Call Date

09/16/2024

Call Agenda

Welcome
Sean Courtney, CDC Division of Laboratory Systems

Mpox Update
Nicolle Baird, CDC Division of High-Consequence Pathogens and Pathology

Oropouche Virus: An Emerging Threat in the Americas/Updated Testing and Reporting Guidance and New Response Plan
Aaron Brault, CDC Division of Vector-Borne Diseases

Call Transcript

Sean Courtney: All right. Good afternoon, everybody. Thank you for joining today's call.
My name is Sean Courtney. And I am in CDC's Division of Laboratory Systems. On the screen is the agenda for today's call. But before we get started, I just want to cover a few housekeeping items and some announcements.

So as you've heard on previous calls, DLS is the CDC Division that works closely with clinical and public health laboratories across the country to support laboratory emergency preparedness and response activities and has been hosting these calls since March of 2020. DLS supports this work across four goal areas, quality, workforce, and training, preparedness and response, and informatics.

CDC's Division of Laboratory Systems launched the ECHO Biosafety Program to develop and engage a community of practice to address biosafety challenges in clinical and public health laboratories. The next session is scheduled for next Tuesday, September 24, and will focus on *Operations: Emergency Response and Contingency Plans*. These monthly sessions are tailored for laboratory biosafety professionals and provide a platform to bridge gaps, build a community of practice, and enhance biosafety.

You can scan the QR code on the slide to register for the next session. To view upcoming sessions and access resources from past ones, visit the ECHO Biosafety Program website. For inquiries, you can contact dlsbiosafety@cdc.gov.

As always, we want to hear from you. So our Training and Workforce Development Branch is interested in hearing more about the education and training gaps that you're currently experiencing. We invite you to send your feedback via email to labtrainingneeds@cdc.gov.

So we'll be sharing slides from today's call along with the audio and transcript. And we'll post them online, hopefully by the end of next week, if not within the next two weeks. You can find them on CDC's Laboratory Outreach Communication System, LOCS, page at the link shown at the bottom of this slide.

And so how to ask a question? During today's call, if you have a question, we'd like you to please use the Q&A button within the Zoom webinar system. Type your question in that box and submit it.

We also ask that you please include your email address so that if we're unable to address your question during the call, that we can follow it up afterwards. If you're from the media, we ask that you please contact CDC Media Relations at media@cdc.gov. And if you are a patient, we ask you to please direct any questions you have to a health care provider.

And I'd like to remind everybody that the information on the slide deck today may contain presentation material from panelists who are not affiliated with the CDC. Presentation and content from external panelists may not necessarily reflect CDC's official position. So please keep that in mind when you go back and look at some of the slides that we post on our LOCS web pages.

And with that, we will get started with our first speaker today. We have Nicolle Baird from CDC's Division of High-Consequence Pathogens and Pathology. And she's going to provide us with an mpox update. So, Nicolle, I'll hand it over to you. Well, I'll flip through. But you can go ahead and just get started.

Nicolle Baird: Thank you, Sean. Sean mentioned I'm Nicolle Baird. So I'm the lead for the Virus-Host Molecular Interactions Team within the Poxvirus and Rabies Branch. Christina Hutson is currently out of the country, so I'll be giving this update in her absence. And I'm going to turn off my video for this just to make sure our connection is not interrupted here. Next slide, please.

So this is the historical geographic distribution of the monkeypox virus clades. Clade II is represented in green, while clade I is represented in purple. As you can see, clade II is endemic, primarily in West Africa, whereas clade I is endemic in Central Africa. Both occur, however, in Cameroon, but are geographically separated by natural barriers. As you are likely aware, clade II is what started the 2022 multinational outbreak. Next slide, please.

So this slide is showing that clade II cases are still occurring in the U.S. So this is the epi curve from May 2022 through August of 2024. And as you can see, the peak of cases occurred in August of 2022, and then we saw a drastic drop in the number of cases. While we haven't seen the same level of cases reached that we saw in August of 2022, we are still seeing consistent levels, averaging about 15 cases per day. Next slide, please.

This slide is zooming in and really taking a closer look at that same epi curve, but now focusing only on 2024. And you can see here that the current median seven-day moving average for confirmed cases is actually less than 15 cases per day and is sitting more accurately at around 4.85 cases per day. And this is down from around 7 cases per day in July.

Of note here, you do see from that bottom little paragraph there that there is an increase in clade II cases for this year as compared to 2023. This is likely attributed to a couple of different things, one being that people did drastically change their behaviors in 2022 and 2023, and a belief that many people have resumed their typical behavior prior to 2022, with the thought that this has sort of gone away. And also with a lot of the clade I messaging that is out there now, physicians may be testing more to ensure that they don't miss an mpox clade I case. Next slide, please.

