Report on the Inadvertent Cross-Contamination and Shipment of a Laboratory Specimen with Influenza Virus H5N1

Centers for Disease Control and Prevention

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Executive Summary

The Centers for Disease Control and Prevention (CDC) conducted an internal review of an incident that involved the inadvertent cross-contamination of a low pathogenic avian influenza (LPAI) A (H9N2) virus culture with a highly pathogenic avian influenza (HPAI) A (H5N1) virus and the subsequent shipment of the contaminated culture to an external high-containment laboratory. The contamination most likely occurred at CDC on January 17, 2014, but was not recognized until the receiving laboratory notified CDC of the contaminant on May 23, 2014. The incident does not appear to have posed a safety risk since the virus was handled solely under laboratory conditions appropriate for containment of HPAI H5N1 virus (biosafety level [BSL] 3 with enhancements [BSL3-E]).

What Happened

CDC's Influenza Division (ID) laboratories had received the H9N2 and H5N1 virus samples as part of ongoing surveillance of human and animal influenza viruses. The virus samples were grown in cell culture and stored for future use. In response to a request from the U.S. Department of Agriculture (USDA), Southeast Poultry Research Laboratory (SEPRL), an aliquot of the H9N2 virus was sent from an ID laboratory to SEPRL on March 12, 2014. Since the H9N2 strain is not a select agent and the ID laboratory was unaware that it had been contaminated, select agent transfer procedures were not followed. On May 23, 2014, SEPRL notified CDC that it had identified an HPAI H5N1 virus (a select agent) in the H9N2 sample. The ID laboratory subsequently confirmed the contamination but did not notify the supervisory chain of command, including branch, division, center, and CDC leadership. The incident was reported to the CDC internal select agent program and to CDC management on July 9, 2014.

Why the Incident Happened

Contamination of the LPAI H9N2 virus culture with the HPAI H5N1 virus occurred at a CDC BSL3-E influenza laboratory. The contamination most likely happened due to the failure of a laboratory scientist to adhere to established best practices and the absence of an approved laboratory team-specific standard operating procedure (SOP) for the work being done. Although several factors contributed to the delay in reporting the incident, the primary factors were 1) a lack of sound professional judgment by those aware of the contamination; and 2) insufficient or ambiguous select agent and institutional reporting requirements. For example, guidance documents from the Federal Select Agent Program and from the CDC internal select agent program do not describe reporting of an unauthorized transfer of a select agent as required reporting of a release.

What Has CDC Done Since the Incident Occurred

In response to this specific incident, CDC has taken the following steps:

- Conducted an internal review (described in this report)
- Closed the laboratory involved until enhancements to safety and security can be implemented
- Included this laboratory in the CDC-wide moratorium on any biological material leaving any CDC BSL-3 or BSL-4 laboratory until adequate, additional approved safety measures are shown to be in place
- Notified USDA's Animal and Plant Health Inspection Service (APHIS), which has since conducted an investigation and issued a report on the incident

These specific actions are in addition to broader actions recently undertaken by CDC to improve overall lab safety and security agency-wide. These broad actions include

- The appointment of a CDC Director of Laboratory Safety to serve as the single point of accountability to improve all laboratory safety protocols, practices, and procedures
- The establishment of an internal Biosafety Working Group under the direction of the CDC Director of Laboratory Safety
- The establishment of an external advisory group on biosafety comprising leading scientists and biosafety experts, which will serve as a work group of the Advisory Committee to the CDC Director
- A review of policies and procedures for laboratory safety and security in all CDC BSL-3 and BSL-4 laboratories

What Additional Steps Are Recommended Moving Forward

Additional recommendations include the following:

- Reassess all CDC laboratory procedures related to H5N1 and other HPAI viruses, including how and where this work is done at CDC and how it relates to CDC's mission
- Develop written, approved policies and procedures to ensure that cross contamination of influenza viruses does not occur in the future
- Institute comprehensive quality control measures across all CDC laboratories through the performance of exclusivity testing of materials (i.e., testing to exclude the presence of other organisms) before transfer to internal and external laboratories. Materials should be accompanied by a written certificate of analysis that describes the tests and methods used.
- Broaden exclusivity testing of incoming samples to ensure the safety of laboratory scientists who work with the samples and their derivatives. For the near term, due to technologic constraints, such testing will likely be limited to agents of highest concern based on the likelihood or seriousness of their presence.
- Ensure that all ID staff are appropriately trained to understand when biosafety events are reportable and to whom they should be reported (both for select agents and non-select agents), and, more broadly, ensure that all CDC laboratory scientists receive such training. A site-specific SOP for event notification should be in place in each laboratory.
- Institute personnel actions as appropriate

