Good afternoon. I'm Commander Ibad Khan and I'm representing the Clinician Outreach and Communication Activity, COCA, with the Emergency Risk Communication Branch at the Centers for Disease Control and Prevention. I'd like to welcome you to today's COCA Call, Clinical Recommendations for Adenovirus Testing and Reporting of Children with Acute Hepatitis of Unknown Etiology. All participants joining us today are in listen-only mode. Free continuing education is offered for this webinar. Instructions on how to earn continuing education will be provided at the end of the call.

In compliance with continuing education requirements, all planners and presenters must disclose all financial relationships in any amount within eligible companies over the previous 24 months, as well as any use of unlabeled product or products under investigational use. CDC, our planners, and presenter wish to disclose they have no financial relationships within eligible companies whose primary business is producing, marketing, selling, reselling, or distributing healthcare products used by or on patients with the exception of Dr. Elizabeth Moulton, who would like to disclose that she is a sub-investigator for SARS-CoV-2 pediatric vaccine trials with Pfizer and that funds went to institution. She would also like to disclose that she's the principal investigator for a pediatric Paxlovid trial with Pfizer. In addition, Dr. Helena Gutierrez would like to disclose that she's on the advisory board for Albireo Pharmaceuticals.

All of the relevant financial relationships listed for these individuals have been mitigated. Content will not include any discussion of the unlabeled use of a product or product under investigational use with the exception of Dr. Elizabeth Moulton, who may discuss off-label use of cidofovir for adenovirus treatment. CDC did not accept financial or in-kind support from ineligible companies for this continuing educational activity.

At the conclusion of today's session, participants will be able to accomplish the following: Describe the current investigation of cases in Alabama and other U. S. states; Discuss the unknown etiology of cases as they relate to hepatitis and adenovirus type 41; And explain how clinicians can best support the investigation through testing and reporting cases in their states.

After the presentation, there will be a Q&A session. You may submit questions at any time during today's presentation. To ask a question using Zoom, click the Q&A button at the bottom of your screen, then type your question in the Q&A box. Please note we receive many more questions than we can answer during our webinars. If you're a patient, please refer your questions to your healthcare provider. If you're a member of the media, please contact CDC Media Relations at 404-639-3286 or send an e-mail to media@cdc. gov.

We have introduced self-knowledge checks throughout the presentation. We hope you enjoy these opportunities to assess your understanding of today's session. Please do not type your answers to the self-knowledge checks into the Q&A box, as this may disrupt the Q&A portion at the end of the session.

I would now like to welcome our presenters for today's COCA call. We're pleased to have with us Ms. Amanda Ingram, who's an Epidemiologist Supervisor working in the Infectious Diseases and Outbreaks Division at the Alabama Department of Public Health. Dr. Helena Gutierrez, who's a medical director for the Pediatric Liver Transplant Program and an Assistant Professor

for the Division of Pediatric Gastroenterology, Hepatology, and Nutrition at the University of Alabama at Birmingham. Dr. Elizabeth Moulton, who's an Assistant Professor of Pediatrics, working in Transplant Infectious Diseases at Baylor College of Medicine in Texas Children's Hospital. And Dr. Jacqueline Tate, who's an Epidemiology Team Lead for the Viral Gastroenteritis Branch in the Division of Viral Diseases at the Centers for Disease Control and Prevention. It is now my pleasure to turn it over to Ms. Ingram. Ms. Ingram, please proceed.

Before I kick things off, there's one last disclaimer we must provide. The following presentation contains content made by external presenters and not by the Centers for Disease Control and Prevention or the Department of Health and Human Services. This presentation is for informational purposes only and should not be construed to represent any agency or department determination or policy. Any mention of a product or a company in the presentation does not indicate endorsement or recommendation by the U.S. government, CDC, or HHS. Next slide.

First, I'd like to take the time to thank the organizers of this call for allowing us to speak about how this investigation began in Alabama from a local and state public health perspective. Next slide.

Between October 12 and November 1 of 2021, four children under the age of six years presented to the emergency department of a large pediatric hospital in Alabama. All presented with symptoms associated with varying degrees of acute liver injury and liver failure, such as jaundice, severe fatigue, nausea, vomiting, loss of appetite, dark urine, and yellowing of the eyes. All were positive for adenovirus, for which is not known to cause hepatitis in otherwise healthy children. Three were later determined to be type 41. None of these children had any significant underlying health conditions of note that would predispose them to developing hepatitis. Medication toxicity was not a factor as only one was taking medications at home. All resided in geographically different areas of the state and had no apparent epidemiologic linkages. Next slide.

By November 4th, the astute clinicians treating children notified public health authorities and an initial conference call was held to discuss observations and next steps. Participants on the call included, not only the clinicians, but epidemiologists, physicians, and laboratorians from local and state health departments, as well as CDC and Wadsworth Center. Following that discussion, available specimens were requested to be sent to Wadsworth Center for genetic typing and, if possible, sequencing. Next slide.

