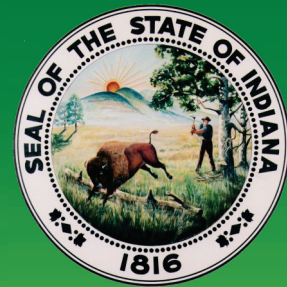


The LAByrinth

Indiana State Department of Health Laboratories Newsletter



Indiana State Department of Health Laboratories

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State Health Commissioner

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Assistant Commissioner
Laboratory Services

Our Mission:

The Indiana State Department of Health Laboratories partners with other public health agencies to provide timely and accurate information needed for surveillance and outbreak investigations to protect and improve Hoosier health.

Hepatitis C Awareness

By Jacalynn Surma



May was Hepatitis C awareness month. The Indiana State Department of Health’s (ISDH) Serology Lab has performed testing for this viral blood borne pathogen for many years, but due to recent updated recommendations by the CDC as well as newly available therapies, this virus is becoming more newsworthy.

Hepatitis C is a liver disease that results from contracting the Hepatitis C virus (HCV). HCV infection is spread primarily from contact with the blood of an infected person. The disease can be classified as either “acute” or “chronic” based on the patient’s symptoms and disease progression. HCV infection is one of the leading causes of liver cancer and cirrhosis, and 75-85 percent of those infected will progress from acute to chronic illness. Since HCV can often have a lack of signs and/or symptoms, it can be difficult to encourage those at risk to get tested. However, even if a person is asymptomatic, they can still be contagious.

So, who is at risk for contracting the virus? The most common method of transmission is via injection drug usage (IDU). Along with the risky behavior of persons sharing needles and syringes, HCV infection can also be easily spread by the sharing of other drug paraphernalia, including accessories used for snorting. Others who are likely to get Hepatitis C virus are those in contact with needle stick injuries in healthcare settings, those who receive unsterile tattoos or piercings, and babies born to an HCV positive mother. The HCV virus can remain viable on surfaces for at least 16 hours, and up to 4 days, making this virus eminently more robust than HIV. HCV can also be spread in unsterile medical procedures, including dental offices, as was recently reported in the national news concerning a dentist in Oklahoma. Persons who may be at less risk, yet should still be aware, are those having sexual contact with an HCV infected person, as well as those sharing personal hygiene items with a HCV infected person, especially where blood may be exposed.

Since Hepatitis C was not discovered until the late 1980’s, and the blood supply was not screened for it until 1992, anyone who received blood products or had medical procedures performed prior to 1992 are considered at risk. The CDC has recently recommended that the baby boomer generation, those born between 1945 and 1965, be tested once for HCV.

The primary method for screening for this virus is through antibody testing. The ISDH Serology Lab screens patient serum samples for IgG antibodies using a Chemiluminescent ImmunoAssay (CIA). This testing is performed on one of two analyzers: the Ortho 3600 or the Ortho ECI. Both instruments can process hundreds of samples per day, and can also test for HIV and Hepatitis A and B.

Inside this Issue:

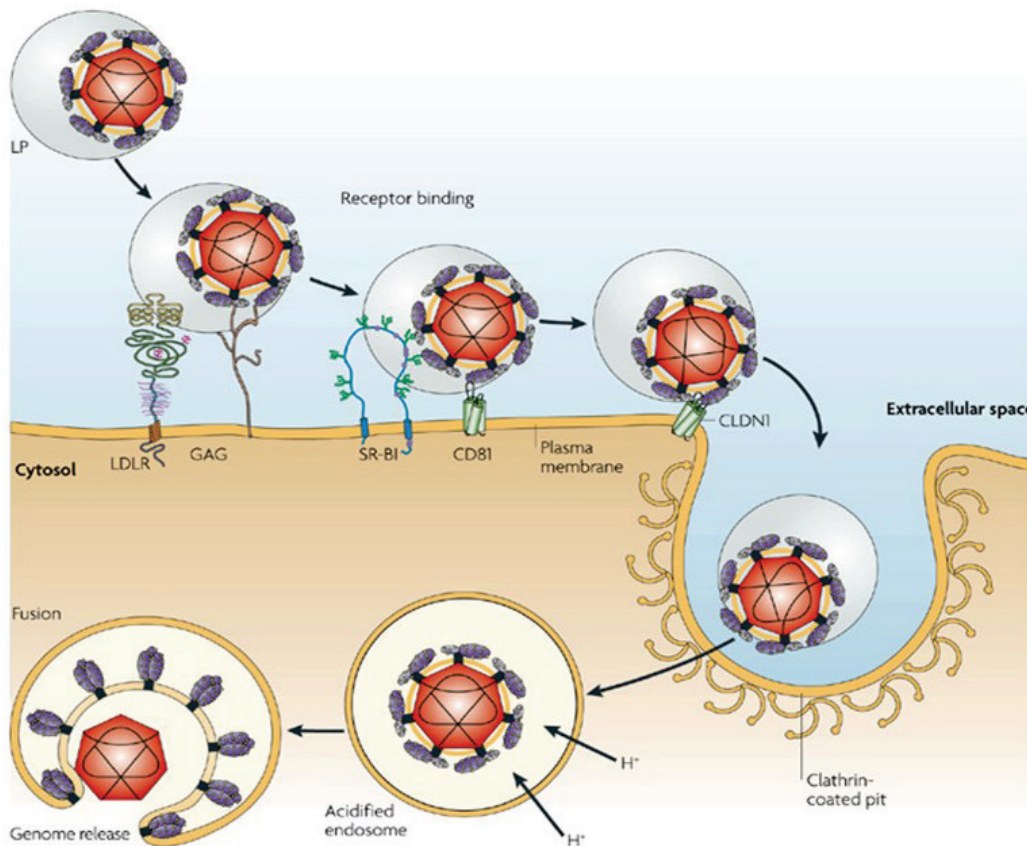
Hepatitis C Awareness	1-2
Heavy Metals in Pharmaceuticals	3
Phlebotomy Training	4-5
2012 Influenza Outbreak	6-7

When a sample is resulted as Reactive by one of the analyzers, if the antibody result is high, no further testing is needed. However, if the antibody result is lower than a certain level, confirmatory testing is indicated. The ISDH lab uses a Nucleic Acid Amplification test (NAAT) to detect viral RNA. This assay is both sensitive and specific, and can rule out falsely positive CIA results, as well as confirming an active infection.

The ISDH Serology Lab tested nearly 27,000 serum samples for HCV in 2012. The primary source of specimens is the Indiana Department of Corrections (IDOC), which supplies 80 percent of serum specimen for HCV testing. This testing, as well as HIV testing, is mandated by Indiana Administrative Code. The majority of the other 20 percent of samples sent to the ISDH Serology lab are sent from Counseling and Testing Sites (CTS). The Serology Lab receives serum samples from over 50 CTS throughout the state. The purpose of the CTS is to prevent the spread of HIV, and since many of the risk factors for HIV and HCV are the same, many of the CTS request HCV testing for their clients.

The DOC sites average a 16 percent positivity rate for HCV, while the CTS average an 11 percent positivity rate. Although not very glamorous, testing the prison population is an effective means of identifying new HCV infections in Indiana.

The ISDH Serology lab, ISDH Epidemiology Resource Center, and statewide CTS programs all have the same goal for HCV: its prevention. The most critical component to this goal is awareness. When someone is made aware that they have risk factors, a person can and should get tested. When a patient is identified to have a HCV infection, they can work with their physician to receive the appropriate treatment regimen. The sooner a virus can be identified and treated, the more likely a person's overall health won't be adversely affected. For more information, please visit the CDC's viral hepatitis page at <http://www.cdc.gov/hepatitis/index.htm>



The Future of Precise Measurement of Heavy Metals in Pharmaceuticals in a Quality Control Arena