Background

In January 2014, a culture of low pathogenic avian influenza (LPAI) A (H9N2) virus was unknowingly cross-contaminated with a highly pathogenic avian influenza (HPAI) A (H5N1) virus at one of the Centers for Disease Control and Prevention's (CDC) influenza laboratories. In response to a standard research request by the U.S. Department of Agriculture (USDA), Southeast Poultry Research Laboratory (SEPRL), in Athens, Georgia, CDC sent an aliquot of the contaminated H9N2 virus culture to SEPRL on March 12, 2014; subsequently, some of the contaminated culture was transferred to other CDC influenza laboratories. This report reviews the circumstances related to this event, outlines how the event may have happened, and provides recommendations for preventing similar incidents in the future. The CDC influenza laboratory where this incident occurred is now closed and will not reopen until appropriate improvements are in place.

Avian influenza viruses

Most avian influenza viruses, such as the H9N2 virus described in this report, cause only mild disease in poultry and are classified as LPAI viruses. Highly pathogenic avian influenza viruses have specific differences in their hemagglutinin proteins that enhance their ability to cause severe illness in poultry. The term HPAI refers to the virulence of the virus for poultry, and not to its virulence for humans. However, some HPAI viruses, including the specific H5N1 virus involved in this incident, are also highly virulent for humans. All exotic avian influenza viruses (those originating outside the United States) are handled at biosafety level (BSL) 3 with enhancements (BSL3-E) conditions, whether they are LPAI or HPAI viruses.

Laboratories

The mission of CDC's Influenza Division (ID) is to improve global control and prevention of seasonal and novel influenza and to improve influenza pandemic preparedness and response. In collaboration with domestic and global partners, the ID accomplishes its mission by building surveillance and response capacity, monitoring and assessing influenza viruses and illness, improving vaccines and other interventions, and applying research to provide science-based enhancement of prevention and control policies and programs. More details on the work of the ID can be found at http://www.cdc.gov/maso/pdf/NCIRDfs.pdf.

The ID has three branches: the Virology, Surveillance and Diagnosis Branch (VSDB); the Epidemiology and Prevention Branch; and the Immunology and Pathogenesis Branch. The cross-contamination incident involved two teams in VSDB, referred to as Team 1 and Team 2 in this report. VSDB conducts comprehensive characterization of human and animal influenza viruses to inform pandemic risk assessment for novel influenza viruses; develops and evaluates candidates for both novel and seasonal vaccine strains; provides expert advice on vaccine strain selection; and develops new methods to identify and characterize influenza viruses. The incident originated in Team 1. Team 2 received the contaminated material from Team 1 for routine antiviral susceptibility testing.

Both the Team 1 laboratory scientist involved in the contamination incident and the Team 1 lead are experienced ID researchers.

Methods Used in Reviewing this Incident

A team of CDC laboratory scientists interviewed all persons who were involved directly with the incident and others who had specific knowledge of the incident and related activities. Each person was interviewed individually; interviews included a standard set of questions, as well as specific questions based on an individual's role and responsibilities. Relevant standard operating procedures (SOPs), protocols, and training records were also reviewed.

Description of the Event

What happened

The Team 1 laboratory received an LPAI H9N2 virus in cell culture and in a clinical specimen (both from a nonfatal human case) from the Public Health Laboratory Services Branch, Center for Health Protection, Kowloon, China, Hong Kong Special Administrative Region, on January 15, 2014. Also on January 15, 2014, the same CDC laboratory received two HPAI H5N1 viruses in cell culture (from two nonfatal human cases that occurred in southern Vietnam in 2012 and 2013) from the Pasteur Institute, Ho Chi Minh City. The H9N2 virus and the two H5N1 viruses were subsequently grown in cell culture, sequenced to compare their hemagglutinin genes to other circulating viruses, and tested by hemagglutination inhibition (HAI) assay to compare their antigenic cross-reactivity to antisera produced against existing pre-pandemic candidate vaccines. All laboratory work at CDC was carried out in BSL3-E facilities as required by USDA. The findings of these studies were presented at the biannual World Health Organization (WHO) Vaccine Consultation Meeting in February 2014.

On January 17, 2014, a Team 1 laboratory scientist inoculated the H9N2 virus from the cell culture into Madin Darby Canine Kidney (MDCK) cell culture (referred to as the first CDC passage H9N2). On the same day and in the same laboratory, the laboratory scientist also inoculated MDCK cell cultures with the two H5N1 viruses. The first CDC passage H9N2 virus was later found to be contaminated with one of these two H5N1 viruses. The H5N1 cell cultures were later confirmed by real-time reverse transcription–polymerase chain reaction (rRT-PCR) not to be contaminated with H9N2.