And next slide. So moving on to clade I, monkeypox virus, specifically looking at clade I and DRC. DRC detected the first case of clade I mpox in the 1970s and really has detected cases every year since. However, there has been a steady rise in cases over the years, since 2021, as you can see in this chart,

particularly looking at the jump in the number of suspected cases from 2022, which was around 5,600, 5,700 to 2023, with over 14,000 suspected cases.

And for 2024, there have been, to date, more than 15,000 suspected cases. It is important to note that these are suspected cases. Not all have been confirmed to be mpox. And only a fraction are actually confirmed at this point.

Suspected cases are based on clinical suspicion, and there are a number of other rash-causing illnesses circulating in the area that could be causing clinical suspicion of mpox, such as varicella, zoster virus, or chicken pox and measles, as well as other rash illnesses. The CDC and other partners are currently working together to provide support to the country and to expand the testing in DRC so that a higher proportion of these suspected cases can be tested. Next slide, please.

The data that we have on monkeypox virus sequences in DRC is limited. There are only 59 sequences available from the past year and only 173 whole genome sequences available total. And that's going back to the 1970s. In many areas, transmission is reported to be dominated by zoonotic spillover, with limited human-to-human spread, so occurring primarily just within households or within close contacts. And this virus has been evolving in the animal reservoir for many years now. And the orange and yellow dots shown on this map here are what we typically see for this virus. However, if we look specifically at the South Kivu outbreak in DRC, this is represented by the red or the maroon dot here, we see a different outbreak history happening.

So this outbreak is happening mostly in adults, as opposed to children or other household spread, and is being caused by what is called a monkeypox virus clade Ib. So this virus has APOBEC3 mutations, which is suggestive that human-to-human transmission has occurred. And we are seeing this confirmed in our epi data as well. And this is different from the 2022 outbreak that was driven primarily by MSM networks. Now we are seeing a high prevalence of cases in female sex workers, further confirming the human-to-human transmission. Next slide, please.

The virus coming from South Kivu is a genetically distinct clade from the traditional clade I, as can be seen in this phylogenetic tree. You see the clade 1b at the top there in the maroon dots. And this clade 1b has a deletion that affects detection by the CDC clade I specific PCR assay.

However, the CDC non-variola Orthopoxvirus assay, or the NVO PCR assay, will still detect this subclade just fine. And this is also the same target of the Cepheid GeneXpert test, so it should detect this 1B as well. Let's see. Sorry, I lost my place there.

We are seeing currently a lot of headlines about this new virus. But the reality is that this Ib has really likely been evolving for several years now prior to the outbreak. Next slide, please.

As I mentioned, clade Ib has a large deletion that prevents detection with the CDC clade I specific assay. The reason for this is that the large deletion is in C3, which is the target of the CDC assay. And while clade I and clade II have over 95% homology, there is one difference between the clades, and it is in the C3 gene. So C3 is not found in clade II.

From previous studies and current epi studies. It's believed that clade Ib is less virulent or pathogenic. And previously, our lab published an animal study where we swapped the complement control protein or C3 between the clades, and so specifically deleted from clade I and compared to the wild-type clade I.

And we saw 100% mortality with the wild-type clade I versus 0% mortality in the clade I with the C3 deletion.

This indicated to us that C3 does play a significant role in pathogenesis. And we are seeing evidence of this decreased virulence with the lower case fatality rate in South Kivu right now. So right now, the case fatality rate in South Kivu has consistently remained below 1%. Next slide, please.

Clade I cases have also been detected outside of DRC. CAR and ROC are both endemic for clade I. However, we are now seeing spread to nonendemic countries, which prompted the release of the new HAN in August that states that Ib has been found spreading from North and South Kivu to bordering countries of the DRC, including Uganda, Rwanda, and Burundi, with Burundi really being the most affected. We've also seen one travel-associated case in Sweden and one in Thailand. The CDC is currently working to support DRC and the neighboring countries in testing and sequencing efforts and vaccine strategy and helping with preparedness efforts as well. Next slide, please, and the next.

So moving on to clade I surveillance in the U.S., our goal is really to identify if any clade I monkeypox virus is circulating within the U.S. However, it is important to note that the IPC and patient care does not change based on clade determination. And as with any mpox cases, early cases require rigorous case investigation and contact tracing.

CDC continues to solicit all mpox specimens from labs using the CDC 510(k) cleared NVO test to be forwarded on to CDC after initial diagnosis so that we can perform clade specific PCR testing and/or sequencing to identify any mutations. And that is really for labs that aren't already doing clade-specific testing or sequencing on their own. We are also happy to support those labs that are able and willing to bring up clade-specific testing in their labs. And CDC collaborates with labs who perform testing that can flag a high likelihood of clade I. Next slide, please.