By Taj Mohammad



Since its inception about two centuries ago in 1820, the United States Pharmacopeia (USP) has been setting standards for pharmaceuticals, which are then enforced by the Federal Food and Drug Administration (FDA). One such standard is measuring of and labeling the drug formulations containing heavy metals. Metals become a part of the pharmaceutical ingredients *via* several routes, which include the starting materials, reagents, catalysts used in the syntheses, reactors, pipes, equipment, metal-based coloring agents and plant or mineral raw materials. The USP has approved a wet chemistry-based method [USP <231>] to assay the metals in pharmaceuticals which has been used since 1905. This method has also been prescribed by the British Pharmacopoeia (BP), Japanese Pharmacopoeia (JP) and European Pharmacopoeia (EP). The wet chemistry method involves precipitating the metals as their colored sulfides using lead sulfide as the precipitating agent. The color of the

produced metal sulfides varies from white, yellow, orange, brown, or black, depending on the metals present. The color is compared with a standard and the amount of metals in the sample is estimated. This old visual test does not provide any quantitative results and lacks sensitivity, specificity and reliability. For example, the method cannot distinguish arsenic versus lead, and provides no information at all on their respective amount. The recovery of spiked metals is poor, often 50 percent or less. Furthermore, this method is perceived as a tedious, time-consuming, cumbersome and labor intensive approach.

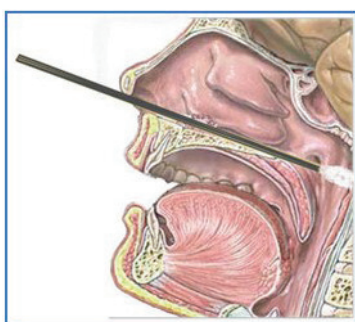
The USP recommends that drug manufacturers replace the wet chemistry method with more sensitive and accurate instrument-based techniques. The USP embarked on this project more than a decade ago; and after several forums has produced the final draft of the instrument-based method [USP <233>]. It expects the new standards to be approved by May 2014. In the initial evaluation of the instruments for accurately measuring metal impurities in pharmaceuticals, the USP short listed the three top instrumental methods, namely flame atomic absorption (FLAA) spectrophotometer, inductively coupled plasma-atomic emission spectrometer (ICP-AES), and inductively coupled plasma-mass spectrometer (ICP-MS). The expected instrumental methods will be the ICP-AES and ICP-MS. These instruments are in the order of their increasing cost, complexities in operation and maintenance, and follow the same trend for sensitivity and versatility. It is worth noting that with the additional equipment included in-line prior to the sample introduction into the plasma, ICP-MS can distinguish and quantitate the different elemental forms of quite different biological properties of a metal. For instance, it can achieve elemental speciation of the highly toxic arsine (AsH_3) from the non-toxic organometallic species arsenocholine ($[\text{As}(\text{CH}_3)_3\text{CH}_2\text{CH}_2\text{OH}]^+$). Another illustration of chemical speciation by ICP-MS is the differentiation and quantitation of carcinogenic hexavalent chromium [also known as chromium-6 or Cr(VI)] from the vital element trivalent chromium [also known as chromium-3 or Cr(III)]. For risk assessment, it will be more important to know the concentration of the toxic form of the element rather than the total metal concentration. From the initial list of several elements of concern [USP <232>], the USP has selected the four metals for mandatory evaluation in pharmaceuticals. These metals are arsenic, cadmium, lead, and mercury.

The ISDH Laboratories' Chemistry Laboratories Division routinely uses the flame- and plasma-based instruments for the trace metal analysis of environmental samples in a variety of matrices. Many consumer products are frequently assayed by these instruments as well. The Blood Lead Laboratory employs the ICP-MS instrument for the detection and quantification of trace toxic metals in clinical samples. Future testing for ubiquitous toxic metals influencing human health will clearly rely upon modern instrumentation.

Phlebotomy Training for Public Health Nurses and Nursing Personnel

By Jyl Madlem

A new training program designed specifically for local health department public health nurses kicked off in April. This program included phlebotomy and nasopharyngeal swab collection training. The purpose of this training is to bridge a gap in the skill set of public health nurses who do not receive phlebotomy training either in nursing school or on the job. In addition to the intended audience of public health nurses and nursing personnel, other public health personnel such as disease investigators and administrators also attended. These trainings have been delivered to nine of the ten preparedness districts throughout Indiana and are ongoing. Jyl Madlem, assisted by Dana Greenwood, Chief Nursing Consultant for the Immunizations Program, delivered these trainings.