Meanwhile, since adenoviruses are not reportable in Alabama, we wanted to learn a little bit more about the virus. We already knew that adenoviruses have two main presentations, respiratory and gastrointestinal. In fact, we routinely work on viral pathogens of this nature. For example, influenza and norovirus. One question we had was, is this just the time of year that adenoviruses are detected more frequently in patients? What we found is according to the National Respiratory and Enteric Virus Surveillance System, no matter the test method, respiratory adenoviruses do not demonstrate discernable seasonal patterns. Next slide.

We also wanted to know which types of adenoviruses were most common. Our cases had more of a gastrointestinal presentation as compared to a respiratory one. However, the top six

adenovirus types identified between 2003 and 2016 by the National Adenovirus Type Reporting System were of the respiratory variety. Type 40 and 41 are known to be the two most common gastrointestinal types. Type 41's not ranked in the top ten, and type 40 didn't even make the list. Next slide.

So, being armed with all this information, ADPH issued a call for cases, the first in February 2022 followed by a second in April. Next slide.

The one in February focused more on adenoviral gastroenteritis in otherwise healthy individuals with unusual presentations. We weren't sold just yet that this issue was impacting only children, so we cast a wide net and asked for cases to be reported regardless of age. By the time April rolled around, we were getting a much more focused picture of what we were dealing with. This call for cases mirrored that of CDC a new case with a focus on pediatric hepatitis. We were wanting to know about illnesses that occurred on or after October 1, 2021 among children less than 10 years of age with an ALT or AST greater than 500 and an unknown etiology for their hepatitis. Meaning they were negative for the viral hepatitis A through E. Next slide.

To capture these reports electronically, ADPH created a REDCap Survey where clinicians could not only report potential cases, but also upload the associated lab results and medical records. A team of physicians would review the submissions to determine if the individual met the case definition. They would then reach out to the submitter if they had any additional questions before making their final decision. Of the nine submissions to date, only three have met the current case definition. Next slide.

Although there have been a few alerts and warnings over the last few years related to emergency department visits among children under the age of 10 reporting hepatitis, jaundice, and liver failure in Alabama Syndromic Surveillance Data, none were clustered like they were during the fall and winter of 2021, where one alert and three warnings were observed between November 21st and December 25th. We also looked more specifically at ICD-10 codes related to unspecified viral hepatitis diagnoses, but only three visits were identified between October 2019 and present and none were since October 2021. Next slide.

To date ADPH has identified 12 cases of pediatric hepatitis from various geographic areas across the state. All were hospitalized. Two required subsequent liver transplantation and, thankfully, none of our cases died. Next slide.

Of those, 83 percent have been female, 83 percent white, and 50 percent Hispanic. Persons of Hispanic ethnicity only represent four and a half percent of the overall population of Alabama. Hopefully the exposure interviews will shed some light on the potential significance, if any. The median age of our cases has been two years with a range of zero to six. Next slide.

As displayed on this epidemiologic curve, all but two or 83 percent of Alabama's cases reported were admitted within a five-month span. Next slide.

Adenovirus was detected in all 12 cases. Of those, 91 percent were detected in whole blood. 45 percent were genomically typed as type 41. And 17 percent were other either type 40 or 41, as

they were tested using a GI panel, which can't distinguish between the two types. None met the criteria for whole genome sequencing. However, there is some additional sequencing being performed, which may shed some light on the relatedness of these samples. It is important to note that adenovirus was not the only viral pathogen detected in these children. Nine or 75 percent of these cases had more than one viral pathogen detected at the time of illness. Other pathogens detected included rhinovirus, enterovirus, respiratory syncytial virus, even coronavirus OC43, human metapneumovirus, and rotavirus A. None were positive for acute SARS-CoV-2 infection for which many of them tested multiple times. However, to confirm past SARS-CoV-2 infection did not play a role in these illnesses, available specimens are being forwarded for serology testing. Although we can't say that adenovirus caused the hepatitis in these cases, we can say there does appear to be an association. Next slide.

Only three were old enough at the time of illness to be eligible to receive the COVID-19 vaccine, of which none had received prior to their illness. According to ADPH's immunization registry, only three had received any sort of vaccine in the six months prior to their illness. Next slide.

So, in the days and weeks to come, ADPH will begin combing through the details of the ED visits identified in the alert in the morning shown on one of our previous slides to see if we can identify any additional cases. We are also continuing to review the medical charts of the newest cases identified. And to date the investigation has been more of a clinical focus as opposed to a public health one. So, we're going to start shifting our focus by conducting interviews with the parents of these children to see if we can identify any common exposures using a standardized questionnaire. Hopefully with all the tools in our tool belt we'll be able to start putting the puzzles -- or putting the pieces of the puzzle together, rather, and figure out what's really going on. Next slide.

So, here's your first self-knowledge check for the day. And it's, which virus was not detected in any of the cases in Alabama? A, adenovirus. B, human coronavirus OC43. C, rotavirus A. D, SARS-CoV-2. Or, E, all of the above. Next slide.

