

Report of the Committee on Transmissible Diseases of Poultry and Other Avian Species

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The Committee met on October 26, 2015 from 1:00 to 6:00 PM and October 27, 2015 from 1:00 to 6:00 PM at the Rhode Island Convention Center in Providence, Rhode Island. There were 57 members and 69 guests present for a total of 126 participants. Chair Dale Lauer presided assisted by Sarah Mason, Vice Chair. The Chair welcomed the Committee members and summarized the 2014 meeting. Noteworthy events include the appointment of Dr. Alberto Torres to the NAHRS Steering Committee, the meeting of the PRD CAP (Poultry Respiratory Disease Coordinated Agricultural Project) & NC 1180 Committee on Wednesday October 28, 2015 and a revised agenda format that would focus on the 2015 HPAI event in the United States. There were no resolutions passed in 2014.

Presentations & Reports—Session 1

2015 HPAI Response and Analysis Presentations:

USDA HPAI Response presented by Dr. Burke Healy, United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), USDA-APHIS-VS, Fort Collins, CO. A summary of the report is included in these proceedings.

California HPAI Response presented by Dr. Annette Jones, California Department of Food and Agriculture, Sacramento, CA. A summary of the report is included in these proceedings.

Minnesota HPAI Response presented by Dr. Shauna Voss, Minnesota Board of Animal Health, Willmar, MN. A summary of the report is included in these proceedings.

Wisconsin HPAI Response presented by Dr. Myron Kebus, Wisconsin Department of Agriculture, Madison, WI. A summary of the report is included in these proceedings.

Use of Ventilation Shutdown for Mass Depopulation of Poultry in Emergency Situations presented by Dr. Eric Gingerich, Diamond V, Zionsville, Indiana. A summary of the report is included in these proceedings.

Report on Highly Pathogenic H5 Avian Influenza viruses in the Americas presented by Dr. David Suarez, USDA-ARS-SEPRL (Agriculture Research Service, Southeast Poultry Research Laboratory), Athens, GA. A summary of the report is included in these proceedings.

Report on the Molecular Epidemiology of the H5 clade 2.3.4.4 in the United States presented by Dr. Mia Kim Torchetti, USDA-APHIS-VS-NVSL (National Veterinary Services Laboratory), Ames, Iowa. A summary of the report is included in these proceedings.

Wild Bird Avian Influenza Surveillance presented by Dr. Tom Gidlewski, USDA-APHIS-WS (Wildlife Services), Fort Collins, CO. A summary of the report is included in these proceedings.

HPAI Epidemiology, USDA Perspective presented by Dr. Brian McCluskey and Dr. Lindsey Garber, USDA-APHIS-VS, Fort Collins, CO. A summary of the report is included in these proceedings.

HPAI Epidemiology, Minnesota Perspective presented by Dr. Michelle Kromm, Jennie-O Turkey Store,

Willmar, MN. A summary of the report is included in these proceedings.

The Monday session adjourned at 6:00 PM. The Committee reconvened at 1:00 PM on Tuesday, October 27, 2015.

Presentations & Reports—Session 2

2015 HPAI Recovery / Moving Forward Presentations

2015 HPAI Assessment, Moving Forward presented by Dr. John Clifford, USDA-APHIS-VS, Riverdale, MD. A summary of the report is included in these proceedings.

USDA HPAI Fall Planning Activities presented by Dr. Patricia Fox, USDA-APHIS-VS, Raleigh, NC. A summary of the report is included in these proceedings.

USDA Biosecurity Perspective presented by Dr. Lee Ann Thomas, USDA-APHIS-VS, Riverdale, MD. A summary of the report is included in these proceedings.

Minnesota HPAI Education and Biosecurity Reviews presented by Mr. Steve Olson, Minnesota Turkey Growers Association, Chicken and Egg Association of Minnesota, Buffalo, MN. A summary of the report is included in these proceedings.

Layer Biosecurity presented by Dr. Travis Schaal, Hy-Line International, Dallas Center, IA. A summary of the report is included in these proceedings.

HPAI Vaccines presented by Dr. David Swayne, USDA-ARS-SEPRL, Athens, GA. A summary of the report is included in these proceedings.

Secure Poultry Supply Plans presented by Dr. Julie Helm, Clemson University Livestock Poultry Health, Columbia, SC, and Dr. Eric Gonder, Butterball, LLC, Goldsboro, NC. A summary of the report is included in these proceedings.

This concluded the reports on the 2015 HPAI Response, Analysis and Recovery Activities.

A number of abstracts were made available to members of the Committee on Transmissible Diseases of Poultry and Other Avian Species. These included:

Broiler Industry abstract by Dr. Deirdre Johnson, Mountaire Farms, Inc., Millsboro, DE was made available at the meeting. A complete report is included in these proceedings.

Table Egg Industry abstract by Dr. Eric Gingerich, Diamond V, Zionsville, IN was made available at the meeting. A complete report is included in these proceedings.

Turkey Industry abstract by Dr. Steven Clark, Devenish Nutrition, Fairmont, MN was made available at the meeting. A complete report is included in these proceedings.

Live Bird Marketing System abstract by Dr. Fidelis Hegngi, USDA-APHIS-VS, Riverdale, MD was made available at the meeting. A complete report is included in these proceedings.

Avian Disease and Oncology Lab (ADOL) Research abstract by Dr. John Dunn, USDA Agricultural Research Service (ARS), Avian Disease and Oncology Laboratory (ADOL), East Lansing, MI was made available at the meeting. A complete report is included in these proceedings.

Abstracts were made available to TDP Committee members for their review, there were no questions or discussion on the abstracts distributed.

AI and Newcastle Disease Subcommittee report was presented by Dr. David Suarez, USDA-ARS-SEPRL, Athens, GA. A summary of the report is included in these proceedings.

National Poultry Improvement Plan report written by Dr. Denise Brinson, USDA-APHIS-VS, Conyers, GA, presented by Dr. Patricia Fox, USDA-APHIS-VS, Raleigh, NC. A summary of the report is included in these proceedings.

NVSL Avian Influenza and NDV Diagnostic Report was presented by Dr. Mia Kim Torchetti, USDA-APHIS-VS-NVSL, Ames, Iowa. A summary of the report is included in these proceedings.

NVSL Bacteriology Diagnostic Report was presented by Ms. Brenda Morningstar, USDA-APHIS-VS-NVSL, Ames, Iowa. A summary of the report is included in these proceedings.

Committee on Salmonella Report was presented by Dr. Doug Waltman, Georgia Poultry Laboratory Network, Gainesville, GA. A summary of the report is included in these proceedings.

Committee Business:

Sub-Committee Report: The Avian Influenza/Newcastle Disease Subcommittee Report as presented by Dr. David Suarez was approved by Committee.

Committee Recommendations: No Recommendations were proposed

Committee Resolutions: Four resolutions were brought before the Committee for consideration. These resolutions

included:

- (1) PCR diagnostics for avian influenza in National Poultry Improvement Plan (NPIP) Authorized Laboratories;
- (2) Use of Highly Pathogenic Avian Influenza Secure Egg Supply Plans, Secure Broiler Supply Plans and Secure Turkey Supply Plans during an HPAI event;
- (3) Use of Ventilation Shut Down for Mass Depopulation of Poultry to Control Highly Pathogenic Avian Influenza;
- (4) Incorporation of poultry industry biosecurity oversight into the National Poultry Improvement Plan (NPIP).

The Committee considered, reviewed and had a thorough discussion of each proposed resolution. Resolution (1) failed. Resolutions (2), (3) and (4) were approved by Committee and submitted to the Committee on Resolutions.

There being no further business the Committee adjourned at 6:00 PM, October 27, 2015.

USDA HPAI Response

Burke Healy, DVM, USDA-APHIS-VS, Fort Collins, CO

The United States experienced an outbreak of Highly Pathogenic Avian Influenza (HPAI) during 2014-2015 that was unprecedented in animal disease history. 21 States with commercial, backyard poultry or wildlife had HPAI detections thought there were none in the Atlantic flyway. 15 States reported commercial (9) or backyard (11) poultry HPAI detections. In total, there were 211 commercial premises with HPAI detections. Most are currently approved to restock.

Economic losses have totaled \$1.6 billion in direct losses and \$3.3 billion economy wide. Costs to federal taxpayers is \$990 Million (\$200 M indemnity; \$600 M other response costs to date). The trade impact has been substantial, with 17 trading partners instituting a total US ban, and 38 partners instituting a regional ban.

HPAI response protocol involves placement of a Quarantine to restrict movement of poultry into and out of an established control area, eradication of the infected flock, monitoring of domestic and wild birds in the control area, virus elimination within affected locations and testing to verify elimination of the disease agent. Depopulation of affected flocks can be achieved using fire-fighting foam, CO2 gas, or a form of ventilation cessation and heating. Disinfection of affected locations follows, with virus elimination as the goal. Farms which cannot be adequately cleaned with conventional methods may be allowed to lie fallow for a period of time sufficient to allow for virus destruction.

Highly Pathogenic Avian Influenza (H5N8) in California, 2015

Annette Jones, DVM, California Department of Food and Agriculture, Sacramento, CA

After watching highly pathogenic avian influenza (HPAI) detections in wild birds and backyard flocks move down the western United States (U.S.) in late 2014 and early 2015, California experienced the first United States spill over into a commercial turkey flock in late January and again into a mixed chicken and duck farm supplying live bird markets in February 2015.

Fortunately, or perhaps unfortunately, California had experienced an outbreak of notifiable avian influenza in 2014 in a duck and quail farm, so most California personnel involved, including laboratory staff, were fairly experienced. Also, fortunately, local United States Department of Agriculture (USDA) personnel had not been deployed to other outbreaks, so California was able to immediately stand up blended Incident Management Teams (IMT's) and quickly establish Incident Command Posts in close proximity to the outbreaks. A long history of emergency response to Exotic Newcastle disease, multiple avian influenza outbreaks, and multiple bovine tuberculosis outbreaks forged close working relationships between federal and state first responders, so integration was seamless.

Because later outbreaks in 2015 were much more significant, this summary will not include the various elements of response replicated there, but will focus on some elements unique to detecting the first positive commercial flock in what became a much larger outbreak.

At the time the first and second flocks were detected, there was hope that these would be the only positive flocks in the U.S., so besides disease control and eradication, there was a premium placed on accurate and optimal early public communication and communication with trading partners.

Besides establishing a blended IMT within hours of the presumptive positive and developing an initial Incident Briefing and Incident Action Plan, a Joint Information Center (JIC) was immediately established to ensure consistent information was shared by all involved parties. The JIC was established in a virtual environment and included USDA, the California Department of Food and Agriculture (CDFA), the California Department of Public Health, the Governor's Office of Emergency Services, Stanislaus County, and the impacted company's Public Relations. USDA also coordinated with the Centers for Disease Control. Before initial announcements, all parties ensured their facts and talking points were correct and consistent and agreed to the timing of notifications. Because

this strain of influenza was not known to be a human pathogen, it was critical that public safety reassurance was provided **BEFORE** any misinformation began to circulate. Getting this right up front is critical! The company wanted to notify their customers before public announcements were made and, true to their ethic, wanted to be transparent, so they publically self-identified. Trade notices, State Veterinarian notices, poultry industry notices, political notices and public notices occurred almost at the same time and in a well-coordinated manner.

With regard to trade, the immediate goal was to minimize inappropriate trade sanctions and protect exports for the rest of the country. Remember, at that time, there was hope that this outbreak would only affect the Pacific migratory flyway. As always, the demand for real-time information from U.S. trading partners was intense. Because a fairly strong IMT was in place, and this was the first U.S. positive commercial flock, and initially was a single flock, a different approach was taken. Initially situation updates were filtered through several management levels before getting to those negotiating trade, but information was changing quickly and the multiple steps lead to delays. After just a couple of days, all agreed that the Incident Commander (IC) should directly communicate with the trade staff. This communication would normally not be considered ideal because an IC needs to be focused on response, but in this situation, it did help drive accurate information more quickly and helped to minimize trade impacts for other states. If the larger outbreak in April had not occurred, this shift in communication could have been very significant and may need to be explored further for future outbreaks.

While this last point is not unique to the first commercial detection, it is a lesson learned that has been a challenge to mitigate. Historically in the face of an initial detection of a highly transmissible disease, all resources have been directed to the Operations Section for depopulation and decontamination. Most recognize the importance of reducing risk where it is known to exist – the “virus factory” embodied by an infected flock - but the risk of missing silently spreading disease elsewhere is often neglected. For these HPAI outbreaks in California, a concerted and successful effort was made to begin epidemiology and surveillance as quickly as depopulation. To accomplish this goal, the Plans Section had to be adequately staffed immediately. In the past, California has struggled to accomplish this goal, but had more success in 2015. Fortunately, no spread was detected from either introduction.

Minnesota HPAI Response

Shauna Voss, DVM, Minnesota Board of Animal Health, Willmar, MN

On March 2, 2015, the Minnesota poultry industry experienced their first introduction of H5N2 Highly Pathogenic Avian Influenza (HPAI) virus in a commercial turkey breeding operation in Pope County. Three weeks later, the second and third cases were identified in commercial turkey operations in a Lac Qui Parle and Stearns Counties. By April 13, there were 13 confirmed cases of H5N2 in the state with new cases were being identified daily. Between March 2, 2015 and June 6, 2015 a total of 110 premises in 23 counties were classified as positive for H5N2 HPAI. Of the 110, 104 were commercial turkey premises (75 commercial turkey flock premises, 23 breeding turkey flock premises and 6 that were characterized as Dangerous Contact Premises), 4 were commercial chicken layer premises, 1 commercial chicken pullet premises, and 1 backyard flock. Over 9 million birds were depopulated during the event.

RESPONSE

The Minnesota Board of Animal Health was the lead response agency for HPAI events in Minnesota and initially utilized a small State Incident Management Team (IMT) to organize the response. However, because state resources were quickly overwhelmed, the Board made a request to USDA to received help through the deployment of USDA IMTs. At the height of the incident, over 600 people from across the country were working on the ground in Minnesota to control and eventually stop the spread of the virus.

As part of response efforts, a 6.2 mile radius control area was established around each infected premises. All premises with poultry within each control area were quarantined and surveillance was performed. Non-infected flocks that were quarantined needed to receive a permit from the Board prior to movement of poultry or poultry products off the farm. Hatching eggs, day-old poults, table eggs and birds moving to slaughter also had to have a permit to move from or into control areas. The Secure Poultry Supply Plans were utilized as guidelines for permitted movement with over 2,555 permitted movement documents issued.

The majority of all HPAI testing in Minnesota was performed at the University of Minnesota, Veterinary Diagnostic Laboratory in St. Paul, MN. A total of 16,451 PCR tests were performed for HPAI between March 1, 2015 and June 30, 2015. Other NAHLN laboratories in the region were utilized for additional surveillance testing. Sample drop-off sites were established in three counties, close to where most of the control areas were located, to collect samples in a biosecure manner and courier them to the laboratory twice daily.

RECOVERY

As of October 16, 2015, 101 out of the 110 Positive Premises have restock agreements signed and 67 of those premises have had their quarantines released. A number of premises have had a delay in restocking due to the nationwide shortage of poults. Six premises have elected to fallow; as a results, those quarantines will be released 120 days after the compost pile was capped.

LESSONS LEARNED

- 1) It is critical to know before an outbreak what resources you have and where you can get more. An Incident Command Post was established at an Emergency Operation Center in Kandiyohi County that had been used for previous other low pathogenic avian influenza response efforts. While we thought we were prepared for depopulation (the state of Minnesota owns a foaming unit and exercises it regularly), the Board was unable to keep up with the number of positive premises. We were also under prepared to handle efficient depopulation of large layer complexes. Water became a precious commodity for depopulation. On April 23, 2015, the Governor declared a Peacetime Emergency which allowed the National Guard to assist in sourcing and delivering water for depopulation efforts.
- 2) Obtaining a rapid diagnosis and being able to effectively complete surveillance activities requires having people trained to collect samples and a way to get those samples to the lab. Because Minnesota has an established Authorized Poultry Testing Agent program, only a small number of people needed Just-In-Time training to collect samples. Having trained personnel who work on the farms to collect samples reduces the likelihood that surveillance crews may contribute to disease spread. In addition, established drop-sites and a courier to bring samples to the lab twice daily facilitated rapid diagnosis and compliance in the required testing protocols.
- 3) Because HPAI is considered a Foreign Animal Disease, USDA will be involved and therefore, National Prem ID numbers and the USDA Emergency Management Response Services (EMRS 2.0) will be used. Valuable time can be saved if there are response personnel familiar with EMRS 2.0 and if National Prem ID Numbers (PIN) are established before an outbreak.
- 4) A key component in a successful response is being able to deliver a consistent message, in a timely manner, to those who need to know. A communications group of state and industry personnel had worked for over a year before HPAI arrived in Minnesota on a plan to communicate about avian influenza. This allowed for a seamless relay of information to the public and ensured a consistent message.
- 5) The 2015 HPAI H5N2 virus was unlike any infectious agent that the poultry industry in Minnesota or the upper Midwest had experienced before. As a result, biosecurity practices employed by producers on farm were often inadequate to prevent introductions. In addition, responders must be certain that they are employing and practicing biosecurity measures that will prevent spread off of infected premises. Due to the numbers of responders during the event, Just-In-Time training on biosecurity will have to be utilized and compliance must be monitored to prevent complacency.
- 6) Unforeseen circumstances will present themselves during any response. Therefore, it is important that your HPAI plan is flexible. For Minnesota's response, it was valuable to have industry partners at the table to provide feedback on current industry practices and to ensure that there were no unnecessary hindrances to business and production practices.

Wisconsin HPAI Response

Myron Kebus, DVM, Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison, WI

The Wisconsin Department of Agriculture, Trade and Consumer Protection's (DATCP), Division of Animal Health, managed a highly pathogenic avian influenza (HPAI) outbreak that included ten different infected poultry flocks. There were 1.9 million birds depopulated, 265 premises tested twice in the control zones, over \$8.6 million paid out in federal indemnity. The farms included a cage free layer farm, caged layer farms, turkey farms, and one backyard flock.

On Friday, April 10, 2015 the Division received notice of a presumptive positive, which was confirmed as H5N2 on April 13, 2015. The first premises was a 200,000 bird cage free layer facility in Jefferson County, in southern Wisconsin. The farm noticed a slight increase in mortality that began on Monday, April 6, 2015 followed by increasing mortalities over the next several days. The next was a small flock of 33 chickens in Juneau County in western Wisconsin was confirmed on April 17, 2015. This flock housed feral ducks that would leave the farm for weeks and return. The third infected premises followed on virtually the same day as the second premises. It was a 130,000 bird commercial turkey operation in Barron County in northwestern Wisconsin. These were followed by four more commercial turkey farms, a caged egg layer farm, and finally a pullet farm confirmed on May 3, 2015.

Along with the ten confirmed positive HPAI poultry infected premises, 265 premises within a 10km control zone of the infected premises were quarantined and 1,445 poultry on those premises were tested twice two weeks later in order to confirm the disease had not spread. The Division evaluated requests for movement of poultry and poultry products from producers within the control areas. The process included conducting an onsite biosecurity assessment of the requesting flock, completing the required testing, and approval by the state veterinarian in the state of destination. The USDA permitting team handled the issuance of the approved permits and captured the information in EMRS. The Division estimated that more than 700 permits were issued during the 2015 spring HPAI event.

Press releases were issued upon confirmation of infected premises, release of control areas, and quarantine release of infected premises. Fact sheets were posted on the DATCP website on topics including: HPAI and human health, poultry industry in Wisconsin, protecting your farm from HPAI, and others. The DATCP Public Information Officers issued a daily HPAI briefing, distributed electronically to over 5,000 subscribers and posted on the DATCP website. All quarantine releases were accompanied by a letter and pamphlet reminding poultry owners to continue to be diligent in regards to biosecurity and protecting their flock from infection. The Wisconsin State Veterinarian issued a Summary Special Order on June 6, 2015 prohibiting poultry movement to swap meets or open shows in Wisconsin unless part of a county, district, or state fair. An additional order, also issued June 10, 2015, requiring participants in poultry shows associated with fairs to certify that no poultry mortalities have been found on their premises within ten days prior to movement of poultry to the fair.

Wisconsin Governor, Scott Walker, declared a State of Emergency for Wisconsin's response to HPAI. This declaration opened the full resources of the state Emergency Operations Center and the National Guard to assist in the response.

By August 8, 2015 all infected premises were released from quarantine and were eligible to restock following guidelines agreed upon between the USDA-APHIS, the State of Wisconsin, and the farm.

Planning continues for the next outbreak. Major concerns are the ability to mobilize enough staff to manage a large incident. Discussions continue with Wisconsin Emergency Management and other state agencies. The Division hosted four poultry workshops statewide that were attended by over 300 producers from all industry sectors. An overview of the outbreak was presented, questions were answered and biosecurity guidelines were discussed.

Use of Ventilation Shutdown for Mass Depopulation of Poultry in Emergency Situations

Eric Gingerich DVM, Diamond V, Zionsville, IN

During the highly pathogenic avian influenza outbreaks during the spring of 2015 in the upper Midwest, many problems occurred that did not allow timely depopulation of turkey and layer flocks. USDA has stated that a flock infected with HPAI should be put down within 24 hours after confirmation. This stops the shed of virus and does not allow the increase in shed rate of HPAI virus seen in the outbreaks if flocks are allowed to remain alive. Ventilation shutdown (VSD) is being considered as one solution should this problem arise again.

During the HPAI outbreaks of 2015, the large number of outbreaks occurring at one time overwhelmed the ability to depopulate flocks on a timely basis using the approved methods of CO2 carts for layers or firefighting foam for turkeys. It is felt that many flocks could have been spared being infected with HPAI had flocks been put down in a timely manner and suppressed the high levels of virus shed from them.