There are currently tests being utilized that do not target a viral essential gene and/or that cross-react with other Orthopoxviruses. So ensuring a testing algorithm that incorporates an accurate test to start is critical. So this would include having the NVO test uses a primary test, since this test targets a viral essential gene and therefore, would not be impacted by circulating mutations. Additional clade-specific testing or sequencing can be performed after that if needed. However, what is most important is not missing a case. And really, using that NVO test would ensure that you would not miss a case.

We are in the process of validating an mpox triplex test for EUA submission to FDA that includes the CDC NVO test along with the CDC clade-specific Ia and clade II tests. This assay would still not detect Clade Ib, however, but, again, you can use an algorithm. For example, if the result is NVO positive, Clade Ia and clade II negative, you can sequence to confirm the clade and subclade. And there are also currently clade Ib PCR tests being developed and in the pipeline.

Next slide, please. I think that's all I have. Thank you. Happy to take any questions as well.

Sean Courtney: All right. Thank you. Appreciate the mpox update. I do see one question currently. And I'm not sure if you can answer it, but I'll pitch it to you. Which is if you could comment on lab staff who only handle specimens, so they do not culture or analyze samples, are they required to have the mpox vaccine booster every two years? Or is there a recommendation on vaccination?

Nicolle Baird: So our guidance hasn't changed really from the clade II outbreak. It's the same guidance. And I believe it's on our website. I'd have to take another look. But it would be preferable to have lab staff vaccinated. But it would still be using the same biosafety conditions, again, that we specified during the 2022 outbreak.

Sean Courtney: Great. Thank you. Another question that just came in, around you mentioned that clade I might have been evolving for the past few years, actually, before this outbreak. Do you know of any factors that could have contributed to that? Would it have been lack of sampling, or just potentially being subclinical?

Nicolle Baird: I can't speak to it. And there's probably someone better to answer that than me. So that's something I can take back and get the answer to for you. But I suspect the mutation, the APOBEC3 mutation has really played a role in why we are now seeing the human-to-human transmission. So I imagine that initially, it was most likely evolving in the more rural regions, again, and seeing zoonotic spillover, but with that mutation, human-to-human transmission was able to be sustained.

Sean Courtney: Great. Thank you. Next one, you also mentioned that there are some clade Ib tests being developed. Are those being developed at CDC, or are they outside of CDC? And if they are outside, are they the ones that we're evaluating?

Nicolle Baird: There are some being developed, I believe, outside of CDC. So I think there are a couple of European countries that are developing a clade Ib test currently. But CDC is also working on one as well.

Sean Courtney: Got it. Thank you. All right. Well, I do not see any other questions at this time, so I'm going to go ahead and say thank you, again, for joining our call today and providing this update on the mpox outbreak that's currently happening.

I would ask if you're able to to hang out on the line. And if any other questions pop up in the Q&A function here within Zoom, you can go in and type responses there if you're able to hang out on the call for a little bit longer. But I do thank you for joining today's call.

Nicolle Baird: Sure, no problem. Thank you.

Sean Courtney: All right. Thank you. All right. And with that, we will move to our next speaker. And we have Aaron Brault from the Dengue Oropouche Emergency Response. And he's going to be providing us an update on the Oropouche virus outbreak. So, Aaron?

Aaron Brault: Well, thanks, Sean. I appreciate the opportunity to talk to your group. Yeah, today I'm going to talk about Oropouche, which is a *Peribunyaviridae* virus.

And we make a habit of working on a very wide diversity of arboviral agents that usually most people have never heard of until lots of people have heard of them. So Oropouche, like Zika, is probably going along that same path in terms of public awareness of this as an emerging threat in the Americas. So I'm going to talk a little bit about the background of the virus, some information on what our testing capacity has been and what we've evolved to in terms of our testing capacity, and our reporting guidance and a new response plan.

And so as Sean mentioned, I'm serving as the lead for the Oropouche Laboratory Task Force, specifically. And I'll get into, principally, the laboratory components, but also some of the reporting components that are involved more heavily with our epi group, which I can either answer or defer those questions to our group. So if I can get the next slide, Sean, that'd be great.

Great. Thank you. So Oropouche virus is an emerging arbovirus in the Americas. It's been known since the 1950s, first isolated in Trinidad and Tobago in 1955, and then subsequently in the Amazon basin. Additional work was done to identify the enzootic transmission cycles. It belongs to the Simbu serogroup in the genus *Orthobunyavirus* in the *Peribunyaviridae* family. As I mentioned, it had been isolated in Trinidad, so in the Caribbean. But it also had been identified to have enzootic transmission cycles going on in South and Central America and a number of different countries, but, not surprisingly also in portions of the Caribbean as it was initially isolated in the '50s but also identified in Haiti in an infected individual, a child in 2014.