On January 23, 2014, a principal investigator at the SEPRL laboratory contacted the Team 1 lead and requested an H9N2 virus that was representative of viruses circulating in China for use as a challenge virus for a study of H9N2 poultry vaccine efficacy in chickens. In response, CDC shipped an aliquot of a second passage of the H9N2 virus to SEPRL on March 12, 2014, not knowing that it was contaminated with H5N1. The material was shipped overnight by commercial courier service using a USDA Animal and Plant Health Inspection Service (APHIS) permit for LPAI H9N2 virus as a Category B virus. Category A infectious substances (such as HPAI H5N1) are packaged similarly to Category B substances, but Category A packaging must pass more stringent durability testing. USDA also requires a permit for shipping any infectious agent that has the potential to cause animal disease (including all animal influenza viruses, domestic or exotic). CDC and SEPRL used valid USDA permits to receive and use the H9N2 virus. In addition, both laboratories have the appropriate USDA permits to receive and use H5N1 viruses.

After shipping the contaminated second CDC passage H9N2 strain to SEPRL, additional CDC work was done with the contaminated first CDC passage H9N2 strain. As a matter of standard operating procedure, Team 1 produced ferret antiserum for an HAI to characterize the H9N2 virus present. The

two ferrets used in the production of the antiserum did not exhibit signs of illness, and subsequent assays showed high homologous H9N2 titers consistent with H9N2 infection. In contrast, ferrets infected with H5N1 virus typically show clinical disease. In addition, Team 2 conducted other studies with cell cultures and mice (see below for description).

SEPRL propagated the contaminated H9N2 virus they had received and conducted studies to characterize the virus, including inoculation of chickens to evaluate its pathogenic properties. They observed mortality in the chickens that was not consistent with H9N2 virus infection. SEPRL performed molecular analyses on the SEPRL virus stock and on the material sent to them by CDC and confirmed the presence of a contaminating virus with a molecular sequence consistent with HPAI H5N1 virus.

On May 23, 2014, the SEPRL principal investigator notified the Team 1 lead that the sample CDC shipped to SEPRL was contaminated with an H5N1 virus and that it was genetically similar to a published sequence of an H5N1 virus from Vietnam. That same day (May 23), Team 1 tested the H9N2 virus sample sent to SEPRL (the second CDC passage H9N2) and confirmed that it was positive by rRT-PCR for an H5N1 virus. On May 28, 2013, Team 1 also tested the first CDC passage H9N2 sample by rRT-PCR. Results showed a 1,000- to 10,000-fold increase in the signal for the H5N1 contaminant in the second CDC passage H9N2 sample when compared to the first CDC passage sample. This finding indicates that the first CDC passage H9N2 had a very low contamination level with H5N1, which increased with the subsequent passage. Sequencing of the contaminating H5N1 virus by Team 1 showed that it was identical to one of the two strains of H5N1 from Vietnam that had been cultured on the same day as the H9N2 virus.

The Team 1 lead did not report the H5N1 contamination to the VSDB branch chief, the ID Associate Director for Laboratory Science, or the ID select agent Principal Investigator. The Team 1 lead thought this was not necessary because the event did not meet the definition of a release of a select agent outside of biocontainment (both the CDC and SEPRL laboratories had worked with the contaminated virus at the required BSL3-E or Animal Biosafety Level [ABSL] 3 with enhancements [ABSL3-E]). SEPRL did not indicate that they planned to report the event to the USDA Federal Select Agent Program. Team 1 also did not immediately notify Team 2 that the H9N2 (the first CDC passage) sample they had received was contaminated with H5N1.

On February 10, 2014 (before the contamination was recognized), Team 2 staff conducted antiviral resistance testing using a neuraminidase inhibition (NI) assay on the H9N2 sample that was provided to them by Team 1. Team 2 also propagated the H9N2 sample in a different cell culture line (subsequently referenced as the Team 2 passage sample H9N2) for mice inoculation experiments as part of a joint collaboration with the U.S. Department of Health and Human Services, Biomedical Advanced Research and Development Authority (BARDA), and a private company to evaluate investigational therapeutics for influenza A and B viruses. The Team 2 laboratory operated at BSL3-E or ABSL3-E levels.