An option to quickly cause death of all birds in a house is to shut off the ventilation fans (VSD) that will allow the heat from the birds to increase rapidly and result in hyperthermic death. A precedent has been set by the United Kingdom's Department for Environment, Food, and Rural Affairs (DEFRA) for use of this method in emergencies. DEFRA set forth guidelines for VSD use in their document "Guidelines for Killing Poultry Using Ventilation Shutdown (VSD) in September 2009 (<http://www.slideshare.net/charmkey5/operating-guidance-ventilation-shutdown-procedure-defra>).

Besides the reduction in shedding of virus, other reasons for deciding to use VSD are that 1) it greatly reduces the time of exposure of workers depopulating flocks using standard methods to potentially zoonotic agents, and 2) reduces the amount of birds suffering from the disease during slower depopulation methods.

It is agreed that VSD is not the ideal method for mass depopulation as it results in longer periods of time for suffering compared to other methods. The decision to use VSD is only to be made after all other more humane methods have been considered and it has been determined that the time taken for other methods will allow the amount of virus to become excessively high and results in undue spread of the disease.

The United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) developed and announced its position on the use of VSD on September 18, 2015. This document contains a decision tree for determining if a particular depopulation situation should use VSD or not. This document is available at the USDA-APHIS website -

https://www.aphis.usda.gov/animal_health/emergency_management/downloads/hpai/ventilationshutdownpolicy.pdf

The VSD process as defined by DEFRA is to raise the temperature in the house to 104F within 30 minutes and to hold this temperature for at least 3 hours. Water is not turned off during the process. Sealing the house is required to help hold heat in the house. Supplemental heat may be required and guidelines are being developed using predictive modeling in different scenarios. More research is needed to make this procedure as humane as possible.

The American Association of Avian Pathologists (AAAP), at their annual meeting in the summer of 2015, approved a position statement drafted by their animal welfare and management committee to approve the use of VSD, with appropriate veterinary consultation, in cases of emergency when deemed necessary in order to control the spread of a foreign animal disease (FAD). The AAAP position statement, FAQs, and background information are

available to AAAP members on the website www.aaap.info under Committees/Animal Welfare/Emergency Mass Depopulation Guide and Avian Influenza Resources.

The American Veterinary Medical Association's (AVMA) Panel on Depopulation will be developing their guidelines for mass depopulation over the next two or more years. More information can be seen at the AVMA website - <https://www.avma.org/KB/Resources/Reference/AnimalWelfare/Pages/Depopulation.aspx>

Highly Pathogenic H5 Avian Influenza viruses in the Americas

David Suarez, DVM, USDA-ARS-SEPRL, Athens, GA

In late 2014 highly pathogenic avian influenza was detected first in Canada and then in the United States associated with wild birds, commercial poultry, and backyard flocks. The H5N1 highly pathogenic avian influenza virus lineage can be traced back to as early as 1996 with the first isolation of virus in geese in Guangdong province, China. The viral lineage had multiple basic amino acids at the cleavage, which is a marker for a virus having a highly pathogenic phenotype. The hemagglutinin gene for all the Asian lineage HPAI viruses can be traced back to these original viruses, but all the other gene segments for this virus have reassorted from the original virus. The drift became so important that a revised nomenclature system was developed to account for all the genetic changes in the virus, the clade system. Each clade is at least 2% different in nucleotide sequence which does correlate with antigenic differences. As the virus continued to mutate, the clade system continued to evolve into different subclades, so that now we have as many as 9 different clades that branch to fourth order, i.e. clade 2.3.2.1. Although we think of highly pathogenic avian influenza as being restricted to poultry, we have detected on at least three occasions where the virus has spilled over from poultry back to wild birds where the virus has persisted in wild birds for several years. This first occurred in 2005, and then again in 2008, and the most recent example occurred in 2014-2015. These were clade 2.2, clade 2.3.2.1, and clade 2.3.4.4 respectively. The most recent virus with a N8 replacing the N1 gene was first reported from South Korea where the virus infected commercial poultry, primarily ducks causing a large outbreak. The timing of the virus infection suggested that wintering birds were infected with the virus and spread the virus to commercial poultry. The virus appeared to move to the wild bird breeding grounds in the summer, which includes breeding grounds in North America (Alaska). The virus appeared to spread among wild birds in North America, and when the birds moved south for the winter, they carried the virus with them and infected poultry in Canada and the United States.

The viruses identified in the Americas all had similar hemagglutinin genes, but some variation was seen in other gene segments. One type of virus, the H5N8, was similar in all eight genes with the viruses that were detected in South Korea and were also closely related to viruses from Russia and Europe. The H5N8 virus was detected multiple times from apparently healthy wild ducks in the United States and was associated with at least two outbreaks in commercial poultry and several backyard birds. Another variant was a reassortment between the H5N8 virus and a North American low pathogenic virus, such that the neuraminidase gene and several internal genes were replaced. This new virus, a H5N2, was also detected in wild birds and commercial turkeys in Canada and the Midwest United States. Several other minor variants have been detected, but most viral isolates have been the H5N8 or H5N2 viruses.

Representative H5N8 and H5N2 viruses have been used in experimental laboratory studies in several different species of birds. Based on these studies several observations can be made. The virus when given by the standard intravenous (I.V.) pathogenicity test is highly pathogenic causing 100% mortality in chickens, confirming that these viruses are highly pathogenic viruses. However, field observations in South Korea, has suggested that the virus was not as virulent in chickens as other H5N1 HPAI viruses. In laboratory challenge studies of chickens it was shown that high doses of virus were needed to infect birds by the oro-pharyngeal challenge model, which more closely matches field exposure. The virus also poorly transmitted to uninfected cage mates by direct contact. However, when a chicken became infected, the bird died. Clinical signs typically were depression and rapid death, but more classic lesions of highly pathogenic avian influenza were seen in some birds including hemorrhages in the legs and petechial hemorrhages in the myocardium, pancreas, and proventriculus. Rarely neurological lesions were observed. In contrast to the chicken studies, mallard ducks were extremely susceptible to infection with both viruses, and the viruses transmitted efficiently to uninfected cage mates. However, no clinical disease was observed in any birds although all experimental birds became infected. Pathology did show some systemic infection, but not enough to cause disease or mortality. Studies with isolates later in the outbreak showed the H5N2 viruses were more infectious to chickens and killed birds faster showing evidence of adaptation of the virus to chickens.

The H5N8 and H5N2 viruses tested in the United States shows that the virus is extremely infectious in mallard ducks without causing clinical disease. Although there are many wild ducks species where the virus has been detected, it appears based on the experimental data that these HPAI viruses are well adapted to many duck species and behave more similarly to low pathogenic avian influenza viruses. This likely explains why this virus lineage has been detected on three different continents, and also suggests the virus is likely to persist in wild birds for a while. Although commercial turkeys and chickens have been infected in the United States and Canada, the

viruses tested did not seem well adapted to gallinaceous poultry and required a high infectious dose. This correlates with field data where the initially infected farms have geographically have been far away from each other. However, later during the outbreak, partly through changes in the virus and inadequate biosecurity, farm to farm spread probably was more important source of spread. Because of the likelihood of persistence of the virus in wild birds for several years, biosecurity will need to remain at enhanced levels to protect the poultry industry.

Molecular Epidemiology of the H5 clade 2.3.4.4 in the United States

Mia Kim Torchetti, DVM, USDA-APHIS-VS-NVSL, Ames, IA

H5N8 virus (H5N8 clade 2.3.4.4) originating from Eurasia (EA) spread rapidly along wild bird migratory pathways in the Eastern Hemisphere during 2014. Introduction of this virus into the Pacific Flyway of North America sometime during 2014 allowed mixing with North American (AM) origin low pathogenicity avian influenza A viruses generating new (novel) combinations with genes from both EA and AM lineages (so-called “reassortant” H5Nx viruses). To date, the H5Nx viruses have been detected in the Pacific, Central, and Mississippi Flyways (Figure 11). These findings are not unexpected as the H5Nx viruses continue to circulate.

USDA’s NVSL collaborated with the USDA ARS Southeast Poultry Research Laboratory (SEPR) and the Influenza Division of the Centers for Disease Control and Prevention (CDC) to generate the analyses for this report. Consensus data from whole genome sequence is used to monitor the virus evolution and assess risk to veterinary or public health based upon presence/absence of specific amino acid substitutions or protein motifs.

All viruses analyzed to date are highly similar, have an HA gene derived from the EA H5 clade 2.3.4.4, and are highly pathogenic in poultry. Both H5N2 and H5N8 were implicated in recent poultry outbreaks. Where there is molecular evidence that independent introductions as well as “common source” exposures are occurring concurrently, further field epidemiologic investigation is warranted.

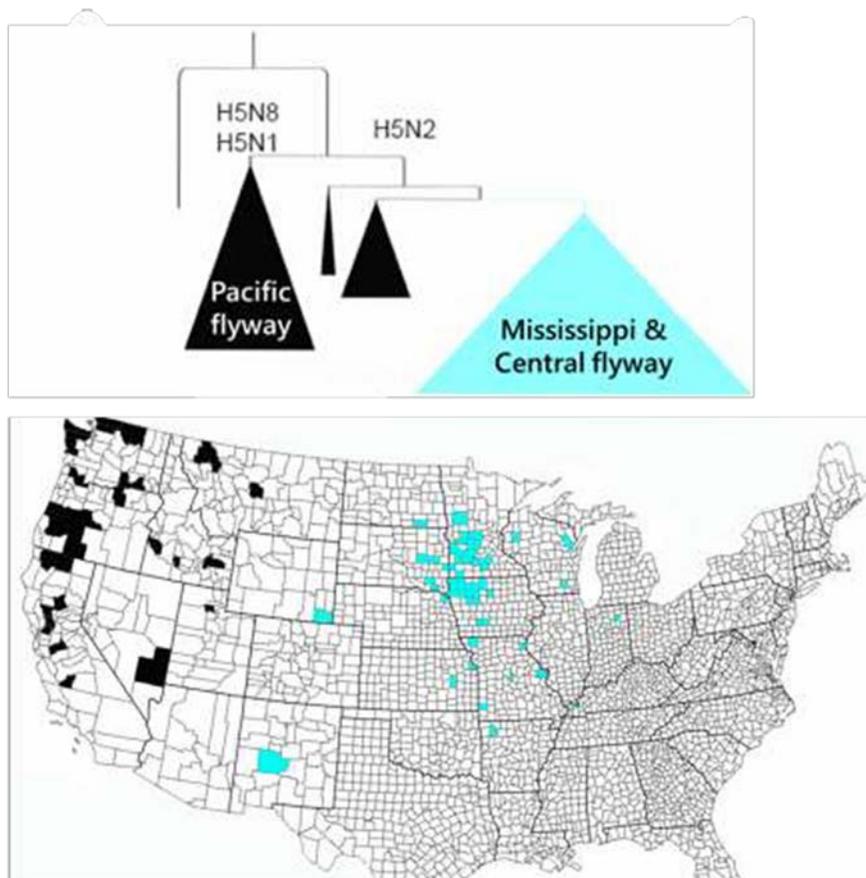


Figure 11. Phylogeny of the PB2, HA, and matrix genes of the H5Nx viruses and geographic distribution by subtype

Poultry events in Pacific Flyway appear to be largely due to point source/independent introductions as

were early Midwest events based upon network analysis and available epidemiologic data. Data for later Midwest events suggest point source as well as “common source” exposures occurring concurrently. States affected last appear to be largely due to common source/human activity.

Presently the risk to human health remains low; molecular markers associated with antiviral resistance or increased virulence and transmission in mammals have not been detected. However, CDC continues virus monitoring.

This analysis includes samples collected between December 2014 to early June 2015 (Figure 12) from 17 States (>240 viruses distributed as in Table 37). While these viruses remain highly similar overall (>99% similar to the index viruses within subtype as well as to the nearest Asian isolate A/crane/Kagoshima/KU1/2014[H5N8]), analytical tools that identify substitutions along the hemagglutinin (HA), neuraminidase (NA) and internal proteins can improve our understanding of the virologic, antigenic, and epidemiologic features of the virus. The section on Diagnostics and Characterization for H5Nx viruses in this report offers further information.

Table 37. Distribution of viruses by region, subtype or virus group, and sector/type with state/county affected and duration from sample collection

Region	Virus subtype or group	layer, commercial turkey, commercial backyard	wild bird+raptor	# states affected	# counties affected	Duration from sample collection	Mode of spread based upon molecular analysis
Midwest H5N2	1			5	16	27 Feb to 20 Apr 2015	independent + limited lateral
	2a		⊙	4	16	6 Apr to 4 May 2015	independent + limited lateral
	2b		⊙	5	18	25 Mar to 4 Jun 2015	ind+lat 76% MN turkey
	2c		⊙	5	22	13 Mar to 25 May 2015	ind+lat 85% IA chicken>turkey
	2d		⊙	4	10	26 Mar to 14 May 2015	ind+lat 91% MN turkey
Pacific	H5N2	⊙	⊙	4	16	8 Dec 2014 to 11 Feb 2015	independent
	H5N8			6	13	7 Dec 2014 to 6 Feb 2015*	independent
	H5N1	⊙	⊙	1	1	7 Dec 2014 to 6 Feb 2015	independent

* not including Indiana BY 5 May 15



Figure 12. Duration of detection from sample collection date by virus subtype/group

Summary of H5Nx Molecular Analysis

All viruses detected to date have an HA gene derived from the EA H5 clade 2.3.4.4 and are highly pathogenic for poultry. Pacific and early Midwest detections appear to be largely independent introductions and later events include potential for human involvement.

Pacific Flyway Findings

- Three different subtypes were detected (Table 37); the EA/AM H5N2 viruses predominated.
- No H5N2 was detected in commercial poultry in the Pacific flyway.
- The H5N8 viruses have wholly Eurasian gene constellations except two from Oregon (Jan 2015) with two North American internal genes (PB1 and PA).
- H5N8 was detected in both poultry and wild bird populations in the Pacific flyway.
- Long branches (representing nucleotide differences) observed by network analysis for all H5Nx viruses in the Pacific flyway are suggestive of independent or point source introductions (Figure 13).
- These findings are consistent with both the movement of the virus in wild bird flyways and the low infectivity in gallinaceous poultry.

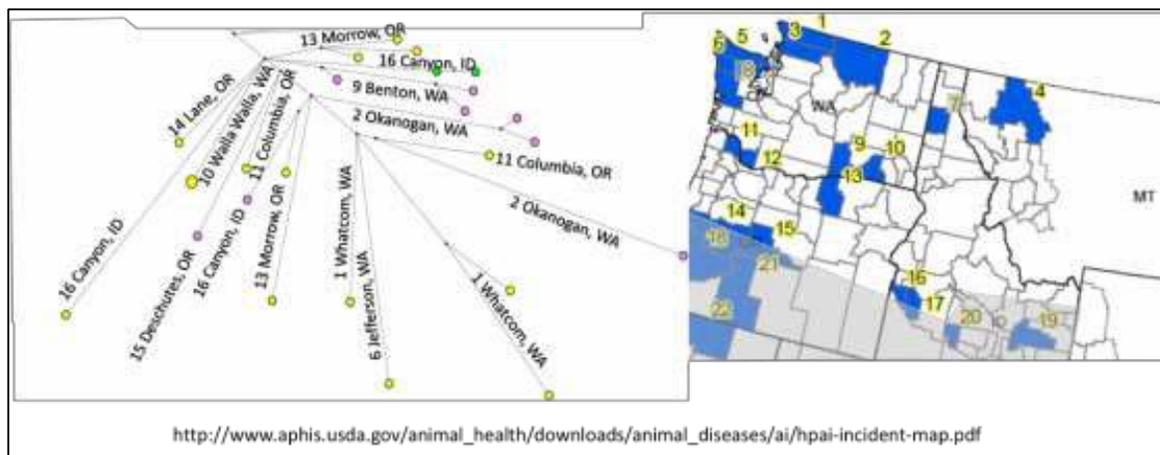


Figure 13. 8-gene network: Selection of 24 Pacific flyway detections spanning 3 States and 13 counties from December 8, 2014, to February 11, 2015; long branches suggest independent or point source introductions (greyed area = H5N8). Numbers on network correlate to map, which is available at web site above; yellow circle = wild bird, purple = backyard, red = poultry. Numbering indicates order of county detection; subsequent detections in positive county are not numbered.

Midwest Findings

- The Midwest viruses cluster into major groups 1 and 2 with four subgroups in group 2 indicated in Table 37.
- Groups 1 and 2a span several States and counties and contain long branches similar to that observed in the Pacific group suggesting largely independent or point source introductions in addition to limited evidence of lateral spread (Figure 14).
- The remaining groups (2b, c, and d) have a mixture of long branches suggestive of independent or point source introductions alongside shorter branches and highly similar viruses consistent with common source or lateral spread. The network and map in Figure 15 demonstrate the relatedness of the 2d.1 subcluster (ex-Stearns cluster), which gained in number and has confirmed epidemiologic links for many of the premises.
 - Minnesota viruses are predominantly group 2b, 2d from turkeys
 - Iowa viruses are predominantly group 2c from layers and turkeys
 - All Midwest subgroups may be found in turkeys compared to layers (Table 37), suggesting there may be increased risk for a broader range of potential exposures
 - Only a single detection of EA H5N8 has been made outside the Pacific flyway (IN); molecular evidence suggests it may not have been present in the Mississippi, but further data are needed.

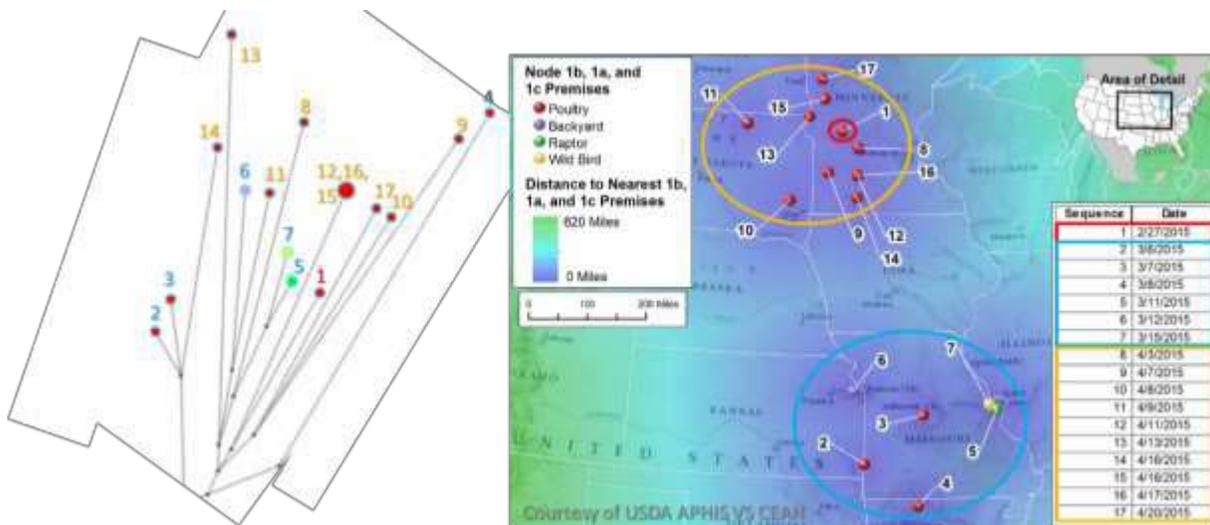


Figure 14. Network analysis (8 gene) of H5N2 Midwest Group 1: 17 detections spanning 5 States and 16 counties from February 27 to April 20, 2015; long branches suggest largely independent or point source introductions with limited evidence of lateral spread. Colored boxes match colored circles on map and colored numbers on network. Yellow circle = wild bird, purple = backyard, red = poultry.

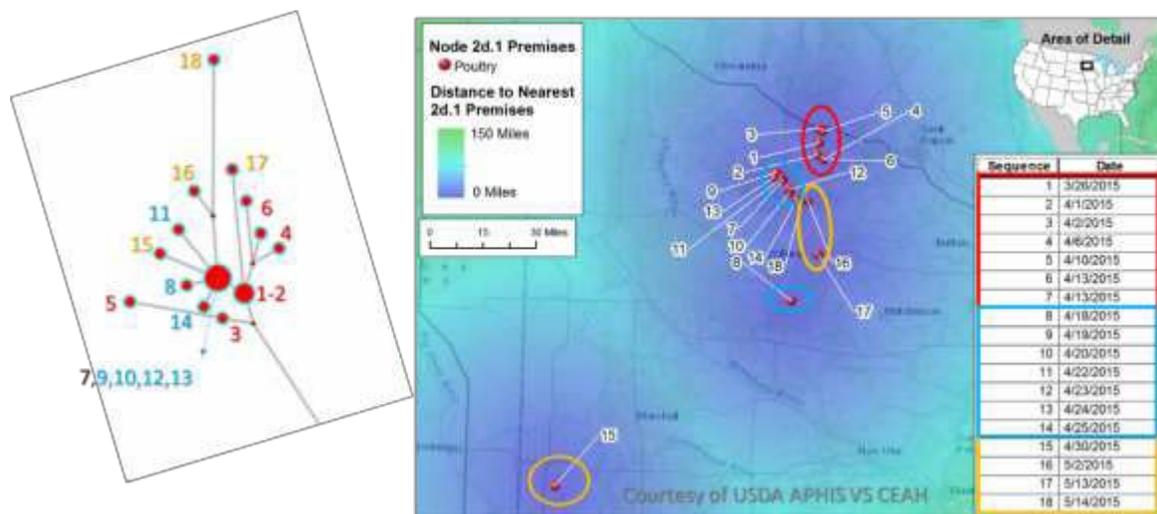


Figure 15. Network analysis (8 gene) of H5N2 Midwest Group 2d.1: 18 detections in single State across 4 counties from March 26 to May 14, 2015; highly similar viruses and shorter branches consistent with common source or lateral spread, viral change is consistent with the date of detection. Colored boxes match colored circles on map and colored numbers on network; red circle = poultry.

