 2012, 2014, where D68 outbreak has been rather stable. It is still unclear whether this represents actual increase in AFM cases or this reflects just better surveillance. We also see that it seems that AFM cases appeared to peak one month later than the D68 cases, which we demonstrated in figure B and C. You can also see in figure A, but this is likely driven by the aggregation of the data. So AFM, we're just looking at national data, whereas BioFire we're only looking at, we're looking at roughly 20 states. So it's not covering all the states and their differential, there's different levels of surveillance depending on the use of BioFire panels in different states.

[00:15:37] But nonetheless, in figure, D and E, as we show there's a, so D is a plot of correlation coefficients between AFM cases and D68 cases, yellow being higher correlation, which is across almost all states, there's a, see positive correlations. And in figure E is, again, we see that there's a noisy but positive correlation, black line is for national and red lines are for each state. So it seems like there, there's a very clear positive correlation between these number of D68 cases and AFM cases which other studies have noted previously, as well, we're here we're looking at upgradable to look at it this at a more granular scale across wide range of states.

[00:16:29] When we look at even more finer scale data here in New, New York and Utah and Colorado, we see that again, the timing of AFM cases match the timing, timing of AFM outbreaks match the timing of D68 outbreak. And we also don't see the lag that we mentioned earlier which again highlights that the lag is probably driven by geographical variation in BioFire deployment surveillance and some sort of aggregation issue not reflecting real biological lag. So that we wanted to know as what is driving the biennial patterns of D68 outbreaks? Why does D68 outbreak happen every two years? So, we adopted the methodology from Margarita. They were sure they use ... it was used to study other enterovirus anyway. So essentially, we can come up with a mathematical model that assumes lifelong immunity. Once people get infected, they completely recover and then they can get ... they cannot get reinfected. And the ... in such model we can show that can match the observed dynamics of D68 quite well.

[00:17:52] So, here the white, white panels hardware, we tried to match the model to the data. And orange is where we try to predict future dynamics. So, in s- in some cases, Missouri and Utah, Colorado, the fits and predictions look very good. You look on Ohio fits look up but predictions are a bit difficult just in prediction, promise short time series can be difficult. But when we try to include more data, we see that we do get a better prediction suggesting that the lifelong immunity from enterovirus D68 infection alone can indeed explain the dynamics, outbreak dynamics, the every biennial patterns of the D68 outbreaks. We want, we do want to stress that what matters here is, our research suggests like primary infection is likely important. So, it does not necessarily preclude the possibility of secondary infections, but as long as secondary infections don't lead to significant amount of transmission that it will be consistent with our model.

[00:19:12] And we further tested these simulations that we could also demonstrate that the, the outbreaks may not continue every two years, that biennial patterns may not necessarily be stable. So when, so when 2020 came, we now had to ask what is the impact of this pandemic on D68 dynamics? What is, what are non-pharmaceutical interventions, social distancing measures going to do to an, to the spread, circulation of D68. Early in 2020, we were seeing decline in enterovirus cases in other countries. Rapid declines. Here I'm

showing Hong Kong, South Korea, Taiwan. By around March, April, they were reporting basically essentially almost no enterovirus cases. In the US, unfortunately, we don't have data, particularly because the D68 prediction algorithm from BioFire relies on a certain, certain versions, but after which became no longer available after their product update. But we can still, since we can still use our mathematical model to try to predict whether an outbreak in 2020 would have been possible. And what if there's decreasing contact rate and therefore transmission rate due to distancing measures? And what, what would that mean in terms of future outbreaks?

[00:20:48] So here we evaluated the possibility of D68 outbreak in 2020 across a wide range of scenarios, first under normal conditions, and while there's a 5%, 10%, and 20% decrease in transmission. So here we see in the very top panel, panel B, we see a yellow strip around September indicating that a large outbreak in 2020 would have been possible. But, as we see, as we introduce, decrease, as we decrease the transmission rate, these strips disappear, suggesting that even a modest 20% decrease in transmission rate could have prevented the outbreak. So, this work, these predictions we initially made in April, around April and March. As we predicted, there were no AFM outbreaks in 2020, though we, we cannot, unfortunately, again, due to the product update, we cannot assess whether there were no D68 outbreaks. So there were certainly decreases in reportings of D68 and I think other people, Kevin, including Kevin, could share their insights from clinical, clinical insights.