The correct answer is D, SARS-CoV-2. Adenovirus was detected in all cases reported in Alabama. There were six other viral pathogens detected, including rhinovirus, enterovirus, respiratory syncytial virus, human coronavirus OC43, human metapneumovirus, and rotavirus A, but at a lesser frequency. SARS-CoV-2 was not detected in any of these cases. So now I'd like to hand the presentation over to Dr. Helena Gutierrez.

Hi, I want to thank the organizers for including me as a presenter in this call. I will be discussing the clinical presentation and evaluation of all our Alabama cohort. Next.

I would like to start by giving some background information on pediatric acute hepatitis and acute liver failure. Next.

Acute hepatitis is defined as inflammation of the liver regardless of etiology. It is basically an umbrella term. With hepatitis you see elevation of liver enzymes, and this reflects damage to the liver. Once the damage is severe, abnormal albumin and INR are also seen. This happens in the setting of impaired liver synthetic function or liver failure. Hepatitis has a wide range of

presentation. It can present asymptomatically as clinical hepatitis. It can present with mild to moderate symptoms of hepatic damage. Here you will likely appreciate jaundice, some abdominal pain and malaise. It can also present in the setting of chronic liver disease or even cirrhosis with ascites, coagulopathy and gastrointestinal bleeding. Lastly, it could also present as an episode of acute liver failure or even acute and chronic liver failure with compromised synthetic function, including hyperammonemia, coagulopathy, low albumin, and hepatic encephalopathy. Next.

The diagnostic evaluation of a patient with acute hepatitis is driven by the history and clinical exam at presentation. The differential diagnosis in the pediatric population is broad, and it can be categorized as infectious versus not infectious causes. Within the possible infectious etiologies, we have many viruses, including the hepatotrophic viruses from A to E and non-hepatotrophic viruses that are named in this table, such as the adenovirus, CMV, enterovirus EBB, HSV, among others. There are also known viral infections, as you could also appreciate in this table. Next.

As previously, mentioned there are also noninfectious etiologies. And these include autoimmune conditions, such as autoimmune hepatitis. Or systemic diseases, such as lupus or juvenile rheumatoid arthritis. They are metabolic conditions and anatomic abnormalities, toxic exposures, and hemodynamic instability or obstruction, which could also cause hepatitis. Next.

Pediatric acute liver failure is an acute onset of liver disease without evidence of chronic liver disease. Here you see biochemical evidence of severe liver injury and, more importantly, you're going to see coagulopathy that does not correct with administration of vitamin K. The definition of acute liver failure in the pediatric population is that a patient's PT has to be greater or equal to 15. Or the INR greater or equal to 1.5 with evidence of hepatic encephalopathy. Or PT greater than 20 or INR greater than 2 with or without hepatic encephalopathy. There's not a lot of systematic data on acute hepatitis, but we do have a significant amount of data on pediatric acute liver failure. So, looking at these in detail, we know that the overall incidence is not determined specifically, but population-wide rates are estimated to be around 500 cases per year. And it is important to know that about 30 percent of these cases are of indeterminant etiology. In 2020, there were close to 500 pediatric liver transplants in the United States, and approximately 20 percent of those transplants had an unknown diagnosis. So, this information speaks about the complexity of this particular diagnosis and the gaps in knowledge that we still have. Next slide.

Now, I will discuss in detail the clinical presentation of our cohort. Next.

We have a total of nine patients with age range from 1.1 to 6.5 years. The majority were below the age of 3. Seven were female, and all patients were of white race and six of them of Hispanic ethnicity. All patients reside in Alabama and no geographic linkage was seen. Next.

Symptoms duration prior to presentation was between a few days to one and a half to two weeks. The most common symptoms were gastrointestinal in origin, including emesis and diarrhea, followed by fever and fatigue. Upon initial physical exam, the majority of these patients had scleral icterus, followed by jaundice and hepatomegaly. Next.

Initial laboratory studies were concerning. Liver enzymes were significantly elevated. Bilirubin, INR, and ammonia levels were anywhere from normal to elevated. The median ALT at presentation was 1,724, with a range between 600 to 4,695. As a reference, a normal ALT value is roughly between 14 to 20. So, the initial median ALT of our cohort was 85 times the upper limit of normal. And those at the upper range had an ALT near 200 times the upper limit of normal. Similarly, the median AST was also significantly elevated at near 2,000, and this is close to 100 times the upper limit of normal. The median total bilirubin was 7 and direct bilirubin was 5.5, median INR was 1.2, and ammonia was 73. Next.

The results of our infectious workup were as following. So, all of our patients tested positive for the adenovirus per whole blood to PCR. The median viral load at presentation was 11,000 copies per ml. Six out of nine patients were positive for EBV per whole blood PCR with a median viral load of 1,680. Five out of six patients had serology done, and they were all negative for EBV, IgM. And four out of five were EBNA positive. All nine patients tested negative for COVID by nucleic acid amplification testing. All nine patients that tested negative for viral hepatitis A, B, and C. Multiple viruses were detected in respiratory PCR panel. This was done in eight patients, and four out of eight were positive for enterovirus and rhinovirus. And one out of eight was positive for either metapneumovirus, RSV, or seasonal coronavirus. Only one patient tested positive for the adenovirus by respiratory PCR. And one out of three patients was positive for stool adenovirus PCR. Next, please.