Other General Findings:

- Over 240 viruses analyzed have been >99% similar to the index case across entire genome within subtype and for HA across subtypes.
- The majority of poultry viruses are nearly identical across the HA1 protein and have a change in the HA1 protein at a putative antigenic site (HA S141P; numbering per mature H5 HA; Table 38). Such substitutions may be more easily sustained in small virus populations (e.g., poultry flock).
- The molecular evidence reported on June 15, 2015, for two viruses that spanned a State boundary between

Diagnosics and Characterization for H5Nx Viruses

Eurasian H5 clade 2.3.4.4 viruses (aka H5Nx), more specifically the “Intercontinental Group A viruses”¹ (icA), were initially detected in the United States during December 2014 and are known to be highly pathogenic to poultry. No other Eurasian H5 viruses have been detected in the United States to date (August 2015). The index viruses are A/gyrfalcon/Washington/41088-6/2014(H5N8) and A/Northern pintail/WA/40964/2014 (H5N2).

Molecular diagnostics for influenza A virus (IAV) used across the NAHLN in the United States have been confirmed to work well to detect these Eurasian H5Nx viruses.² As a primary surveillance tool, the NAHLN H5 assay is broadly reactive and not intended to distinguish geographic lineage or pathotype. NVSL also uses a highly specific H5-icA assay³ developed by SEPRL, which targets the Eurasian H5 clade 2.3.4.4 gene and conducts Sanger sequencing protocols to generate partial HA/NA sequence directly from the sample for confirmation, pathotyping, and subtype determination. Select viruses are also processed for in vivo pathotyping in specific pathogen free chickens. Results from in vivo testing is specific to the species tested (e.g., chickens).

Additionally, whole genome sequencing is conducted to monitor viral evolution. Both Ion Torrent and MiSeq technologies are used. A brief summary of the procedure for IAV follows. All eight segments of isolates were amplified using gene-specific and universal primers for each segment. The cDNA was purified and cDNA libraries were prepared for the Ion Torrent using the IonXpress Plus Fragment Library Kit (Life Technologies) with Ion Xpress barcode adapters. Prepared libraries were quantitated using the Bioanalyzer DNA 1000 Kit. Quantitated libraries were diluted and pooled for library amplification using the Ion One Touch 2 and ES systems. Following enrichment, DNA was loaded onto an Ion 314 or Ion 316 chip and sequenced using the Ion PGM 200 v2 Sequencing Kit.

Analysis of sequence data includes phylogeny of all eight segments, determination of amino acid substitutions across the HA1 protein, and network analysis of three gene segments (PB2, HA, MP). Phylogenetic trees are generated using neighbor-joining algorithms with a kimura-2 parameter nucleotide substitution model. Amino acid differences in the HA1 portion of the HA protein compared to the A/gyrfalcon reference virus with potential virologic significance are annotated based on previous experimental studies with HPAI H5 viruses that have demonstrated changes in virus phenotype using various in vivo and in vitro systems. The NA and internal protein genes are aligned to H5N8 and H5N2 reference virus genomes using MUSCLE (i.e., A/gyrfalcon/Washington/41088-6/2014 and A/Northern pintail/WA/40964/2014) and screened for the presence of amino acid substitutions or protein motifs that have previously been associated with either poultry or mammalian host adaptation.

¹ 2015 Lee et al, Intercontinental Spread of Asian-origin H5N8 to North America through Beringia by Migratory Birds, epub ahead of print *J Virol* <http://jvi.asm.org/content/early/2015/04/02/JVI.00728-15.long>

² Influenza A protocols including Spackman 2002 targeting the matrix, VetMax Gold AIV and the H5 subtyping assays (2008 and 2014 protocols)

³ The H5-icA assay protocol is available from SEPRL and positive control is available from NVSL for standard user-fee; note that this assay has a very narrow in spectrum specific to H5 clade 2.3.4.4 viruses and should be used in conjunction with the NAHLN H5 assay, not as a replacement

Avian Influenza Wildbird Surveillance

Tom Gidlewski, DVM, USDA-APHIS-WS, Fort Collins, CO

This presentation describes the national surveillance plan for avian influenza virus (AIV) in wild waterfowl. Collaborating entities include the USDA, APHIS, Wildlife Service (WS), National Wildlife Disease Program (NWDP) and VS; the United States Geological Survey (USGS); the United States Fish and Wildlife Service (USFWS); and the National Flyway Council. This national level surveillance directly supports the United States Interagency Strategic Plan for Early Detection and Monitoring for Avian Influenzas of Significance in Wild Birds (2015), and is based on the Surveillance Plan for Highly Pathogenic Avian Influenza (HPAI) in Waterfowl in the United States (2015).

HPAI Surveillance Goals

The goals of this surveillance effort are: to maximize our ability to detect AIV in wild waterfowl so that we can identify the distribution of avian influenzas in the United States; to detect spread of influenzas to new areas of concern; to provide a flexible surveillance framework to monitor wild dabbling duck populations for reassortments of

influenzas and introductions of new viruses; and to estimate apparent prevalence of important influenzas once detected.

The plan focuses on sample collection at the watershed level (sub-regional watersheds) and specific watersheds have been identified for sample collection. This selection is based on areas that have high mixing of wild bird populations (sometimes from multiple flyways) and historic low pathogenicity AIV presence. This allows targeted sample collection in high priority watersheds where AIV dynamics will likely be indicative of what is also occurring in nearby areas that are not sampled. If the targeted numbers of samples are collected from dabbling ducks within each specified watershed, we will be able to determine with 95% certainty whether the avian influenza viruses of interest are present at the time of the surveillance.

2015 Implementation Plan

1. SPECIES AND SAMPLE NUMBERS:

- a. The target number applies only to dabbling ducks.
- b. Target species is dabbling ducks. The Fulvous Whistling duck is not taxonomically a dabbling duck but because of its foraging habits it is included in the same functional group for purposes of this surveillance plan.

Target Species by Functional Group Dabbling Ducks:

American Green-winged Teal	Mallard
Northern Pintail	American Black Duck
Wood Duck	Blue-winged Teal
Cinnamon Teal	Northern Shoveler
Gadwall	American Wigeon
Mottled Duck	Muscovy Duck
Fulvous Whistling Duck	

- c. Captive-reared and released ducks that are subsequently live-captured or hunter harvested may be swabbed like any other dabbling duck and will be counted in the watershed target numbers.
- d. In biological year 2015 (BY2015: April 1, 2015 through March 31, 2016) approximately 41,000 wild bird samples will be collected nationwide.

2. WHAT TO COLLECT:

- a. The target sample numbers represent samples collected from agency harvested birds, hunter harvested birds and live wild birds.
- b. One cloacal and one oropharyngeal swab will be collected from each wild bird sampled. Cloacal and oropharyngeal swabs will be combined in the same tube of media.

3. WHEN TO COLLECT:

Sample collection will occur during three different time periods during BY2015. This differs from previous sampling protocols in an effort to capture wild migratory bird movements at different times of the year. A month of overlap has been added to the seasons to allow flexibility in reaching the targets. Birds sampled in August or December may be counted toward the target for either season.

- a. *Summer breeding season* (May-August),
- b. *Fall migratory season* (August-December),
- c. *Over-wintering season* (December-February)

4. WHERE TO COLLECT:

Target watersheds for HPAI sampling are at the watershed level (sub-regional watersheds). This is a departure from our previous AIV collection protocols which allowed samples to be collected anywhere within a state. There is flexibility in watersheds and seasonal targets. The program has modified targets in approximately half of the states in response to logistical feedback from the field.

5. SAMPLE SUBMISSION:

All samples will be submitted to one of seven approved National Animal Health Laboratory Network (NAHLN) laboratories. Samples will be screened to determine if type A influenza virus is present; if the test is

positive, the sample will be further analyzed using H5 and H7 specific assays. Samples testing H5 or H7 positive at a NAHLN laboratory will be sent to the National Veterinary Services Laboratories (NVSL) for confirmatory testing and final diagnosis.

6. PERMITS:

The NWDP has a “blanket” scientific collecting permit from USFWS that includes all states, except Hawaii, for the swabbing of most species collected as live birds or hunter harvest. Agency harvest for the sole purpose of disease sampling is not permitted.

7. STATE AGENCIES, TRIBAL AGENCIES and USFWS:

Close collaboration with state and tribal game agencies, and the USFWS is vital. Sample collection should include efforts by federal, state, tribal, local, university and non-governmental participants. Local expertise should be utilized to assess the watersheds and targets in this plan and determine adjustments that are needed. It will be necessary for state wildlife agencies and Wildlife Services programs to communicate their sampling plans in the various watersheds in order to optimize sample collection goals throughout the summer, fall, and overwintering seasons.

8. REPORTING FIELD DATA:

Each participating agency, university, or other entity is responsible for entering field data directly into the APHIS Veterinary Services Laboratory Submission System website (VSL) (<http://vsapps.aphis.usda.gov/vslabsub/login.do>) within 24 hours of submitting samples to the laboratory. Once the field data have been entered into the system, results will be entered online and available for viewing. Collectors and submitters can also run reports and queries. Positive cases as well as the total number of birds sampled are posted on the website. (https://www.aphis.usda.gov/wildlife_damage/downloads/JULY%202015%20-%20JUNE%202016%20WILD%20BIRD%20POSITIVE%20HIGHLY%20PATHOGENIC%20AVIAN%20INFLUENZA%20CASES%20IN%20THE%20UNITED%20STATES.pdf)

9. MORBIDITY AND MORTALITY SURVEILLANCE:

Morbidity/mortality events should be investigated regardless of the time of year, species involved, or the number of samples already collected in the state. Morbidity/mortality samples do not count towards meeting the watershed targets and are not entered into the VSL database. Morbidity/mortality events have a different disease risk associated with them and the data cannot be analyzed in the same way as apparently healthy birds (live-capture and hunter harvest).

The USGS National Wildlife Health Center (NWHC) in Madison, Wisconsin is the primary partner for performing diagnostics related to mortality events and can provide guidance on the investigation, sampling, and diagnostics for observed avian mortality. Contact at 608-270-2480, NWHC-epi@usgs.gov

State veterinary diagnostic laboratories may also be used in morbidity/mortality investigations rather than the NWHC and should be contacted directly. Additional information is available on the USDA APHIS AI website. (https://www.aphis.usda.gov/wps/portal/aphis/ourfocus/animalhealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_animal_health%2Fsa_animal_disease_information%2Fsa_avian_health%2Fct_avian_influenza_disease).

HPAI Epidemiologic Findings, USDA Perspective: What They Tell Us about Prevention and Control

Brian McCluskey, DVM, Lindsey Garber, DVM, USDA-APHIS-VS, Fort Collins, CO

Since the expansion of HPAI viruses into commercial poultry occurred in January 2015, APHIS Veterinary Services (VS) has initiated a number of epidemiologic and laboratory based investigations to better understand the factors associated with HPAI virus transmission. These investigations include:

- **Field-based observational studies with data collected through surveys and site visits;**
- **Geospatial analyses;**
- **On-farm sampling efforts; and**
- **Phylogenetic investigations.**

With the data from these reports, APHIS concludes that there is not substantial or significant enough evidence to point to a specific pathway or pathways for the current spread of the virus. This is further supported by the molecular analysis of the virus.

In a case series investigating 81 turkey farms across the Midwestern United States, we found turkey farms typically follow biosecurity protocols, which are established by the company with which they work. Common procedures include spraying vehicle tires with disinfectant at the farm entrance, requiring visitors and employees to wear coveralls and disposable boot covers (or dedicated footwear) before entering the barns, using disinfectant footbaths at barn entrances, using rodent control, and caring for younger birds before caring for older birds. The objective is to establish a clean-dirty line where outside contaminants are not carried into the barn.

Fomites, such as equipment, are probably playing a role in this outbreak. In the majority of cases in this study, feed trucks, live haul loaders, pre-loaders, and other items were shared by multiple farms. While equipment sharing makes economical and logistical sense, it also increases the risk of lateral spread of HPAI between farms. Wild birds, another possible route of disease transmission, were observed inside barns on 35 percent of the farms, with the frequency ranging from daily to occasionally.

While most of the 81 farms surveyed had biosecurity protocols in place, only 43% of case farms reported that biosecurity audits or assessments were conducted on the farm by the company or a third party. Farms can decrease their HPAI risk by verifying that biosecurity procedures are being followed properly.

We conducted a case-control study focused on egg layer flocks in Iowa and Nebraska. A number of risk factors for HPAI introduction and factors associated with lowering the risk of introduction were identified in our multivariable analysis at both the farm and barn levels. At the farm level, being located in an existing control zone was highly associated with farm status. Rendering dead birds was a risk factor; 39% of case farms (compared to 13% of control farms) reported that the renderer came onto the farm. Although a similar percentage of case and control farms reported that garbage trucks came to the farm, 61% of case farms (compared to 23% of control farms) reported that the garbage trucks came near the barns. Having visitors change clothing was protective. Visits in the past 14 days (see prior report for the definitions of time periods for data collection) by a company service person were associated with farm status.

At the barn level, three variables remained statistically significant in the final multivariable model. Having a hard-surface barn entry pad that was cleaned and disinfected was protective when compared with all other levels combined (i.e., not having a hard surface, or no cleaning or no disinfection). Dead bird disposal within 30 yards of a barn remained a statistically significant risk factor. Although we identified a ventilation type that was protective, we are continuing to analyze that data due to a number of related factors that influence the effect of ventilation type.

We investigated the potential for airborne transmission by multiple methods. When aerosol exposure indices and distance measures were assessed together, the effect of the aerosol exposure index was often no longer statistically significant. These two variables are by nature correlated, as distance is an inherent part of the aerosol exposure index in addition to wind direction and speed. As a result, it was not possible to separate their effects in this analysis, and we were not able to determine with certainty whether aerosol transmission was responsible for a farm becoming infected. Other mechanisms associated with proximity could also have resulted in HPAI spread between nearby farms. Findings from these and other studies form the basis for recommendations on prevention strategies at the farm and barn level.

HPAI Epidemiology: Minnesota Perspective

Michelle Kromm, DVM, Jennie-O Turkey Store, Willmar, MN

In 2015, an unprecedented outbreak of highly pathogenic avian influenza (HPAI) occurred in the United States, greatly impacting the turkey industry in the Upper Midwestern United States. A case-control investigation was initiated by industry, government, and academic partners to describe epidemiologic features of the outbreak on turkey operations in the Upper Midwest. A comprehensive questionnaire was developed and administered to farm managers and supervisors to review farm biosecurity, litter handling, dead bird disposal, farm visitor and worker practices, and presence of wild birds on operations two weeks prior to HPAI confirmation. Case farms were HPAI-infected farms associated with a turkey company and control farms were non-infected farms with turkeys of similar stage of production associated with the same company.

The final analysis included 63 (37 case farms and 26 control farms). Of the case farms, 21 (57%) were company farms, 5 (14%) were contract farms, 4 (11%) were lease farms and 7 (19%) were independent farms. The control farms were either company (73%) or contract (27%) operations. The median size of case and control farms was 56,930 (range: 7,200 – 315,000) and 51,847 (range: 7,200 – 328,148) birds per farm, respectively.

Multivariable modeling through backward selection identified several factors associated with increased odds of case status, including: close proximity of the farm to other turkey operations, field work nearby in the 14 days prior to the outbreak, rendering of dead birds, and wild mammals observed near turkey barns. In a sub-analysis

separating early and late periods of the outbreak, early period factors identified that actively working a nearby field in the 14 days prior to the outbreak and a high level of visitor biosecurity were associated with increased odds of case farm status, while high level of worker biosecurity had a protective effect. Late period factors associated with increased odds of case farm status included a non-asphalt road being used by vehicles coming onto the farm and use of a vehicle wash station or spray area, while wild birds observed near dead bird disposal was associated with reduced risk of case farm status in the late period. Commonly shared equipment such as feed trucks and bird moving equipment were not found to be risk factors in this study; however, a USDA observational study associated shared equipment with increased risk for an HPAI introduction (USDA-APHIS-Epidemiologic and Other Analyses of HPAI-Affected Poultry Flocks: July 15, 2015 Report). Study results indicate that the initial introduction of the virus likely occurred through both environmental and between-farm pathways and the outbreak was perpetuated by multiple factors. These factors need to be further evaluated to prevent future large-scale outbreaks.

Table 1. Factors associated with case farm status (from multivariable analyses)

Multivariate Model	No. of Controls (%)	No. of Cases (%)	Variables	P-Values	Odds Ratio (95% CI)
Full Period	10 (38.5)	21 (56.8)	Tilled in last 14 days	0.02	6.46 (1.36 – 30.78)
	9 (34.6)	4 (11.1)	Wild mammals near barns	0.06	0.14 (0.02 – 1.06)
	15 (57.7)	30 (81.1)	Render dead birds	0.02	9.80 (1.46 – 65.96)
	3 (11.5)	23 (62.2)	Close proximity to other farms	<0.01	46.14 (5.96 – 357.55)
Early Period ^a	4 (40.0)	15 (83.3)	High visitor biosecurity	0.07	7.92 (0.88 – 71.41)
	7 (70.0)	5 (27.8)	High worker biosecurity	0.05	0.07 (0.01 – 0.96)
	3 (30.0)	12 (66.7)	Tilled in last 14 days	0.05	13.88 (1.04 – 184.85)
Late Period ^b	12 (75.0)	17 (94.4)	Non-asphalt roads	0.10	10.05 (0.65 – 156.49)
	10 (62.5)	18 (94.7)	Use of vehicle wash/spray stations	0.06	12.40 (0.94 – 163.52)
	12 (75.0)	6 (31.6)	Wild birds near dead bird disposal	0.02	0.12 (0.02 – 0.72)

^aFarm proximity in the area could not be included in the early model because no control farms were in high farm proximity area. Therefore, proximity of farms alone may be a comparable or better predictor of being a case in the early period than the set of variables together in the multivariable model shown. However, that cannot be determined with the given data.

^bThe model shown was the result of not including farm proximity in the area in the multivariate model selection process. When high farm proximity is included, the model reduces to only including the high farm proximity variable. Therefore, similar to the early model, proximity of farms alone in the an area may be a comparable or better predictor of being a case than the set of variables together in the late period multivariable model shown here.

2015 HPAI Assessment: Moving Forward

John Clifford, DVM, USDA-APHIS-VS, Riverdale, MD

Current Status: This outbreak has been unprecedented in the annals of animal health in the United States. APHIS has confirmed H5/H7 highly pathogenic avian influenza in a total of 232 premises: 211 commercial operations and 21 backyard flocks. Approximately 50 million birds have been culled as a result of the presence of these HPAI strains. Current response activities are well over half a billion dollars for depopulations, indemnity, and cleaning and disinfection. According to a Congressional Research Service Report released late this summer, the value of egg-laying hens lost is \$1.04 billion, and \$530 million for turkeys. That's about \$1.6 billion dollars in direct losses. This outbreak has had a far-reaching impact on the larger economy as well, including the lost business incurred by sectors that work with the poultry industry, such as feed and trucking. The economy-wide impact is estimated at \$3.3 billion. At the height of the outbreak, the response involved over 3,600 State and Federal responders, including: over 700 APHIS employees deployed; 200 State personnel; and 2,900 contractors.

Key Issues:

Trade Impact. With respect to trade, 17 trading partners have suspended all U.S. poultry and poultry products. The major closures are China (\$391 million), Russia (\$153 million (already shut off under preexisting trade

restrictions), and South Korea (\$123 million). Trade has continued from areas of the United States not affected by HPAI. U.S. poultry and poultry product exports to these 38 trading partners in 2014 were valued at \$4.4 billion.

Travel Impact. Veterinary Services employees traveled to Asia, Africa, the Middle East, and Europe to provide updates on the status of the U.S. response to HPAI and discuss potential impacts on trade of that response. The meetings provided VS with the opportunity to clarify the host country's previous misconceptions on U.S. policy. In many cases, the countries agreed to lift remaining bans, consider regionalization, and allow the use of vaccines, but each country provided specific follow-up requests for official written requests from the United States and further information on our protocols and plans for HPAI response. Many host countries were sensitive to the global aspects of HPAI and receptive to a multi-pronged approach for contingency. They also demonstrated great interest in the scientific aspects of the HPAI situation.

2015 Assessment and Lessons Learned: Through the spring and summer, we engaged in weekly planning and information-sharing calls with State and industry partners and participated in several conferences and workshops to plan for fall. All of these activities have helped us identify gaps and lessons, and address them in time for possible detections this fall. APHIS has spent numerous hours developing a comprehensive planning document that we provided to Secretary Vilsack on August 15th. This is a "living document," and we have continued to refine it. A version of the plan was released to the general public on September 18th. We have conducted considerable outreach to ensure that States, industry, and producers are aware of our efforts. Our planning activities assumed a worst-case scenario beginning in September 2015, with HPAI occurring simultaneously in multiple sectors of the poultry industry throughout the Nation.