[00:22:07] So what does this mean in terms ... what, what is going to, what is going to happen for future outbreaks? Would, would we get more D68 AFM outbreaks in '21, 2021, 2022? We do not have data for D68. But we want to allude to a study led, led by Rachel Baker in our group, which directly tries to answer this question for generally for endemic diseases, this paper where she focuses on RSV, but the general conclusions will still hold. So this figure I ... summarizes her findings very well. I want to point that figure C first, so where loose trips are showing when non-pharmaceutical interventions are put into place, and red lines are showing model predictions. So before the outbreak, RSV show, it was showing very clear annual epidemic patterns. But, when non-pharmaceutical interventions were put in place due to COVID, the cases of RSV declined rapidly. What this means is that we are, normally we would have an outbreak in 2020. But since we're missing the outbreak, we are not letting the population develop immunity that would otherwise have developed in the population. So, we're letting the susceptibility to increase as we see in the blue dashed line here, which is more susceptible. And as susceptibility and population increases, eventually, when we lift the intervention measures, we can get a larger outbreak in the future.

[00:23:55] But there is large uncertainty in when, how large this outbreak will be, which is shown in the top row of panel A and B. And it, there's also which is showing the, the peak epidemic size, as you can see, it could range from one to nine. So it's one being the same ratio and nine being nine-fold increase in endemic size. And there's also large uncertainty in when this, the next epidemic will happen, which is shown in the bottom row peak date, as you can see could happen anywhere between 2021 and 2025. And these depend on how long these interventions are put in place and how much, how strong these interventions are. But the key takeaway is that putting interventions on preve-, on preventing an outbreak is good for now, but it will cause the population of susceptibility to continue to increase because we're missing outbreaks and that could lead to greater outbreak in future.

[00:25:05] So in summary, our study was able to demonstrate strong spatial temporal correlation between D68 and AFM cases, providing ecological support for their causality. And we also predicted that a major D68 outbreak would have been possible in 2020 under normal conditions. But, decrease in contact rate due to current intervention measures likely prevented D68 outbreak and possibly AFM outbreak. But, this means that the population of susceptibility will start to build up and at some future, we could be getting a larger outbreak. So we all wanted to thank the collaborators and people at, especially people at CDC and the, the

New York and Colorado Department of Public, Public Health Departments that also shared their data. Thank you very much.

Dr. Kevin Messacar: [00:26:10] Thank you so much, Daniel. That was really, really great. And will be a great jumping off point for questions in the panel. Encourage the audience again to keep putting your questions in the chat and we'll try to address them as they come in. I will tackle the first couple questions but first, I'd like to introduce the rest of the panel. I'd like to start with Dr. Adriana Lopez who is an epidemiology at the Centers for Disease Control and Prevention on the acute flaccid myelitis team that's been working on this for many years now and is one of the nation's experts in the epidemiology of AFM. Dr. Charles Chiu, who is an MD, PhD infectious disease physician and researcher, clinical microbiologist, and sequencing guru galore at, at UCSF has done a lot of the work in sequencing EV-D68 isolates and now you may see him doing a lot of the sequencing for some of the SARS-CoV-2 variants and brings a very unique perspective, from molecular epidemiology side of things and the clinical micro side of things as well.