The training program consisted of a didactic portion, a discussion regarding billing for services, and demonstrations of proper nasopharyngeal swab collection procedures. Proper site selection for venipuncture was discussed as well as patient identification, proper order of draw, and allowable tourniquet time. In addition, hemolysis, capillary collection, applicable regulations, risk management, exposures, competency evaluations and required bloodborne pathogen training were explained. A phlebotomy procedure was reviewed and provided to attendees as well.

Once the didactic portion was complete, the hands-on laboratory training began. This part of the training allowed attendees to practice phlebotomy skills on training arms and hands until such time as they felt competent to attempt to collect a sample on another person. Jyl Madlem assisted attendees during this part of the program by providing tips for successful venipuncture and critiqued performance.

During the hands on phlebotomy practice session, attendees also worked with Dana Greenwood to practice proper nasopharyngeal swab collection. Dana also provided helpful tips and critiqued performance.

In order to assess the efficacy of this training program, a pre-test was used to assess current knowledge of attendees at the start of the program. A post-test was administered at the end of the didactic training to assess learning from the program. Results indicate an average increase in learning of 44.2% across all nine completed preparedness districts (See Figure 1).

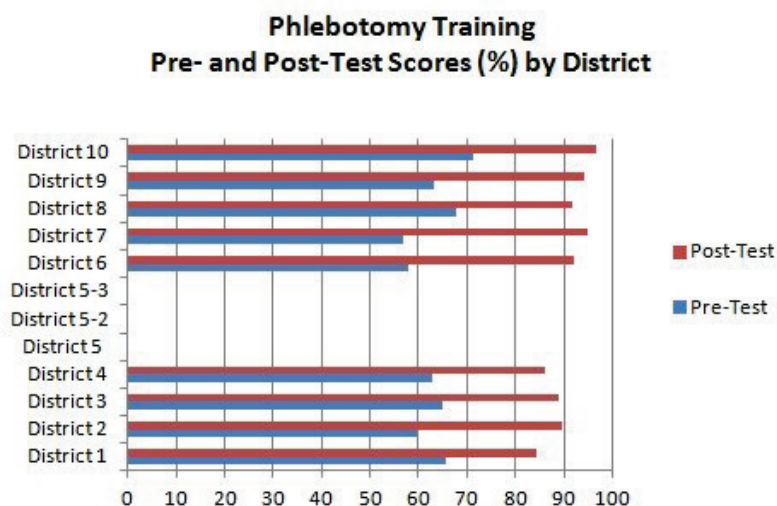
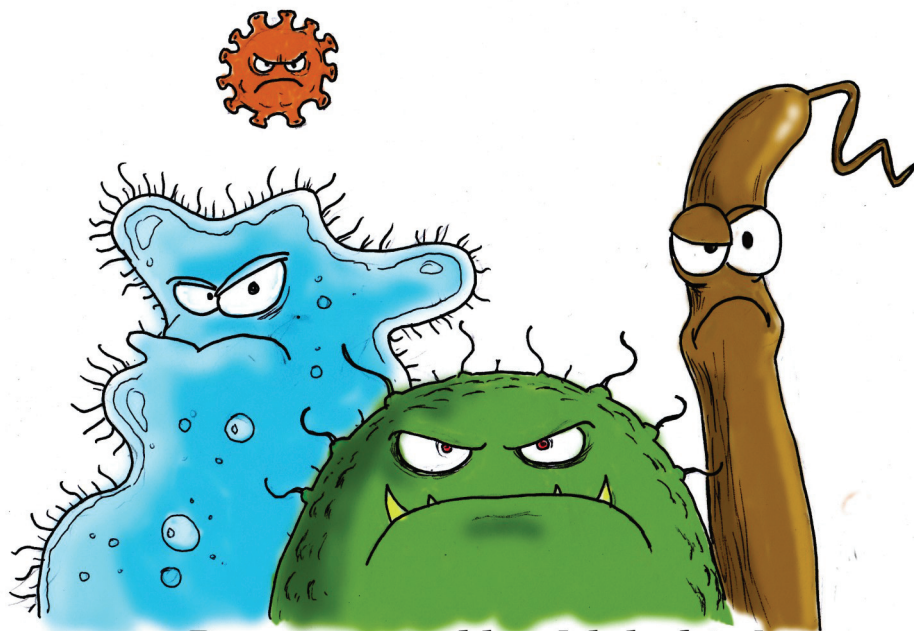


Figure 1: Pre and-Post-Test Scores by District

Evaluations received from this training program yielded many positive comments from attendees. Among the comments on the evaluations were “This training is one of the few training sessions I’ve attended in recent years that was really worth my time.”, “We’re going to start collecting serology specimens.” and “Jyl’s helpful hints were terrific!”.

