Transcript

Date of call: 06/26/2023

Welcome
Sean Courtney
CDC Division of Laboratory Systems

SARS-CoV-2 Variants Update
Natalie Thornburg, CDC Coronavirus and Other Respiratory Viruses Division

H5 Update
John Barnes, CDC Influenza Division

Mpox Outbreak Update
Christina Hutson and A.D. McNaghten, CDC Mpox Response

FDA Update
Timothy Stenzel, U.S. Food and Drug Administration

Diagnostic Challenges during Outbreak of Fungal Meningitis Associated with Epidural Anesthesia Performed in Matamoros, Mexico — 2023
Anastasia Litvintseva, CDC Division of Foodborne, Waterborne, and Environmental Diseases

Sean Courtney: All right, good afternoon, everybody. Thank you for joining us for today's LOCS call. My name is Sean Courtney. And I am a health scientist in CDC's Division for Laboratory Systems. On the screen is the agenda for today's call. And before we get started, I want to actually cover a few announcements and some general housekeeping items. I'm trying to move some things here.

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. We've 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 and data science.

As always, we'll be sharing slides from today's call along with audio and transcript. And we'll post them online hopefully by the end of next week or within the next two weeks. You can find them on CDC's LOCS page at the link shown on this page.

And so we want to hear from you. Our training and workforce development branch is interested in hearing more about the education and training gaps you're currently experiencing. And we invite you to send your feedback via email at labtrainingneeds@cdc.gov.

And so we're excited to announce that the CDC/APHL Next Generation Sequencing Quality Initiative has released three tools for each of the 12 CLSI quality system essentials. They're available to download at the link shown on the slide and can be found with a simple keyword search or by using the page's search filters. The NGS Quality Initiative is committed to developing new tools and continuously improving existing products. If you'd like to learn more about the initiative, please visit the NGS Quality Initiative website or contact NGSquality@cdc.gov.

As always, if you have any questions during the presentation today, we ask that you please use the Q&A function within Zoom. So that we can address it during the call and to please not use the chat function. Also, please include your email. So that we can follow up if we're not able to answer your question during the call. And if you're from the media and have questions about the presentation or would like to follow up with the speaker, we like to request that you please contact CDC Media Relations at media@cdc.gov.

And lastly, I'd like to remind everyone that these slide decks may contain presentation material from panelists who are not affiliated with CDC. And presentation content from external panelists may not necessarily reflect CDC's official position. Please keep that in mind when you go back and look at some of the slides that we post on our LOCS web page.

And with that, I'd like to introduce our first speaker for today. We have Natalie Thornburg from CDC's Coronavirus and Other Respiratory Viruses Division. And she'll be providing us with the SARS-CoV-2 variants update. Natalie, I will turn it over to you. And I will stop sharing.

Natalie Thornburg: Great, thank you.

Sean Courtney: All right, you should be good to go.

Natalie Thornburg: All right, so this is just a screen share from our CDC data tracker, genomics data tracker, that updated last Friday. I think since I last talked with you, we've changed the cadence of our reporting and have dropped back to biweekly. So we're updating it every other week.

So we updated last week. And then we'll do it a week and a half from now. And we also changed the binning of data. So previously, the data had been in biweekly bins-- or weekly bins-- and now, they're in biweekly bins. And the main reason for that is just with continued decreased case counts, we are having decreasing numbers of specimens and in order to remain to maintain power to calculate weighted estimates and to do the Nowcast modeling, which estimates proportions of lineages into the present, it was important to generate those biweekly data bins, just for statistical power.

It's good news, though, that we've had continuing downward and low case count trends since, really, January of this year. And thus far, we're still maintaining low percent positivity. So everything-- this is the snapshot right here of the national proportions of lineages. So on the left side are weighted estimates. Those are generated from actual sequences generated. And then on the right side is Nowcast.

So Nowcast is a model. And it's modeled into the present as a reminder. So the weighted estimates, the actual sequences that are generated, are used to calculate growth rates. And then those growth rates are used to predict current proportions of circulating lineages.