[00:27:19] I introduced Dr. Daniel Park, who will stay on, and our last panelist will be Dr. Heba Mostafa from John Hopkins who I just met today, but I know has done a lot of work in the Epidemiology Surveillance and Sequencing of Enterovirus isolates through the efforts of John Hopkins. So, I think we have a great panel today. Hopefully that can address a lot of your questions. I think a lot of the answers are going to be unknown for the future but using Gen ... Daniel's data as a jumping off point we'll try to ge- generate some good discussions of preparedness and what we can do looking forward to the seasons that come. I will start with the first few questions that came up in the chat that had to do with the EV-D68 signature on the BioFire platform. And I'll kind of start from the very basics from scratch on this clinical testing platform by BioFire. For those who aren't as familiar so BioFire is a commercial company that provides clinical testing platforms. Their respiratory pathogen panel is a multiplex PCR panel that tests for several respiratory viruses and a few bacteria. And one of the targets on there is in enterovirus, rhinovirus target for that family of viruses that are still genetically similar. They do not report out whether it's a rhino virus, whether it's eno- enterovirus or that type of enterovirus.

[00:28:48] However, they realized as they started to develop their trend program, which is a program by which they get de identified data from centers who sign on to get their testing results and provide epidemiologic real time monitoring of the pathogens that are going through your system region, the country that this could have some, implications to improve our surveillance for enterovirus D68, which many of us know does not have a clinically available test. After the 2014 outbreak, the CDC really was the only place you could get testing for EV-D68. After that, several institutions developed their own EV-D68 specific PCRs but in general is not clinically available. And so, the epidemiologic data has been mostly nationwide, looking at when we see EV-D68 show up when we see AFM cases show up at Daniel data digs into the signature which getting back to it. What BioFire did in the paper that I posted there which was not part of this effort which was prior to this effort was, they took these EDRV known samples, they ran an EV 68 specific PCR to know which ones of them were EV 68, which ones were not, and then use some machine learning to look into the intricacies of the melt curves for the rhino entero targets that make up case by case basis. But, on the larger epidemiologic scale was able to tell us kind of in a heat map way when he EV 68 was showing up in which regions of the country, at which time all the way back to when the RPP testing had started with BioFire. That allowed us to do a more granular analysis of the association between AFM cases in those regions and EV 68 circulation. The really unfortunate part that Daniel slid in there at the end that I put into the comments in, in response to the second question, was that this signature was recently lost as the panel was upgraded to the RPP 2.1 that includes the SARS-CoV-2 target, now has lost the ability to have this EV 68 signature. So, we can learn from the patterns in the past, but we aren't going to be able to continue to use that with the same level of robustness in the future. So I will stop hogging the panel discussion there. But Dr. Mostafa, or any of the others that ask those questions, does that answer your questions as far as the BioFire signature? And again,

I'd refer you to that paper by Myers and, and JCB.

Dr. Heba Mostafa: [00:31:40] Thank you. Yeah, this was great.

Dr. Kevin Messacar: [00:31:45] All right. I've got to scan the chat real quick, because I was talking. Let's start with the first question that I see coming in after that. Is there any way to use these tools to predict the potential evolution of the virus during a downturn? Would you expect increased variation away from the current B3 and A2 and D for those who aren't aware? There's a little bit of discrepancy in the literature of what to call A2 versus D, sub clades would they be limited due to lack of cases and resulting immunological selection? What a great question and I'd start if any of the panelists want to jump in otherwise, I, I pointed towards Charles to, to start, he's done a lot of work on variants for SARS-CoV-2 these are RNA viruses, what would we expect to happen during a low circulation year as far as changing of claims and evolution? What would your experience suggest?

Dr. Charles Chiu: [00:32:43] Yeah, it's a great, it's a great question. And, you know, I, I would, I would say right off the bat, that we probably don't know for sure what's going to happen with regards to evolution. And part of the reason why it's, it's very difficult is that as you know, there have been very few actual cases. You know there's been a sharp reduction in cases overall in general for RNA viruses, and entero- enteroviruses, RNA viruses. For RNA viruses you would expect that the, the emergence of, of, of, of mutations and selection, and essentially ev- evolution of, of of variation typically occurs if you have ongoing replication. So the more cases, in general, the more likely it is that, that we're going to see variation. I would expect that during this downturn, that there probably is going to be less variation. So, I would be there would ... But, the other thing I, I have to caution about is enteroviruses are very, there, there's a lot about enteroviruses in particular that we don't really know. Al- although the, you know, the some of ... the work that you know, Daniel presented is fantastic.

