APHL All Member Call:
Highly Pathogenic Avian Influenza A(H5N1)
May 29, 2024

Call Summary

Contact for Questions or Suggestions
APHL Influenza Contact: fluquestions@aphl.org

Situational Update on Highly Pathogenic Avian Influenza (*Todd Davis, Ph.D., MSPH, CDC*)
USDA has reported 67 dairy cattle herds with confirmed H5 across nine states. There have been reports of positive cases in other animals as well, including alpaca herds in Idaho. CDC and USDA are working together to update recommendations on livestock exhibitions and attendance at county and agricultural fairs, which typically focus on swine influenza viruses, but we are adding additional language on highly pathogenic avian influenza.

A human case of conjunctivitis was identified from a dairy farm worker in Michigan last week. Michigan is monitoring individuals that are exposed to animals infected with H5 using a text-based reporting system. The individual reported eye redness with discharge and some discomfort, but no respiratory symptoms were reported and the illness has resolved. The nasal pharyngeal (NP) swab was negative while the conjunctival swab was positive for H5.

CDC was able to isolate the virus from the conjunctival swab and has also sequenced the full genome; the genetic analysis is described in a technical report. The virus was identified as clade 2.3.4.4b with each individual gene segment closely related to genotype B3.13 viruses detected in dairy cows, as per USDA testing. No amino acid changes were identified in the hemagglutinin (HA) gene sequence from the Michigan patient specimen compared to the HA sequence from the case in Texas and only minor changes were identified when compared to sequences from cows. There were no molecular markers associated with reduced susceptibility to oseltamivir or baloxavir. Across the genome there were no specific molecular markers associated with mammalian adaptation. One notable change is described in the technical report, but to summarize, it is a mutation that has been identified in more than 99% of all viruses from cattle but was not found in the previous human H5 case from Texas. There is a preprint publication from USDA that describes in more detail why this mutation is important, but it is associated with mammalian adaptation in avian influenza viruses that cause increased replication, fitness and mammalian adaptation in mice models.

Laboratory Testing Updates (*John Barnes, Ph.D., CDC)*
CDC was granted FDA enforcement discretion on conjunctival specimens to be tested with the H5 subtyping kit. FDA wants PHLs to remain testing conjunctival specimens as a pair with NP swabs to mitigate the risk of misdiagnosis in a patient with conjunctivitis and no corollary respiratory symptoms. Conjunctival specimens should be collected and transported using the same swabs and transport media as NP swabs (i.e. viral transport media (VTM)). CDC is also required to notify FDA within 7 days of any complaints, regarding the performance of the assay under those conditions. The enforcement discretion is until August 1, 2024, with the possibility of extension if appropriate.

In summary, conjunctival swabs are only recommended for testing in patients showing signs of conjunctivitis and should always be paired with a NP specimen. All other elements of the test remain the same (e.g. extraction platforms, PCR master mix, etc.) so at this time CDC plans to utilize this enforcement discretion and will not update the instructions for use.

The summer surveillance guidance has been finalized. Essentially, PHLs are being asked to continue with the seasonal guidance and all three National Influenza Reference Centers (NIRCs) will be active. This means PHLs should continue to submit up to the following every other week:

- 6 Influenza A(H3N2)
- 4 influenza A(H1N1)pdm09
- 4 influenza B/Victoria

If you are in a state with an Influenza Sequencing Center (ISC), submit to the NIRC first and then sequencing remaining specimens for your surveillance.

CDC is working with commercial laboratories to encourage them to submit influenza positive specimens to PHLs, especially those without subtyping results. If you have contacts with clinical and commercial laboratories, please reach out and encourage them to submit influenza specimens to public health for surveillance.

CDC recognizes many states will not reach the surveillance goals due to low levels of influenza activity. The enhanced surveillance request is an effort to prove that we are not missing cases of influenza A(H5).