Now, just taking a look at this, it looks like there's a lot of colors and a lot of lineages. But there's really-- even though there's a lot of lineages on the data tracker at the moment, they're very, very similar. So really, everything that's circulating right now are XBB viruses. XBB viruses, as a reminder, I will go ahead and scroll down to the dendrogram on the data tracker, XBB viruses down here were recombinant viruses that were descended from BA.2 parental lineages, so two different BA.2 viruses, we combined to make XBB viruses. And everything that's circulating right now are XBB.

Some of them don't look like XBB viruses because they have aliases right now like FE.1.1, EU.1.1. But alias or nickname are assigned whenever too many numbers are added on to the lineage. So even those that don't look like XBB viruses, FD.2, EU.1.1 are indeed XBB viruses.

Many of these viruses, actually, even though they descended from different parental lineages, have either very similar or identical spike sequences. So a couple of new lineages were broken out on the data tracker last week. Those include EU.1.1. EU.1.1 is a lineage-- is a sublineage of XBB.1.5, which was the major circulating lineage in the winter. FE.1 is a sublineage of XBB. Its full name is XBB.1.18.1.1.

And it contains an additional substitution at residue 456 in spike. And then, XBB.1.5.68 was also broken out on the data tracker. And so you can see those three here. They're predicted to be just above 1% of circulating viruses, about 1% of circulating viruses nationally.

So regionally, we're seeing similar, very similar to what we are observing nationally. We're seeing a lot of heterogeneity with lineages. But it's less heterogeneity as far as spike sequences go. So very similar lineages and similar proportions of lineages are circulating in most regions.

All regions have been seeing a decrease in the parental XBB.1.5 lineage. And in every region, it's dropped below a 50% threshold. And that is really the update, the end of my update for the SARS-CoV-2 genomics current situation.

Sean Courtney: All right, thank you for that update today, Natalie. I do not see any questions for you in the chat right now. But I know you have to drop for another call. So if we receive any later, though, I'll make sure to pass them on to you, so we can try to get them answered. But really appreciate you joining today's call and providing us this update. So thank you.

With that, let me get the screen back up. All right, so next up, we have Dr. John Barnes from CDC's Influenza Division. He's going to be providing us with an H5 avian influenza update. And I want to note that he also does not have slides today. So John, turning it over to you.

John Barnes: Yes, thanks, Sean. As you're aware, CDC and our partners are continuing to monitor and respond to the global outbreak of highly pathogenic avian influenza, influenza H5N1. This is the current clade, 2344B has been detected in wild birds across the U.S. in 49 states and the District of Columbia. And it's caused outbreaks in 47 states, affecting more than 58 million commercial poultry and backyard flocks.

Although these viruses primarily infect different types of wild birds and domestic poultry, H5N1 can also infect other animals. And we've seen a wide geographic distribution of H5N1 circulation in mammals as well. For the most part, these have been mammals who are likely to consume or otherwise be in close contact with infected birds. And so reports of sporadic H5N1 virus infections are not unusual or unexpected, given the widespread H5N1 virus infections of wild birds.

So far, there have been 13 human cases reported since 2022 with two additional cases since the last time we gave this update. Both individuals in these last two cases were from the UK, where poultry-- and they were poultry workers who were asymptomatic. Timing and exposure of these cases suggests that one of the workers would likely had contamination of the nose and throat from material that he inhaled.

Although it's unclear that the second case was from contamination or a true infection. There was no human-to-human transmission of any-- that's been identified in any case thus far. CDC still continues to believe that the current risk to the public of H5N1 remains low. However, influenza viruses are very unpredictable and can evolve rapidly.

So continued comprehensive surveillance and frequent reassessments of these viruses are critical to determine the public health risk along with the ongoing preparedness efforts. Because of this, we continue to take the situation seriously. As we've mentioned in the past, we've been undertaking efforts over the past several months to assess and update our preparedness plans and rapidly address any gaps that may have been identified.

CDC continually conducts genetic analysis of viruses to identify changes that may impact disease severity and transmissibility, antiviral susceptibility, and diagnostic test performance. CDC, along with our state and local public health partners, continues to actively monitor people in the United States who have been exposed to infected birds or poultry for 10 days after exposure. To date, more than 6,500 people in 52 jurisdictions have been monitored since 2022.

So, in 2022, the Influenza Division tracked more than 50 human infections with avian influenza A viruses reported to WHO from seven countries and four WHO regions. As you probably know, the CDC has developed a candidate vaccine virus that is nearly identical to the current H5N1 virus circulating. And, therefore, would provide good protection against circulating viruses. The CVV has been shared with vaccine manufacturers at this point.