As part of the workup, we looked into the possibility of autoimmune hepatitis. Five out nine patients had elevated IgG, and four out of nine patients had one positive autoantibody either ANA or SMA in the low range, so anywhere from 140 to 180. Taking this into consideration along with the liver biopsy findings and the clinical course, none met the criteria for autoimmune hepatitis. We also looked into the possibility of an underlying metabolic condition. Serum amino acids were checked in five patients, and the results were either nonspecific -- which can be seen in the setting of severe illness -- or normal. Similarly, urine organic acids were checked in five patients. And, again, results were reassuring. We also obtained an acyl-carnitine profile in four patients, and we found one patient to have nonspecific abnormalities, and three patients had normal results. Alpha-1 antitrypsin 7n deficiency was evaluated by obtaining phenotypes, and five out of five patients had normal, paranormal phenotype. We looked at, for the possibility of Wilson disease, which typically does not present this early, but ceruloplasmin levels were obtained and weren't normal. Acetaminophen levels was checked in seven patients, all within normal levels. And not written here, but all nine patients had liver ultrasounds, which did not show any type of anatomical or vascular abnormality. Next.

Six out of nine patients had a liver biopsy. All biopsies had variable degrees of acute inflammation from lobular to interface to portal. There was some canalicular cholestasis that was appreciated, and spotty hepatocyte necrosis was also appreciated in some biopsies. Fibrosis was minimal to not present. None of the biopsies demonstrated confluent necrosis, which is typically seen in the adenovirus hepatitis. Additionally, adenovirus was not seen in immunohistochemistry. Neither was identified as viral inclusions in electromicroscopy. EBER in situ hybridization was negative on all patients that had a liver biopsy done. And adenovirus PCR was positive in liver tissue in two out of three patients. However, it isn't clear if this is secondary to adenovirus in liver tissue or contamination from blood at the time of liver biopsy. Next.

The clinical course of our patients was significantly different between the patients who had severe hepatitis and those who are acute liver failure. So, six patients did not progress to acute liver failure and only had severe acute hepatitis. This cohort had statistically lower liver enzymes upon presentation with a median ALT of 911 and a median AST of 1,626, versus 3,854 and 4,000 in the group with acute liver failure, respectively. The median viral load of adenovirus was also significantly lower. It was 9,262 versus 55,340 copies per ml. All of these patients recovered with their native livers. At clinic follow-up, with a median of 38 days, three out of five patients still had adenovirus viremia with a median of 2,000. Our second group of patients either presented with acute liver failure or progressed into liver failure. The median peak of INR was 8.8. And the median peak of ammonia was 169 for this group. One patient recovered spontaneously and is doing well with a native liver. Next.

But two patients presented with acute hepatitis and progressed to liver failure. Their adenovirus median viral load at presentation was significantly higher when compared to those patients who recovered spontaneously. The median viral load for this group was 63,000 versus 7,465 in the group, in the other group. Both patients received cidofovir. Despite treatment, their adenovirus viral load remained elevated with a median peak close to 100,000. Both patients developed hepatic encephalopathy and ascites. One patient required continuous renal replacement therapy; one patient required total plasma exchange. One patient met criteria for hemophagocytic lymphohistiocytosis and subsequently started on etoposide and dexamethasone. Both patients were ultimately transferred to another quaternary hospital for possible extracorporeal liver support to serve as a medical bridge to liver transplant. This was done in the setting of the adenovirus uncontrolled viremia on the possibility of these being a contraindication for liver transplant. However, these, both patients subsequently underwent successful liver transplant, and they are both currently doing well. Next.

A measurement for liver dysfunction includes: ALT and AST, INR, Serum albumin, B and C only, or All of the above. Next.

The correct answer is D. The coagulation proteins needed in the blood clotting cascade are primarily produced in the liver. Severe liver injury leads to a reduction of the liver synthetic function and, therefore, decreased synthesis of clotting factors, which translates into elevation of INR. Albumin is produced by the liver and, therefore, serum albumin is low in liver failure. Thank you. Next.

Hello everyone. I'm delighted to be able to talk a little bit about adenovirus and what was previously known about adenovirus hepatitis as well as what is recommended for testing in these cases of acute hepatitis of unknown etiology. Next slide.

So, adenoviruses are a family of double-stranded DNA viruses. And there are many different types of adenoviruses, which are grouped into species, which are denoted by letters. I'll draw your attention to species C, which is the group of adenoviruses that was previously known to be associated with hepatitis, usually in immunocompromised hosts. The other virus type that you've already heard mentioned is adenovirus 41, and that's in the species F. And that typically causes gastroenteritis in infants and young children. Next slide.