Phlebotomy training for public health nurses and nursing personnel will be an ongoing training provided to local health department personnel by the ISDH Laboratories. District 5 trainings have been scheduled for May 25, July 23 and July 30.

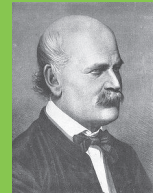
DON'T INVITE UNINVITED GUESTS



**Practice good health habits!
Wash hands. Exercise. Eat Healthy.**

Fun Fact:

Prior to the late 19th century, handwashing and basic disinfection techniques were not practiced in hospitals and medical wards. The doctors would go from patient to patient (even after cadaver examinations) without washing hands, cleaning instruments, or changing clothes. The doctors of that time considered their dirty, bloody coat to be a status symbol. Medical instruments were wiped off on their clothes and even their shoes. A Hungarian physician, **Ignaz Philipp Semmelweis (1818-1865)** noted the high mortality of childbirth in doctors’ ward compared to midwives’ ward. He put forth the idea of washing hands in chlorinated lime solution, and as a result, the mortality in his hospital dropped dramatically. Semmelweis tried to convince the medical establishment of his findings, unfortunately he was ignored and laughed at. Some of the doctors were offended at the suggestion that their hands were unclean, challenging their social status as gentlemen. Semmelweis tried without success for years, until he had a breakdown and was committed to mental asylum, where he was beaten by the guards and died from infection. His antiseptic technique was not widely accepted until more than 20 years later, when Louis Pasteur finally proved that germs can be transmitted from one organism to another.



2012 Influenza A (H3N2)v Outbreak in Indiana: Highlighting Partnerships between Human and Animal Surveillance



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BACKGROUND

The Indiana State Department of Health (ISDH) Laboratories maintains a strong relationship with ISDH Epidemiology for year-round surveillance to detect circulating seasonal and novel influenza. As a result of these enhanced surveillance efforts, Indiana was the first state in 2011 to detect cases of human infection with influenza A(H3N2)v virus. Over the years, the Indiana Board of Animal Health (BOAH) and the ISDH have developed a cooperative partnership for enhanced swine influenza (SI) surveillance. Though BOAH does not perform routine influenza testing for the purpose of surveillance, they maintain constant communication with swine industry stakeholders, such as Indiana swine veterinarians. In order to be alerted when influenza case numbers are higher than normal or otherwise unusual in clinical presentation.

Purdue University's Animal Disease Diagnostic Laboratory (ADDL) is a member of an SI surveillance project coordinated through the USDA's National Animal Health Laboratory Network (NAHLN). Swine are automatically and anonymously sampled in the project and samples collected from these swine are tested for influenza A virus by PCR; additional testing is done on PCR positive samples to further characterize the virus.

The ISDH and BOAH, along with local health departments, develop robust partnerships in order to maintain communication and coordinate their investigations of zoonotic diseases that may result in a public health event. In the summer of 2012, the investigation of influenza A(H3N2)v at Indiana county fairs and the Indiana State Fair underscores the importance of these relationships.