On June 23, 2014, the Team 2 lead sent an email to the Team 1 lead and a Team 1 laboratory scientist informing them that NI testing of the H9N2 virus they had received had produced atypical results. The Team 2 lead was concerned that a laboratory error had occurred during the Team 2 passage of the virus, and requested that the original H9N2 cell culture prepared by Team 1 (the first CDC passage), as well as the Team 2 passage sample be sequenced to confirm the subtype. On the same day, the Team 1 lead and a Team 1 laboratory scientist telephoned the VSDB Branch Chief to report that the first CDC passage H9N2 cell culture was cross-contaminated with a Vietnam clade 1 H5N1. Subsequently, the VSDB Branch Chief informed the Team 2 lead by telephone of the contamination. The VSDB Branch Chief then

contacted all teams in the branch and confirmed that no team, except for Team 2, had worked with the contaminated H9N2 sample. As of June 23, 2014, all contaminated H9N2 stocks had been destroyed or were secured in select agent approved freezers.

On July 2, 2014, the Team 1 lead informed the ID Director about the contaminated H9N2 shipment to SEPRL and the events that had followed. The ID Director requested additional information and on July 7, 2014, notified the ID select agent Principal Investigator. On July 8, 2014, the ID Director, select agent Principal Investigator, Associate Director for Laboratory Science, VSDB Chief, and Team 1 lead met and agreed that the incident should be reported. The ID Director and select agent Principal Investigator notified SEPRL on July 9, 2014, of their intention to report the incident to the Responsible Official (RO) for the CDC internal Select Agent Program and did so the same day. The CDC RO immediately reported the incident to the CDC Federal Select Agent Program, Division of Select Agents and Toxins. CDC senior leadership also was notified on July 9, 2014.

Except for the shipment to the SEPRL ABSL3-E laboratory, the contaminated H9N2 stocks were not transferred outside of CDC. The Team 2 staff who worked with the contaminated stocks either destroyed or transferred all material to select agent freezers. All vials of the contaminated H9N2 samples have been accounted for. All animals that were infected with the contaminated H9N2 material were humanely euthanized; animal carcasses and tissues were placed in a metal discard pan and left in the laboratory for CDC animal care personnel to autoclave and incinerate according to standard operating procedures. All work with the contaminated H9N2 virus, including work done in the Team 2 laboratory, was done under BSL3-E or ABSL3-E conditions, and no human or other animal exposures with H5N1 are known to have occurred.

<u>How it happened</u>

The Team 1 laboratory scientist involved in the incident indicated that the following procedures were used: inoculation of the MDCK cell culture with the H9N2 virus; decontamination of the biosafety cabinet (BSC) using a standard protocol; and then inoculation of the MDCK cell culture with the two H5N1 viruses. A minimum of 1.5 hours (30 min for inoculation of H9N2; 30 min for decontamination of the BSC; and 30 min for inoculation of the two H5N1 strains) would have been required to process the specimens as described by the Team 1 laboratory scientist. Additional time would have been needed for set up time at the BSC, movement of materials from the BSC to the incubator, showering out of the BSL3-E laboratory suite, and other actions required to complete the work.

The card key readers indicated that the Team 1 laboratory scientist entered the BSL3-E suite at 10:13 AM, and accessed the freezer with the H5N1 and H9N2 virus strains twice (at 10:50 and 10:54 AM). The Team 1 laboratory scientist manually signed out of the BSL3-E suite at 11:45 AM. A total of 51 minutes elapsed from when the Team 1 laboratory scientist accessed the agents in the freezer to when this staff member exited the BSL3-E suite. During the 51 minutes, the Team 1 laboratory scientist also would have been required to shower out of the suite and change into street clothes. Therefore, the time that this staff member performed the cell culture work was substantially less than the 1.5 hours that would have been required if the protocol had been followed.

Because there is no written documentation (i.e., a laboratory notebook or other notes), it is not possible to say conclusively what actions the Team 1 laboratory scientist did and how they were done. However, the 1.5 hour-protocol could not have been followed during the 51 minutes that the Team 1 laboratory scientist was in the BSL3-E suite. When interviewed, the Team 1 laboratory scientist acknowledged

being rushed to attend a laboratory meeting at noon. The Team 1 laboratory scientist also indicated being unable to specifically remember the events since they had taken place almost 6 months previously. The Team 1 laboratory scientist further described following a "best practices" protocol for temporal separation of LPAI and HPAI virus propagation; however, this laboratory did not have a written, approved laboratory team-specific SOP for the work that the Team 1 laboratory scientist was doing.

The Team 1 lead, the Team 1 laboratory scientist who conducted the cell culture inoculations, the VSDB Branch Chief, the ID Director, and others noted the Division's heavy work load at the time of the incident. The ID laboratories were under substantive pressure to generate data for the upcoming WHO Vaccine Consultation Meeting in February 2014. The ID typically provides 50%-75% of the data reviewed at these meetings.