So, to start I'll talk a little bit about adenovirus hepatitis. And this is a paper that was looking in the literature and collected the cases of fulminant adenovirus hepatitis. And to be included in this cohort, you had to have adenovirus detected from the liver itself directly. They had a total of 89 patients in this cohort, and that included nearly two-thirds of which were pediatric age. It's worth pointing out that 98 percent of these patients had an underlying condition. The most common of which was actually a preexisting liver transplant, but also bone marrow transplant, other solid organ transplants, and neonates would be included. These patients presented with fever as a very common symptom in 92 percent. Whereas, diarrhea and jaundice really occurred in the minority of patients. Of the 68 patients that had histopathology reported in the literature, 94 percent of them demonstrated necrosis, 72 percent had intranuclear inclusions, and 21 percent had smudge cells. And I'll show you some images of this later. And then of the various methods of detecting adenovirus in the liver, nearly two-thirds were positive by culture. 60 percent were positive by immunohistochemistry, meaning 40 percent were not. Electromicroscopy was positive in 54 percent. And then in a few cases, they either used PCR in situ hybridization. And then, finally, it's worth noting in these, you know, severe cases of fulminant adenovirus hepatitis, the survival was only 27 percent, and that's likely related to the severe immunocompromising conditions that these patients had. Next slide.

Along those same lines, in immunocompromised patients, the main stay of treatment for adenovirus is actually reduction of immunosuppression, and that was done in about half of the patients. Of -- there's a smattering of other patients that were treated with cidofovir as a possible antiviral with activity against adenovirus or IVIG as a supplement to provide antibodies against viruses in general. Of the patients who received liver transplant, 12 of whom required retransplantation, which had about a 50 percent survival. And out of those 12, there was only one case of recurrent adenovirus hepatitis. Next slide.

This is a separate study that was looking at 12 consecutive cases of adenovirus hepatitis and the histopathology that is seen there. In these samples, the biopsies are denoted by B, and A is for autopsy. They saw necrosis in all of the samples throughout. And that could either just be spotty necrosis, or it could be up to massive necrosis. Interestingly, in these cases they actually didn't see a ton of inflammation. Over half the cases had very minimal inflammation. And possibly that could be related to the underlying immunocompromising conditions that many of these patients have, such as bone marrow transplant or liver transplant perhaps preventing a robust immune response. In almost all of the patients, they saw viral inclusions. And all but one were positive by immunohistochemistry. Next slide.

So here on the left of the screen, you can see the kind of classic smudge nuclei that are common with adenovirus, the characteristic with adenovirus infection. And on the right you can see a more well-developed viral inclusion that actually has a central eosinophilic component. Next slide.

And this just depicts the variation that you can see in necrosis. Either kind of focal necrosis with the white arrow, versus more confluent coagulative necrosis that can occur with more severe infections. Next slide.

So, switching gears a little bit to focus on adenovirus 41. One of the questions that came up was about whether or not adenovirus 41 is really restricted to the gastrointestinal tract or whether you have viremia. And one study has looked at this. In Brazil, they took children hospitalized with gastroenteritis. And of the 110 patients who were positive by adenovirus in the stool, 80 had positive -- had sera that they could test. And a little over almost two-thirds of them showed positive results in the serum. About half of those patients had concordant results. Meaning that the stool and the serum adenovirus typing matched. And of those, 80 percent of them were species F. And so that includes 40 and 41, suggesting that viremia may occur in the setting of regular routine gastroenteritis. Next slide.

So, along those lines, in these cases where we know at least some of the patients have been associated with adenovirus 41, the CDC has recommended testing for adenovirus in a variety of specimen types. Nucleic acid amplification testing, such as PCR is preferred. Because typically that is more sensitive than antigen testing or cultures. For the blood specimen, the recommendation is to test whole blood, if possible, to plasma based on the fact that, when we received several patients for transplant and tested them with the plasma, despite them having previously tested positive on whole blood, they were negative. And then, when we went back and tested whole blood samples on them, they were indeed positive, suggesting that at least some of these patients, the whole blood may be more sensitive than plasma. So that's the reason for that recommendation. Adenoviruses commonly caused respiratory infections, so we want to definitely test a respiratory specimen. And adenovirus 41 typically causes gastroenteritis, and so testing the stool specimen is recommended. If a liver biopsy is being performed because it's clinically indicated or there's tissue from an explant or from an autopsy, there can be additional testing done on that as well. Next slide.

So, taking this kind of sample by sample. For whole blood, recommendation is to test with a quantitative adenovirus PCR. And this is probably not available in many local centers. And there are two reference laboratories, both ARUP Laboratories and Quest Diagnostics that offer this testing. And so, for example, with ARUP, the results will be reported at, as either 1,000 to 10 million copies per ml. But the number you also want to look at is the log of the copies per ml. And the reason for that is, is that changes of less than half a log can reflect the variability that's inherent in this testing mechanism as opposed to necessarily a real clinical change in the level of the virus in your patient. And for both of these labs, it's important not to freeze the whole blood sample prior to sending it for testing. Next slide.

For respiratory testing, your adenoviruses is part of many of the respiratory viral panels or multiplex PCR testing. If you're looking for adenovirus specifically, there are three laboratories that offer testing that should detect adenovirus 41. And those include Eurofins-Viracor, which offers both a qualitative and a quantitative adenovirus test, depending on the specimen type. And then ARUP Laboratories and Quest Diagnostics also offer testing as well. Next slide.