[00:33:50] It's, it, it still is the fact that for many ou- outbreaks, we don't know exactly for sure why is it that certain clades, or, or certain genetic lineages of the virus will, will tend to be predominant, say in a given outbreak versus another one. And it's, it's actually several reasons of one of which could include simply random genetic drift. So, what we call the founder effect, where essentially it just happens to be in a susceptible population. If you have one particular everything else being equal, if you have, for instance, several enterovirus strains that all have the capacity of, of causing AFM the ... and, and, really is, in some cases, it can be random, where you just happen to have an outbreak in the right place with, with the susceptible population.

[00:34:38] So I would say that that we currently don't know exactly, I would predict that probably the we're not gonna see much of a difference, although that certainly could be it, that certainly may be wrong you know, given, given, as I said before the difficulty in kinda predicting exactly what clades of enteroviruses will be associated any given outbreak. But I do think that it ... the the question is really, I think important in that it really highlights the need to actually do genomic surveillance. And so, so Dr. Messacar, Kevin mentioned about that, currently, that, you know, without currently, it's gonna be difficult to do su- surveillance with the BioFire because we don't have the capacity of identifying enteroviruses. But I would actually go a step beyond that and saying that without doing genomic sequencing, we, we, we won't have a way to identify clades. And so, I think ultimately it's, it's going to be really important to continue to do genomic surveillance for of enterovirus D68 as we are currently doing genomic surveillance of SARS-Coronavirus-2, for instance.

Dr. Kevin Messacar: [00:35:44] Dr. Mostafa, do you have anything to add? I know you've done a lot of work on mole- ... molecular epidemiology of...

Dr. Heba Mostafa: [00:35:50] Yeah, so I, I wanted to also like follow up on Dr. Chiu's explanation of the first half of the question which is mainly about the tools that can differen- that can actually differentiate between

the different clades. And the clarify a little bit the different options we usually have. So the diagnostic approaches in the clinical lab of enteroviruses, relies on detecting kind of a conservative area in the genome which is the five prime UTR region. And this region usually does not differentiate between enteroviruses and rhino viruses. And this is the same approach that the BioFire essay also uses. So, to be able to look at the genomes and identify D68 versus others or also differentiate the different sub clades we need to look into a more like species or type a specific region in the genome which has been the VP1 region. And the, there are like the classical method to do this with Sanger sequencing which which has been like not very frequently done for enteroviruses, unfortunately.

[00:37:09] And this is why we don't have a lot of data about enterovirus surveillance nationwide. This BioFire method for concluding signatures is actually very it was like very elegant because like this is a, this is an essay which is used clinically. So you can really have a lot of data if this sensitivity is acceptable, which I think it is. Then the other thing that I wanted to highlight is that those clades or sub clades data that we have, were mainly then based on that VP1 sequence, but then there aren't too many whole genomes available also to give us more information. So, there is actually about to be done and then implement us surveillance wise to like, provide better understanding of, of this of like the circulation, the evolution and the different types that are circulating in different places.

Dr. Kevin Messacar: [00:38:10] Thank you so much. I think if there's one thing we all are going to agree on, on the panel is that we need better molecular epidemiology and, and sequencing of, of enteroviruses in general. For those of you who haven't seen it before the next screen website any XP, SPR AI N website, does molecular epidemiology for a series of emerging pathogens and has included enterovirus D68 in that, and they take all of the publicly available, sequenced isolates via DP1 one or whole genome and throughout the globe and do a nice phylogenetic analysis and geographic analysis of which streams are circulating where. However, that's extremely limited by where sequences are coming from. So, if things aren't getting sequence, they're not going to show up there. And I think Dr. Mostafa and Dr. Chiu both made that point very strongly that we need more sequencing of more isolates.