The inventory of influenza A(H5) subtyping kits is 750 currently in stock at IRR. We are building another approximately 1200 kits and will kick off another round of manufacturing of subtyping kits to ensure there is enough capacity for additional testing.

Michigan Testing Activities and Conjunctival Swab Validation (*Marty Soehnlen, Ph.D., MPH, HCLD(ABB), Michigan Public Health Laboratory)*

Michigan has over 20 dairy facilities and multiple poultry facilities that are considered positive for influenza A(H5). There are 284 herds of cattle in Michigan and not all of them are choosing to have screening or testing done. So far over 20 poultry facilities have been culling their flocks. There has been consistent sampling of wild animals around the farms that are impacted and there have been numerous animals that have been determined to be positive. Animal testing is all done through the veterinary diagnostic laboratory and USDA.

Two cats have tested positive as well, from households of farm workers from different dairy farms that were impacted. In one of those cases, the owner has not presented for testing and is currently refusing calls from the local health department. The second case had 3 cats in the household. All three cats became ill and symptomatic. The first cat from the household died (positive for H5 and negative for rabies). The other two cats are improving and one has resolved neurological symptoms.

The state PHL is providing collection supplies to local health departments, who can order additional collection kits online through a special inventory system. The state lab provides advice and collection instructions to clinical laboratories for what specimens are accepted. To date, they have not had issues getting the correct paired specimens.

The positive human case had paired NP and conjunctival swabs. The testing performed at CDC confirmed the conjunctival swab was positive, the NP, which was also testing in Michigan, was negative. The conjunctival specimens are only being tested using the H5 subtyping assay, but NP specimens are going through their full influenza testing algorithm. They start with biofire, followed by typing and sending both positive and negatives

at this time down to CDC. Any specimens with presumptive positive results have a re-collection from the patient and the specimen will follow BSL-3 inactivation before it is worked on the rest of the way through the algorithm.

They have tested 60 patients and will likely continue testing more. They have started a rolling verification of the conjunctival specimen type. They are testing the conjunctival swab and sending to CDC for confirmatory testing, that way they can match back results and appending that to our standing validation for influenza testing. This approach was approved by our laboratory director and other PHLs may consider a similar approach.

We are not issuing written reports for conjunctival specimens, but instead are providing verbal reports to epi and the formal report comes from CDC. Once they have enough numbers to complete our verification, they will issues formal presumptive positive reports.

The conjunctival specimens are being split before sending to CDC, with the intent to take as much of the leftover negatives and work with APHL to help prioritize other states or jurisdictions that may need access to that material since it is difficult to obtain.

Questions and Answers (Q&A)

Q: Where were the alpacas located that tested positive?
A: Idaho.

Q: Is the current H5N1 virus expected to be responsive to Tamiflu?
A: Yes.

Q: Was illness associated with the alpacas?
A: Yes, there was morbidity and mortality in the alpacas.

Q: Does FDA enforcement discretion apply only to testing performed at CDC or does it apply to PHLs using the CDC assay as well?
A: This would apply to all laboratories using the CDC assays. The enforcement discretion allows PHLs to utilize the specimen type as FDA approved and you can go through your verification process for CLIA rather than doing it as an LDT.

Q: Are there positive controls available as conjunctival swabs or will PHLs need to spike for verification?
A: CDC does not have conjunctival specimens to share for positive controls and PHLs will likely need to spike samples for their verification processes.

Q: Is an independent validation or verification required for each individual facility?
A: Yes, each lab should work with their CLIA director to determine how best to complete as done for any new test you would be bringing onboard.

Q: Is there a number of samples that should be used for the verification?
A: CMS has not provided a specific number of samples required for verification.

Q: If a lab receives an NP and conjunctival swab, but have not yet verified the conjunctival swab as a specimen type, should the PHL test the NP swab and send the conjunctival swab to CDC?
A: While PHLs are in the verification process, CDC can provide a diagnosis on the conjunctival swab alone, but it would be best to also send as a paired specimen to CDC.