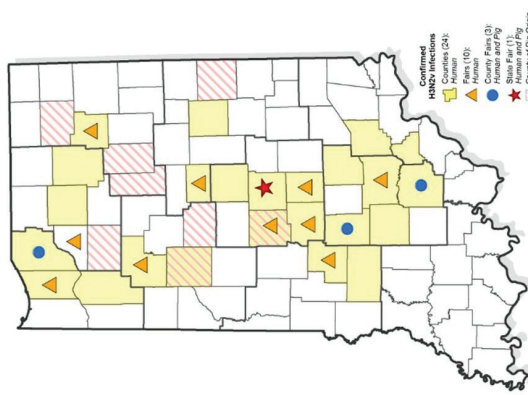
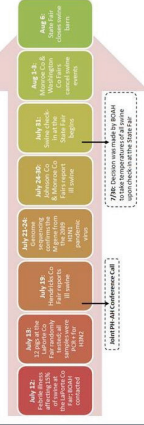


Figure 1: Confirmed H3N2v infections by county in the state of Indiana.

INVESTIGATION & DETECTION OF H3N2v

On July 11, 2012 a fair veterinarian was requested to examine an ill pig in the show barn at the LaPorte County Fair. The following day, the fair veterinarian reported a dead piglet (up to 107 lbs). The Board of Animal Health was notified of the situation. Initial and coprological samples were randomly collected from 12 pigs dispersed throughout the swine barn. Testing was performed on both asymptomatic and symptomatic pigs from different herds. Samples were sent to the State Animal Disease Diagnostic Laboratory, where they were tested by matrix-RTP-PCR for the presence of influenza A and by H and N subtyping-RTP-PCRs. All 12 were identified as H3N2.

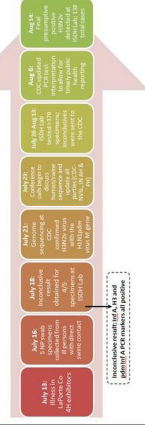
Outbreak Timeline: Swine



On July 19, a joint public health and animal health call was coordinated to discuss both swine and human testing results. Pig samples were forwarded to the National Veterinary Services Laboratories (NVSL) where RTP-PCR testing determined that 12/12 samples contained the matrix (M) gene from the 2009 pandemic H1N1 influenza virus. Samples with the strongest Ct value were sequenced using the Ion Torrent whole genomic sequencer.

At the same time swine testing was being conducted, ISDH, in collaboration with county health departments, was coordinating human testing. Diagnostic specimens were collected from people reporting influenza illness (ILI) that had been in contact with pigs at the fair. Real-time RTP-PCR testing at the State Department of Health Laboratory indicated a presumptive positive for influenza A(H3N2)v specimens were forwarded to the Centers for Disease Control and Prevention (CDC) for RTP-PCR confirmation and genetic sequence analysis.

Outbreak Timeline: Human



Sequence data was obtained directly from the clinical specimens. Comparison of the sequence obtained by NVSL on the pig samples and by CDC on the human specimens indicated virtually identical sequences, demonstrating virus transmission between humans and pigs. BOAH and ISDH requested notification of all fairs with swine and human illnesses.

Together, the ISDH and BOAH obtained close to 400 clinical specimens and 47 swine samples from county fairs across Indiana and the State Fair as part of this dual investigation.

INVESTIGATION & DETECTION OF H3N2v

Influenza PCR results, swine testing

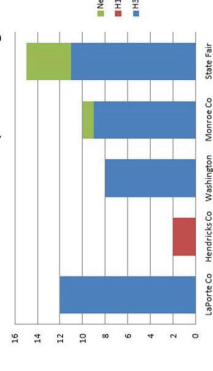


Figure 2: Results of influenza PCR testing conducted at the Purdue University Animal Diagnostic Disease Laboratory.

In addition to PCR testing, BOAH and attending veterinarians, utilized an additional diagnostic tool for exhibition swine at the Indiana State Fair that were showing clinical signs consistent with influenza virus. This new test, Flu DETECTSM SWINE, is a 15-minute Rapid Immuno Migration test designed to detect influenza A viral antigen. Because the specificity of the test is 100%, BOAH felt confident in taking the action necessary to mitigate disease spread to protect the health of both swine and humans.



Figure 3: Results of testing conducted by BOAH and ADDL from pigs in the swine barn at the State Fair.