Contamination of the H9N2 sample with H5N1 could have occurred at several time points. However, it most likely occurred during inoculation of the cell culture material with the first CDC H9N2 cell culture passage because of the following reasons:

1. The original H9N2 cell culture and specimen that CDC received were tested by rRT-PCR; no evidence of H5N1 was detected. In addition, it is unlikely that the Center for Health Protection, Kowloon, China, Hong Kong Special Administrative Region, Hong Kong (the source of the original H9N2 virus), would be working with the Vietnamese/Cambodian-specific clade of H5N1 that was identified in the contaminated stock.
2. The time to inoculate the MDCK cell cultures was at a minimum 40 minutes less than the time required if the "best practices" protocol had been followed.
3. The rRT-PCR results of the first CDC passage H9N2 strain compared with the second CDC passage of the same strain showed a 1,000-10,000-fold increase in the signal for the H5N1 contaminant in the second CDC passage. This finding indicates that the first CDC passage H9N2 sample had a very low contamination level with H5N1.
4. Molecular sequencing of the first CDC passage H9N2 sample indicated that the H5N1 contaminant was identical to one of the two H5N1 strains (Vietnam strain VP39) that the Team 1 laboratory scientist had worked with on the same day (January 17). SEPRL also found that the contaminating virus in the sample they received was genetically similar to a published Vietnam strain of H5N1. Subsequent analysis at CDC of the SEPRL sequences showed that they were identical to the Vietnam VP39 strain of H5N1.
5. To rule out other sources of contamination, a phylogenetic tree was constructed that included other virus strains from Vietnam and Cambodia that were manipulated in the same laboratory 2 weeks before and after the contamination incident. This analysis showed that the other virus strains propagated during that time period belonged to other clades.

The Team 1 laboratory scientist described first inoculating cell cultures with the H9N2 virus followed by inoculation of cell culture with the two H5N1 viruses. This sequence of events would not explain how the H9N2 cell culture was contaminated. Possible explanations for contamination of the H9N2 virus with H5N1 include

1. **The laboratory scientist handled both virus subtypes in the BSC at the same time**.
2. **The laboratory scientist handled the H5N1 cultures first followed by the H9N2 cultures.** The H5N1 cell cultures were confirmed by rRT-PCR not to be contaminated with H9N2. Lack of appropriate decontamination between the two inoculations could explain the contamination.

3. **Use of the same reagents for procedures with both virus subtypes.** The laboratory scientist suggested that the buffer solution (phosphate buffered saline [PBS]) used in the hemagglutination assays could have been the source of contamination. PBS is used for serial dilutions during preparation of hemagglutination titration plates. However, it is unlikely to have been the source of contamination because the process would involve the reagent being opened, poured into a multi-channel pipettor basin in the BSC, closed, and returned to the reagent shelf before the flasks with viruses would be opened. Although there is no written, approved protocol for reagent use in the influenza laboratories where the incident happened, an undated guidelines document for influenza laboratories in another building requires separate reagents for each influenza subtype.
4. **The contamination occurred in the incubator.** This is unlikely because flasks used to culture both the H9N1 and H5N1 strains were not vented and the flasks were not opened until the time they were harvested. Further, the H5N1 cell cultures were harvested 2 days prior to the H9N2 cell culture.

Findings

Factors contributing to this incident include the following:

Incident-related findings

1. **The failure to follow best laboratory practices to prevent cross contamination.**

The laboratory scientist who performed the cell culture virus inoculations acknowledged that the amount of time spent in the laboratory suite was not sufficient to perform the procedures according to established best practices. This staff member could not recall any specific deviation from the accepted practices on that day, and it could not be determined exactly how the contamination occurred.

2. **The failure to notify the supervisory chain of command in a timely manner that an HPAI-contaminated virus stock had been shipped to an external entity and another CDC influenza laboratory.**

Although guidance on what constitutes a release of a select agent may be ambiguous and there was some confusion regarding whether the incident was reportable, sound professional judgment would have warranted that the Team 1 lead report it immediately to a supervisor. Email exchanges between Team 1 and SEPRL do not indicate that select agent concerns were discussed. However, even if a select agent had not been involved, the recognition that transfer of a cross-contaminated virus had occurred would have warranted that the Team 1 lead report the incident to the VSDB Branch Chief immediately. The VSDB Branch Chief then could have informed the other ID branch laboratories to ensure that the contaminated virus stock was not being used and was under select agent inventory control.