Q: Is there any language that should be included on reports, especially if conjunctival swabs and NP swabs could have different results?
A: Enforcement discretion is to mitigate risk of missing a case. The specimen results can be reported on its own. If conjunctival swab results are discrepant from NP swab (as it was in MI) that person is still considered to be positive based on that result alone.

Q: In severe human cases associated with clade 2,3,4,4b are there hallmark mutations that should be monitored for or are there good references to review those details?
A: Yes, there are typical mutations, primarily in the polymerase genes, that are often associated with mammalian adaptations, likely leading to increased severity and replication in the lower respiratory tract. Refer to published articles describing mutations to be on the lookout for and the CDC H5N1 Genetic Changes Inventory for more information.

Q: How should conjunctival swabs be verified as a specimen type?
A: Labs can use conjunctival matrix that is negative for influenza to serve as either negative controls or spiked positive controls. CDC is working with a company to develop a synthetic positive control as well and hope to have more information in the near future. There is purified RNA available commercially that may be used as a positive control.

Q: Will CDC send additional kits for verification purposes?
A. The H5 subtyping kits have enough material for 1000 tests and this should be sufficient for verification and testing. CDC is manufacturing additional kits.

Q: Are additional extraction platforms being added for use with the H5 subtyping kit?
A: No, there are several extraction platforms already validated to use with the kits and adding more would not be a quick process.

Q: Is CDC working with manufacturers to automate the H5 assay in the event of surge?
A: CDC is working with commercial vendors and manufacturers to potentially develop their own tests, but those would not necessarily be covered under CDC's 510(k) assay.

Q: Is CDC IRR open to expanding access to local PHLs?
A: Possibly. CDC will take this into consideration on a case-by-case basis.

Q: Is there an update on replacing the H5 kits with acceptable H5b primers?
A: CDC is currently building the H5b components in house and there should be an update in June on the progress.

Q: Should PHLs wait to verify the conjunctival swab type until there is a working H5b primer in the H5 subtyping kit?
A: No, you do not need to wait. The H5a and influenza A targets are enough to confirm presumptive positivity within your laboratory.

Q: Is CDC going to work with commercial labs to ensure that they only send appropriate specimen types to PHLs? Many commercial labs use media collection reagents which are not compatible or approved for testing using the CDC assay (UTM, Media containing GITC, etc.).
A: CDC is working with commercial labs to understand what specimens they would be willing to share with PHLs and will develop guidance based on that information. Some specimens may have to be for surveillance purposes only.

Q: Does a positive conjunctival sample and a negative respiratory specimen rule out H5?
A: No. That is what was seen in the Michigan case. A positive conjunctival specimen is a positive case.

Q: Are there other resources that cover molecular markers of illness severity in humans?
A: There are a number of review articles and publications that have looked at H5 specifically. The GISAID FluSurver can be used to search for molecular markers that are associated with phenotypic changes in H5N1 viruses.

Q: If PHLs do not have negative conjunctival swabs, how can they proceed with the verification process?
A: Consider a rolling verification process as described by Michigan, or use conjunctival specimens that may be positive for other pathogens (e.g. residual adenovirus specimens from hospital laboratories).

Q: What level of biosafety PPE is used in the rabies lab at Michigan?
A: BSL-2+; working in a biosafety cabinet and using full respiratory protection (PAPRs or medically fitted N-95 masks).

Q: Were all of the humans that were tested in Michigan symptomatic?
A: yes, Everything that is collected is from an individual with known exposure to dairy or poultry with symptoms.

Q: Is any swine testing occurring?
A: Yes, USDA has active surveillance programs in swine and feral pigs.

Q: Can APHL consider developing a process with large commercial laboratories to de-identify residual swabs and distribute them to PHLs to help respond to these types of public health issues?
A: APHL can look into this.

Next call: June 12, 2024 at 3:00 pm