So this is a little bit out of date. But as of August 1, 2024, there's greater than 8,000 confirmed Oropouche cases that have occurred in the Americas. This is a pretty big expansion. Brazil has periodic outbreaks. But this is a sizable outbreak.

The PAHO identification in their latest bulletin had this number, a little over 9,500 cases in terms of confirmed cases. Principally, these are in areas that are nonendemic for Oropouche virus. So the virus has kind of expanded its geographic range into other areas and other countries. So we're up to six different countries that have undergone Oropouche locally acquired infections, most notably Cuba.

So in the U.S. and Europe, there have been a number of travel-associated cases. Here I list this as 30. We're probably pushing 70 now, if I look at the most recent surveillance data and our diagnostic data. But definitely in excess of 50, and I believe we're going to be reporting out approximately 70 human cases associated with travel to areas with known Oropouche transmission. They've been identified in travelers principally from Cuba. But we have had at least one traveler from Brazil that has demonstrated to be positive on return to the United States. Can I get the next slide, Sean?

So as I mentioned, the virus was initially isolated and identified in Trinidad and Tobago in the 1950s and then subsequently in the Amazon basin. And so most of the work done looking at the transmission cycles of Oropouche virus have been done in Brazil in establishing two different transmission cycles, both the sylvatic cycle, so kind of a jungle habitat cycle, if you will, that involves a number of different vertebrate amplification hosts. Most notably the three-toed sloth and other non-human primates that manifest viremias that are sufficiently high to infect subsequent vectors, namely mosquitoes, *Ochlerotatus serratus* mosquitoes, that bite these individuals that are viremic, they extrinsically incubate the virus, and then subsequently, after that extrinsic incubation period, are subsequently capable of transmitting the virus onto another vertebrate amplification host with this tangential sort of accessory cycle that can involve wild and domestic birds, as well as different rodents potentially could be contributing to that cycle as well.

Periodically, of course, you have this enzootic cycle that spills over into an epidemic epizootic sort of cycle in which humans will manifest sufficiently high viremias in order to infect other vectors. Vectors that have been incriminated in these peridomestic transmission epidemic cycles involve *Culex quinquefasciatus*, which is the house mosquito, which is a common mosquito in Brazil and the United States as well, where they can then subsequently transmit from human to human with, of course, that extrinsic incubation period and the intervening vector cycle.

Additionally, and most notably, a very important vector during these epidemics are biting midges or no-see-ums, if you will. And these are *Culicoides* species, most typically *Culicoides paraensis*, which has a distribution which is largely in many, many portions of the Western hemisphere and is present in many of the areas that are undergoing active Oropouche transmission right now, although we have very scant data on the vector distribution in Cuba. And we're trying to enhance that by looking at data that might be coming out of Guantanamo Bay as well. So I will take the next slide if I could. Thanks.

All right. So Oropouche infection is a pretty nondescript arboviral infection. We could say this about most all of the arboviruses that we work on. Clinical illness is very similar, as I mentioned, to other arboviruses. The symptomatic rate is actually quite high. So unlike Zika virus, for instance, where 80% of cases are predicted to be asymptomatic, in this case, with Oropouche virus, about 60% or in excess of 60% of people become symptomatic with a relatively short incubation period of 3 to 10 days, which is also fairly typical of other arboviral infections.

Along that same theme, clinical presentation is very similar to other arboviral infections that are caused by these epidemic waves that we see in other arboviral infections like dengue, Zika, and chikungunya virus, which co-circulate in many of these same areas, of course, making clinical diagnostics all the more difficult. Acute onset of fever, chills, headache, very common, myalgia and arthralgia, which of course, are common symptoms of the aforementioned arboviruses as well. Other symptoms include retro-orbital pain, photophobia, vomiting, diarrhea, rash, conjunctival, infection, and abdominal pain. Clinical laboratory findings can include lymphopenia and leukopenia and elevated liver enzymes.

One of probably the most distinctive criteria for or presentations of Oropouche virus is that you have that initial disease presentation, and then after either as little as a few days or a few weeks, we can actually see recurrence of those symptoms. And this occurs in a high proportion of cases. Approximately 70% of cases can demonstrate a kind of recurrence of these symptoms. And that's fairly diagnostic for Oropouche compared to other arboviral infections. Next slide, please.

So Oropouche typically results in fairly mild illness, but as we can see with many of other arboviruses as well, we can see significant or severe clinical manifestations in some portion of the individual. So if you get the denominator high enough, we can actually see very severe clinical illness in some individuals with hemorrhagic manifestations being one potential, as well as neuroinvasive disease. So we see meningitis and meningoencephalitis, which can result in occipital pain, dizziness, and other neurological presentations with clinical laboratory findings include pleocytosis as well as elevated protein in the cerebrospinal fluid.