H3N2v CASES IN THE UNITED STATES

State	Human (n=2011)	Swine (n=2012)
Arkansas	0	1
California	0	4
Illinois	2	218
Indiana	0	12
Iowa	3	1
Michigan	0	32
Minnesota	0	6
Missouri	0	5
Nebraska	0	1
North Dakota	0	1
South Dakota	0	1
West Virginia	0	0
Wisconsin	0	20
Total	5	305

OUTCOMES/RECOMMENDATIONS

Risk Management Practices for Swine Exhibitions

1. Establish a relationship with a veterinarian who is willing to be on-call for the show and work to develop a plan for the identification and response to an influenza-like illness in swine.
2. Exhibition organizers should inform exhibitors and animal caretakers that people experiencing influenza-like symptoms should not be in contact with swine for at least 24 hours after the fever ends.
3. The following should be discouraged within the animal areas: eating, drinking, smoking, sleeping, and bringing in pacifiers, baby bottles or eating/drinking utensils.
4. An adequate number of hand washing stations should be maintained during the exhibition. Signage to encourage use of these hand washing stations should be posted.
5. Barn hygiene should be monitored and maintained. Special attention should be paid to areas where the public may have access.

Influenza Risk Management Recommendations for Show Organizers

1. BOAH strongly recommends that all swine should be vaccinated for influenza prior to the opening day of the exhibition.
2. Exhibition organizers should take steps to limit the amount of time swine are congregated on the exhibition grounds; ideally, not more than 72 hours.
3. Swine should be observed daily for signs of disease or infection; if temperature is 103.0 F or higher, remove pig from the exhibition grounds.
4. BOAH recommends that exhibition swine are identified with USDA approved 640 radio frequency identification (RFID) tags.



CONCLUSIONS

The strong relationship between ISDH, BOAH and various local health departments highlights the value of cooperative partnerships at the state level. These partnerships allowed for easy intra-agency communication and facilitated timely collection and testing of samples from both human and swine to minimize the spread of infection. From these partnerships, new recommendations for swine exhibitions were created and approved to decrease the likelihood of zoonotic transmission in the public setting.

Indiana Public Health receives National Recognition for Influenza Outbreak Poster

Indiana public health received national recognition for our poster demonstrating how the ISDH Lab's strong and ongoing relationship with both ISDH Epidemiology and the Indiana Board of Animal Health (BOAH) paid off in important public health benefits during the 2012 outbreak investigation of influenza A (H3N2)v at Indiana county fairs and the Indiana State Fair. Katie Masterson, Virology/Preparedness Laboratory Supervisor, was the lead author of the prize-winning poster entitled "2012 Influenza A (H3N2)v Outbreak in Indiana: Highlighting Partnerships between Human and Animal Surveillance" which was presented by Dr. Judy Lovchik, ISDH Laboratories Director, and won first place at the 2013 Association of Public Health Laboratories Annual Meeting in Raleigh, North Carolina. Co-authors of the poster included Mark Glazier, Dr. Judy Lovchik, David Dotson, Shawn Richards and Dr. Jennifer House from ISDH, and Dr. Brett Marsh from BOAH.

Using enhanced laboratory surveillance methods, Indiana was the first state in 2011 to detect cases of human infection with H3N2v virus. Subsequently BOAH and ISDH developed a cooperative partnership for enhanced swine influenza (SIV) surveillance which enabled prompt preventive action during the 2012 Indiana State Fair, when further spread of the variant influenza virus was mitigated by limiting exposure of both pigs and humans to infected swine.

Of the 400 clinical specimens received in that outbreak, ISDH detected and investigated 138 cases of human infection with H3N2v from 24 Indiana counties involving 14 Indiana fairs. With analysis at the Indiana Animal Disease Diagnostic Laboratory, the BOAH detected 40 cases of swine infected with influenza A (H3N2) from 14 Indiana counties involving five Indiana fairs. Concurrent confirmation of both swine infections with H3N2 and human infections with H3N2v provided an invaluable link in detecting this shared influenza outbreak.

The impact of this strong relationship between ISDH, BOAH and local health departments highlights the value of cooperative partnerships at the state level. These partnerships allowed for easy intra-agency communication and facilitated timely collection and testing of samples from both human and swine to minimize the spread of infection. From these partnerships, new recommendations for swine exhibitions were created and approved to decrease the likelihood of zoonotic transmission in the public setting.

<-- The Poster is on page 6.

The LAByrinth

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