For stool adenovirus PCR, adenovirus 40/41 testing is available on some gastrointestinal multiplex PCR panels. And then for reference labs, Eurofins-Viracor offers a qualitative adenovirus test that will detect all of the different serotypes of adenovirus. And then it's also worth noting that adenovirus 41 does not grow on a routine viral culture because it needs a special cell type. So viral culture, especially, is not good for this testing. Next slide.

For testing liver tissue specifically, there are a couple of options. Eurofins-Viracor offers a qualitative test, which is what we use for our liver transplant recipients. When they need a liver biopsy, they'll take a needle core, and they can, are able to give a qualitative result about whether a virus is present. And they also offer a quantitative test on a slightly larger piece of tissue, which would be helpful for a native liver that's explanted to assess whether or not adenovirus is perhaps present. Both ARUP Laboratories and Quest Diagnostics also offer qualitative testing on tissue as well. Next slide.

As far as adenovirus typing, the recommendation is, if you have enough volume, to prepare one aliquot for diagnostic testing and save one aliquot set aside for adenovirus typing should the diagnostic testing be positive. For the whole blood, again, it shouldn't be frozen for diagnostic testing, but for typing it should be frozen, ideally at minus 70 or colder, if that's available. If you have any patients with acute hepatitis who are testing positive for adenovirus, if there were either aliquots that were saved or any residual clinical specimens, those should be kept frozen at minus 70 until it can be arranged to be sent for adenovirus testing through the state public health laboratories. Next slide.

So, a brief self-knowledge check. A three-year-old, previously healthy boy developed vomiting/diarrhea six days ago. Vomiting improved, but he has poor appetite and diarrhea continues. Parents noticed last night that his eyes appeared slightly yellow, and this morning he is jaundiced prompting presentation. His ALT is 2,000, AST 2,500, and total bilirubin is 8. Testing for hepatitis A, B, and C is negative. What would be the best test of the following to evaluate for adenovirus in this patient? A, adenovirus quantitative PCR on plasma; B, adenovirus quantitative PCR on whole blood; C, adenovirus qualitative PCR on whole blood; D, B and C only; Or, E, all of the above. Next slide.

The correct answer is B. And the reason for that is because we've seen on several patients that adenovirus is detected in the whole blood, but has not been detected in the plasma in patients presenting with adenovirus-associated hepatitis. The quantitative PCR measures the level of the adenovirus present in the blood. And, in general, for adenovirus infections, higher levels of viremia correlate with more severe disease. And the trajectory of the adenovirus viremia can be monitored over time, ideally using the same laboratory and specimen type, such as whole blood. And this is especially useful in patients who have severe hepatitis or acute liver failure where you are thinking about transplantation. Next slide.

In searching the literature, there's very little known about the differences between plasma and whole blood. What is known in this small study, when the whole blood and plasma were both positive, they correlated nicely with a correlation coefficient of 0.98. However, they did have one sample that was positive in whole blood at quite a high level, at 33,000 copies. The two samples that were positive in plasma were only positive at a low level. Next slide.

And, finally, I'll just close on some basics for infection control and prevention. So adenoviruses are non-enveloped viruses, and makes them difficult to inactivate with an alcohol-based hand sanitizer, which is why washing hands with soap and water is so important. Adenoviruses, again, may remain viable on skin fomites and environmental surfaces for an extended period of time, and so it's important to consider contact and droplet precautions if you have children with acute

hepatitis for the duration of their illness, unless an alternative etiology is identified. Next slide. Next slide. And with that, I will turn it over to Jacqueline Tate from the CDC.

Thanks, Dr. Moulton. Today I'm going to talk about the nationwide investigation of pediatric hepatitis of unknown etiology. Next slide, please.

A Health Alert Network Health Advisory, known as the HAN, was issued on April 21st.It notified U. S. clinicians and public health partners about the cases investigated in Alabama, recommended adenovirus testing in children with hepatitis of unknown etiology, and requested possible cases of hepatitis of unknown etiology in children less than 10 years of age to be reported. Next slide, please.

Other countries around the globe have seen an increase in cases of pediatric hepatitis of unknown etiology. Most notably the United Kingdom. In a technical brief issued by the UK Health Security Agency on May 6,163 cases of non-A through E hepatitis in children less than 16 years of age with transaminases above 500 were reported from January 1st through May 3rd of this year. Most cases occurred in children less than five years of age with a gastroenteritis-type prodrome followed by the onset of jaundice. Adenovirus was detected in 72 percent of the cases that were tested. And of the 18 cases that were successfully subtyped, all were type 41F. Community transmission rates of enteric adenovirus have been increasing in the UK since the end of 2021. SARS-CoV-2 was the next most common pathogen identified and was detected in 18 percent of cases. Additional testing identified a smaller number of cases with other viruses. The leading hypothesis in the UK is that a cofactor affecting young children is rendering normal adenovirus infections more severe or causing them to trigger immunopathy. However, investigators in the UK continue to examine the role of SARS-CoV-2 and are working to rule out any toxicology component. Next slide, please.