CDC's existing influenza surveillance systems are well equipped to rapidly detect cases of avian influenza A virus infection in people. Human infection with novel influenza A virus is a national notifiable disease.

And CDC thoroughly investigates every novel influenza case in collaboration with our state and local health departments.

Seasonal influenza viruses-- virus detection assays are used in US states in 170 laboratories globally. And all can detect novel influenza A viruses. There are also specific diagnostic assays to detect current H5N1 virus or H5 viruses that is available in all 50 states and 129 international laboratories.

Additionally, most commercial assays for human influenza virus testing are likely to detect H5N1. And CDC has been working with testing-- commercial testing manufacturers to ensure testing capacity in the event of widespread human cases. As one additional measure, CDC has been working with clinicians, clinical labs, and state public health labs to increase surveillance of novel influenza A, including H5N1, among people who have had severe respiratory disease in the summer months when influenza circulation is lower and influenza testing may not be as commonplace.

CDC is asking that during these late spring and summer months, influenza A positive samples from ICU that are not subtyped in the clinical lab please be submitted to state public health laboratories for subtyping analysis. I should also mention that if you have a BioFire or multi-respiratory panel like the BioFire or Qiagen panels and those have no subtype but are influenza A positive, those are viruses that we desperately want to see in the public health lab for further analysis.

Of course, CDC continues to monitor seasonal influenza activity, particularly in the southern hemisphere right now, as well as other detections of novel influenza. Earlier this month, Brazil reported a human case of variant H1N1v in a 42-year-old woman with an exposure to swine. The illness was severe and ultimately fatal. And the patient-- the patient did have significant underlying health conditions. No human-to-human transmission has been identified at this point.

But we should want to point out that please be aware that any un-subtypeable or non-subtype assay viruses, this is why we want them into the public health labs. So they can be followed up on. CDC continues to monitor sporadic cases of H9N2, H5N6 in China. And while H9N2 cases have been typically mild, H5N6 have been really severe and often fatal.

All recent cases have been associated with exposure to poultry. And no human-to-human transmission has been identified at this point. Thank you for your continued support and prevention and control of seasonal and novel influenza.

Sean Courtney: All right, thank you for those updates today, John. Really appreciate you joining the call. There were a couple of questions that popped up. And so maybe you can answer them here. And the first one is, have there been any cases seen in domesticated birds?

John Barnes: Yes, there have been backyard poultry that had been a bunch of cases of H5N1. That’s been a new feature—a new feature of this particular outbreak that has been more prevalent over the last couple of years.

Sean Courtney: Great, thank you. The next question is, have there been any laboratory-acquired infections of avian influenza, for example, with animal testing?

John Barnes: Not to my knowledge.

Sean Courtney: All right, great. Thank you. And those are the only questions I see currently in the Q&A box. So again, thank you for joining the call today and for providing this update. And if you can hang around if you have the time and if any additional questions pop up, if you could just answer them within the Q&A function, that would be great. I think there was a follow-up. And there was a question about if there were any birds like parrots that have also been seen.

John Barnes: Oh no, most of the-- that I know of, most of the virus have been detected in backyard poultry. I'm not sure of any like parrots, or birds that are normally kept in cages in the house have been affected as of yet.

Sean Courtney: All right, great, thank you. All right, thank you. I appreciate the updates again. Thank you, John. And up next, we would like to please welcome Dr. Christy Hutson from CDC's Mpox Response. She'll be providing us an update today on the mpox outbreak. And Christy, I am handing it over to you.

Christy Hutson: Thanks, Sean. And right as this started, my power went out. So I'm on my hotspot. So let me know if you can hear me okay?

Sean Courtney: Yes, you sound good. Thank you.

Christy Hutson: Okay, great. Thanks, Sean. All right, so good afternoon, I'm Christy Hutson. I'm the Branch Chief of the Poxvirus and Rabies Branch. And we also have A.D. McNaghten on with us as well, who is the Deputy Incident Manager of the Mpox Response. So we're just giving a brief situational update today. Next slide.

So this is just an overview of the cases over the last few months. And you can see that as of last week, June 20, 2023, we had a seven-day average for starting on June 8 at one case per seven-day average. And that trend has continued for the last several weeks. Next slide.