3. **The lack of an approved, written SOP to prevent cross contamination when multiple influenza virus subtypes are handled in a single laboratory on the same day.**

The laboratory scientist who performed the cell culture virus inoculations described appropriate procedures for 1) spatial and temporal separation of multiple subtypes handled in the same BSC on

the same day; 2) decontamination procedures for the BSC between inoculations of different virus subtypes; and 3) processing LPAI viruses prior to HPAI viruses. The ID High Containment Laboratory (HCL) has undated guidance for these procedures; however, the laboratory where the incident occurred had no written, approved protocol for these procedures.

The ID has a robust infrastructure of SOPs. The VSDB has over 500 documents in their Quality Management System (not including ID procedures). A cursory review found two SOPs from two different teams for inoculation of MDCK cells: one procedure described inclusion of negative controls, while the other did not. Given the large number of SOPs, a comprehensive review to identify redundant (and possibly conflicting) SOPs for the same procedures was beyond the scope of this investigation.

4. **The lack of required record keeping to document procedures and processes conducted in the laboratory.**

There were no written records to document that the procedures were performed as described by the Team 1 laboratory scientist, including the order in which the viruses were inoculated, which reagents were used (e.g., lot numbers could have been traced for contamination), or how the BSC was decontaminated. The only relevant records were worksheets documenting what was done with the cell cultures following inoculation.

5. **The lack of SOPs for quality control of influenza material transferred to CDC laboratories or external laboratories.**

Team 1 did not routinely perform exclusivity testing (i.e., testing to exclude the presence of other influenza strains) on virus preparations prior to transfer. Team members did not suspect that the H9N2 preparation was contaminated and confirmed only that the intended strain was present. Best practices would mandate that a more rigorous quality control system be in place to ensure that the specimens shipped from the BSL3-E laboratory were tested to identify contamination with other influenza viruses used recently in the BSL3-E laboratory. A certificate of analysis documenting these findings, as well as other quality control information, should have been required.

The failures to obtain transfer authorization and to follow proper shipping procedures for a select agent were unintentional results of not identifying the presence of the contaminating strain. The procedures followed would have been appropriate for a pure H9N2 strain. The contaminated preparation was also used in an animal facility space at CDC, which was appropriate for containment of the H5N1 as well as the H9N2, but was not registered for select agent use.

6. **The lack of record keeping documenting the transfer of specimens from the BSL3-E laboratory to other laboratories inside and outside of CDC.**

The contaminated preparation or its derivatives were still being used by other teams in the ID as late as June 23, 2014—a month after SEPRL notified the Team 1 lead of the contamination and the contaminated preparation had been confirmed to be present in CDC stocks. This lack of action not only could have created hazardous situations, but also could have resulted in erroneous data being obtained and reported. Team 1 had also planned to ship the contaminated H9N2 to the Department

of Infectious Disease, St. Jude Children's Research Hospital. Although the material was not shipped, a record of destruction was not available.

7. The heavy workload for ID personnel, underscoring the need for prioritization of programmatic activities.

All ID personnel who were interviewed discussed the pressure of accomplishing many competing programmatic activities that were required of them.

8. The lack of direct oversight regarding select agent work being done in the ID.

The ID had submitted paperwork to the CDC internal Select Agent Program to amend their registration so the VSDB Branch Chief could be the select agent Principal Investigator for the branch. However, currently the ID select agent Principal Investigator is the branch chief for the Immunology and Pathogenesis Branch.

<u>Incident response-related findings</u>

9. The lack of awareness and understanding of select agent reporting requirements.[1]

The Team 1 lead confirmed on May 23, 2014, that the H9N2 preparation was contaminated with H5N1. The unauthorized transfer of a select agent to SEPRL should have been reported to the CDC RO immediately. The delay in reporting does not appear to have been deliberate on the part of this staff member. The Team 1 lead indicated that, rather than select agent concerns, initial thoughts were scientific professional concerns that a colleague at SEPRL had wasted resources due to the Team 1 error. After recognizing that there might be select agent issues, the Team 1 lead believed that a report was not necessary because no release had occurred; the H5N1 virus had been handled only in BSL3-E, select agent registered facilities. Further, communications between the Team 1 lead and SEPRL did not include discussion of any select agent reporting requirements. However, the Team 1 lead should have recognized that the movement of the H5N1-contaminated H9N2 preparation to CDC laboratories that were not select agent registered was an unauthorized intra-facility transfer that did not meet the requirements of select agent regulations. In addition, transfer between facilities (CDC and SEPRL) required preauthorization on APHIS/CDC Form 2 which was not obtained, and represented an unauthorized inter-facility transfer.