Future HPAI Planning: Promoting improved on-farm biosecurity practices in order to prevent future HPAI cases to the greatest extent possible; improving HPAI surveillance in wild birds as a means to provide "early warning" risk information to States and industry; expanding Federal, State and industry response capabilities, including availability of personnel, equipment, and depopulation, disposal and recovery options; improving our capabilities to rapidly detect HPAI in domestic poultry and to depopulate affected flocks within 24 hours to reduce the environmental load of HPAI viruses and their subsequent spread; streamlining the processes for payment of indemnity and the cost of eliminating viruses so that producers receive a fair amount quickly, to assist them in returning to production; enhancing our ability to communicate in a timely and effective way with producers, consumers, legislators, media, and others regarding outbreaks and other information; and making preparations to identify and deploy effective AI vaccines should they be a cost beneficial addition to the eradication efforts in a future HPAI outbreak. This plan builds upon the Foreign Animal Disease Preparedness and Response Plans (FAD PRoP) and Continuity of Business/Secure Food Supply plans that were already in place and used during the 2015 outbreak.

USDA Federal Fall HPAI Planning Activities

Patricia Fox, DVM, USDA-APHIS-VS, Raleigh, NC

The Fall 2015 HPAI Response Plan was published on September 18, 2015, but is a living document, and thus, subject to change. It was developed based on lessons learned in the recent HPAI outbreak, and supplements but does not replace the "Red Book." The Plan assumes a worst-case scenario of 500 infected premises for the fall of 2015 with no zoonotic spread. Four key areas are covered: 1) Preventing or reducing future outbreaks; 2) Enhanced Preparedness; 3) Improved and streamlined response capabilities; and 4) Preparing for the potential use of AI vaccines.

Enhanced biosecurity has been identified as an important way to prevent future outbreaks. Risk factors associated with poor biosecurity were identified in epidemiology studies. Educational materials and a biosecurity self-assessment tool have been developed with Iowa State University and US Poultry and Egg Association and are available on the USDA Avian Influenza website. An interim rule will be published soon requiring that in order to receive indemnity for an infected flock, a producer must self-certify that biosecurity procedures were in place and followed. This is the first step towards requiring stronger accountability for producers in prevention of infection.

The Interagency Strategic Plan for wild bird HPAI surveillance was published in June, 2015. According to the plan, 40,000 samples from wild birds will be collected between July 2015 and July 2016. Plans include stakeholder announcements and web posting if any new findings of HPAI occur.

In order to improve state & industry response capabilities, surveys were sent to these groups asking for details on their current readiness for response.

To enhance diagnostic laboratory preparedness, NAHLN labs reviewed and updated their staffing plans, surge capacity plans, and barcoding and shipping protocols.

Capacity and training for deployed Federal personnel have been increased by plans to hire 350 VMOs,

AHTs and support personnel. In addition IMTs will be reconstituted and expanded and NAHERC will be used in the future.

Steps are being taken to improved capacity for depopulation and disposal. Towards this end, Federal and State rules on carcass disposal have been researched and compiled, and maps created showing landfill, incineration & rendering facilities in various states. The National Veterinary Stockpile (NVS) has improved its inventory of depopulation and disposal equipment, assessed water and carbon sources for composting and updated their inventory of PPE and other response supplies.

To improve public communications in an outbreak the agency is hiring additional Public Information Officers (PIOS) and is working on message development and dissemination. Plans included deployment of a site manager to each affected facility.

Modeling studies indicate that rapid detection, depopulation and disposal have the greatest impact on reducing outbreak size and duration. In order to increase the speed of detecting affected premises, the agency now accepts presumptive positives at a NAHLN lab as sufficient for depopulation. In addition, they are implementing the antigen capture immunoassay to identify suspect cases. A decision could be made to depopulate if clinical signs present in a flock with an antigen capture test positive result.

The agency has put forward a goal of depopulating affected flocks within 24 hours of positive test. Firefighting foam or CO2 gas are preferred methods, but we are prepared to use ventilation shutdown.

To speed the completion of cleaning and disinfection of infected sites, dry cleaning and heating are now preferred for virus elimination. A flat (per bird) rate is being developed for C/D (dry cleaning/heat) payments to producers.

Streamlining of indemnity payments to affected producers will be achieved by allowing Electronic submission of flock inventories. Indemnity calculators will be continually reevaluated. In order to obtain indemnity for an infected flock, the owner and grower (if applicable) sign an **Appraisal and Indemnity Request Form**. The form includes self-certification that a biosecurity plan was in place and being followed when the outbreak occurred. The producer agrees to the current calculator values for the birds and to the process (interim rule language) for the splitting of payments between owners and growers. In addition the producer agrees to provide documentation to allow verification of inventory and expected contract value for the flock. **This is the only document needed to depopulate. A signed 1-23 form or flock plan will NOT be required.** There will be one document outlining all of the payment processes, including a flat rate payment for virus elimination (formally C&D) based on number of birds and facility type. The VS 1-23 form will be used for all items that must be destroyed (birds, eggs, feed, corn, items that cannot be C&D'd). We expect very limited use of Cooperative Compliance Agreements (only for depopulation and disposal activities).

In preparation for the potential use of AI vaccines, two companies were awarded contracts for vaccine manufacture on October 13, 2015. Additional "requests for proposals" (RFPs) will be released quarterly. No current decision has been made to use vaccination in a future HPAI outbreak. Vaccine use would require careful consideration of the efficacy of the vaccine, any impacts of using HPAI vaccine in the field, and the potential trade impacts. Vaccination, if approved, would be part of an eradication effort, not a replacement for it.

USDA Biosecurity Perspective

Lee Ann Thomas, DVM, USDA-APHIS-VS, Riverdale, MD

Biosecurity is a broad term to mean that encompasses those operational or structural measures or procedures intended to protect humans or animals against disease. While standard biosecurity efforts practiced by the poultry industry may have been sufficient in the past, evidence of farm-to-farm spread of the HPAI virus strain circulating in the Midwest shows that stricter biosecurity is needed.

To facilitate stricter biosecurity, APHIS has developed educational materials including Spanish translations and a biosecurity self-assessment checklist, which are available online through the U.S. Poultry and Egg Association. As of October 23, 2015, 531 self-assessments had been completed. The majority were submitted by layer (270), broiler (118), pullets (77), or turkey (70). The number of responses "in progress" indicates the efforts that producers are taking to improve biosecurity, although additional efforts are still needed.

Additionally, APHIS is publishing an interim rule on HPAI indemnity that will contain a provision requiring all future HPAI-affected commercial poultry producers to self-certify that biosecurity procedures were in place at the time HPAI was detected. This represents the first step in creating a system of greater accountability for biosecurity. Following this, we will collaborate over the next year with stakeholders to design a biosecurity auditing system. An

industry-driven initiative, an addition to the National Poultry Improvement Plan, or a third party auditor is possible approaches.

Minnesota HPAI Biosecurity Education

Steve Olson, Minnesota Turkey Growers Association, Buffalo, MN

One of the last steps required for poultry farms to resume business is to meet the requirements outlined in USDA's Restocking document. These requirements include biosecurity practices. The Education Committee of the Minnesota Turkey Research and Promotion Council (Council) initiated an HPAI and Biosecurity Education program to provide information and an opportunity for dialog on HPAI and farm-specific biosecurity. The Council, with financial support from the Minnesota Board of Animal Health, hired veterinarians to provide education on HPAI to growers that had infected flocks and to review their biosecurity. A veterinarian met one-on-one with the grower, farm manager or flock supervisor. A fact sheet with information on HPAI was provided and discussed during the meeting, followed by a tour of the farm. Reviews were conducted, in almost all cases, on farms that had restocked. This enabled a more valuable review of the implementation of biosecurity practices. The intent of these meetings was educational. Feedback from growers has been overwhelmingly positive.

The Process:

A team of poultry veterinarians (and one swine veterinarian) made slight modifications to an existing biosecurity review tool. The tool was uploaded to iAuditor application. Smartphones and/or iPads were used for the onsite review. The iAuditor application enabled the reviewer to complete the report on-site. For each question/area of review, the reviewer identified whether the practice was Safe or At-Risk. The reviewer was able to photograph Safe and At-Risk practices to clarify with the flock supervisor and enter notes/comments into the report. Reports were sent electronically to the farmer owner and flock supervisor.

As of October 2, 2015, 58 reviews had been completed which included 63 farms of the 104 HPAI introductions on Minnesota turkey farms. Some farms conducted a review with their veterinarian but are not included in this project because the review tool was slightly different. Other farms had not yet restocked.

The review tool is available to all Minnesota turkey growers on our members-only website. Dr. Sally Noll with the University of Minnesota's Animal Science department will be providing a summary report and publishing findings as fact sheets through the University of Minnesota Extension.

The review team commented during a debrief that they were very impressed with the commitment of the growers, farm managers and flock supervisors to take the necessary steps to prevent future introductions by building upon their existing biosecurity practices.

Commercial Layer Biosecurity

Travis Schaal, DVM, Hy-Line International, Dallas Center, IA

The biosecurity practices at Midwest layer complexes before spring 2015 was adequate to efficiently produce large volumes of eggs. As the layer industry consolidated into the central US over the last 20 years, major infectious disease challenges (Marek's, infectious bursal disease, *E. coli*, avian encephalomyelitis, *Mycoplasma gallisepticum*, fowl pox, infectious laryngotracheitis, infectious bronchitis, and Newcastle disease) were adequately controlled through robust vaccination programs. The biosecurity of complexes was likely never fully challenged by a devastating pathogen such as highly pathogenic avian influenza (HPAI) until the recent outbreaks. It should be noted that *Mycoplasma synoviae* has often been found on layer complexes, and moved between sites, displaying some potential weaknesses in on-farm biosecurity. Several complexes and off-line producers had incorporated seemingly stringent biosecurity practices such as "shower in/shower out" for personnel, but these practices appear to have failed in preventing some sites from becoming infected during the 2015 highly pathogenic avian influenza (HPAI) outbreak.

Layer complexes have an exorbitant number of inputs and outputs on a daily basis including: contract crews, farm personnel, lunches, tools, personal vehicles, pullet trucks/carts, crews and carts to deplete of end of lay hens, feed trucks, feed ingredients, farm deliveries (UPS, FedEx, USPS, etc.), office supplies, egg packing materials, liquid egg hauling equipment, manure removal equipment, manure, dead bird disposal, etc. A single complex may receive over 100 semi deliveries of feed ingredients per day to produce feed on-site, and more than one hundred personnel to work in houses and egg processing/packing plants. Addressing all of these factors presents an enormous task for egg companies. Making the task more difficult is the potential sharing of equipment,

staff, and contract crews between locations and farms. Much attention has been given to vehicle tire disinfection at farm the perimeter, but this practice does not address the risk of dirty vehicle bodies, boxes, cabs, and contaminated drivers.

Manure handling and spreading a major risk factor in spread of disease between layer operations. Belted style housing has become more common allowing for a drier manure product that can be land applied throughout the year. Traditional high-rise caged housing is usually cleaned out on a less frequent basis. Manure handling equipment (semis, trailers, loaders, tractors, etc.) and personnel to handle the manure present challenges for cleaning and disinfection due to complexity and size of machinery, and frequent trips on and off a complex. Furthermore, land application of manure between poultry sites on windy days increases the risk of contaminating other nearby poultry houses and vehicles with infectious organic matter. Manure handling equipment and personnel may be shared between complexes and even between companies introducing major risk if no specific interventions are taken to effectively decontaminate vehicles and people between sites.

Contract crews used for moving pullets, vaccine application, flock depletions, and other tasks present a unique challenge for the layer industry. Crew work is sporadic on any given site and activities are time sensitive. This creates a labor surplus during "downtime" when the crew is waiting between tasks and locations. Third party crews have established a niche market by providing ample labor supplies to achieve a given task, on demand; however, cross over between companies exists as crews seek out work to provide full-time employment. Policies requiring downtime between companies are common corporate biosecurity measures (usually 72 hours from other poultry exposure), however, crews cannot maintain staffing without constant work opportunities and may not adhere to requested downtime. If contract crews are not truthful or forthcoming about previous bird contact they increase risk to the industry. Crew clothing, footwear, and personal vehicle traffic all present risks to disease introduction to a site. Often, contract crew staff resides in a specific geographic region where they may interact with farm staff from other companies or people from other industries such as swine, turkey, or broiler production. Cross traffic between farm staff and contract crews at their homes, gas stations, churches and school functions should not be overlooked.

In addition to potential operational biosecurity gaps, the unprecedented spread of HPAI virus may have been due to geographic and meteorological events after large scale viral amplification on layer complexes. Some negative farms may have received contaminated "wind plumes" or dust particles that travelled some distance on the wind from infected premises. Although modeling presented wind-borne transmission of the virus, filtration of air for layer complexes would be a major financial investment that would be better spent on structural and operational measures to decrease links to other poultry facilities.

Quality and auditing programs on layer facilities have been focused on Food and Drug Administration (FDA) *Salmonella* requirements (SE Rule) and welfare compliance, but both of these types of audit programs are limited in scope and relevance to operational biosecurity. Attention should be focused on biosecurity programs that address a hazard analysis and critical control (HACCP) style program for individual layer facilities to address specific risk factors that may introduce pathogens on each site. Programs must be maintained with standard operating procedures and adequate staff training. Clear demarcation of farm AND barn clean/dirty lines is paramount. Color coordinated clothing and footwear allow for a simple visible inspection. Addressing vehicle traffic may require structural accommodations, taking into account seasonality (example, truck washes in Iowa winters). Staff should police their actions and all levels of the corporate structure must buy in to the biosecurity program. Short cuts should not be tolerated and addressed accordingly.

High Pathogenicity Avian Influenza Vaccines

David Swayne, DVM, USDA-ARS-SEPRL, Athens, GA

Since 1959, there have been 36 epizootics of high pathogenicity avian influenza (HPAI) in the world with 31 of 36 epizootics using stamping-out programs leading to rapid eradication. Five of the epizootics have used vaccines as a means to control the disease and reduce infection pressure and spread of the disease. If used properly, vaccines can be an effective tool in control that can lead to eradication. However, field outbreaks of H5N1 HPAI have occurred in vaccinated flocks from both failure of the vaccines (i.e. vaccine efficacy) and failure in administration or immune response of the target species (i.e. vaccination effectiveness). Antigenic drift in field viruses has resulted in failure of protection by classic H5 vaccines strains in Mexico, China, Egypt, Indonesia, Hong Kong and Vietnam. This challenge has been met by developing new vaccine strains that provide protection against ever changing HPAI viruses. In a comprehensive assessment of AI control methods under the World Organization for Animal Health (2002-2010), >113 billion doses of AI vaccine were used in poultry in 15 countries. The majority of vaccine (>91%) was used in China while significant amounts were used in Egypt, Indonesia, and Vietnam. The

other 11 countries used less than 1% of the vaccine. Inactivated AI vaccines accounted for 95.5% and live recombinant virus vaccines for 4.5% of vaccine used. Since 2010, Bangladesh (H5N1) and Mexico (H7N3) have begun HPAI vaccination campaigns.

In 2015, U.S. Department of Agriculture began experimental vaccination studies to assess vaccines as a potential tool for future use in control of H5N8 and H5N2 HPAI outbreaks. Initial studies indicated the historic USDA H5 vaccine bank strains could provide protection from mortality, but varied greatly in their ability to reduce the number of poultry and the quantity of oral and cloacal replication and shedding of challenge virus; i.e. raising concerns at the ability of the heterologous H5 vaccines to reduce infection and the spread of field HPAI virus. Three licensed technologies have shown the greatest potential for use: reverse genetic laboratory generated H5 low pathogenicity avian influenza (LPAI) virus for inactivated vaccine (rgH5-inactivated), recombinant herpesvirus of turkey with H5 hemagglutinin gene insert (rHVT-H5) and recombinant alphavirus RNA particle vaccine with H5 hemagglutinin gene insert (RP-H5). The favored H5 inserts are from a homologous clade 2.3.4.4 H5 HPAI virus with cleavage site altered to LPAI virus. Among all experiments, the rgH5-inactivated vaccine (clade 2.3.4.4) gave the best results in preventing mortality and reducing North American clade 2.3.4.4 HPAI challenge virus shedding in chickens and turkeys, either in single or prime-boost regimes. The rHVT-H5 (Clade 2.2) and RP-H5 (clade 2.3.4.4) worked best in a priming vaccine application followed by booster vaccinations with rgH5-inactivated or RP-H5. The reduction in virus shedding was associated with hemagglutination inhibiting antibodies. In young birds, the RP-H5 may require a higher vaccine dose for and optimal protective response. *In ovo* applications are most promising with rHVT-H5. Collectively, studies support a prime-boost regime for initial optimal protection.

Secure Poultry Supply Plans and the NPIP

Eric Gonder, DVM, Butterball LLC, Goldsboro, NC

The Secure Poultry Supply Plans represent a major step forward in advancing business continuity in the face of an outbreak of highly pathogenic avian influenza (HPAI). However, there is a need for a mechanism to incorporate changes in the Plans as the disease, the industry, and control mechanisms continue to evolve. This requires a collaborative effort of Federal, State and Industry segment participants.

The National Poultry Improvement Plan (NPIP) presents a structure suitable to addressing these issues moving forward. Previous experience with the low pathogenic avian influenza/notifiable avian influenza plans within NPIP and the collaborative structure of NPIP should allow the organization to fill this role suitably and provide industry segment specific advice to the Secretary of Agriculture on future modifications of the Secure Poultry Supply Plans.

NPIP is also uniquely structured and reasonably experienced in the development and execution of biosecurity procedures by industry segment. Expanding those efforts into the future control of HPAI would likewise represent an expansion of NPIP's mission, but it is uniquely structured to address that issue as well. One suggestion currently under discussion is to create a Subpart E "Biosecurity" in the NPIP Program Standards to be addressed for the subparts wishing to participate. There will surely be other suggestions.

Comparison of Operational Plans from the Secure Poultry Supply Plans (Egg, Broiler, Turkey)

Julie Helm, DVM, Clemson Livestock Poultry Health, Columbia, SC

The Secure Poultry Supply Plans consist of the Secure Egg Supply Plan, the Secure Broiler Supply Plan and the Secure Turkey Supply Plan. The detailed plans, biosecurity check lists and movement permit examples can be found at:

- Egg -- <http://secureeggssupply.com/>
- Broiler -- <http://www.securebroilersupply.com/>
- Turkey -- <http://www.secureturkeysupply.com/>

The Plans are meant to be used as a tool to help guide decisions on moving poultry and poultry products from negative premises during a highly pathogenic avian influenza (HPAI) event to allow for business continuity. The plans make specific science- and risk-based recommendations that emergency decision makers (e.g. Incident Commanders) can use to rapidly decide whether to issue or deny movement permits of table egg, broiler and turkey industry products during an event. The plans outline surveillance, biosecurity, and cleaning and disinfection practices for moving product into, within, or out of a HPAI Control Area.

These plans are living documents and will be updated as needed. The original risk-based recommendations were based on past HPAI H5N1 events. New risk assessments, as observed in the recent HPAI

H5N2 event, will continue to be evaluated and added in future updates and will change some of the procedures.

The Secure Egg Supply Plan is 10 years old and was initially developed as a business continuity model in the era of "Stamping Out" in which whole zones of infected and non-infected premises would be depopulated as a way to control an outbreak. The Secure Egg Supply Plan is the most complete of all the plans. All the risk assessments (for HPAI H5N1) and the movement permit guidance were completed for the poultry and products listed in the plan. The Secure Broiler and Turkey Supply Plans began development a few years ago. Both plans were still evaluating risk assessments and developing guidance when the 2015 HPAI event started in the U.S. The permit guidance for broilers and turkeys was released prematurely because of the need to move these birds and products.

The three plans are similar in guidance, but also contain unique features based on the different management styles and perceived risks between the three different industries. The three plan working groups include members from academia (University of Minnesota, Iowa State University), USDA-APHIS-VS-CEAH, USDA-APHIS-VS, industry veterinarians, commodity groups (United Egg Producers, National Turkey Federation) and State officials.

All three plans require negative testing of flocks to move birds or products off of the facility and to move within or out of the Control Area, with the exception of a few egg products in the Secure Egg Supply Plan. Samples consist of oropharyngeal swabs (including swabbing the choanal cleft) and testing with the real-time reverse transcription-polymerase chain reaction (RRT-PCR or PCR for this summary). The numbers of PCR tests (pooled tube of oropharyngeal swabs) is always determined on the number of dead or sick birds in each house. The sample size is either 5 or 11 dead or sick birds for one pool of BHI broth. Target sampling the dead birds first and then sampling sick birds to fill the PCR pool.

The discrepancy between sampling 5 or 11 birds in the plans is taking in consideration that the 11 bird pooled sample in 5.5 ml of BHI broth was only authorized by the National Veterinary Services Laboratory in 2013 versus the previous method of a maximum 5 bird pool sample in 3 ml of BHI broth. Sampling of 11 birds in a pool is preferred as it increases the confidence level of detecting the virus.

The number of negative PCR tests needed prior to moving birds or products will vary in the plans. No testing is required to move pasteurized liquid eggs or inedible egg products to a non-poultry facility since there is no threat of spreading the avian influenza virus. One negative PCR test is needed from table egg layers for moving non-pasteurized liquid eggs, dry eggshells and wet eggshells or for placing turkey poults in a brooder house within a Control Area. Two negative PCR tests prior to move is needed for most of the birds and products, including testing table egg, broiler and turkey breeders to move hatching eggs & turkey semen; testing table egg layers to move table (eating) eggs; and testing the meat broilers and turkeys to move to slaughter. Some bird movement was not considered at the time and is not listed in plans, but will be developed and added to the plans (e.g. spent table egg layers or spent breeders moving to slaughter or rendering).

There are two options for frequency of sampling prior to move. One PCR pool collected on the 2 consecutive days before moving or two PCR pools collected 24 hours before moving. The latter option has a slightly higher confidence level of detecting the virus and requires less potential outside visitations to the farm.

Table eggs and hatching eggs require a holding period prior to moving off of the facility. A one day hold is needed when first starting up a Control Area before washed & sanitized table shell eggs can move from a table egg layer farm to a storage/holding facility, but not allowed to move into the egg market. A two day hold is needed for hatching eggs on all breeder farms before moving to the hatchery, and washed & sanitized table shell eggs and nest run eggs on table egg layer farms before moving to the egg processing plant.