I did want to give this audience an overview of the sort of testing landscape in the U.S. And this is information that the FDA has maintained. It's really helpful to look at the number of tests being used across the United States. So you can see that there is one FDA-cleared test which is held by CDC, which was used from the start of the outbreak by the LRN laboratories as well as four commercial laboratories.

And then there are currently six EUA tests that are approved. I have the link there for those who would like to go there. And then on the right, you can see those six EUA-authorized tests. And there's also 87

laboratories across the country that have notified the FDA that they are running a mpox diagnostic test under their CLIA approval. Next slide.

Another nice measure of the current mpox cases across the country are our wastewater testing. And so this is done-- this is actually a web page on the CDC internet site-- or sorry, on the CDC web page where you can see the amount of testing that's being done, so a total of 463 sites. And I pulled this number on June 21. So, the total number of sites fluctuates a little bit.

But as of that date, we had two sites in Chicago that had intermittent sites over the last four weeks, and then one site in Texas, and one site in Kansas. And as of that date, there were no consistent detects in the past four weeks at any site across the country. And again, this is available for public viewing on the CDC web page. Next slide.

We also wanted to update the Chicago cluster that some of you have heard about. This cluster did initiate a HAN from Chicago, as well as from CDC. And as was just put in the chat, there was an MMWR that was released last week that describes the investigation into that cluster. So, this is the case trend from Chicago. And you can see that like much of the country, after July, August, cases started to decline. And then once we got to May, we did see an increase in mpox cases. Next slide.

So, from March 18 through June 12, Chicago identified 40 laboratory-confirmed cases. All associated with this cluster were males with a median age of 36 years. And 22 were vaccinated with two doses of JYNNEOS. One of them was vaccinated with one dose of ACAM.

Five were partially vaccinated with one dose of JYNNEOS. And then 13 in this cluster were unvaccinated. 11 of these individuals were living with HIV. And 10 were vaccinated with two doses of JYNNEOS or one dose of ACAM and had well-controlled HIV. And the median time from the second dose of JYNNEOS vaccination to mpox diagnosis was 8.4 months. Next slide.

So I'm looking at a little bit more at the details from that cluster. Individuals with two doses of JYNNEOS or one dose of ACAM had self-limiting illness and overall lower prevalence of mucosal lesions. And none of these individuals were hospitalized. Individuals with two doses of vaccine had a higher number of sexual partners in the three weeks before symptom onset compared to those that were partially or unvaccinated.

We did do preliminary sequencing and found that the isolate associated with that cluster is the typical one, B.1 of clade 2B, which is the predominant variant of the mpox outbreak. And there were no mutations found that would confer increased pathogenicity. And it's important to note that this investigation is ongoing. However, we have not seen any similar clusters across the United States that we are aware of at this time. Next slide.

So just some key messages. We have seen the number of new mpox cases in the U.S. trending down since late August. And this is likely because of a combination of vaccination, behavior changes, as well as

possible infection acquired immunity. And while this trend has continued, we do remain-- we do continue to urge health departments and clinicians to remain vigilant, institute appropriate infection prevention and control measures, and notify the public health authorities of suspected cases.

Although-- sorry, last bullet, although vaccine-induced immunity is not complete, vaccination continues to be one of the most important prevention measures. And CDC expects that there may be new cases among previously vaccinated people to occur. But it seems that people who have completed their two dose JYNNEOS vaccine series may experience less severe symptoms than those who have not. And I believe that's my last slide. I'm happy to take questions.

Sean Courtney: All right, thank you for that update, Christy. And apologies, I think the paper just wanted to switch slides on me, so I apologize for that. So there is one question currently in the Q&A. And it is-- does the wastewater positivity rate precede clinical outbreak reporting of human cases? Or does it reflect positivity rate of mpox following clinical case reports?

Christy Hutson: So generally, what we've seen is that when we have the wastewater detects, there are known cases in that jurisdiction. And the shedding-- again, this is viral DNA, not necessarily viable virus-- but shedding of viral DNA from individuals can be for several weeks. If they're immunocompromised, it can actually be for several months before they clear the infection.