[00:39:07] I will call on Dr. Lopez next because the CDC and others I think, have recognized that our enterovirus surveillance in, has been a pretty passive system for a long time through the National Enterovirus Surveillance System, but has started to beef up efforts, including engaging some additional surveillance networks to get better, enterovirus D68 at least surveillance. So, Dr. Lopez, you want to talk about NBSN and some of the other efforts of CDC?

Dr. Adriana Lopez: [00:39:35] Sure. Thanks, Kevin. Yeah, so as Kevin mentioned, we are we have put together there's a new vaccine surveillance networks that has been looking at it's an active prospective population-based surveillance network. And it's for acute respiratory illness in children less than 18 years of age. And what happens is we enroll kids who are hospitalized or visit emergency departments at seven medical centers across the US. And specimens collected from these patients and then tested. And this was originally for acute respiratory illness, and then we have added on AFM a couple of years ago. And so, they test specimens from most of the sites test specimens from July through October to look for enterovirus circulation and typing. So, they'll test and some sites will test all specimens for EV-D68 specifically, others will test only those that are enterovirus, rhinovirus positive, and then look at those for testing.

[00:40:40] So that's what we've been using in terms of active surveillance kind of to look and monitor circulation of enteroviruses to help kind of act as a possible alert for us for AFM. So, we've been using that for the past few years. And with 2020, you know, I don't know if many of you know with the world, as Brian mentioned, there weren't many cases in 2020, as we had expected or anticipated. We had a total of 31 confirmed cases. When in 2018, we have 238. So 2020 ended up being more like a non-peak year. And we looked at our data

from our NBSN sites to see what was going on in terms of enterovirus circulation, and EV-D68 circulations. We did see some sites had EV-D68 detected. But this was very site specific, not all of the sites had it. And there were low numbers, though. But what was interesting was just the numbers that we did see were a little higher than what we'd see in non-peak years, but much lower than what we saw in 2018.

[00:41:55] So it was interesting to look at that. And they continue testing for EV-D68 through December, just to see if maybe the pandemic had caused a shift, and circulation, but they didn't have any detections in December. And we're also looking at our National Enterovirus Surveillance System to see if there are ways that we can improve that, that is a passive system. One of the main reporters to that system is CDC. So, the specimens that we could get put into that system, but it really depends on what labs are reporting to that system. So, hopefully, we can increase our participation to help boost that system as well.

Dr. Kevin Messacar: [00:42:43] Great, thanks so much, Adriana. For those who are submitting questions and feel comfortable with it, try to hit the button to send it not only to all panelists, but to the audience too, because I'm getting a bunch of really interesting comments, but I don't think everyone can, can see them. So I don't think people are meaning to send them privately. But if you don't mind, sending your comments to the whole audience that'll help us follow along. I'll take the next question which didn't go up publicly. But it's from Dr. Amary Fall, who, for those of you who have not come across his work is one of the few people in Africa, in West Africa doing molecular work with enteroviruses and with EV-D68, has put out some nice papers that this is not an isolated North American or European problem. This is really showing up throughout the globe. And he asked the question that I think is on all of our minds after watching Daniel's presentation is, you know, with the low in 2020, what do we expect in terms of this coming back? And I'm going to take this in a different direction a little bit, because I think we have talked about the year to year variations.

[00:43:49] And my personal take on this is that enteroviruses don't like even numbers, there's no reason why they came in 2014, '16, '18, they just need warm bodies that are susceptible to circulate. And so certainly, the fact that we missed the big circulation year in 2020, would mean that there are more susceptible children out there in 2021, 2022, and really depends on what mitigation strategies are in place. So, I'm going to pose the more challenging question to the group with other respiratory viruses, for example, RSV and perhaps starting to maybe see a little with influenza that were socially distanced away, didn't see normal circulation periods. We're starting to see some signs of non-seasonal emergence, particularly with RSV in Australia, where they had a, a fairly large RSV outbreak that was not in their typical RSV season. So, when we see enteroviruses come back because I will answer Carlos' title of this presentation, 'Are enteroviruses going to come back?' Yes, they will. We don't know which one, we don't know when.