Additionally, in healthy women in Brazil, and I believe this came out in the Emerging Infectious Diseases over the weekend, at least a couple of deaths have been associated with otherwise healthy non-pregnant women. So next slide, please.

So Oropouche in pregnancy, as was the case with Zika virus, this has really kind of honed our efforts and enhanced our epidemiological concern regarding Oropouche transmission and the potential that it can result in adverse pregnancy events. We would put the caveat here that we have fairly limited data on the frequency of potential negative impacts on pregnancy and vertical transmission. So based on this limited data set in Brazil, vertical transmission has been defined as a definite possibility. Several pregnant individuals with evidence of vertical transmission have been identified, in which it's been

suspected that transmission to their fetus has occurred and potentially resulted in congenital abnormalities, including microcephaly.

So individuals that were symptomatic with Oropouche infection and had positive test results were the source of this information. And tissues from stillborn infants, as well as one infant born from microcephaly, have been tested positive by RT PCR, which is, further, that connection between infection and potential fetal negative impact. However, currently we do not know what the frequency of negative impacts on pregnancy could be or the timing.

In terms of, as you recall, with Zika virus, it was infection in the first trimester was really critical for developing microcephaly in expectant mothers. And we don't have that information at present for Oropouche virus infection, nor do we really have a good handle on what the relative frequency of those adverse pregnancy events could be. Can I get the next slide, please?

So I'll shift a little bit in terms of identifying what our suspect case definition was or has been. And it's an evolving process, obviously. So as we see expanding geography of the current outbreak, this is subject to change, given that one of the criteria for a suspect case definition is an individual having either traveled to or having a close epidemiological association with an individual who has traveled to one of these areas with known Oropouche viral infection within two weeks of travel, for instance, with an abrupt onset of fever, headache, or at least one of the following symptoms, myalgia, arthralgia, or photophobia, or retro orbital pain or other symptoms of neuroinvasive disease.

Initially we ruled out to limit the number of COVID cases, principally, the presentation of respiratory symptoms. But on the next slide, I'm going to show we've kind of backed off that. Because we've identified a portion of individuals with confirmed infections have presented with respiratory symptoms. And then these are also individuals that we would generally like to see tested negative for dengue. So that generally is associated with reflux testing. So an individual with very compatible symptoms that is dengue negative, with then reflex Oropouche viral testing. However, if individuals have a strong epidemiological association with either travel or a travel-associated case, we do not require that dengue virus negative test results be acquired before we would accept that for viral testing for Oropouche virus. And the next slide, please.

So as I mentioned, we've updated the interim guidance for testing criteria and revised the suspect case definition. We removed the no respiratory symptoms given that 14% of us travelers have reported cough or sore throat. So that's out. And we do consider individuals with respiratory symptoms as part of the testing algorithm.

And we also added, and I have listed here as anticipated CLIA. As of September 10, this is a validated CLIA, validated test for use within our division here within Fort Collins for an acute molecular test for Oropouche virus between 0 and 7 days by RT PCR. So previously, we had used this as a surveillance test. And now most of the samples will likely be submitted as actual CLIA diagnostic testing. It's simplified the specimen submission process in terms of our interim guidance and revised testing algorithm, which I'm going to cover on the next slide if I could, Sean.

Great. So initially when this became an issue of concern, Oropouche transmission in Brazil and subsequently in Cuba, we had at our disposal a CLIA-approved clinical diagnostic test for neutralization testing, which of course, is utilizable at about seven days post symptom onset. And that's what we had

as reportable back to patients and health care providers, including the use of a neutralization test, a plaque reduction neutralization test, using the prototypical Trinidad 1950s isolate.

And then that was also available in a certain number of select state public health laboratories. As I mentioned, as of September 10, we now have molecular testing capabilities for acute specimens, both serum and CSF, that can be tested by molecular tests for Oropouche viral RNA within that time window. And before that, we conducted molecular testing based on a surveillance protocol using the same assay, which has subsequently received CLIA validation.

And so as we mentioned, the previous surveillance testing was not reportable back to patients or to health care providers. But that information was anonymized, I should say, and reported back to the state public health laboratories for epidemiological consideration and utilizing that information to avoid potential autochthonous transmission and reduce the likelihood of that from happening. So next slide, please.

So our testing algorithm, based on the bringing on September 10 of our molecular assay for CLIA so that that data can be reported back to patients and clinical care providers, is that between 0 and 7 days we test by RT PCR using the clinical test. Viremias can actually be fairly high. And so unlike many of the other arboviruses that we work on, pathophysiologically, we actually see fairly high viremia magnitudes with fairly low CT values. So in the order of 16 to 17, which is a very high RNA load in the serum. Although we do have the capacity to test CSF, we don't have as much information given the limited number of CSF submissions that we've received to date.