The VSDB Branch Chief also indicated that when first informed of the incident on June 23, 2014, immediate thoughts were not about the potential select agent issues. The VSDB Branch Chief was

[1] Centers for Disease Control and Prevention (CDC), Division of Select Agent and Toxins (DSAT), and the U.S. Department of Agriculture, Animal and Plant Health Inspection Services (APHIS), are responsible for ensuring implementation of the select agent regulations (42 C.F.R. Part 73; 9 C.F.R. Part 121; 7 C.F.R. Part 331). The select agent regulations require that the CDC/APHIS Form 3 be used for reporting of theft (unauthorized removal of select agent or toxin), loss (failure to account for select agent or toxin), or release (occupational exposure or release of an agent or toxin outside of the primary barriers of the biocontainment area) of a select agent and toxin. Entities are required to notify APHIS or CDC immediately upon discovery and file a Form 3 within 7 calendar days of an incident. In some instances, after discussion with the entity Responsible Official, CDC/DSAT or USDA/APHIS may determine that filing a Form 3 is unnecessary.

most concerned about ensuring that the virus had not been distributed to any branch laboratories other than the Team 2 laboratory, and that it had been handled only under BSL3-E conditions.

The Team 1 lead further discussed the contamination event with the VSDB Branch Chief on July 2, the first day they were both in the office after June 23.

The Team 1 lead also informed the ID Director of the incident on July 2. Based on the information provided by the Team 1 lead, the ID Director thought there was not yet enough information to make an informed decision regarding the need to report the incident. The ID Director met with all the principals on July 8, the first date that they were all available, and reported the incident the next day after notifying SEPRL.

10. The need for ID event notification and select agent training

The Office of Infectious Diseases (OID) developed a flow chart dated February 20, 2013, that provides guidance on "immediate notification of management following potential exposures to infectious organisms or development of related symptoms among individuals working in or visiting CDC laboratories." Prior to this incident and not as a result of it, ID developed an influenza-specific notification flow chart that was finalized on May 7, 2014. Neither of the two notification flow charts includes situations other than potential exposure, injury, or illness to help individuals recognize when other events are reportable.

Unlike the ID Influenza Immunology and Pathogenesis Branch Chief, the VSDB Branch Chief was not a select agent Principal Investigator. Both the Team 1 lead and the Team 1 laboratory scientist involved in the incident had completed the required online CDC internal select-agent training. This program includes a series of scenarios that test knowledge of reporting requirements, but a scenario similar to this incident was not included in the required training. Some organizations, including ID, have adopted the concept of having a dedicated safety officer; these individuals have been able to provide didactic training for their staff.

Recommendations

The following actions and recommendations relate to

- The ID laboratory
- All CDC infectious disease laboratories
- CDC and other organizations

The ID Laboratory

Immediate:

Remediation actions

1. Test all cultures and other preparations done by the Team 1 laboratory scientist involved in this incident to identify any other cross contamination. Initially, preparations that had been done during the year before the incident, as well as those subsequent to January 17, 2014, should be tested. If any cross contamination is found, additional testing of all preparations may be warranted. All buffers and reagents that were used at the time of the incident should be discarded (if any are still in use). This work should be done by a team other than Team 1.
2. Test all preparations which were transferred by ID over the last year to other laboratories (both within and outside CDC) for cross contamination with multiple subtypes of influenza. The ID should propose a reasonable strategy for performing this testing, taking into account the procedure-dependent risk of contamination, any testing already performed, and the number of preparations that this may involve. The ID Director should submit a plan to the CDC Director for consideration. If any cross contamination is found, additional testing of additional viral cultures and other biologic preparations may be warranted. Preparations shipped with a certificate of analysis may not require rechecking.
3. Institute SOPs in all ID laboratories for processing of multiple subtypes of influenza. This action should include finalization of the HCL guidelines that are not currently in an approved SOP.
4. Prioritize activities to allow laboratory scientists to complete mission-critical work and minimize the need to complete lower priority work at the same time. Determine which, if any, activities can be eliminated without significant impact on the Division's mission.
5. Designate the VSDB chief as an additional select agent Principal Investigator.
6. Institute mandatory daily record keeping to document the conduct of procedures such as cell culture inoculation and the materials used. Every procedure should have a checklist that should be completed along with the experiment.
7. Develop and implement SOPs that ensure strict measures are in place to prevent inadvertent cross contamination of influenza subtypes by requiring temporal separation when processing LPAI and HPAI virus subtypes. Optimally, processing of LPAI and HPAI virus subtypes should be done in separate laboratory rooms (i.e., spatial separation). This may require additional coordination between branches, such as utilizing sign-up sheets (as practiced in the HCL) and planning studies further in advance than is generally practiced.
8. Institute personnel actions as appropriate.