Cases continue to be reported globally. Two hundred and thirty-two cases in children less than 16 years of age have been reported from 14 countries in the WHO Euro region. Over two-thirds of cases occurred in children less than five years of age. 6 percent required liver transplant, and one death has been reported. Of those that have been tested for adenovirus, 60 percent were positive, with the highest positivity of 69 percent seen in whole blood specimens. 12 percent were positive for SARS-CoV-2 by PCR. And 14 of 19 tested had antibodies to SARS-CoV-2. Seventy-two cases have been reported by 12 additional countries globally. Next slide, please.

In the United States, we have received preliminary reports of 180 patients under investigation through May 18, 2022. These patients have been reported from 36 jurisdictions, geographically distributed across the country and are not concentrated in time. Most, over 90 percent, have been hospitalized, and 8 percent required liver transplantation. Five deaths are under investigation. Adenovirus has been confirmed in over 50 percent of patients under investigation, with the highest positivity seen in whole blood specimens. Next slide, please.

While we think that adenovirus may be the cause of these reported cases, we are also exploring other potential exposures, including medications, other infections, toxins, food, water, et cetera. We are keeping an open mind and focusing on hypothesis generation. We will continue to share

information as we learn more and will focus the investigation based on our findings. Next slide, please.

We currently have several working hypotheses. The first is that these cases are associated with adenovirus, and specifically adenovirus type 41 infections with or without a cofactor. We are examining whether we are seeing an unusually large enteric adenovirus season that may be unmasking a phenomenon that has always occurred and may be magnified by a lack of exposure to adenovirus during the COVID-19 pandemic that has resulted in a higher number of susceptible children. There could also be altered susceptibility or host response to adenovirus due to lack of exposure during the COVID-19 pandemic. Co-infection with another pathogen, such as SARS-CoV-2. Prior exposure to SARS-CoV-2. Or exposure to a toxin, drug, or environmental exposure. Or there may be a novel variant of adenovirus. Finally, there may be other causes alone without adenovirus, such as long COVID. A toxin, drug, or environmental exposure. Or a new pathogen. Next slide, please.

There is some evidence supporting the role of adenovirus infection, and specifically viremia as seen in more than two-thirds of patients across many countries. Limited data show greater viral load in children who required transplant. The affected age group is the typical age group infected with adenovirus. And this age group may have increased susceptibility due to lack of exposure during the pandemic. Some patients reported a preceding gastrointestinal illness. And there is no clear alternate hypothesis so far. Next slide, please.

However, there is also some evidence that questions the role of adenovirus. Adenovirus is not a well-recognized cause of hepatitis in healthy children, and the pathology is not consistent with typical adenovirus hepatitis in immunocompromised children. Evidence of viral infection has not yet been detected in the liver. Metagenomic studies in the UK did not find adenovirus infection. Limited sequencing data is indicated in multiple strains of adenovirus. Next slide, please.

Our investigation in the United States, we're using a case definition of children less than 10 years of age with elevated transaminases, who have an unknown etiology for their hepatitis with or without any adenovirus testing results. So independent of adenovirus testing since October 1st, 2021. State public health departments should be notified of any cases meeting this definition. Next slide, please.

For each identified patient under investigation, we are requested a detailed chart abstraction to collect information on the clinical course of illness and results of any tests that have been performed. We also have a questionnaire that collects information on a wide variety of potential exposures. We are also requesting some additional lab testing. Next slide, please.

A standard diagnostic workup for acute hepatitis should be done locally per the treating clinicians. Pursue a diagnostic workup for all likely causes of acute pediatric hepatitis, including a comprehensive infectious workup, immunological workup, et cetera. CDC is recommending including adenovirus testing in children with acute hepatitis. We are still investigating the potential relationship between adenovirus infection and acute hepatitis, so consider ordering a clinical PCR adenovirus test for all of the following specimens, if available. A respiratory

specimen. A stool specimen. And a blood specimen with a preference for a whole blood specimen. Next slide, please.

There are three types of testing for the epidemiologic investigation. Diagnostic adenovirus testing on respiratory stool and blood specimens. If you do not have access for testing for these specimens locally, contact your state public health laboratory. Save all adenovirus positive specimens for typing. And save any liver tissue from liver biopsy for native liver explant or autopsy, following local clinical diagnostic review. Next slide, please.

Additional guidance for testing of specimens was provided in an updated health advisory issued on May 11th. Links for this information can be found on the cdc.gov website. Next slide, please.

The self-knowledge check. For patients under investigation, clinicians should considering -- should consider ordering a clinical PCR adenovirus test for which of the following specimens, if available: A, respiratory specimen; B, a stool specimen or rectal swab; C, a blood specimen; Or, D, all of the above. Next slide, please.

The answer is D, all of the above. Because we are still investigating the potential relationship between adenovirus infection and acute hepatitis, clinicians should consider ordering a clinical PCR adenovirus test for all of the following: The respiratory specimen, a stool specimen, and the blood specimen. Next slide, please.