We reflex to neutralization testing for clinical diagnostics at seven days and beyond. However, if an individual is negative at day 6 or 7 by molecular testing, we will enact plaque reduction neutralization testing as well. For non-pregnant individuals and neutralization positive, this is a 90% threshold of a 1 to 20 neutralization titer would be considered adequate to diagnose infection. However, for pregnant individuals, we would prefer to see paired specimens for those patient individuals where we'd like to see a four-fold or greater increase in the neutralization titer between the acute and convalescent, which would be taken two weeks subsequent to the acute sample phase. Can I get the next slide, please?

All right. So, ArboNET, in terms of reporting, we have two new event condition codes, one for both noncongenital and congenital infection, 50290 and 50291, respectively. However, during this transition period, we understand that there can be some difficulties in terms of adapting reporting profiles. And so in the absence of having that capacity, we would request that jurisdictions continue to use the condition 10072, which is, quote, "other arboviral infections." Next slide, please.

So as we can say for most of the 200-plus arboviruses that we're responsible for within our division, Oropouche virus results in a disease that for which there is no vaccine or specific treatment is available. So treatment has consisted of largely supportive, with acetaminophen as the most favored treatment, as a first-line treatment for joint pains and fever in travelers returning with suspected Oropouche infection. Aspirin or other NSAIDs are not recommended given the potential exacerbations of hemorrhagic manifestations. However, patients that do develop significant symptoms or more severe disease manifestations should seek hospitalization and can be hospitalized for supportive care in many cases. Next slide, please.

So guidance and resources from CDC, especially for pregnant individuals we released several weeks ago and updated a travel health notification for pregnant individuals traveling or to reconsider travel to

areas where the most intense transmission has been observed, namely in Cuba, where we've seen most of our travel-associated cases, as well as Brazil. So next slide, please.

In addition to the travel health notification, we've also released health alert notifications for notifying clinicians and public health authorities of the potential transmission of Oropouche in the Americas. It advises testing of travelers returning to impacted areas, potential signs and symptoms, revises awareness of possible vertical transmission, and providing clinical guidance in terms of travel, travel plans for pregnant individuals, and highlights preventative measures for preventing autochthonous or local transmission in individuals that have been confirmed to be travel-associated cases back to the United States and to kick start, if you will, surveillance activities so that we can try to either mitigate or prevent subsequent transmission autochthonously in the United States. So next slide, please.

All right. So we've posted additional web content on our CDC Oropouche pages that are available here, both interim clinical considerations for pregnant individuals as well as testing and reporting guidelines that I've highlighted here. And can I get the next slide, please?

All right. So in addition, I'm going to cover a little bit of information on a response plan, in states in the United States and states and territories. So this is a response plan that's been published on our web page. It serves as a reference and not a prescriptive and is not fully comprehensive. It's subject to change, obviously, as we get more epidemiological information, as we get more information on the potential impacts and frequencies of adverse effects on pregnancy, as well as the timing that could result in adverse effects on pregnancy, that this response plan and recommendations would be modified.

So it includes recommendations for preventing infections in travelers, including pregnant individuals, travel-associated cases that include suspect, probable, and confirmed cases, assessing local transmission of individuals, and then, of course, response in terms of responding and how state public health laboratories or local public health administrations should be responding to single and/or multiple cases, travel-associated cases in an area and potentially subsequent autochthonous transmission cases. And next slide, please.

So the response plan also includes vector maps. And you can see here the *Culicoides paraensis*, as I mentioned. It's got a pretty wide distribution in many areas of the new world, the Western hemisphere. And we have gaps in our distribution. You can see here that most likely Arkansas probably has *Culicoides paraensis*, but none have been reported.

So we're trying to fill in those maps presently and identify potential risks for locally acquired infections that could be predicated by individuals returning that have sufficiently high viremia as to establish these sorts of epizootic or epidemic transmission cycles. But also *Culex quinquefasciatus*, or the house mosquito, which is a common vector of a number of other arboviruses, has been demonstrated to participate in this potential epidemic cycle. And you can see here, it is a very wide distribution in many parts of the United States.

So a large area of the U.S. is potentially at risk for locally acquired infections that we're trying to keep tabs on. We're also making recommendations on entomological surveillance activities. Most vector control districts are geared toward collecting mosquitoes rather than midges. We utilize light traps, but we require specialized meshing for the mosquito traps, because the images are so small comparatively

to mosquitoes. And that is included in the instructions for modifying those light traps that we've provided on our web page. So, again, next slide, please.

So ongoing priority activities are numerous. We're developing interim guidance for pregnant individuals. As we get more information on the frequency and/or timing that could be associated with adverse pregnancy events, that guidance will be changed. Drafting responses so that we can investigate suspected locally acquired infections, and the potential for local transmission and response thereof, and working with our state public health partners in order to get accurate reporting information. So we can assess that risk accurately, also, the ongoing discussions about interim case definitions and the potential that this would be considered a nationally notifiable illness.