[00:44:52] But when they come back, would you expect them in our temperate areas where they typically show up in the summer to fall, that they will show up again in that specified period? Or should we be expanding our surveillance as, you know, CDC did with some of their surveillance networks to look for non-seasonal circulation? Could they show up in the winter, could they show up in a season that we weren't really prepared for it? And anyone who wants to jump on that high grenade feel free.

Dr. Heba Mostafa: [00:45:21] Maybe I can start by just giving a little bit of of our own data from the clinical lab for the season. So you're right that this is a very unique flu season or spider flu season that turns out to be really not the flu season. So we have not seen any influenza or RSV until recently, when we started to see like very, very few, like maybe three RSV and three flu. But we didn't stop seeing grind/anthro positives. So we have been seeing positives for the whole year. The total numbers have been less than a conventional closet here. But also the amount, the total number tested was also less because the clinics were closed, and many practices were closed. But it was fascinating that the, the extended respiratory panel entero-rhino was like the, the main, the main target detected then followed by a ve- by very few cases of adding virus.

[00:46:23] We have been of entero-rhino viruses we started to do this, this in 2019 and we continue to look at 2020 specimens. And it's actually remarkable because you ... as, as I mentioned those essays from the extended panels, they actually don't differentiate between rhino and enteroviruses. So we started actually our algorithm by testing those positives with a little bit of a more specific PCR essay that can be more specific for enteroviruses, and very, very a small percentage of those was positive with this more specific essay indicating that the main virus circulating is actually rhino and then within those positives, we sequenced them with Sanger there's the method to, to type. And it was interesting to see basically, also, a large percentage of them were tied to rhino. And then within the very teeny-tiny numbers of that type and to entero we saw more enterovirus, D68 and 2020, than in 2019.

[00:47:37] But it's still like a small number. So we, we ... I, I should say we had enterovirus D68, circulating in 2020. So why, why didn't we see outbreaks and so on? I think it's still because of the unique pattern of the year with social distancing and everything in place, but it's also remarkable that it has been circulating. I will give, give the chance to somebody else to jump in.

Dr. Adriana Lopez: [00:48:06] Well, I just wanted to say that with the NBSN sites, there are a couple sites that do test year-round. So, they're looking at it as well. But, we're also starting a project with our emerging infections programs and two sites to do some enterovirus surveillance looking at more severe neurologic illness, but that will also be helpful in terms of looking at enterovirus circulation.

Dr. Kevin Messacar: [00:48:34] And I would just add our local data, we tested over 400 specimens in 2020 and, and didn't have a single and those are all EVRV positive in the Bihar data have a single EV-D68 positive good me had been spotty like was seen in and BSN where certain areas saw a small amount of circulation. But, nationwide, we didn't see a large outbreak. Dr. Mostafa, you brought up another question that I'll bring Daniel and Adriana into which is something that no one's been able to explain to me thus far. I have my own theories. But why did rhinoviruses emerge first? When we saw respiratory viruses start to re-emerge after the initial lockdown, it ... could this be explained by the are not of the various viruses, SARS-CoV-2 being you know the highest and continuing to circulate with the lack of immunity out there, rhino virus could potential being less but, or less than SARS-CoV-2, but, EV 68, potentially less than that. Why do we see rhinoviruses emerge, but we didn't see as much enterovirus disease?

Sang (Daniel) Woo Park: [00:49:44] I unfortunately, don't know either. It's something that we've all been wanting to know but, I've heard hypotheses above maybe something to do with some of the viruses being enveloped and some of them not being enveloped. But I have not any answers for why rhinovirus are. But it seems like it is a very persistent pattern across different countries, not just US. We see it in Asian countries, European countries that even when before think even when other respiratory pathogens didn't come back when other respiratory pathogens are basically died out, the rhinoviruses seem to be persisting in these countries.