The plans describe in detail the specific biosecurity requirements listed for trucks and drivers moving birds and eggs and product-specific biosecurity for pre-movement flock isolation periods and procedures at the breeder farm, hatchery, grow-out farm and table egg farm.

Permitted movement requirements include traceability information, normal flock production parameters (e.g. mortality, egg production), truck & driver biosecurity measures, product-specific biosecurity measures, completed epidemiology questionnaire with no dangerous contact to infected premises, any holding or isolation requirements, and any testing requirements. The State Animal Health Official of the Destination State receives a copy of movement permit within 24 hours of issuance. Examples of permits are located in the plans or as supplemental information on-line.

The Secure Poultry Supply Plans were used successfully during the 2015 HPAI H5N2 event. Initially, the State Animal Health Officials potentially receiving birds and products from the Control Areas were unfamiliar with the details of the plans and wanted a uniform method of procedures for interstate movement out of a Control Area. The Highly Pathogenic Avian Influenza (HPAI) permitting working group was formed on April 16, 2015 at the request of the National Assembly of State Animal Health Officials. The charge of the working group was to develop

a document summarizing the recommendations for permitting interstate movement of poultry and eggs from a HPAI Control Area, to include frequency of surveillance testing, number of tests per premises and biosecurity procedures for movement. The recommendations, which contain guidance procedures from the Secure Poultry Supply Plans, were finalized on May 20, 2015, and approved by the National Assembly. The USDA-APHIS-Veterinary Services, Surveillance Preparedness and Response Services has incorporated the working group's recommendations into a critical response activities document entitled "Testing Requirements for Movement from the Control Area" and included it as part of the FAD PReP Materials and References for HPAI Response & Policy Information: 2014-2015 Outbreak.

Recommendations for interstate permitted movement of poultry and eggs out of or within an HPAI Control Area (Infected and Buffer Zones), include:

1. Delay moving live poultry (including hatching eggs) after a new Control Area is established until such time as the Control Area testing of *commercial premises is completed.
2. States should avoid placing additional restrictions on interstate movement of poultry and poultry products from outside of the Control Area in HPAI affected States. These recommendations do not supersede existing state regulations or requirements.
3. Traceability information is required for the premises of origin and premises of destination [each premises will need a Federal Premises Identification Number or (USDA's Emergency Management Response System) EMRS will create one].
4. The flock has normal flock production parameters as described in the Secure Poultry Supply Plans (Egg, Broiler and Turkey).
5. All movement should follow biosecurity procedures for Truck and Driver and Product Specific Biosecurity as described in the Secure Poultry Supply Plans (Egg, Broiler and Turkey).
6. The premises of origin is not an Infected, Suspect or Contact Premises (refer to *Section 5.5, Epidemiological Investigation and Tracing in USDA's HPAI Response Plan*).
 - a. The Incident Commander should determine the need for an epidemiology questionnaire if the flock has normal production parameters and negative tests.
 - b. Receiving State may require information from the epidemiology questionnaire prior to granting permission to move.
7. Egg Movements:
 - a. Hatching eggs should follow the two day holding procedure as described in the Secure Poultry Supply Plans (Egg, Broiler and Turkey), provided the Control Area testing of commercial premises is completed (refer to #1), and should use the recommended testing procedures (refer to #8).
 - b. Table eggs (non-hatching eggs) should follow the two day holding procedure as described in the Secure Poultry Supply Plans (Egg, Broiler and Turkey) and the recommended testing procedures (refer to #8).
8. Testing of poultry should consist of a minimum of two 11-bird AI negative PCR pools per house.
 - a. The sample size consists of one pool of 11 dead/sick birds sampled per 50 dead birds per house.
 - b. Frequency of sample collection:
 - i. Collect all pools within 24 hours prior to movement, or
 - ii. Collect one set of pools within 48 hours prior to movement and the second set of pools within 24 hours prior to movement.

*Commercial poultry premises defined from NPIP §146

1. Meat type chicken slaughter plant (broilers) – 200,000 or more chickens are slaughtered in an operating week (*all the broilers that feed that plant are considered commercial*),
2. Table egg laying premises – 75,000 or more chickens on a premises,
3. Meat type turkey slaughter plant – 2 million or more turkeys are slaughtered in a 12-month period (*all the turkeys that feed that plant are considered commercial*),
4. Commercial meat waterfowl/upland game bird slaughter plants – 50,000 or more birds are slaughtered annually (*all the birds that feed that plant are considered commercial*),
5. Raise for release waterfowl/upland game bird premises (e.g. hunting purposes) – 25,000 or more birds are raised annually on a premises, and
6. Breeder flocks that produce any of the above birds.

Broiler Industry Report

Deirdre Johnson, DVM, Mountaire Farms, Millsboro, DE

Broiler Production: Production thus far in 2015 is ahead of the same period for 2014 by 4.7%. Average broiler age and weight are increased. Average feed cost is reduced from 2014.

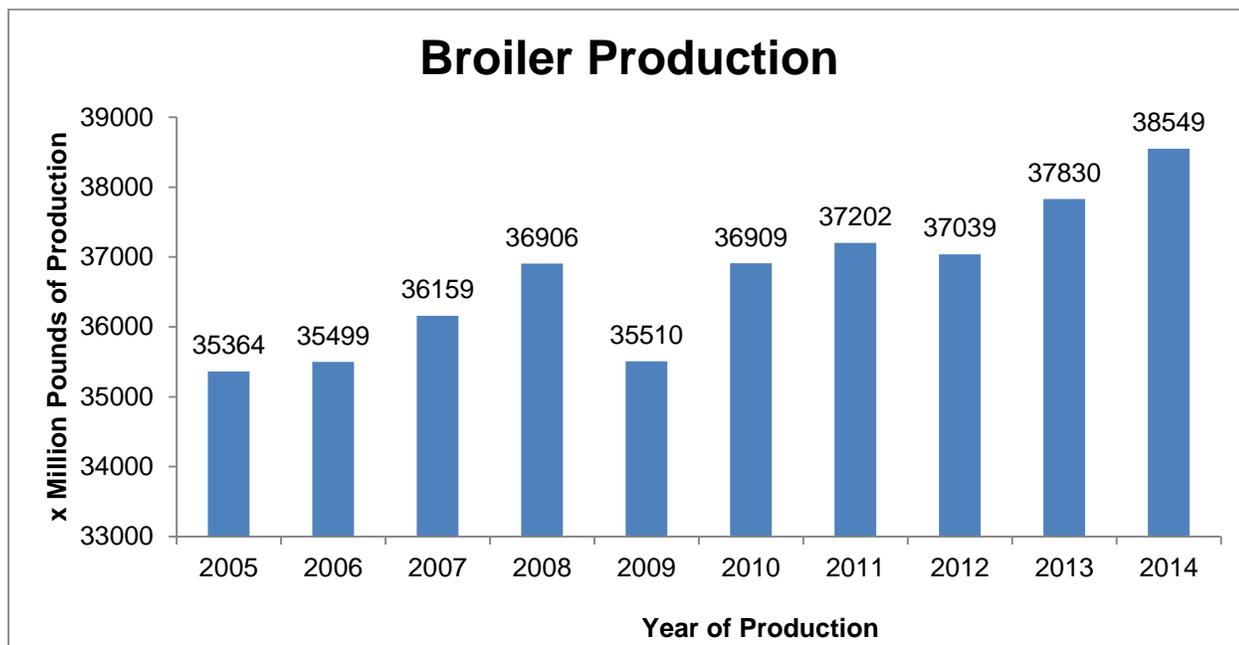
Mortality: First week mortality has increased from 2014. Increased removal of hatchery antibiotics may be contributing to this increase. The same trend was reported last year. Chick quality/early mortality ranked third in the 2015 Association of Veterinarians in Broiler Production (AVBP) poll as displayed later in this report. Total mortality thus far in 2015 is increased compared to the previous two years. This was reflected in all weight classes but more pronounced in the heavier broiler class. This same trend was reported last year.

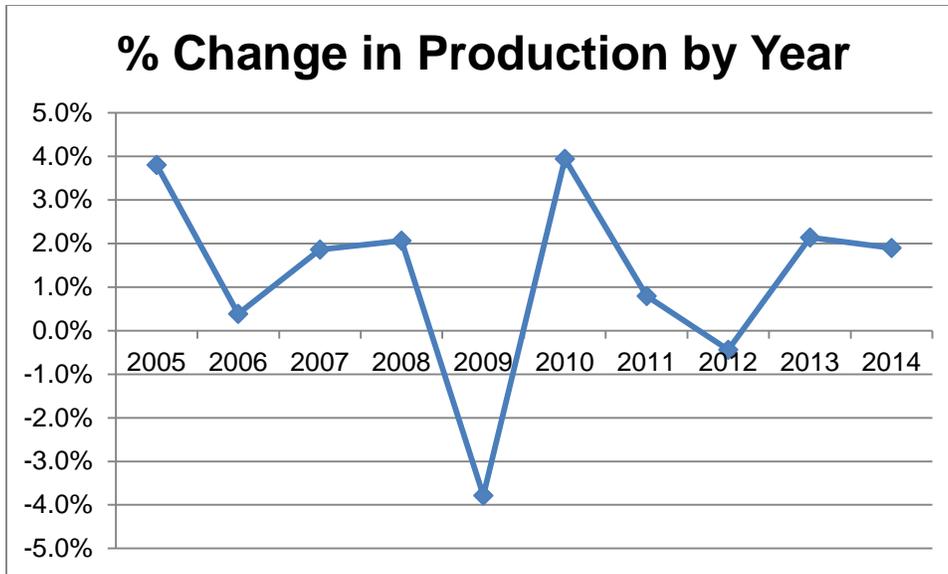
Condemns: Whole Body Farm Condemns + Parts Condemns increased from 0.592% in 2014 to 0.654% in the first half of 2015. Septicemia/Toxemia and Infectious Process account for the majority of this increase.

Key Broiler Health Issues: Even though Highly Pathogenic Avian Influenza (HPAI) has not been detected in commercial broilers, it was the top ranked disease issue in 2015. This same ranking affected the non-disease issues as biosecurity efforts have increased to prevent the introduction of HPAI into commercial broilers. Coccidiosis ranked second amongst broiler Veterinarians as a major disease concern. Historically, it has ranked first. This reflects not only the actual frequency of diagnosis but also the cost and challenge of maintaining effective anticoccidial programs. *Eimeria maxima* was the coccidial species most often mentioned by broiler Veterinarians. Necrotic enteritis ranked fifth as a disease issue and would often be associated with inadequate control of *E. maxima*. Infectious bronchitis ranked fourth on the poll. This disease continues to be a challenge, whether due to new strains or failure of vaccination programs to protect completely against existing strains. Further results for the 2015 AVBP disease poll are displayed later in this report.

Key Non-Disease Broiler Issues: The highest ranked non-disease issue was the biosecurity around HPAI prevention. 74% of the broiler Veterinarians forced ranked this issue first and the remainder of those surveyed ranked it second. Like last year, antibiotic free (ABF) issues ranked high. This is due to increased production and demand for ABF poultry by both customers and broiler production companies. Like last year, the loss or lack of effective drugs and increased regulation by the USDA and FDA ranked high. Poultry welfare issues ranked fifth in the poll. All results are displayed later in this report.

Supporting Data:





	2013	2014	2015
Average Age	49.0	49.3	50.2
Average Broiler Weight	6.44	6.52	6.66
Feed Ingredient Cost/Ton (All Broilers)	348.44	289.50	255.25
First Week Mortality	1.15	1.26	1.48
Total Mortality	3.92	4.36	5.23
Mortality (3.6-4.4 lbs)	3.32	3.59	4.16
Mortality (4.4-5.2 lbs)	3.00	3.51	3.74
Mortality (5.2-6.0 lbs)	4.24	4.25	5.72
Mortality (6.0-6.8 lbs)	3.65	4.06	5.40
Mortality (6.8-7.5 lbs)	4.24	4.98	5.36
Mortality (>7.5 lbs)	4.58	5.04	5.86
WB Farm + Parts Condemns	.525	.592	.654
Septox Condemns	.129	.150	.171
Airsac Condemns	.099	.125	.127
IP Condemns	.031	.039	.047
Leukosis Condemns	.004	.001	.001

As in previous years, AVBP membership was polled concerning disease and non-disease issues. Topic issues were force ranked for both areas. All disease and non-disease issues were also rated in a second graph for each issue. AVBP is comprised exclusively of Veterinarians employed full-time by US broiler companies. The

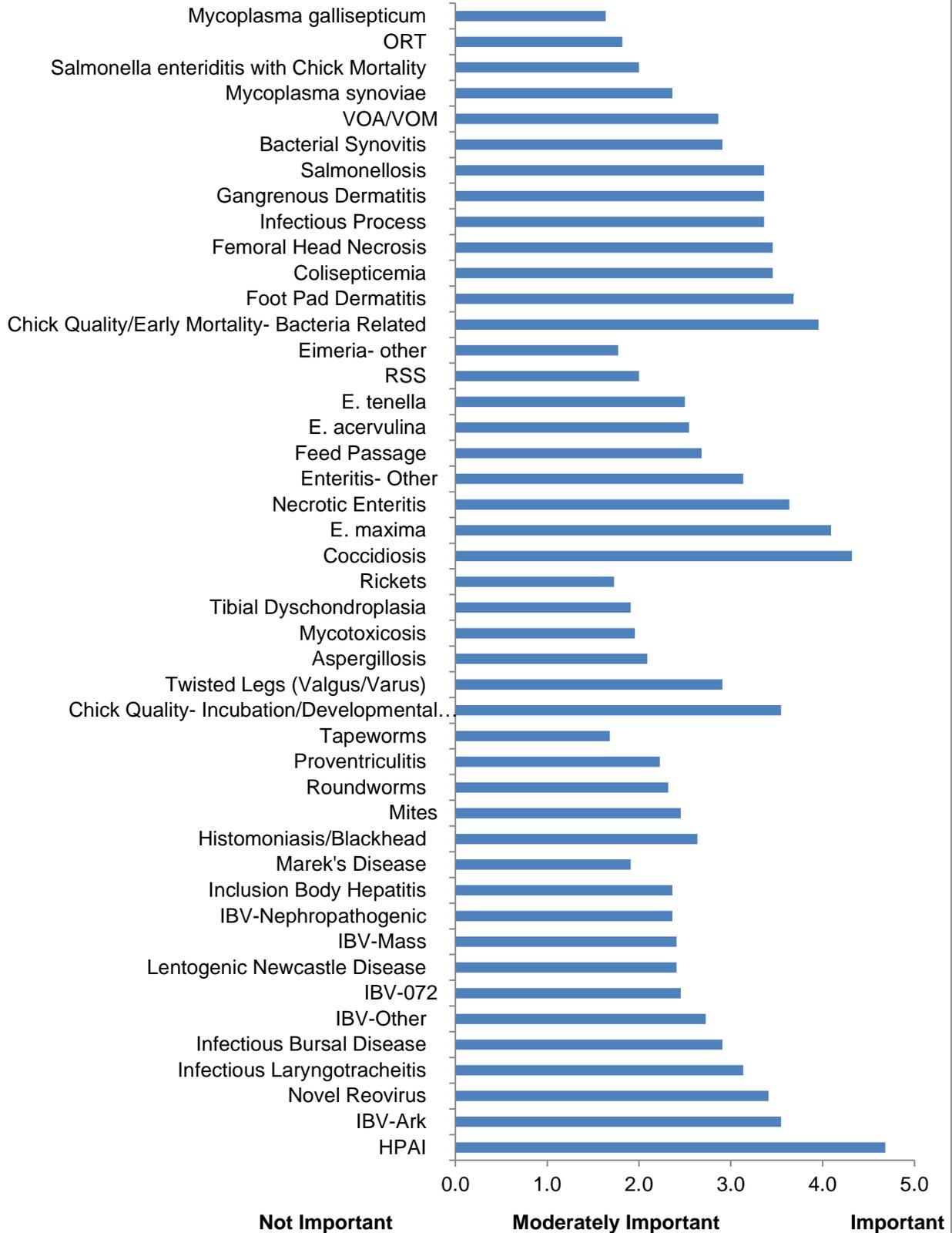
Veterinarians responding the 2015 survey represented 62% of the membership and 84% of USA broiler production.

Ranking:

Top Disease Issues	Composite Forced Rank	Mean Rank
HPAI	1	1.45
Coccidiosis	2	2.74
Chick Quality and Early Mortality	3	4.91
Infectious Bronchitis- Respiratory	4	5.09
Necrotic Enteritis	5	5.65
Novel Reovirus	6	6.04
Grangrenous Dermatitis	7	7.17
Colibacillosis	8	7.3
Bacterial Osteomyelitis of the Legs	9	7.35
Infectious Bursal Disease	10	8.3
Infectious Laryngotracheitis	11	8.35
Vertebral Osteomyelitis	12	8.35
Infectious Bronchitis- Nephropathogenic	13	10.26
Mycoplasmosis	14	10.74
Marek's Disease	15	12.74

Top Non-Disease Issues	Composite Forced Rank	Mean Rank
Biosecurity- HPAI Threat	1	1.35
Antibiotic-Free Issues (Customer or Media)	2	2.48
Increased Food Safety Regulation by USDA	3	2.87
FDA-Drug Availability/VFD Implementation	4	3.3
Poultry Welfare (Internal Programs/Activist Threats)	5	3.61
Meat Quality (White Stripping, Woody Breast)	6	4.65
Exportation Issues (Drug, MRLs, Paws, AI, etc.)	7	4.83
Increased Environmental Regulations	8	5.91

Disease Issue Ratings



Non-Disease Issues Ratings

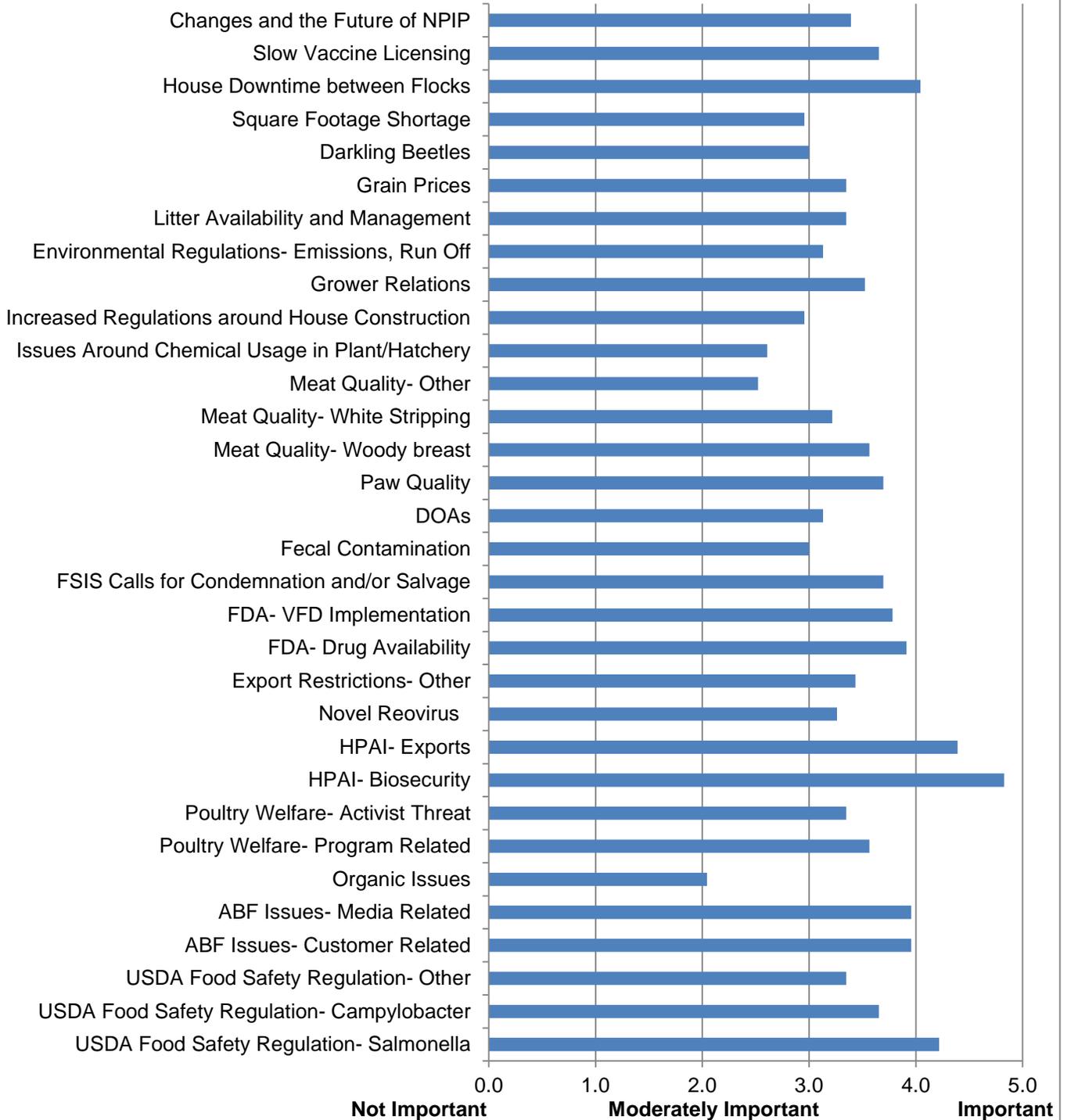


Table Egg Industry Report - October 2014 to October 2015
 Eric Gingrich, DVM, Diamond V, Zionsville, IN

The past year's most significant event was the devastating outbreak of highly pathogenic avian influenza (HPAI) in the central US. The outbreak began in commercial layers in Wisconsin in mid-April and ended in mid-June in IA. During this period of time, Iowa lost about 26 million layers, Minnesota 3.6 million, Nebraska 3.5 million, South Dakota 1.3 million, and Wisconsin 1.1 million for a total of 35.6 million. This is about 10% of the nation's egg layer population. Also, about 5 million pullets were lost to the disease or eradication effort.