So in most of the cases, we do see the case occur and then we see wastewater detects around the same time as those cases or afterwards. There have been some instances where there haven't been known cases. So there's been jurisdiction outreach to let them know that we have intermittent detects. And so that they can reach out to their population to see if there's any undiagnosed cases perhaps, and other vaccine urging, and those other key messages in that area.

Sean Courtney: All right, thank you. I appreciate that. Another point just came up. Are vaccines recommended for healthcare workers or ER clinic workers who treat these patients?

Christy Hutson: So we're following ACIP recommendations. And I'm actually going to-- because I don't remember what the most recent one was for healthcare workers. So I'm going to go find that so I don't misspeak and put it in the chat for everybody. Unless A.D., you know that off the top of your head.

A.D. McNaghten: I don't know that off the top of my head.

Christy Hutson: We'll find it and put it in the chat.

Sean Courtney: OK, great. Thank you. I appreciate that. All right, I do not see any other questions right now. So I want to, again, just thank you both for joining today's call and providing an update on the mpox outbreak here domestically. So thank you for joining us.

And with that, we are going to move to our next presenter. And we have Dr. Tim Stenzel from the Food and Drug Administration. And he's going to be providing us with FDA's update and also does not have slides to share. So Tim, handing it over to you.

Tim Stenzel: Thanks, Sean. Hopefully you can hear me OK.

Sean Courtney: Yes, we can. Thank you.

Tim Stenzel: All right, just a brief update, not significant changes from the previous update, the alerting authorities for a COVID test remains in place. And we are still receiving and processing EUA applications according to the current priorities, which is mainly government funded efforts at this point. We are encouraging all previously authorized kit developers to come in with a full application, either a de novo if it's not been granted before, or a 510K if there's a previous test that has been submitted that you can use as a predicate.

So the bottom line is we're really encouraging kit manufacturers to come in with a full application to get full authorization. So they can stay in the market after the end of the emergency authority. And then for mpox, very similar. The 564 authorities remain in place. Christy gave a good update of available tests.

We are still accepting EUA kit manufacturer submissions if they were previously given the green light based on the published priorities. And there are some that we're continuing to work with. So there hopefully will be some additional EUA authorizations for mpox. That's pretty much it, Sean. I know there's a question in the chat, in the Q&A about tests that have been authorized for saliva.

So we have EUA-approved molecular saliva tests. We have not approved saliva antigen tests. So hopefully that answers that question. Available for any questions.

Sean Courtney: Great, thank you. Yeah, that was actually the one that I was going to ask you. So I appreciate you already jumping to answer that one. And thank you for today's update. I do not see any additional questions in there. So I guess just, again, thank you for joining today's call. And if any additional questions pop up within the Q&A as we continue, if you could just address them if you're available, I would appreciate that. So thank you. Thank you for your update today.

All right, and moving to our last speaker today, we have Dr. Anastasia Litvintseva from the Mycotic Diseases Branch in CDC's Division of Foodborne, Waterborne, and Environmental Diseases. She'll be providing an update on the recent fungal meningitis outbreak. Dr. Litvintseva, handing it over to you.

Anastasia Litvintseva Hi, Sean. Hi, everybody. Yeah, thank you for inviting me. So my name is Ana Litvintseva. I'm the Senior Advisor for Research at the Mycotic Diseases Branch at CDC. And I'll be telling you about the diagnostic challenges about-- during recent outbreak of fungal meningitis associated with epidural anesthesia performed in Matamoros, Mexico. Next slide, please.

So this is a current and rapidly developing outbreak. It has been covered in the media. So some of you have probably heard about this outbreak. It is associated with two clinics in Mexico performing cosmetic surgeries. And it affected people from four different countries. The majority of patients come from the United States and Mexico. However, a small number of people from Colombia and Canada also might have been implicated. Next slide, please.

So we first heard about this outbreak on May 8, where we learned about two unusual cases of meningitis in Texas associated with epidural anesthesia. Although it took some time to identify the etiological agent. Actually, the first real indication of what was causing the outbreak came on May 19 where the Mexican lab was able to detect *Fusarium solani* DNA in one of the patients by real-time PCR.

Fungal etiology has been suggested from early on for a variety of reasons. One of them because all other tests were negative and also because Mexico just recently experienced another outbreak of fungal meningitis associated with epidural injections in other part of the country. Again, diagnostics was slow and challenging. And only on May 28, the first laboratory in the United States was able to confirm *Fusarium solani* in CSF by metagenomics. That was done by University of California, San Francisco. Next slide, please.