Dr. Kevin Messacar: [00:50:32] Yeah

Dr. Heba Mostafa: [00:50:32] that fortunately [

Dr. Kevin Messacar: [00:50:33] crosstalk 00:51:00]-Adriana and you could circulating around-

[00:50:34] [laughs].

[00:50:35] [laughs].

Dr. Adriana Lopez: [00:50:36] Yeah, unfortunately, I don't really have any additional [laughs] questions.

Feedback on what Brian said, it's hard to really know why but we're, you know, we'll continue thinking about it and try and explore different things. But, yeah, as of right now, we're not just not sure.

Dr. Kevin Messacar: [00:50:59] And I want to to make a quick practical point in question two, after two and Mostafa, there's a few comments coming in on the kind of poor quality of the genomic data the sequences coming out on EV 68. And also make the point that we talked a lot about EV 68 today, but we know that other non-polio enteroviruses, also can cause AFM so need to be looking for them and aware of them. But on a practical question, for specimens coming to you to be able to recover whole genome, what can the clinicians on the call do in terms of what are the best specimens to collect? What media to put them in? What transport how to get them to you with the best chance of getting the highest quality data once they hit your lab?

Dr. Charles Chiu: [00:51:49] So maybe I can tackle that. So I, I think Kevin, you had two questions there. The first question is with regards to the metadata the importance of metadata and sequence databases. I'll, tackle the other question, which is on a sample acquisition, I mean, so typically our interest is so for genome sequence recovery, it's really we want to, we want to obtain essentially full length intact RNA. And because RNA is, is quite labile new typically, we would like a specimen, ideally, to be essentially frozen as soon as possible or, or currently for clinical for clinical essays. We typically the specimen stability requirements are that they'd be frozen within six hours of collection, and that's not always possible. But, but for us to report say, a clinical result, either from met- from a meta genomic test or from a direct PCR test it should be frozen within six hours and, and kept frozen.

[00:52:48] In terms of the actual sample type we have been primarily looking at nasal swabs in either UTM or VTM although I, I do I'm aware of we've, we've actually also been doing some studies of saliva similarly, as there's been an interest in moving in potentially moving the saliva. We haven't seen I think we haven't seen the same, the same trends as with SARS-Coronavirus-2 where we're actually seeing actually higher viral loads in saliva. So but what would you think that there may be comparable viral load so saliva might be an interesting sample type to validate especially because you know, many groups are collecting saliva, you know, for SARS-CoV-2, at least in, in outpatients or in asymptomatic infection.

[00:53:33] So and it's an easier sample to collect than a nasal pharyngeal swab. So with regards to, as long as it hasn't been thought, on multiple freestyles, or have been in your earlier quoted, or use other purposes, I mean, typically we want essentially as fresh to sample as possible freshly frozen for genomic sequencing. With respect to the actual titers we've been successful. So we use a variety of methods to, to actually detect enteroviruses ... to sequence a genome of enteroviruses. And that's primarily because enteroviruses can be the titers can be very low. And, and really depends ... especially in, in acute flaccid myelitis patients where the- their presentation may be several days, in some cases, weeks after their onset of symptoms.

[00:54:22] So because the titer are very low we've adopted a variety of methods to do genome sequencing. So, they include a tiling amplicon method where you essentially design primers across the genome, and run PCR of multiple PCR reactions at the same time. That's the idea. We also use we also have a spiked primer enrichment method that was developed in my lab, where we essentially enrich for enterovirus D68 sequences, and we increase the sensitivity of metagenomic sequencing. And then there's another method, which is a capture probe method for enriching as well. In general, if, if the cycle threshold by PCR is less than 32 we've been quite successful in getting the entire genome, if it's above 32, then we can some cases get partial genome sequencing, or we may as Dr. Mostafa commented on, we may choose to restrict our sequencing to say the VP1 gene region for typing purposes.