Increasing the amount of CLIA tests that are approved, as I mentioned, we have a plaque reduction neutralization testing capability, and a molecular test which covers our diagnostic window pretty well. So I'm actually pretty pleased with where we are sitting in terms of diagnostic reporting of patients, with a good overlap between the neutralization and our acute phase molecular testing. Although, in terms of identifying additional timing in say, acute pregnant women, or women that are within that 7-, 8-day window that might be neutralization test negative, PCR negative, then developing additional tests like IgM testing for timing of infection would be potentially useful, although we don't have very good information on the persistence of IgM following infection. And as many of you know, other bunyaviruses, like Jamestown Canyon virus, can have very extended, long IgM persistence, which can confound our ability to time those infections. So more to come on that, we're actively working on developing a MAC-ELISA and potentially Luminex technology for the capacity to be enhanced for IgM testing as well.

We're also working on increasing our throughput capabilities. So we probably have the capability right now in-house of testing about 1,000 samples, or up to 1,000 samples a week during that acute phase of illness and serum and CSF. But we are working with our laboratory services group in Atlanta and our colleagues in the Dengue Branch in Puerto Rico to increase using robotic extraction methodologies, et cetera, to enhance that throughput capability when and if that is potentially necessary.

So with that, I think that was my last slide, Sean. And I'm perfectly happy to answer any questions that are posed to me. Thank you.

Sean Courtney: All right. Thank you for that update, Aaron. Really appreciate it.

There were a few questions that came in, so we'll try to get through some of them. I had some myself. But I'll work on the ones that other people asked.

All right. So the first one was if there's been any reports of co-infections, such as with dengue or other arboviruses.

Aaron Brault: I have not seen any specific reports of co-infections. I would be shocked if that wasn't happening in many areas where we are seeing active dengue virus transmission in the Western hemisphere. One of the potential problems that we're running into is that samples are reflexed for Zika testing if they're dengue negative.

So if a patient presents with very classical dengue symptoms, which are very congruent with those of Oropouche, then they would be considered a Zika infection. So I think that that is something we

probably need to keep on our radar, that testing a portion of dengue positive cases that we have acute samples by PCR might be a good idea in terms of expanding that time window where we might see positivity by molecular testing, but also where we could be missing this clinical exacerbation by having co-infections. So it's a very well-taken point and something we've definitely been considering.

But that's kind of the limitation that we have right now is the vast majority of cases in many of these areas have been dengue. For instance, our surveillance activities have been to identify dengue negative patients, that then we would queue in for Oropouche testing. Thanks.

Sean Courtney: Thanks. I think that brings up a good point, a good question, I guess, also. Is there any consideration for multiplex testing to incorporate Oropouche with dengue or Zika tests.

Aaron Brault: Yeah, absolutely. That's part of the throughput platforms that are being looked at, was to take the triplex or some of these other assays and multiplex that in with Oropouche. So that would help address that question. Yes.

Sean Courtney: Great. Thank you. Next question is do you have any information on a timeline to add details for the Oropouche testing within the CDC Test Directory?

Aaron Brault: In terms of updating the web page or getting the Test Directory modified specifically for Oropouche testing, that should be online now. So yeah, that should be available currently. And if not, please let me know, and I can follow up on that.

But yeah, the Test Directory, it takes us a little while. So there's probably a bit of a lag with our checks and balances for getting our web pages updated. But you can reach out using those test codes and request testing currently. But there might be a bit of a lag in terms of getting our web pages updated., though.

Sean Courtney: Great. Thank you. What about other specimen types? Have you looked at them, or is it detectable in urine or other specific types?

Aaron Brault: Yeah. We focused on serum and CSF for obvious reasons. We are interested in other specimen types, but those will probably potentially be considered in terms of a surveillance methodology in order to get sufficient samples to validate those clinically. So currently, we have not evaluated urine and other specimen types, given the very limited number of clinical samples that we've actually received to test.

But we're banking negative specimens that we can do molecular contrived samples for that purpose, to start that kind of evaluation purpose for the molecular testing. But yeah, it's definitely something that's on our radar. But we wanted to get our capacity for diagnostic testing up and running and provide that service immediately.

Sean Courtney: Great. That makes sense. Next question, and I think you kind of touched on it a little bit during your talk, so maybe you can just expand on it here. Was this the testing provided at the Atlanta or the Fort Collins or both? And also, whether there any state public health labs you know of that currently have any CLIA-validated LDTs for testing.