Thank you. And I will now hand the presentation back to the moderator.

Presenters thank you so much for providing our audience with this timely information. We will now go into our Q&A session. Please remember that to ask a question using Zoom, click the Q&A button at the bottom of your screen, then type your question. Please note, we receive many more questions than we can answer during our webinars. For our first question, this is open to all our presenters. Please identify yourself and answer the question as you see fit.

Aside from these severe cases that were discussed, is there a general increase in milder hepatitis cases of unknown etiology?

This is Jackie Tate. I can take that question. Right now we're looking at various sources of data, syndromic surveillance data and other data sources to get a sense of what the underlying trends are in pediatric hepatitis, particularly pediatric hepatitis of unknown etiology. We're still evaluating that data and working on refining our case definitions. Right now we're not seeing any notable trends, but we will continue to look at the data and report what we find. Thanks.

Thank you very much. Our next question asks, since these cases are quite young, will the clinicians that presented on them, can they answer if there will be more information collected about the mother's health care during her pregnancy?

This is Jackie Tate. I can take that one again. Currently we have quite a detailed case investigation form. We are not collecting information on the mothers per se. Most of the information that we are collecting is about the children themselves.

Thank you very much. The next question is regarding sort of the global information. Have, globally has more common exposure sources been identified yet, looking across the world?

This is Jackie Tate. I will take that. The other country that has quite extensive information and has done, looked at a lot of cases and has a lot of cases is the United Kingdom. They have also not identified any common exposures among their cases. Adenovirus is also their leading hypothesis. They have detailed exposure questionnaire similar to what we're using in the United States. And they've not yet reported on anything, any exposures that they think may be likely to be associated with these cases.

Thank you very much. The next question is regarding some of the case studies that were shared. Were there any remarkable findings in stool samples?

Hi, this is Dr. Gutierrez. As you, as everybody might remember, so out of the nine patients that were covered, only three of them had stool samples, and they were all negative except for one. And we did not see any other pathogens during, on these stool samples.

Thank you for that. Regarding the patients who were transplanted, of the percentage that you shared, how many of them were adenovirus positive?

Hi, this is Dr. Gutierrez again. So all of them. All of the nine patients from our cohort were adenovirus positive. So the patients who needed a transplant had an initial presentation of viremia.

Thank you very much. Another question related to transplanted patients. What were the pathological findings in the livers that were removed?

This is Dr. Moulton. I'm happy to take that. So one patient had lymphohistiocytic lobulitis with a predominant of CD8 positive T-cells and about 20 percent had hepatocyte loss. The other patient had 80 to 90 percent necrosis with I think a slightly different inflammatory infiltrate. Neither of them actually had evidence of hemophagocytosis on the explant.

Thank you very much. Can you all please speak to the possibility of active infection versus reactivation when encountering a positive whole blood PCR?

This is, again, Dr. Moulton. I'm happy to take that. I think adenoviruses, certainly thinking about reactivation is important. You know, these patients often have some kind of preceding GI symptoms, which made us think more along the lines of an acute infection. And then the other question that happens a lot is about whether or not we're detecting the virus in the liver. And I think because this is sort of a newish strain of adenovirus, not one that we typically think of causing hepatitis, some of the tools that we would usually use, like immunohistochemistry, you know, may not be as optimized for this specific strain of adenovirus. And in talking about the pathology, especially on those explants and reviewing them with our pathologists, you know, if the pathology had stained positive for adenovirus, then it would be a different interpretation, and it would be a diagnosis of adenovirus hepatitis. But right now, you know, we still are kind of

working on improving our tools to better understand exactly what's going on in the liver of these children.

Thank you. Our next question asks, earlier it said that the patients were, did not meet the criteria for whole genome sequences. What would be the criteria for the patients to meet, to ensure whole genome sequences conducting?

Yes, this is Amanda. I can take that question initially. And maybe Jackie wants to take the tail end if I miss something. But what we were told is that there needed to be a CT value less than 33 for it to even meet the criteria. And we didn't have enough virus in order to be able to test the specimen for whole genome sequencing.

Great, thank you. And we have time for one last question. And the question asks, are there any hypotheses that the presenters have about why the pathological findings were not consistent? Or at least appear in some cases not be consistent with the adenovirus here?

This is Dr. Moulton. I'm happy to start with that. I think part of the problems is, as I mentioned, is the tools. If we had staining that was positive for adenovirus, that would change the interpretation of the pathology, where we're often seeing kind of an inflammatory infiltrate, but not necessarily evidence of virus. It also raises the question about whether or not you could have an adenovirus infection, say in the gastrointestinal tract, causing gastroenteritis and leading to an immune response to the adenovirus that is then causing a misdirected immune response against the liver for some reason. But at this point we don't have a good answer about is the virus in the liver itself causing, triggering this versus perhaps infection in other sites that are leading to an inflammatory response leading to liver injury.

Thank you very much. At this point I want to thank everyone for joining us today with a special thanks to our presenters. Next slide please. And next slide.

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