So this is an update on cases from last week. Again, all cases have been linked to having procedures, epidural anesthesia in two clinics in Matamoros, Mexico, Clinica K-3 and River Side Surgical Center. Both of them are now closed.

This is the information-- the number of cases is not recent. If you want to see the recent case count, please go to the CDC fungal meningitis website. The only thing I would like to point out from this slide is that, unfortunately, the number of deaths from these outbreaks has increased to six. Next slide, please.

As I said, four countries were implicated, majority of cases are from Mexico and the US. Patients in the US-- patients from 20 states have been involved. But the vast majority of cases came from Texas. Next slide, please.

This slide shows the laboratory summary of what we know about the outbreak as of now. All of the confirmed and probable cases had very characteristic CSF chemistry characteristic of fungal meningitis with low glucose, high protein, and highly elevated white blood cell counts. And just for your reference, in normal CSF, there should be zero, close to zero white blood cells.

Unfortunately, there are no cultures as of this moment. All confirmed and most probable cases that were tested with this test had highly elevated beta-d-glucan levels in their CSF. And there were a couple of confirmations from PCR and metagenomics in the U.S. and Mexico, which all identified *Fusarium* or *Fusarium solani* species DNA in the CSF of these patients. Next slide, please.

And as I mentioned, very interestingly, we don't know the exact connection yet. But in November 2022, Mexico experienced another outbreak of fungal meningitis linked to the same fungal species, *Fusarium*

solani. That outbreak happened in a different part of the country in Durango. And that was associated with OB-GYN surgery specifically with labor and delivery anesthesia.

It was a pretty bad, deadly outbreak. Over 80 patients were identified with meningitis. And unfortunately, nearly half of them died. Next slide, please.

Fungal meningitis due to filamentous fungi is extremely rare disease. And outbreaks are exceedingly rare. The only other big outbreak that happened in this country was the 2012 multistate outbreak of fungal meningitis due to *Exserohilum rostratum* associated with injection of pain medication.

And pretty much everything that we know about the diagnostics and treatment of fungal meningitis due to filamentous fungi, we learned from this other outbreak. Next slide, please. So as far as the laboratory diagnostics of mold meningitis, again, I said it's challenging and very little is known. Next slide, please.

So the best option is obviously culture. It is the gold standard. Having an isolate is really helpful for being able to perform whole genome sequencing, for example, to see whether the Durango and Matamoros outbreaks might have been related. Unfortunately, this case, there are no isolates from this outbreak. And overall, the sensitivity of culture for fungal meningitis due to mold, filamentous fungi, is very low. Back in 2012, only 14% of patients were positive by culture. Next slide, please.

The best method that we have in our arsenal right now is beta-d-glucan detection by Fungitell test produced by Cape Cod Associates. This test detects fungal cell wall polysaccharide beta-D-glucan that is present in most fungal cells. It's a reasonably common test. It is FDA-approved for testing serum. It is not FDA-approved for testing cerebrospinal fluid. However, several commercial laboratories are offering BDG testing on CSF.

And it was very helpful during the 2012 outbreak. All confirmed cases back in 2012, and to my knowledge, all confirmed and probable cases in this outbreak, are positive by beta-d-glucan in CSF with very high titers. The limitations of this test is that, unfortunately, it cannot identify species. Although it can tell that whether it's fungus or not a fungus.

Unfortunately, doesn't work for all fungi. For example, *Mucoromycetes* and a few other fungal groups have different polysaccharides in their cell walls. And therefore, it does not work for those fungi. But it does work well for *Fusarium*, *Aspergillus*, and other molds. And it's known to generate false positives as well. Next slide, please.

Our next best option is pan-fungal PCR, which works by amplifying fragments of fungal DNA present in body fluids. And then it's followed by Sanger sequencing. In the absence of culture, this is a method that can actually identify genus, identify the pathogen down to genus, or sometimes even species complex level.

It's pathogen agnostic. Therefore, it can identify multiple fungi present in the specimen. The laboratory that offers clinical testing by pan-fungal PCR on body fluids, which is located in the University of Washington, they use multiple gene targets to increase sensitivity. And again, it showed decent sensitivity back in 2012.