[00:55:15] But in, in general, we prefer that the psychotherapy will be less than 32, although we have been successful in recovering genomes up to 38. So but, but that's really sort of sketchy or, your ability to recover

genomes at that level. The, the second question that you had which is really relevant on in terms of clinical metadata in our experience was SARS-CoV-2, one of the major issues is not the issue of not being able to get clinical metadata, it's the issue that it, it really depends on who's doing the sequencing and how we're doing sequencing. So, for instance public health agencies are doing sequencing for surveillance methods, there are often lots of restrictions regarding the meta data that you can actually provide and collect.

[00:55:57] So, in, in some cases, we've had difficulty for instance, in even providing collection date. Because collection date is thought to be potentially yeah, you know, an ide- identifying information. So, I, I think that this is a challenging not only with respect to enteroviruses, but with respect to viral sequencing in general, in terms of providing metadata. I completely agree with everyone that metadata is critical for being able to understand the, you know, the clinical relevance and importance of the sequencing information. In fact, arguably, sequence information without metadata is, is, is it's close. It's very, very, not very useful. But the what really needs to be done is more coordination between hospitals who can provide clinical metadata link to say this the genomic sequence and patient information and more coordination between hospitals and county and state and national public health agencies as a way to provide a, a more centralized network for for not only doing the sequencing, but also acquiring the metadata.

Dr. Kevin Messacar: [00:57:00] Thank you so much, Charles. So we're hitting the top of the hour. So, to briefly summarize for our clinicians out there, I, I ... the points I would pull out from Charles is, particularly of interest to this group. And AFM cases, we're already behind the eight ball and that those patients are typically around a week after their respiratory prodromal onset, so that's when viral titers are going to be lower. So you want to get that sample as soon as you suspect AFM. Currently, the gold standard specimen is a nasal pharyngeal specimen that's the deep brain tickler, specimen. Until we get better data we are conducting a study at our site funded by CDC to look at the shedding curve of EV 68. So doing serial swabs of children over 21 days to see how long they shed virus for. And as part of that we're actually going to try to validate the intermittent swabs so not all the way back, but a little more shallow. And a lot of us were being done for SARS-Cov-2, and we're also getting saliva.

[00:57:55] So, people are interested in collaborating. Dr. Hai Nguyen-Tran here is coordinating a study which you should have sample to collaborate with others, it would be really nice if we had less invasive sampling, if possible. And if I had to summarize this whole discussion, we started out with a, a wonderful presentation on predictions for the future. But I think even Daniel will tell you our predictions are only as good as the assumptions they're built on. And there we can't perfectly predict the future, I think Adriana will say we can prepare for what could be coming. So, I think preparedness is a different discussion than prediction and that we try to use the best data that we can to predict seasons that come. We really can control our preparations. Then I think the CDC on the public health side, this Working Group, the parents group is all working to do our best to, to prepare, if this does come back, that we're more ready to get good specimens, to get better sequencing of those specimens, to get better information to hopefully in the future provide better care in terms of treatment and prevention for these kids.

[00:59:02] So, I appreciate our entire panel for all of your expertise and your contributions. What a wonderful way to start the talk with Robin Roberts in the great success and having an ICD code. Remember in October, you can now code a patient at diagnosis with a diagnosis code for AFM which is going to help all of our future efforts. And I would encourage all of you guys new to the Working Group to join these monthly calls. It's a really great collaborative group we look forward to working together with all of you in the future. And I will pass it back to Carlos to close things out.

Dr. Carlos Pardo: [00:59:39] The only thing that I like to add is our great thank you to all panelists, to Daniel, to Charles, to Heba, to Adriana, to Robin, and Kevin thank you so much for playing the role of moderator. And

to all of you thank you for participating in this virtual forum. Again please keep in your calendars that every Thursday the third Thursday, the next one that we are going to have is on April 22nd, at 2:00 Eastern time, and I appreciate that there were many participants from other areas of the world. I can see participant from Japan, from Africa, from Netherlands and Europe. Thank you so much for being available for this meeting. And we hope that you can continue joining us for the next virtual symposium. And stay safe and thank you, all of you, for participating in this panel and symposium. Bye now.