Required training

1. Ensure that all ID staff are appropriately trained and understand when events are reportable and to whom (both for select agents and non-select agents).
2. Revise the current management notification policy regarding potential exposures or illness to include other criteria for notification (e.g., unauthorized transfer of a select agent).
3. Ensure effective and routine training for all appropriate division personnel on select-agent regulations and requirements for compliance.

Required documentation

1. Review all SOPs in the ID branches to ensure they are up to date and reflect current best practices. Ensure that written, approved SOPs are provided to all laboratory scientists.
2. Ensure that the electronic select agent inventory is accurate and up to date. Current procedures (i.e., faxing information from the BSL3-E and updating the information later) may lead to discrepancies between paper and electronic inventories. Update procedures to eliminate discrepancies.

Longer-term recommendations:

1. Reassess all procedures for working with H5N1 and other HPAI viruses, including how and where this work is done at CDC and how it relates to CDC's mission.
2. Establish comprehensive quality control measures to include exclusivity testing of materials prior to transfer to internal and external laboratories. Materials should be accompanied by a written certificate of analysis that describes the tests and methods used.
3. Broaden exclusivity testing of incoming samples to ensure the safety of laboratory scientists working with the samples and their derivatives. For the near term, due to technologic constraints, testing will likely be limited to agents of highest concern based on the likelihood or seriousness of their presence.
4. Establish Division-wide specimen database(s) to better communicate what specimens are available, what testing has been done and still needs to be done, and where all passages of virus samples are located. Access to data regarding select agent specimens should be limited to individuals with Security Risk Assessment (SRA) approval.
5. Enhance training for select agent compliance, including scenario-based training for laboratory scientists and leadership to understand when events are reportable. Implement regular staff meetings with the RO to receive updates and advice regarding select agent issues.
6. Identify additional select agent Principal Investigators as necessary to ensure that all staff who work with select agents are under the supervision of a select agent Principal Investigator for the project.
7. Review all ID SOPs to ensure consistency and reduce redundancy.

All CDC Infectious Disease Laboratories

1. Ensure that all CDC laboratories handling infectious materials have procedures and training for staff to identify and protect against exposure to potential sources of unrecognized hazardous agents, e.g., exposures that could occur through contamination or misidentification of cultured materials or through clinical materials which may contain unsuspected agents. The laboratory-specific biosafety manual should clearly identify situations in which extra precautions are warranted and describe appropriate procedures to minimize risk.
2. All laboratories should develop written SOPs describing laboratory safety requirements for accepting specimens.
3. Ensure that laboratory scientists are appropriately trained to understand when events are reportable and to whom (both for select agents and non-select agents). A site-specific notification SOP should be in place in each laboratory.

CDC and Other Organizations

1. CDC Internal Select Agent Program. The level of staffing for the internal CDC select agent program is inadequate and not comparable to other entity select agent programs, especially those of the size and complexity of the Roybal campus. There are currently only four individuals in the select agent program at the Roybal campus, including the CDC RO and one person dedicated to security issues. The CDC RO has responsibility for the entire Roybal campus as well as supervisory responsibility for three team members in Atlanta and for the RO at CDC's Ft. Collins facility. The CDC RO also provides guidance to the ROs at the CDC Chamblee and Anchorage campuses.
   a) Additional support should be provided for an individual dedicated to training to allow the internal Select Agent program to provide didactic training, including the development of reporting scenarios.
   b) Administrative support is needed to ensure that the RO and staff can provide site-specific training for laboratories.
2. Table top exercises and drills. Exercises and drills that can help enable CDC laboratory scientists to recognize when events are reportable to management should be conducted. These practices should not be limited to select agents, but should include all laboratories that work with BSL-3 or BSL-4 pathogens.
3. CDC's Division of Select Agents and Toxins (DSAT) and USDA/APHIS. Guidance documents[2] from the Federal Select Agent Program do not describe reporting of unauthorized transfers as a release. Additional guidance to ROs from DSAT and APHIS on reporting of incidents similar to the one described in this report would be helpful to CDC and to all entities registered with DSAT/APHIS.

[2] The Information Document for Report of Theft, Loss, or Release of Select Agents or Toxins (http://www.selectagents.gov/resources/APHIS-CDC_Form_3_Guidance_Document-English.pdf); the Select Agents and Toxins Theft, Loss or Release, APHIS/CDC Form 3 (http://www.selectagents.gov/resources/APHIS-CDC_Form_3_Notification_of_Theft_Loss_or_Release_Fillable-English.pdf); and the Select Agents and Toxins Theft, Loss or Release Information Document (http://www.selectagents.gov/resources/CompleteTHEFT%20LOSS%20%20RELEASE%20guidance%20document%20June82010_FINAL.pdf).