Aaron Brault: Yeah. No, sorry if I didn't make that clear. Currently, in terms of the approved testing, is just here in Fort Collins in the Arboviral Diseases Branch for the molecular and the neutralization testing. For molecular, we would like to roll out the higher throughput potentially with surge capacity in Atlanta and San Juan in Puerto Rico. For CLIA-approved and reportable testing for the molecular assays, although that has not happened yet. So they're in the validation process.

So in terms of other CLIA-approved state public health laboratories, I did reach out to a number of our state public health laboratory partners. And I know that New York has got a neutralization test that they are validating currently. And they anticipate that will happen fairly soon.

And Florida is doing the same for molecular testing. So we've had some active discussions with them. So I would anticipate that in the coming weeks a number of other state public health laboratories will be rolling on diagnostic test capacity. But at present in terms of molecular testing, I think it's just Fort Collins. And I would anticipate that other select number of state public health laboratories will have neutralization capacity very shortly.

Sean Courtney: Great. Thank you. Thanks for that clarification. All right. Next question, I probably could have asked before. What about looking at amniotic fluid RNA for the test as well?

Aaron Brault: Yeah, that's one of the specimen types that we've been banking in terms of negative samples for generating contrived specimens. It's just we had to prioritize getting that on board. And so doing a validation on those samples will be something we will reverse engineer into our CLIA-approved testing, potentially. We did not purposely want to do that for our surveillance activities because it would put us in a real conundrum in terms of identifying potential acutely infected individuals for which we could not report back that information.

From a diagnostic perspective, it's definitely something we'd like to do. It will provide us a lot of epidemiological information in terms of timing and risk during pregnancy. But we just onboarded the diagnostic last week, and we're actively looking at doing that now.

IgM testing, I think, will be potentially useful in terms of timing infection, with the caveat that it would be good to have some information on IgM persistence. And then also, testing potentially CSF from neonates, that might be-- and we did that for Zika virus, so analogous to that situation.

Sean Courtney: Great. Thank you. All right, last two questions, and I'll let you go. But this has been fantastic, so thank you. One that I had was actually has the test been evaluated for asymptomatic cases, or is it strictly for symptomatic patients?

Aaron Brault: Yeah, this is specifically for symptomatic cases, so we do want to refrain from testing asymptomatic individuals, given that the positive predictive value would be diminished by testing asymptomatic individuals. And I think, like we saw with Zika, that could usurp and really overload the state and our testing capacity, as well as a number of our state public health laboratories partners, in terms of our testing capabilities. So we do want to prioritize symptomatic individuals.

Sean Courtney: All right. Perfect.

Aaron Brault: Yep.

Sean Courtney: All right. And then the last one, if you're at liberty to share, what are the molecular targets that the CDC assay uses for Oropouche detection. and if there's any data to suggest that these regions are conserved within the currently circulating strains?

Aaron Brault: Yeah. So I'll address the second part of that first. So we're targeting the S segment. This is the Naveca, the modified Naveca primer set, which is the minor groove binding protein probe configuration. And in terms of in silica analysis and as well as analysis of cross-detectability of different strains of Oropouche in our hands, it works quite well. So inclusivity is very good.

Exclusivity, it will pick up other Peribunyaviruses, like Jatobal and Iquitos virus. So we are doing some sequencing confirmations on cases as well, although those other agents are not particularly-- Jatobal is not known to be a human pathogen. And Oropouche, although it does result in clinical illness, has not been known to cause these large outbreaks. So we want to keep that on our radar in terms of the capacity.

In terms of providing specifics regarding primer proconcentrations and those bits of information, we are running it as a single plex assay. And for us to specifically share our assay conditions will probably be more along the lines of our throughput assay as we go through our infectious disease test review board so that can be shared outside of the agency. But yeah, the Naveca primers that are primer and probe sets that are distributed by PAHO work quite well. So that would be our recommendation, that that's a very viable option for state public health laboratories to go.

Sean Courtney: Excellent, excellent. Thank you so much. All right, Aaron, I really appreciate you joining our call today. This was a really fantastic update. So thank you.

Aaron Brault: My pleasure. Thank you.

Sean Courtney: Yeah. And thanks to both of our speakers today. I thought they were fantastic updates on both the mpox and Oropouche outbreaks. So it was a really great call.

Yeah, let's move on. OK. Yeah. So just as a reminder, our next call is going to be held on Monday, October 21, from 3:00 to 4:00 PM Eastern time. This is the third Monday of each month.

Let us know, please, if you have any suggestions for future topics for calls, as we look forward to continuing to discuss these and other important matters for your laboratory and testing community's needs. As we mentioned at the beginning of the call, we will post the audio, transcript, and slides from today's call on the website within the next week or so. And as always, you can follow CDC on social media through the various links shown here.

And yeah, thanks everybody for joining the call today. And we look forward to seeing you next month. Have a good one. Bye.