A complicating issue was the inability to depopulate flocks on a timely basis leaving them to shed HPAI virus at a high rate and allowing increased spread. The lack of sufficient manpower, MAK (Modified Atmosphere Killing) carts, and/or CO2 supplies was responsible. From this issue, ventilation shutdown (VSD) is to be added as a possible depopulation method during the depopulation decision process according to USDA.

Many problems with biosecurity were found after close evaluation of the practices used by the farms that broke with the disease. A significant amount of investment in vehicle wash and baking stations, Danish entry type entryways for employees, visitors, and crews, hard surface pads outside of house entries, gates to control traffic, etc. Interviews of employees also took place to make sure they have no connections with other poultry operations in their households or off-work activities.

It is felt that with the improvements in biosecurity, greater awareness of the threat of HPAI, and more timely depopulation of flocks that are infected, that widespread HPAI losses as experienced this year will not occur again.

Other than HPAI, overall health of the national table egg layer flock continues to be very good. There are no other major clinical disease problems occurring at this time. This is due to the several resources and practices available to the industry:

- Continued availability of high quality vaccines
- Flock supervision from professional, well-trained flock service technicians
- Readily available veterinary technical assistance from primary breeder, vaccine company, diagnostic laboratory, feed additive suppliers, and consulting veterinarians
- High quality nutrition provided by professional nutritionists
- Housing of a majority of layers in environmentally controlled facilities in cages without exposure to litter
- Use of sound biosecurity practices.
- Continual surveillance for foreign animal diseases or potentially highly pathogenic agents such as Newcastle and avian influenza by our state and federal laboratory system

A poll of the Association of Veterinarians in Egg Production (AVEP) was conducted within the last month. The members were asked to rate a list of common diseases of caged and cage-free pullets (23 and 24 conditions listed respectively) and caged and cage-free layers (32 and 34 conditions listed respectively) as to their prevalence and their importance in their area of service on a scale of 0 to 3 with 0 = not seen, 1 = seen but not common, 2 = commonly seen, and 3 = seen in a majority of flocks. For the importance question, they were asked to give a value of each disease to a company in their area of service on a scale of 0 to 3 with 0 = not important issue for flock health or economics to 3 = very important issue for flock health and economics. 22 members of the total membership of 100 answered the survey.

To follow are the results of prevalence and importance of chick issues:

	Caged Pullets		Cage-Free Pullets	
	Prevalence	Importance	Prevalence	Importance
Yolk Infections	1.39 (1.19)*	1.35 (1.13)	1.50 (1.14)	1.44 (1.14)
Starveouts	1.61 (1.25)	1.24 (0.93)	1.31 (1.14)	1.38 (1.08)

* 2014 survey results are in parenthesis

Yolk infections and starveouts are associated with hatch egg quality, hatchery sanitation, and hatchery management of incubation, sanitation, chick processing, holding, and delivery. Compared to last year's survey, these problems appear to be on the rise again.

The survey revealed the following top 5 diseases of concern occurring in US for growing pullets excluding chick yolk infections and starveouts:

Top 5 Caged Pullet Diseases	Top 5 Cage-Free Pullet Diseases
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Prevalence	Importance	Prevalence	Importance
1 – Coccidiosis (0.98)	1 – Coccidiosis (1.65)	1 – Coccidiosis (1.50)	1 – Coccidiosis (2.00)
2 – tie Post SE Bacterin Hepatitis & Infectious Bursal Disease (IBD) (0.78)	2 – E. coli (1.59)	2 – Piling (1.25)	2 – Piling (1.94)
	3 – tie IBD & Marek's (1.47)	3 – E. coli (0.94)	3 – E. coli (1.63)
4 – tie Infectious Laryngotracheitis (ILT) & E. coli (0.56)		4 – Necrotic enteritis (NE) (0.94)	4 – ILT (1.56)
	5 – ILT (1.29)	5 – IBD (0.88)	5 – NE (1.56)

Note that none of the caged pullet diseases are prevalent above the 1 category so these conditions are not common. Coccidiosis and secondary necrotic enteritis remains the number 1 disease concern in pullets. It is a problem in caged pullets as well with vaccine usage as an intervention on the rise. Piling issues continue to plague the cage free pullet grower. SE bacterin induced hepatitis syndrome can result in up to 7 percent mortality starting 2 weeks after the administration of SE bacterin. It has a genetic susceptibility base as it has not been seen in one strain of birds. The cause of this problem continues to be unknown at this time. Infectious bursal disease (IBD) is its subclinical form may lead to immunosuppression after the maternal antibody has subsided. The use of the recombinant HVT-vectored IBD vaccine has greatly aided those sites with problems. Infectious laryngotracheitis is causing losses of pullet flocks in enzootic areas.

To follow are the top 5 diseases for caged and cage-free layers from the survey:

Top 5 Caged Layer Diseases		Top 5 Cage-Free Layer Diseases	
Prevalence	Importance	Prevalence	Importance
1 – E. coli (1.67)	1 – E. coli (2.28)	1 – Cannibalism (1.83)	1 – E. coli (2.22)
2 – Cannibalism (1.50)	2 – Cannibalism (1.89)	2 – E. coli (1.78)	2 – Cannibalism (2.06)
3 – Calcium Depletion (1.39)	3 – MG (1.72)	3 – Ascarids (1.28)	3 – MG (1.78)
4 – tie Gout & <i>Mycoplasma gallisepticum</i> (MG) (1.28)	4 – Coccidiosis (1.61)	4 – Piling (1.17)	4 – Cocci (1.72)
	5 – Calcium Depletion (1.56)	5 – tie Mites & Coccidiosis (1.06)	5 – tie Fowl Cholera & Piling (1.44)

Colibacillosis continues as the #1 disease problem in caged and cagefree flocks and is a problem mainly of young flocks with mortality rates of 0.5 to 4% per week starting shortly after housing can occur. It is felt that this condition is most often secondary to upper respiratory challenges with MG, *Mycoplasma synoviae* (MS), ammonia, infectious bronchitis (IB), etc. in early lay. It also may be a primary problem if water lines are contaminated with *E. coli*. The overall prevalence and importance of colibacillosis was about the same as last year. A post-molt colibacillosis syndrome is also seen in some flocks due to declining immune system function, an ascending infection of the reproductive tract, upper respiratory infections, etc. The live *E. coli* vaccine, introduced in mid to late 2006, has been increasingly used successfully as both a preventative and as a treatment in the face of an outbreak in most areas. Some producers are now applying the live *E. coli* vaccine by eyedrop during the growing period to assure that each bird receives a dose.

Cannibalism was shown to be an important issue in both cage and cagefree layers. In cagefree production, the 10-day or younger rule for beak trimming results in longer beaks than desired compared to a beak trim at 4 to 8 weeks and may result in an increase in incidence and severity of cannibalism. The increasing use of large colony cages may also increase the level of cannibalism.

Calcium depletion continues to maintain high importance in caged flocks and is normally associated with either late onset of switching to lay feeds with high levels of calcium or low feed intake during early production with the lack of proper formulation to account for the low feed intake. This condition will be an ongoing issue with

increasingly higher egg production rates accompanied with lower feed consumption through improvements in management and genetics.

Focal duodenal necrosis (FDN) dropped out of the top 5 conditions for caged layers this year. Apparently, preventative measures are working and the prevalence is low. Visceral gout came into the top 5 list this year in caged production for the first time. This condition is normally associated with kidney damage due to calcium toxicosis during a time when the bird cannot rid it from the kidneys (immature birds) such as feeding layer feed too early. Coccidiosis is an important issue for both caged and cagefree layers indicating problems with developing immunity during growing.

Mycoplasma gallisepticum (MG) continues as an issue in multi-aged facilities and is successfully controlled in most cases through vaccination. Each complex must customize its vaccination program to control the strain on the farm. Ts-11 and 6/85 live vaccines are used for controlling mild strains of Mg while F-strain live vaccine is being used to control more pathogenic strains or where the Ts-11 or 6/85 vaccines are no longer effective. The live pox-vectored recombinant Mg vaccine is being used in a variety of situations and appears to be useful in low challenge situations. Vaccine failures with all vaccines are somewhat common and the unit must resort to medication programs using tylosin or tetracycline antibiotics before alterations in the immunity program are made. Most all operators are now applying the F-strain vaccine by eyedrop rather than spray in an effort to increase its efficacy.

An external parasite, the Northern Fowl Mite, has fallen in the list compared to last year's survey. The use of effective treatments has apparently had this effect. Spray treatment of caged layers is difficult due to the configuration of equipment but the feeding of elemental sulfur may have led to this decrease. Elemental sulfur in dust baths is being used very successfully in cage-free flocks. Insecticidal treatment of pullet moving trucks and equipment may also have had an effect.

The AVEP survey also asked about other issues and diseases of concern on a scale of 0 to 3 with 0 = no concern, 1 = some concern, 2 = moderately concerned, and 3 = very high concern. The opinions of the 20 respondents is as follows:

Issue	Average 2012	2013	2014	2015
Avian Influenza (AI)	1.55	2.00	2.19	3.00+
Lack of Effective Treatments	2.15	2.43	2.56	2.14
SE and FDA Egg Safety Rule	2.55	2.29	2.31	2.29
<i>S. heidelberg</i> and Egg Safety Rule	2.45	1.90	2.13	2.05
Welfare in General	2.33	2.15	2.31	2.21
Beak Trimming	1.70	1.50	1.88	1.91
Disposal of male chicks	1.40	1.25	2.00	1.64
On-Farm Euthanasia	1.95	1.80	1.88	1.73
Molting of Layers	1.60	1.35	1.31	1.27
Banning of Cages	2.60	2.35	2.69	2.27
Adoption of Enriched Cages	N/A	2.11	2.44	1.86
Supply of Useful Vaccines	1.20	1.05	1.56	1.45
Number of Responses	20	17	16	22

The concern for AI is self-evident. The lack of effective treatments for diseases such as colibacillosis, necrotic enteritis, ascarids, *Capillaria spp.*, spirochetosis, fowl cholera, etc. is a very high concern and a welfare issue for the diseases that can cause much suffering due to illness. The list of antibiotics that can be used in egg layers is quite short – bacitracin, tylosin, and chlortetracycline. The lack of an anti-parasitic product for used in controlling ascarids during lay, or other nematodes, is especially troublesome as these conditions are becoming increasingly common in cage-free production. Amprolium continues to be available to prevent and treat coccidiosis. Hygromycin is also now approved for use in egg layers in production for roundworms, *Capillaria spp.*, and cecal worms but there is nothing for treatment of organic layers. Also, there is an increase in usage of non-antibiotic, preventative feed and water additives containing probiotics, prebiotics, and fermentation metabolites.

Concern for *Salmonella enteritidis* (SE) and its consequences continues due to the ongoing possibility of human outbreaks as occurred with the egg recall of 2010 involving two Iowa operations in August 2010. The Egg Safety Rule was implemented on July 9, 2010 for flocks over 50,000 layers. Flocks of between 3,000 and 50,000 joined the program on July 9, 2012. Inspections by FDA are ongoing. The prevalence of SE is at an all-time low based on certain states monitoring results. A moderate degree of concern for adding other serotypes to the plan is apparent.

The FDA Egg Safety Program entails obtaining chicks from NPIP SE Clean breeders, rodent and fly monitoring and control programs, biosecurity, cleaning and disinfection of premises, training of persons involved, testing of manure samples at 14-16 weeks, 40 to 45 weeks, and 6 weeks after molt. If any of the manure tests are positive for SE, egg testing must take place. The producer funds all testing and compliance efforts. Laboratories have managed to gear up to handle the increased testing load this requires. Producers with a manure positive swab test are holding eggs from the market until after the test results of eggs are obtained. The use of DNA based tests are now being used that minimize the time of testing from the formerly required 10 days for culture to as low as 27 hours with the new tests. There is no provision in the program for compensating a producer who has an egg-positive flock and does not have a pasteurization or hard-cooking plant that will take their eggs. Producers are greatly ramping up measures to reduce risk of SE infection by increased use of vaccines, intestinal health feed additives, rodent and fly control measures, and biosecurity practices as was intended by the plan.

The possible addition of *Salmonella heidelberg* (SH) to the FDA Egg Safety Plan has the industry questioning why and how this will be initiated. SH in humans has not recently been attributed to eggs and the prevalence of SH in humans has dropped since the late 1990's to 2011 from 1 per 100,000 population to 0.35 per 100,000 in CDC figures from FoodNet. Also, there is no breeder program as there is for SE and it may take five to 10 years before one can be fully assured of a clean product once a breeder program is started. Also, no specific SH vaccines are available as they are for SE. It is estimated that a much higher contamination rate of flocks with SH is present compared to SE.

Poultry welfare concerns continue to be of high to very high concern due to continued activities by activist groups. The increase in concern over day old male euthanasia has come about by some companies stating they are going to require egg products from flocks where day old male euthanasia is not used.

The transition to low density enrichable cage and cagefree egg production in California due to the regulations of Prop 2 has gone well with the California consumers paying a 60 cent premium for this decision. Several houses in the Southwest and Midwest were converted to comply with the CA regulations.

Vaccine use continues to be the mainstay of disease prevention in the egg layer industry second to biosecurity. The supply of useful vaccines continues to be adequate and appears to be keeping up with the layer industry needs. It will be interesting to see if this good supply of vaccines continues with the consolidations now occurring in the poultry vaccine business.

This is the third year that the AVEP members have been asked for their ideas as to research needs for the layer industry. A summary of the top 5 responses of the 21 responding members is as follows:

Research Need Area	Number of Respondents
1 – Enteric conditions (FDN, reovirus, spirochetes, cocci, non-specific enteritis, etc.)	12
2 – Avian influenza control/prevention – biosecurity, depop, disposal, vaccines, etc.)	7
3 – Mass depopulation methods	6
4 – Effective treatments, antibiotic or non-antibiotic	5
5 – Internal parasites control methods	3
5 – Improved recombinant vaccines with multiple antigens	3
5 – Post SE bacterin hepatitis in pullets	3

For the second year in a row, the egg industry, not affected by AI, has experienced unprecedented profits for the past 12 months. And again the reason is due to AI, last year due to AI in Mexico and this year for AI losses of egg layers in the US. For the first 9 months of 2015, the average egg producer according to the Egg Industry Center has made over \$14 per bird. Normally, the average for a 10-year period is \$1 per bird. Low feed prices for the period from October 2014 through September 2015 has aided greatly in assuring high profits.

Iowa (32.6 million) continues to be the lead state in egg layer numbers even though they had significant losses due to AI earlier in the year. Iowa is followed by #2 Ohio (30.8 million), #3 Indiana (26.0 million), #4 Pennsylvania (23.8 million), #5 Texas (15.6 million) and #6 California (12.9 million) according to the National Agricultural Statistics Service for August 2015. Total commercial egg layer numbers were 272 million in August 2015, down from 296 million in August of 2014.

Turkey Industry Report – Current Health and Industry Issues Facing the US Turkey Industry

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In preparation for this report to the USAHA Committee on the Transmissible Diseases of Poultry & Other Avian Species, the subcommittee chairman, Dr. Clark, surveyed turkey industry professionals and veterinarians representing (n=25) U.S. turkey production regarding the health status of turkeys produced in August 2014 through August 2015. The turkey industry reports several disease challenges for this 12 months varying by geographic regions within a state and across the United States. This report will list (Table 1) the challenges by disease and issues. Of particular interest in 2015 are issues with lack of efficacious drugs, *Clostridial dermatitis*, avian influenza, *Salmonella* and *Colibacillosis*. Most notable, avian influenza moved from 28th rank (score 1.5) in 2014 to 4th (score 3.1) in 2015.

The “**lack of approved efficacious drugs**” continues to be the top health issue (Table 1). The withdrawal of the New Animal Drug Application (NADA) for enrofloxacin in 2005 for use in poultry leaves the industry with no adequate therapeutic response to *colibacillosis* (ranked #3, unchanged since 2009), or **fowl cholera** (ranked #11 from #12). In July 2011, the sale of roxarsone was suspended; September 30, 2013, the FDA marketing authorization NADA was withdrawn. The sponsor of Penicillin-100 Type A medicated article (in feed administration) withdrew the approval (NADA) June 30, 2015. Issues over the use of antibiotics in animal agriculture remains a major concern for the turkey industry and for all of animal agriculture.

Clostridial Dermatitis (CD), also referred to as **Dermatitis** or **Cellulitis**, remains a major disease issue across all geographic regions; as the survey average decreased slightly to a score of 3.3 (from 3.5 in prior year) and ranked #2 (no change), from 3.6 (#2), 3.8 (#2), 3.9 (#2), 4.0 (#2), 3.8 (#2) and 3.3 (#3) in 2013, 2012, 2011, 2010, 2009 and 2008, respectively. Analysis indicates range of concern; 46% of respondents score CD a 4 and 5 (severe), 38% score it a 2 and 1 (mild); severe (4-5) versus mild (1-2) scores were 50%, 62%, 76% and 32%, 27%, 20%, respectively for the prior three years (2014, 2013, 2012). CD is most commonly seen in, but not limited to, commercial male turkeys nearing market age. Opinions vary as to risk factors and potential causes of the problem. Some of the key areas to control CD include: early recognition; removal of mortality 2-3 times per day; medicating affected flocks with appropriate antimicrobials; promptly managing all water spills and wet litter. There has been limited success with vaccinating at-risk flocks with autogenous bacterins and toxoids. For some, a novel litter amendment has shown limited success.

Poult enteritis of unknown etiologies has decreased in importance, to position #12 from #10, with a score of 2.3 (from 2.4). **Turkey Coronavirus (TCV)**, as a defined cause of enteritis, was ranked #32 (Table 1), down from #27, with 119 reported cases (Table 2). Majority of TCV cases were limited to one geographic area.

Protozoal Enteritis, attributed to flagellated protozoa, *Cochlosoma*, *Tetratrichomonas* and *Hexamita*, ranked #22 (score 1.8), relatively unchanged over past years. Several types of protozoa are associated with enteric disease of turkeys. Protozoal enteritis can present with general signs, including dehydration, loss of appetite (off-feed), loose droppings (diarrhea) and watery intestinal contents. Flagellated protozoa include *Cochlosoma*, *Tetratrichomonas* and *Hexamita*. *Eimeria* and *Cryptosporidia* are non-flagellated protozoa. *Cochlosoma* and *Hexamita* are associated with enteritis, primarily in young turkeys, especially in the summer months. There are field reports of co-infections with *Cochlosoma* and *Tetratrichomonas*, or *Cochlosoma* and *Hexamita*, or flagellated protozoa and *Eimeria*.

Single age brooding has been implemented during the last several years to assist in managing diseases on turkeys farms, especially enteric diseases. Historically, production systems included 2 - 3 different ages on a single farm site reared in separate barns, from day-old to market age. The trend is to isolated, specialized brooding facilities. All production is separate hen and tom rearing. The brooding phase for commercial turkeys is rearing about 0 – 5 weeks of age, then the flock is moved to specialty finisher or grow-out barns. Single age brooding may be termed all-in/all-out or single-age or brooder hub. Single age brooding systems can operate in two ways. One option rears the turkeys to slaughter age at the same farm site, without other ages on the farm. Another system of single age brooding involves farm sites dedicated to brooding, then at 5 weeks of age birds are moved to a separate site for finishing. In 2015, 61% of brooding was single age, compared to 42% in 2008. Single age brooding is more common in the Southeastern U.S. than the Midwest states. Conversion to single age brooding started in late 1990 following the emergence of Poult Enteritis Mortality Syndrome (PEMS) in North Carolina; advantages became obvious and it has expanded to other areas of the U.S. **Tunnel ventilation** of finisher (grow-out) barns is becoming more popular method to minimize heat stress; in 2015, 26% of the industry finisher production is tunnel ventilated, compared to 11% in 2008.

Late mortality ranked 6th (2.7) health issue and changed from #4 the prior year. Late mortality may be defined as mortality, in excess of 1.5% per week, in toms (males) 17-weeks and older; mortality is not diagnosed to a specific disease or cause. Late mortality may be associated with physiologic or biomechanical deficiencies following early rapid growth in heavy toms achieving genetic potential; aggressive behavior noted in mature toms; leg problems and/or hypertension.

Leg problems (#10, prior year was #6) are ranked among the top concerns of the turkey industry. Leg problems are a common complaint, such as, spiral fractures of the tibia or femur. Leg Problems may be defined as

lameness, particularly in toms, several weeks prior to slaughter. Leg problems are attributed to various conditions (refer to Table 1), including, pododermatitis, fractured femurs, fractured tibia, osteomyelitis (OM), tibial dyschondroplasia (TDC), spondylolisthesis, "Shaky Leg", chronic reovirus infection, etc.