Initially, the sensitivity of conventional PCR in 2012 *Exserohilum* meningitis was nearly 30%. And then it increased to 47% when the assay was converted to real-time format. It has a few limitations. It's highly dependent upon the DNA extraction. And for fungal meningitis, DNA extraction is really challenging because unlike some cases of meningitis, more conventional meningitis caused by *Cryptococcus*, or *Histoplasma*, or other yeasts that circulate in cerebrospinal fluid, filamentous fungi do not circulate.

Therefore, obtaining these fungal fragments hyphae for extraction is challenging. The best method that worked so far is targeting free circulating DNA present in CSF. And that, unfortunately, requires larger volume of CSF for testing. Of course, presence of DNA doesn't always indicate infection. And PCR is highly sensitive to contamination. Some results, especially with unusual pathogens, can be hard to interpret. Next slide, please.

The newly developed metagenomics next gen sequencing is a very promising method that, unfortunately, is only offered by a single center in University of California, San Francisco. So this method relies on direct sequencing and analysis of DNA. Again, it may be even more sensitive than pan-fungal-- not sensitive, it might be more informative than pan-fungal PCR, because in many cases, it can identify a pathogen down to species level.

And it's pathogen agnostic. And it interrogates the entire genome. If pan-fungal PCR relies on specific primers and targets, this method can detect any traces of pathogen's DNA. And because there is no amplification, there is lower chance of contamination and higher confidence in the result-- in the results. The main drawback of this method, again, high volume of CSF is needed because DNA extraction is really challenging. And it's a very complex method that's only available at a single center right now. Next slide, please.

And the final method I wanted to mention is the method that's only available in Mexico. Very interestingly, our Mexican colleagues, before this outbreak, already had a *Fusarium*-specific real-time PCR developed that specifically can amplify and measure the level of Fusarium in body fluids. It is a commercially available kit in Mexico.

It was the first method to detect *Fusarium solani* in this outbreak. And it appears to be sensitive. The limitations are that it's, again, only available in Mexico. And there is very little we know about this test, including the performance characteristics. And we were not able to obtain PCR primers and probe sequences, which appear to be proprietary. Therefore, we don't know how specific this method can be. Next slide, please.

So with that, I wanted to finish with a compilation of these different websites. If you would like to learn more about the outbreak, there is a link to the CDC fungal meningitis website, a couple of HANS, and a clinician-focused presentation. Thank you. Happy to address questions.

Sean Courtney: All right, thank you for that update today. Really appreciate you joining the call. There's a couple of questions that came in while you were talking. So I'll read you a few of them if you're able to answer. The first one was wondering if the actual epidural was contaminated.

Anastasia Litvintseva: We don't know this yet. This is a hypothesis, most likely, that the pathogen was injected-- it was linked to contaminated medicine. But there is no confirmation yet.

Sean Courtney: All right, thank you. The next question is are post-mortem samples usable for culture? Have any of the deceased undergone autopsy with culture attempted?

Anastasia Litvintseva: To the best of my knowledge, there have been autopsies. The specimens have been sent for culture. Unfortunately, there are no positive cultures yet. But in theory, it should be possible to culture this organism from tissues. But unfortunately, all negative so far.

Sean Courtney: All right, great, thank you. I do not see any additional questions at this time. So I just want to go ahead and thank you again for joining our call today. Really appreciate this update and joining this LOCS Call. So thank you, Dr. Litvintseva. All right, we're going to end a little early today. So I just want to, again, thank all of our callers and all of our speakers today.

As a reminder, we typically hold these calls on the third Monday of each month. And they're scheduled for one hour. Our next call is scheduled for Monday, July 17, from 3:00 to 4:00 PM Eastern time. Please let us know if you have any suggestions for topics for future calls as we look forward to continuing to discuss these hot topics and to answer any of your laboratory and testing community needs.

And as we mentioned before, we will post the audio transcript and slides from today's call on the website within the next two weeks. As always, you can find CDC on Facebook, Twitter, Instagram, and LinkedIn.

And please follow these to stay up to date with the latest news and recommendations. And again, we just want to thank you all for joining us today. And we continue to be very grateful for your work. And we will talk to you again on Monday, July 17. Thanks, everybody. Have a great one.