Turkey Reovirus Digital Flexor Tendon Rupture (TR-DFTR) was recognized as a newly emerging disease in 2011. Since then multiple unique reoviruses have been isolated and identified as the cause of tenosynovitis and digital flexor tendon rupture in commercial turkeys. Clinical signs in young flocks are reportedly mild to nonexistent, but can develop into lameness and/or abnormal gait in older flocks, starting at about 12 weeks of age. Affected flocks may also report an increased incidence of aortic ruptures and poor flock performance (weight gain, uniformity). Research continues into pathogenesis, virus characterization, diagnostics and epidemiology. Research indicates that the turkey arthritis reovirus is distinct from the recently identified novel reovirus causing arthritis in chickens, and more similar to the turkey enteric reovirus. TR-DFTR was added to the survey in 2011 and ranked #11 (Table 1) with 106 "confirmed" cases or flocks (Table 2). In 2015 TR-DFTR ranked #19 with 146 cases (150 in prior year). Multiple companies have implemented autogenous reovirus vaccination programs to induce the maximum production of antibodies and resulting transfer of maternal antibodies. Results show a significant reduction in associated clinical signs in those poult placed from vaccinated flocks. A commercial turkey lighting program of 4-8 hours of continuous dark in a 24-hour period has also been recommended. The combined efforts of breeder vaccination, commercial farm biosecurity and flock management appear to be controlling this disease. Increased recognition of TR-DFTR in 2014 - 2015 confirmed that the reovirus has mutated into three distinct strains.

Blackhead, also known as Histomoniasis, decreased to position #13 (#11 prior year). It is one disease with no efficacious drug approved for use in turkeys. There were 55 reported cases of blackhead (Table 2) an increase from 61 the prior year, and a record 108 in 2010. Histomoniasis occurs regionally and seasonally in turkeys, and can result in significant mortality. Dimetridazole was extremely efficacious and previously approved for use in turkeys for the prevention and treatment of blackhead; it was banned in 1987. The lack of any legal treatment for Histomoniasis is of concern, especially in the case of valuable turkey breeder candidate flocks. Losses to blackhead have been severe in several areas of Europe, and sporadic cases are occurring in North America. On April 1, 2015, the sponsor announced that it would discontinue marketing nitarsone, by fall 2015, and would request withdrawal of the approval for the drug by the end of 2015. Nitarsone is approved for the prevention of Histomoniasis (blackhead disease) in turkeys and chickens, and is the only approved animal drug for this indication. Nitarsone will cease to be available in the 2016 growing season.

Heat stress ranked #18 following another hot summer, compared to #29 the prior year. **Poult Enteritis Mortality Syndrome (PEMS)** ranked #30 versus #34 previously, *Ornithobacterium rhinotracheale (ORT)* ranked #7 versus #9 previously. **Avian Metapneumovirus (AmPV)** ranked #25 versus #35, with a few atypical cases limited to the Midwestern U.S. *Bordetella avium* continued as a significant respiratory disease challenge in several geographic regions; bordetellosis ranked #8 (2.5 score) in 2015 compared to #5 (2.9) the prior year.

Mycoplasma synoviae (MS), infectious synovitis) infections, ranked #27 (#25, prior year), are one cause of synovitis. It may be present in flocks 10-12 weeks of age with typically low mortality and low morbidity. There were 24 cases of MS reported (Table 2). The primary breeders have remained free of *M. gallisepticum (MG)*, *M. meleagridis (MM)* and MS. Sporadic, but increasingly frequent infections with Mycoplasma, both MG and MS, often in association with backyard poultry and broiler breeder flocks is an ongoing concern, having the greatest impact when a breeder flock is infected and has to be destroyed. There were 31 cases of MG reported (Table 2).

In the Winter/Spring of 2015, an unprecedented outbreak of **Highly Pathogenic Avian Influenza (HPAI)** struck Western and much of the Midwestern United States. Turkey flocks in eight states were affected by H5N8 and H5N2 strains of HPAI, with H5N2 accounting for the vast majority. In total, 153 farms commercial turkey or turkey breeder flocks were infected, resulting in the loss of over 7.75 million turkeys, in addition to over 40 million chickens (layers and broiler breeders). USDA has classified this outbreak as the worst incident of animal disease in US history. The virulence of the H5N2 was like nothing seen before and its impact was unprecedented. As available studies and observations note, the route of introduction was not limited solely to infection from wild migratory birds. HPAI entered farms on personnel, vehicles and blown dust. Onset of the 2015 H5N2 strain of HPAI was subtle with birds often asymptomatic until several days after infection, followed by the sudden, rapid onset of mortality. All infected flocks have been depopulated. Cleaning and disinfection followed by a required 21-day fallow period of turkey houses (barns) has substantially impacted turkey production in certain regions of the U.S.

In response, the turkey industry, along with APHIS, state governments, and other stakeholders has worked to review and improve disease monitoring, prevention with a primary focus on biosecurity enhancement, and response. Many lessons have been learned, and data is still being analyzed for any information that might help mitigate future introductions of the virus, which some expect could happen during the fall 2015 or spring 2016 migratory seasons. Vaccines have been developed for the prevention of H5N2 HPAI and APHIS has developed a

plan of action for the deployment. Although the agency has not yet approved vaccine usage, APHIS has committed to stockpiling the vaccines in the event that the decision is made to use them in the future.

In light of the HPAI outbreaks in the West and Midwest, the industry accelerated work on developing the **Secure Turkey Supply** plan or STS (www.secureturkeysupply.com). STS includes Federal and State Transport (FAST) Plan for Movement of Commercial Turkeys in a High Pathogenicity Avian Influenza (HPAI) Control Area, and Turkey Risk Assessment. Draft versions of the Plan were utilized in regions affected by HPAI, and were instrumental in many instances where movement and shipping of turkeys and turkey products were at risk. The goal of the Plan is to facilitate business continuity and economic survival of participating non-infected turkey operations in a Control Area after a detection of HPAI, and to help assure the continuous availability of safe turkey meat to consumers.

Regarding disease surveillance, the industry has continued to voice strong support for the maintenance of the **National Poultry Improvement Plan (NPIP)** in the face of increased government spending cuts. NPIP is a vital state-federal-private partnership for the turkey industry, as well as the broiler and egg industries, and APHIS has continued to show strong support for the program, having hired additional staff for the program in 2014, and maintaining their offices in Conyers, Georgia, instead of moving it to the Washington, D.C. area. NPIP has been additionally helpful in addressing certain aspects of disease control and eradication in the HPAI outbreak. The industry is also supportive of federal efforts to update and modernize ARS' Southeast Poultry Research Laboratory in Athens, Georgia. To date, only \$45-million (or approximately 1/3rd of the ARS request) has been allocated, with an additional ~1/3rd portion approved, but still pending Congressional budget passage.

Two of the industry's top priorities continue to be the health of turkeys and ability to utilize approved drugs, especially in light of recent avian influenza outbreaks and increased scrutiny from special interests regarding antibiotic resistance. The first related guidance, in regards to drug utilization, was published in 2003, Final Guidance #152, **"Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to their Microbiological Effects on Bacteria of Human Health Concern"**. Since then there has been a great deal of discussion around antibiotic resistance leading to numerous efforts by the Food and Drug Administration's Center for Veterinary Medicine (FDA/CVM): In 2012, the Guidance for Industry (GFI) #209 **"The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals"** was published. On December 11, 2013, the FDA finalized Guidance for Industry #213, **"New Animal Drugs and New Animal Drug Combination Products Administered in or on Medicated Feed or Drinking Water of Food Producing Animals: Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209"**. In 2015, FDA/CVM published the finalized VFD regulation and mandates the rules and responsibilities of licensed veterinarians in prescribing and administering medically important antimicrobials in feed. Guidance #213 established procedures for phasing out the use of medically important antimicrobials for production purposes in alignment with Guidance for Industry #209 and proposed changes to VFD drug regulations. Final implementation is scheduled for December 2016, no drugs listed as "medically important" that are exclusively labeled for production purposes can be used. Drugs that are used, must prove, through data, that they are used for at least one of the following: prevention, control, or treatment and only be administered via a prescription from a veterinarian. All 26 animal drug manufacturers have agreed to comply. In conjunction with this guidance, the Veterinary Feed Directive (VFD) increases the veterinary oversight of the administration of drugs. The rule incorporates many of NTF's comments. Specifically, (1) Category I Type A medicated articles can continue to be utilized by unlicensed feed mill; (2) the rule continues with the concept of veterinary oversight as opposed to continued supervision; (3) record keeping is required to be kept for two years rather than the one year that was proposed, and NTF supported; (4) veterinarians don't have to be licensed in each state, but do need to be compliant with each state's rules in which they practice; (5) though "standing VFDs" were not defined, they were discussed in the rule and approved as long as they are within the rule's defined expiration date requirements; (6) though there are not uniform VFDs, the rule requires the application sponsor to provide all the information a veterinarian would need.

In addition to guidance from the FDA, antibiotic resistance has been a key focus throughout the Obama Administration. Last year, the CDC released a report on antibiotic resistance calling for immediate action to address the issue. Following this report, the **President's Council of Advisors on Science and Technology (PCAST)** published a report on antibiotic use in human medicine and agriculture -- Combating Antibiotic Resistant Bacteria (CARB). The report included an Executive Order calling for a national response to antibiotic resistance through the establishment of a Presidential Advisory Council run by HHS in consultation with USDA and the Department of Defense. In March 2015, this group established a National Action Plan to ultimately (by the implementation date in the year 2020) achieve the five goals laid out by the Administration. USDA's Food Safety Inspection Service (FSIS), Agricultural Research Service (ARS) and Animal and Plant Health Inspection Service (APHIS) are working with FDA/CVM to collect better data to inform these goals. The industry continues to discuss what data should be collected with these Agencies and how it will be done. Both the FDA and The Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria will hold public meetings at the end of September 2015 to further discuss the concepts

for developing measurements and targets for data collection.

For the last 15 years, the US animal agriculture industry has been continually challenged with numerous attempts to ban the use of antibiotics in livestock and poultry. The current attempt at the federal level is with the 114th Congress' **Preservation of Antibiotics for Medical Treatment Act** of 2015, introduced into both the House and Senate [H.R.1552; S.621], otherwise known as PAMTA 2015. The Senate version is titled S. 621 **Preventing Antibiotics Resistance Act** (PARA) and is "to amend the Federal Food, Drug, and Cosmetic Act to preserve the effectiveness of medically important antimicrobials used in the treatment of human and animal diseases." The legislation would disallow use of medically important antimicrobials for nontherapeutic uses. The turkey industry opposes PAMTA, a bill that would devastate the ability to protect animal health by unnecessarily and inappropriately removing several classes of important antibiotics from the market. The turkey industry welcomes honest discussion of science-based, pragmatic options which preserve animal welfare while providing consumers' assurance the use of these vital, safe and effective medications is professional, judicious and does not jeopardize their effectiveness in human medicine.

In August of 2014, the Food Safety and Inspection Service (FSIS) published the final **New Poultry Inspection System** (NPIS) rule, which will modernize the inspection of turkeys and other poultry in the United States. In establishments that volunteer to transition to the new inspection system, FSIS inspectors will be allowed more flexibility to patrol the plant and provide scientific oversight to ensure the plant is meeting the required food safety performance standards. Federal inspectors will be stationed at the end of the production line to verify every poultry carcass meets the federal regulations, and plant employees will have an expanded role in inspecting carcasses for quality standards on the inspection line. The first turkey plants began their transition to the new system in the summer of 2015, and additional plants will continue to transition through 2015 and 2016.

In 2014, turkey **production** slightly decreased to 7,217,056,000 from 7,277,536,000 pounds (live weight). Overall domestic per capita **consumption** for turkey products decreased from 16.00 lbs in 2012 to 15.80 in 2013. The preliminary number for 2014 is 15.90 lbs turkey consumption per capita, which is the lowest level since 1988. **Live production** in 2014 decreased to 237,500 million head with an average live weight of 30.40 lbs. In 2013, 240.000 million head were produced with an average live weight of 30.34 lbs. (Reference: National Turkey Federation Sourcebook, October 2015).

Table 1. Turkey health survey (August 2014 - 2015) of professionals in US turkey production ranking current disease issues (1= no issue to 5 = severe problem). N=25

Issue	Score Average (1-5)	Score Mode (1-5)
Lack of approved, efficacious drugs	4.4	5
Clostridial Dermatitis (Cellulitis)	3.3	5
<i>Colibacillosis</i>	3.2	5
Avian Influenza	3.1	5
<i>Salmonella</i>	3.0	3
Late Mortality	2.7	3
<i>Ornithobacterium rhinotracheale</i> (ORT)	2.6	3
<i>Bordetella avium</i>	2.5	2
Cannibalism	2.4	3
Leg Problems	2.4	3
Cholera	2.3	2
Poult Enteritis of unknown etiologies	2.3	1
Blackhead (Histomoniasis)	2.3	1
Coccidiosis	2.2	2
Tibial Dyschondroplasia (TDC, Osteochondrosis)	2.1	2
Round Worms (<i>Ascaridia dissimilis</i>)	2.0	2
Breast Blisters and Breast Buttons	2.0	2
Heat stress	2.0	2
TR-DFTR (Turkey Reovirus Digital Flexor Tendon Rupture)	1.9	1
Newcastle Disease Virus (NDV)	1.8	1

Osteomyelitis (OM)	1.8	1
Protozoal Enteritis (Flagellated)	1.8	1
Bleeders (aortic, hepatic ruptures)	1.8	1
Fractures	1.6	1
Avian Metapneumovirus	1.5	1
<i>Mycoplasma gallisepticum</i> (MG)	1.5	1
<i>Mycoplasma synoviae</i> (MS)	1.5	1
Shaky Leg Syndrome	1.5	1
<i>Erysipelas</i>	1.4	1
PEMS (Poult Enteritis Mortality Syndrome)	1.4	1
Necrotic enteritis	1.3	1
Turkey Coronavirus	1.3	1
H3N2 (H1N1) Swine Influenza	1.2	1
<i>Mycoplasma iowae</i> (MI)	1.2	1
Spondylolisthesis (Kinky-Back)	1.1	1
<i>Mycoplasma meleagridis</i> (MM)	1.1	1

Table 2. Turkey health survey (August 2014 - 2015) of professionals in U.S. turkey production.

Disease	Number of cases by year						
	2015	2014	2013	2012	2011	2010	2009
Blackhead (Histomoniasis)	55	61	52	80	89	108	67
<i>Mycoplasma synoviae</i> (MS)	24	41	75	49	39	56	38
Turkey Coronavirus (TCV)	119	43	420	221	70	91	3
Turkey Reovirus Digital Flexor Tendon Rupture	146	150	39	131	106*	n/a	n/a
<i>Mycoplasma gallisepticum</i> (MG)	31	17	45	n/a	n/a	n/a	n/a

*One respondent noted that their operation processed over 300 flocks with varying degrees of severity, but not included in the reporting of 2011 confirmed cases; Turkey Reovirus Digital Flexor Tendon Rupture (TR-DFTR).

Table 3. Turkey research priorities (August 2014 - 2015) of industry professionals in turkey production (1= low to 5 = high).

Issue	Score Average (1-5)	Score Mode (1-5)
Disease	4.0	5
Food Safety	3.9	5
Welfare	3.5	4
Poultry Management	3.2	3
Nutrition	3.1	3
Waste Disposal	2.4	2
Processing	2.4	2
Environmental	2.1	2

Table 4a. Percentage (%) of brooding (commercial; farm) production is all-in/all-out (single-age; brooder hub); average of respondents (n=25).

Year	Percentage (%)
2015	61.4
2008	42.1

Table 4b. Percentage (%) of finisher (grow-out; farm) production is tunnel ventilated; average of respondents (n=25).

Year	Percentage (%)
2015	25.7
2008	11.3

Table 5. Eighteen (18) in-feed FDA approved medications for turkeys listed by label indication.

Subtherapeutic (Production)	Therapeutic (Prevention, Control, Treatment)
Bacitracin Zinc	Amprolium
Bacitracin Methylene Disalicylate	Bacitracin Methylene Disalicylate
Bambermycin	Chlortetracycline **
Chlortetracycline **	Clopidol
Neomycin + Oxytetracycline **	Diclazuril
Oxytetracycline **	Fenbendazole
Ractopamine	Halofuginone ^
Virginiamycin **	Lasalocid
	Monensin
	Neomycin + Oxytetracycline **
	Nitarsone
	Sulfadimethoxine + Ormetoprim ^**
	Oxytetracycline **
	Zoalene (DOT)

^ Not currently marketed.

** Deemed "Medically Important" per FDA Guidance for Industry #209 and #152.

Roxarsone and Penicillin approvals withdrawn September 30, 2013, and June 30, 2015, respectively.

Live Bird Marketing System (LBMS) Avian Influenza (AI) Program Working Group Report

Fidelis Hegngi, DVM, USDA-APHIS-VS, presented by Patricia Fox, DVM, USDA-APHIS-VS, Raleigh, NC

Since 1986, States in the Northeast have been monitoring live bird markets for the presence of avian influenza (AI) viruses that may pose a threat to the commercial poultry industry. Beginning in 1994, low pathogenicity avian influenza (LPAI) H7N2 proved to be endemic in live bird markets in the northeastern United States. In 1999, the U.S. Department of Agriculture (USDA) established a LBMS working group to support States wanting to eliminate persistent LPAI H7N2 in the live bird markets. On October 20, 2004, the USDA Animal and Plant Health Inspection Service (APHIS) published uniform program standards to prevent and control H5 and H7 LPAI subtypes in U.S. live bird markets. The standards cover licensing, AI testing, recordkeeping, sanitation, biosecurity, surveillance, inspection, tracebacks, premises registration, traceouts when positives occur, and response to positive facilities. The standards apply to live bird markets, auctions, and small sales, as well as to producers and distributors who supply the markets. The standards are currently being implemented.

States are responsible for enforcing the LBMS LPAI program standards. State participation is voluntary. Participating States enact regulations for compliance of their live bird markets, producers, and distributors. All markets, producers, and distributors supplying the markets must be registered or licensed with the State and must allow Federal and State inspectors access to their facilities, birds, and records. These facilities must also have written biosecurity protocols in place. APHIS coordinates and administers the program and also provides personnel and resources to assist States in implementing and ensuring compliance with program requirements.

In February 2015, the LBMS working group held its annual business meeting in Sacramento, CA, to address LBMS AI prevention and control program concerns. More than 50 participants representing 23 States attended, including 16 APHIS Veterinary Services representatives; 2 university representatives; 25 State

Department of Agriculture representatives; 9 LBMS/poultry industry stakeholders; and 4 representatives from animal health diagnostic laboratories. Participants discussed the program's progress, shared ideas for continued development, and agreed on further implementation.

In addition, the working group discussed:

- (1) The Avian Health line item budget;
- (2) An overview of Canada's HPAI H5N2 experience;
- (3) The National Import and Export Services (NIES) information needs for international reporting;
- (4) The VS guidance document on indemnity requirements and process issues/procedures for flock plans, compliance agreements, and indemnity claims in cases of H5/H7 LPAI infection in poultry;
- (5) The Washington State experience on vvIBD/ILT/LPAI;
- (6) An update on the National Veterinary Services Laboratories (NVSL) AI surveillance testing that included current nationwide findings and recommended AI diagnostic tests and reporting of results to include a network algorithm for AI, a timeline for testing schemes for samples, and discussion on weak positives at National Animal Health Laboratory Network (NAHLN) laboratories;
- (7) Observations on global occurrences of HPAI H5N8 and other IAV of interest;
- (8) Wild bird surveillance projected for 2015 and beyond;
- (9) An update on the Zoetis Flu Detect AI rapid test;
- (10) An update on the NPIP program and the announcement of the 2015 Official State Agency (OSA) and the General Conference Committee (GCC) meeting in Salt Lake City, UT;
- (11) A review of NPIP authorized laboratories for past, present, and future;
- (12) The National Animal Health Monitoring System (NAHMS) Layer 2013 Study Results;
- (13) The VS perspective on the California 2014 LPAI H5N8 incident;
- (14) Salmonella in baby poultry sold at feed stores;
- (15) The 2015 Biosecurity for Birds (BFB) website/webinar and other outreach/education successes;
- (16) The 2015 Bird Health Awareness Week Webinar and Twitter entries;
- (17) Social media/advertising/Purina and Tractor Supply Partnership/education /outreach needs and future of BFB educational materials;

Special presentations were given on State avian influenza incidents in late 2014 and early 2015, including challenges and lessons learned in California, Delaware, Maryland, New Jersey, Oregon, and Washington. In addition, personnel from the USDA Agricultural Research Service (ARS), Southeast Poultry Research Laboratory (SEPR), discussed research on HPAI H5N8 and H5N2.

In fiscal year (FY) 2015, USDA's BFB campaign continued its efforts to educate the backyard poultry community about ways they can help protect and maintain the health of their birds. The campaign consisted of a photo contest with hundreds of entries, the annual bilingual calendar, Bird Health Awareness Week in February, two webinars and concurrent Twitter chats, fair packages, and social media outreach. Social media continues to be a major outreach tool. The Healthy Harry Facebook page has more than 5,000 likes (an increase of 1,000 likes) and the Healthy Harry Twitter account has more than 1,500 followers (an increase of 400 followers). The campaign launched three new Healthy Harry videos on YouTube in FY 2015: a biosecurity video, a live bird market video, and an NPIP video, each with at least several hundred views.

In FY 2014, approximately 140,987 tests were conducted for AI surveillance in the LBMS. Surveillance in the LBMS remains a high priority in FY 2015. Approximately 38,878 tests have been conducted for AI surveillance in the LBMS for the first full quarter and partial second quarter. Tests included agar gel immunodiffusion, real-time reverse-transcriptase polymerase chain reaction (rRT-PCR), antigen capture immunoassay, and virus isolation. For virus isolation and rRT-PCR, each sample may represent 5 to 11 individual swabs pooled for a composite single sample/test.

Since USDA initiated the H5/H7 LPAI LBMS prevention and control program in 2004, we have seen a marked decline in the incidence of LPAI viruses in the U.S. LBMS. The number of LBMS H5 and H7 AI positive premises has decreased steadily. In FY 2015, USDA detected only one LPAI H5N1 virus in a New Jersey live bird market. The virus was characterized as H5N1 North American lineage LPAI based on partial HA/NA sequence and cleavage site analysis and is different from the Eurasian/AM H5N1 virus recently detected in a wild bird in Washington.

HPAI in the Live Bird Marketing System –General Guidance

USDA will handle findings of HPAI in any component of the LBMS the same way it handled detection in a commercial poultry facility. This includes the finding of HPAI in LBMS environmental samples or when birds are no longer on a LBMS premises. Specifically, premises with presumptive positive HPAI results must be quarantined and inventoried. An epidemiological investigation should be conducted that includes all components of the LBMS. Rapid

and diligent traceback and traceforward investigations of movements from infected hauler, dealer, and wholesaler premises must be implemented. This tracing will aid in the control of the spread of HPAI virus and limit the impact of the outbreak.

Infected premises will be depopulated and cleaned and disinfected in accordance with the guidelines available in the HPAI Response Plan: The Red Book (www.aphis.usda.gov/fadprep). The results of the epidemiological investigation will determine if additional components of the LBMS, such as haulers' trucks and dealer and wholesaler facilities require depopulation, disposal, and cleaning and disinfection. Control areas will be drawn around infected premises, according to the HPAI Response Plan: The Red Book.

Avian Disease & Oncology Lab (ADOL) Research Update

John Dunn, DVM, USDA-ARS-ADOL, East Lansing, MI

Employing Genomics, Epigenetics, and Immunogenetics to Control Diseases Induced by Avian Tumor Viruses: *Improved chicken genome assembly to aid genetic and biological studies*. The chicken genome provides the blueprint for the underlying biology of all traits including those that are agronomically important such as growth, reproduction, health, and well-being. In collaboration with investigators at Washington University School of Medicine in St. Louis, MO, we used advanced sequencing technologies to increase the coverage and length of sequence contigs of the chicken genome assembly. This tool will allow scientists and commercial companies to conduct more complete and accurate studies to identify specific genes and pathways that will result in precision breeding and rearing of chickens with superior agronomic performance. As chicken is the primary meat consumed, this will benefit consumers and society by reducing the amount of feed and waste produced, and increasing health and well-being of reared birds.

Host genetics/epigenetics play a critical role in control of vaccinal response to Marek's disease (MD), an avian tumor virus-induced disease. Since the introduction of MD vaccines in the 1970s, the influence of host genetics on vaccine protective efficacy has been grossly overlooked by the vaccine and poultry industries. We have provided strong experimental evidence that host genetics contributes up to 83% of MD vaccine protective efficacy. This finding lays the foundation to search for the genetic and epigenetic mechanisms underlying the biological pathways that modulate vaccine protective efficiency. Further studies are likely to provide the knowledge needed to develop new and improved vaccines for not only more effective control against MD but also against other pathogens that will be highly protective in chickens of varying genetic backgrounds. This finding will directly benefit the poultry industry by significantly reducing economic losses due to disease control, improve animal welfare, and provide consumers with safe poultry products.

Genetic and Biological Determinants of Avian Tumor Virus Pathogenicity, Transmission, and Evolution

Characterization of Marek's disease virus (MDV) field strains. It has been nearly 20 years since a comprehensive set of MDV field strains have been solicited from poultry companies for pathotyping. Although MD condemnation rates in broilers have been dropping, there has also been increasing use of the most protective vaccine (CVI988/Rispens) in both broiler and layer operations, which may be masking an increase in virulence of circulating MDV field strains. We analyzed samples to determine whether the virulence of field strains has plateaued in recent years or if increasing virulence is causing industry to become more reliant on Rispens vaccination. We were unable to isolate any viruses significantly more virulent than field strains collected 20 years ago, which suggests that current management and vaccine practices have slowed the evolution of MDV.

Global gene expression in skin tissue of chickens infected with MDV. The feather follicle epithelium (FFE) is the only anatomical site where fully infectious enveloped cell-free MD virus particles are produced and released into the environment. The molecular mechanism of virus replication, assembly and dissemination is not known. Using state-of-the-art RNA sequencing technology, global gene expression profiling was conducted between the skin tissues of control and MDV-infected susceptible chickens. Data analysis revealed substantial changes in the expression patterns of both host and virus genes in the infected skin tissues when compared to the control uninfected samples. To our knowledge this is the first study to provide direct insight into the pathogenesis of MDV in the epithelial cells of the skin leading to the production of fully infectious virus particles. This study will be the base for the development of specific recombinant vaccines to block the production and dissemination of such virus particles into the environment.

Role of natural killer cells in MDV-induced protection. To shed light on the possible role of natural killer (NK) cells in vaccine-induced protection, we collected tissue samples from control and vaccinated chickens and conducted NK cell-specific gene expression analysis. Data obtained revealed activation of NK cells and up regulation of NK cell-specific genes in the vaccinated birds. Additionally, immunohistochemistry analysis showed

that the number of activated NK cells was increased in the tissues of vaccinated birds in comparison to the control chickens. Higher expression levels of a NK cell activation marker (CD107a, a cell surface protein) suggested that NK cells, an essential component of the innate immune system, play a critical role in the vaccine-induced immunity against MDV infection. Understanding the mechanism of vaccine-induced protection will help to design effective recombinant vaccines against newly evolved and highly pathogenic strains of MDV.

Effect of MDV infection on structural changes and gene expression pattern within comb tissue of affected chickens. The chicken line 6₃ (MD-resistant) exhibits an unusual necrotic dermatitis of combs, wattles, and toes under natural condition that is exacerbated by MDV-infection. We investigated the effect of MDV-induced immune suppression on structural changes and gene expression pattern within comb tissues of lines 6₃ and 7₂ (MD-susceptible) at 21 days post infection. Gene expression analysis revealed that many immune-related genes were all up-regulated in the necrotic combs of MDV-infected line 6₃. The expression levels of these selected genes were much lower in the combs of the susceptible line that displayed no visible necrotic damage. Staining for MDV antigens did not detect any viral proteins in the combs of either line but a massive infiltration of macrophages and sub-populations of T cells into the necrotic tissues. Further analysis also revealed thinning and erosion of epidermis within the connective tissues of the necrotic combs. Gram stain of the sectioned frozen comb samples exposed the presence of *Staphylococcus* bacteria species. This is the first study to shed light on the unintentional consequence of line selection that could negatively affect the immunological competence of the birds against immunosuppressive agents.

Pathotyping of bacterial artificial chromosome (BAC) clones of recombinant MDV. The cloning of MDV genome as an infectious BAC clone has led to major advances through our ability to study individual gene function by making precise insertions and deletions in the viral genome. MDV BAC clones are likely to replace wild type MDV field strains used in all aspects of MDV research due to advantages that include 1) precise manipulation of the viral genome, 2) viral genomes that are stable and can be maintained independently of propagation in eukaryotic cells, and 3) shipping BAC-cloned viruses is significantly easier and cheaper than shipping cell-associated viruses. We acquired virulent MDV BAC clones that have been generated by researchers around the world and produced a standardized virulence rank. Clones were pathotyped to compare virulence rank to prototype field strains using the standard pathotyping assay and the results indicated viruses derived from BAC clones encompassed all three virulent pathotypes (vMDV, vvMDV and vv+MDV). Although these clones were found to not be able to replace the current MDV strains used in traditional pathotyping, their full characterization, side-by-side comparison, and broad range of virulence makes them excellent candidates as standardized reagents in most other future and ongoing MDV studies.

Protective efficacy of a BAC clone of a recombinant strain of MDV containing reticuloendotheliosis virus (REV) long terminal repeat (LTR). Vaccination is used worldwide to control MD, but increasingly virulent field strains can overcome this protection, driving a need to create new vaccines. The use of recombinant DNA technology has greatly increased the ability to generate new vaccine candidates. We recently developed a recombinant vaccine candidate by inserting the long terminal repeat (LTR) region of a reticuloendotheliosis virus (REV) into a very virulent MDV strain. This recombinant did not cause disease in susceptible chickens. We then analyzed the ability of the recombinant vaccine candidate to protect against challenge with a very virulent plus MDV strain (vv+MDV) following vaccination *in ovo* at 18 days of embryonation. The passage 70 recombinant vaccine candidate protected the chickens against lymphoid tissue atrophy but did not demonstrate the same level of protection against MD lesions as the most effective commercially available MD vaccine. The recombinant vaccine candidate may be a useful candidate to include in a multivalent vaccine program since it allows for easy manipulation to include genes encoding antigens of other avian pathogens.

Interference among turkey herpesvirus (HVT) vectored vaccines. HVT has been widely used as a vaccine for MD since the 1970s. Because HVT is a safe vaccine that is poorly sensitive to interference from maternally derived antibodies, it has also been developed as a vector vaccine for Infectious bursal disease, Newcastle disease, Infectious laryngotracheitis, and avian influenza. Unfortunately, vaccine companies and producers have found that these HVT vector vaccines interfere with each other when mixed together, reducing the protection against one or more of the vectored diseases. We vaccinated chickens with each of the commercial HVT vector vaccines and found differences in the replication rates among the vaccines. When two of the vector vaccines were administered simultaneously, it was rare to find both vaccines replicating within the birds, instead only one of the two vectors was typically detected. These findings provide a preliminary explanation as to why mixing HVT vector vaccines leads to reduced protection against the vectored diseases.

Avian Influenza/Newcastle Disease Subcommittee Report

David Suarez, DVM, USDA-ARS-SEPRL, Athens, GA

A review of World Organization of Animal Health (OIE), Food and Agriculture Organization (FAO), World Health Organization (WHO), Pro-Med Mail reports, and other sources were reviewed to provide an overview of avian influenza outbreaks of consequence worldwide for the past year.

The goose/Guangdong/96 H5N1 highly pathogenic avian influenza outbreak continues with several notable changes in the past year. The virus remains endemic in China, Vietnam, Indonesia, Egypt, and Bangladesh. The virus has also been reported in poultry from several other countries in Asia including Bhutan, Laos, North Korea, Israel, Palestine, and Iran and wild bird isolates were reported from Kazakhstan and Russia. This lineage of virus continues to antigenically drift, and viruses are characterized up to fourth order clade designations. However, little sequence information is available for these outbreaks, so it is difficult to determine the molecular epidemiology connections between the outbreaks. The most significant new outbreak of H5N1 is in West Africa where Nigeria, Niger, Burkina Faso, Cote D'Ivoire, and Ghana. The outbreak in Nigeria in particular is widespread throughout the country. Controls efforts in Nigeria include indemnity payments to affected farmers, but reports of insufficient payments and other logistical difficulties continue to hamper control efforts. The lack of a strong veterinary surveillance system in any of the countries likely masks the true extent of infection. The FAO has requested international support to help control the outbreaks. Another country with multiple outbreaks was India. India reported three separate outbreaks, in both poultry and wild birds that geographically were widely distributed. Official reports describe the virus as being under control, but under reporting cannot be ruled out.

The H5N8 clade 2.3.4.4 virus that has caused such severe problems in the United States and Canada has also been found in many other countries. The virus has continued to be a problem in South Korea where it was first reported in early 2014 and new outbreaks were just recently reported. Outbreaks in Japan, Netherlands, United Kingdom, Germany, and Italy had reports of wild bird and poultry introductions in November 2014. Later reports from Hungary in Feb 2015 and Romania in wild bird in April 2015 were also reported. The outbreaks did not spread widely and were contained. H5N8 outbreaks and reassorted H5N2 viruses were also reported in Taiwan, where they have continued to cause outbreaks.

The closely related H5N6 2.3.4 viruses have been reported in several countries in 2014-15 including China, Vietnam, and Hong Kong. These viruses share a hemagglutinin gene that is closely related, but distinct from the H5N8 viruses, but it has a similar internal gene cassette. This virus lineage has been reported in poultry and in wild birds. Of some concern is the reports of several human infections with this lineage of virus in China and Hong Kong.

Other HPAI outbreaks have been reported. Taiwan has had a continuation of the Mexican origin H5N2 outbreak and a new H5N3 outbreak in 2015. Germany also reported a H7N7 HPAI outbreak in July 2015 on one farm. This outbreak is presumed to be caused by a low pathogenic avian influenza virus mutating to the highly pathogenic form of the virus. A similar but unrelated H7N7 HPAI outbreak occurred in the United Kingdom in June 2015. Both outbreaks appear to be contained. Mexico continues to suffer with H7N3 HPAI in spite of widespread vaccination. Some official reports from Mexico to OIE of outbreaks in backyard flocks were reported in 2015, but based on unofficial industry results, outbreaks in commercial poultry are common.

Two significant avian influenza zoonotic events occurred in 2015. First, the H5N1 outbreak continues to cause sporadic human cases. However, Egypt reported a large increase in human cases in 2015 with 136 cases and 39 deaths. The reason for this large increase in cases is unclear. The Chinese H7N9 outbreaks continues to follow a predictable pattern with the third wave of human infections occurring in 2014-15 season, and new human cases are already being reported as part of the 4th wave in the 2015-16 season. Currently there are 680 confirmed human cases with 271 deaths, almost all occurring in China. Most human infections are still linked to exposure to poultry in live poultry markets, and the principal control tool is to close markets associated with human deaths. Poultry surveillance continues within China, but with relatively few reported detections of the virus. Because the virus does not cause disease in poultry, little effort is being made to control the infection in poultry. Sporadic human cases of H9N2 continue to be reported, but disease severity in humans remains low. The H9N2 continues to be a major problem for poultry production in the Asia and the Middle East, and vaccination for control is commonly used.

Virulent Newcastle disease virus continues to be a major poultry disease pathogen in Asia, Africa, South America, and Mexico despite the heavy use of vaccination. Previous work has clearly shown that homologous vaccination provides increased protection as measured by levels of viral shedding. However when looking at protection from mortality in the laboratory, the commonly used vaccines, like B1 or LaSota, provide excellent protection. Studies were performed to compare LaSota vaccine and reverse genetics vaccines that are homologous to the challenge virus. In an attempt to show clinical differences in mortality, a high challenge dose

with an early challenge was used. Vaccinated birds are usually challenged 3 weeks after vaccination, but in these studies birds were challenged either 1 or 2 weeks after vaccination. This earlier challenge did create statistically significant differences in mortality with better results seen with the homologous vaccines. It is recommended for better control of NDV that homologous vaccination be used.

National Poultry Improvement Plan 2015 Report

Denise L. Brinson, DVM, USDA-APHIS-VS, Conyers, GA, presented by Patricia Fox, DVM, USDA-APHIS-VS, Raleigh, NC

The National Poultry Improvement Plan is a Federal-State-Industry cooperative program. There are 49 Official State Agencies, one US Territory Official Agency and 98 Authorized Laboratories. Official NPIP disease monitoring classifications include: U.S. Pullorum Typhoid Clean, U.S. *Mycoplasma Gallisepticum* Clean & Monitored, U.S. *Mycoplasma Synoviae* Clean & Monitored, U.S. *Mycoplasma Meleagridis* Clean, U.S. *Salmonella Enteritidis* Clean and Monitored, U.S. Sanitation Monitored, U.S. Salmonella Monitored, U.S. Avian Influenza Clean, U.S. H5/H7 Avian Influenza Clean for poultry breeding flocks, and U.S. H5/H7 Avian Influenza Monitored for commercial (production) poultry flocks.

Pullorum-Typhoid Status: There were no isolations of *Salmonella pullorum* in commercial poultry in FY2011, FY2012, FY2013, FY2014 or FY2015. There were no isolations of *Salmonella pullorum* in backyard birds in FY2013, FY2014 or FY2015. There have been no isolations of *Salmonella gallinarum* since 1987 in any type of poultry in the US. U.S. Pullorum-Typhoid Clean participating hatcheries include: 237 egg and meat-type chicken hatcheries, 45 turkey hatcheries, and 734 waterfowl, exhibition poultry and game bird hatcheries.

U.S. Pullorum-Typhoid Clean Participating Breeding Flocks and Number of Birds are listed below:

- Egg-Type Chickens** - 261 Flocks with 5,617,813 birds
- Meat-Type Chickens** - 6108 Flocks with 129,022,446 birds
- Turkeys** - 562 Flocks with 23,510,786 birds
- Waterfowl, Exhibition Poultry, and Game Birds** - 6,397 Flocks with 2,191,933 birds
- Meat-Type Waterfowl** - 90 Flocks with 161,824 birds

Avian Influenza Status: In FY2015 (July 1, 2014-June 30, 2015), there was one isolation of Low Pathogenicity Avian Influenza in commercial poultry in the US:

H7N3 isolated in a California commercial turkey flock

Table 1: 2015 NPIP U.S. Avian Influenza Clean and U.S. H5/H7 Clean Participating Breeding Flocks; and U.S. H5/H7 Avian Influenza Monitored Participating Commercial Flocks:

Subpart	Flocks	Birds	Tests
Egg-Type Chicken Breeders	280	5,785,681	22,794
Table-Egg Layers-Commercial	6,223	1,035,237,331	144,587
Chicken Breeders	9,082	140,170,728	477,681
Chickens-Commercial	111,282	8,823,120,888	1,403,096
Turkey Breeders	1,107	28,359,997	58,001
Turkeys-Commercial	21,798	259,805,524	214,361
Waterfowl, Upland Game birds, Ex. Poultry	6,487	2,353,757	412,284
Upland Game birds, Waterfowl, Raised for Release Upland Game birds, Raised for Release Waterfowl-Commercial	3,064	45,526,914	40,960

Total	159,323	10,340,360,820	2,773,764
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<i>Mycoplasma gallisepticum</i> , <i>Mycoplasma synoviae</i> , and <i>Mycoplasma meleagridis</i> positive breeding flocks - National Poultry Improvement Plan FY2015				
	WEGBY	Egg-Type	Meat-Type	Turkeys
<i>M. gallisepticum</i>	51	0	13	1
<i>M. synoviae</i>	32	0	43	2
<i>M. meleagridis</i>	0	0	0	0

Authorized Laboratories Activities: The National Veterinary Services Laboratories issue a group D Salmonella check test, Salmonella serotype proficiency check test Mycoplasma serology, and an Avian Influenza check test for the Agar Gel Immunodiffusion test annually for Authorized Labs of the NPIP. Laboratory training provided to the authorized labs included a Salmonella Isolation and Identification Workshops, a Mycoplasma Diagnostic Workshop, and an Avian Influenza Diagnostic Workshop during FY2015.

NVSL Avian Influenza and NDV Diagnostic Report

Dr. Mia Kim Torchetti, USDA-APHIS-VS-NVSL, Ames, IA

NVSL AVIAN INFLUENZA and NEWCASTLE DISEASE ACTIVITIES REPORT – FY 2015

The National Veterinary Services Laboratories (NVSL) in Ames, IA, in coordination with the National Animal Health Laboratory Network (NAHLN), received avian samples for testing of avian influenza (AI) and avian paramyxovirus serotype-1 (APMV-1) in fiscal year (FY) 2015 (10/1/14 to 9/30/15) arising from National Poultry Improvement Plan (NPIP) and Live Bird Market (LBM-BYD) surveillance programs, foreign animal disease (FAD) investigations, import and export activities, wild bird surveillance, and other diagnostics. The majority of the samples are received for confirmation testing, but it is currently not possible to separate confirmations from other testing due to limitations of the laboratory information management system and inconsistent information received on submission forms.

In December 2014, detection of highly pathogenic (HPAI) Eurasian lineage H5 2.3.4.4 influenza viruses associated with a wild bird mortality event and raptor mortality in Whatcom County, Washington marked the beginning of the largest animal health emergency in the U.S. The NAHLN and NVSL played a crucial role in the response effort; NVSL received 1625 outbreak samples for confirmatory and first line testing between 12/08/2014 and 6/17/15 (**Table 1a**: poultry=1065, wild bird=560). Information regarding the response, epidemiology, and virus information can be found at [this link](#).

Assay Updates. Molecular diagnostics for influenza A virus (IAV) used across the National Animal Health Laboratory Network (NAHLN) in the U.S. were confirmed to work well to detect these Eurasian H5Nx viruses. As primary surveillance tools, the NAHLN H5 and H7 assays (both 2008 and 2014 protocols) are designed to capture broad virus diversity and do not distinguish geographic lineage or pathotype. Virus subtype and pathotype can be expedited using other molecular methods such as Sanger sequencing to generate partial HA/NA sequence directly from the sample where sufficient viral RNA is present.

Import and Export Testing. Pet bird and psittacines made up the majority of import testing, while export testing was conducted in petbirds, psittacines, columbiformes, and poultry (~400 tests per year). All import and export samples tested for FY2015 (n= 1721) were negative for AI and ND (**Table 1b**).

Live Bird Marketing System (LBMS), Backyard Birds and Exhibition Birds. As part of the ongoing LBMS surveillance for presence of AI and APMV-1, the NVSL tested 1579 specimens in 398 submissions from 32 states (AL AR AZ CA CT DE FL ID KS LA MA MD ME MN MT NC NE NH NJ NY OH OK OR PA RI TN TX UT VA WA WY) by virus isolation in embryonated chicken eggs and, when appropriate, by real-time RT-PCR (rRT-PCR). All remaining LBMS surveillance specimens were tested at the State level. In FY2015, AIV (n=41) and APMV-1 (n=90) was isolated from specimens tested. For low pathogenic avian influenza a single North American lineage H5N1 LPAI virus from chickens was detected in a NJ live bird market. Other non-H5/H7 AIV are listed by H-type in **Table 2**. Ninety APMV-1 viruses were isolated from 11 states (CT DE FL MA MD NE NJ NY PA RI SC). Pathogenicity of representative APMV-1 isolates obtained from birds was determined by the intracerebral

pathogenicity index (ICPI) test and/or by analysis of the deduced amino acid profile at the fusion protein cleavage site. All were characterized as low virulent (lentogenic pathotype) strains.

Commercial Poultry. Surveillance for AI in commercial poultry is conducted under provisions of the National H5 and H7 Low Pathogenicity Avian Influenza Control Program implemented in September, 2006. The majority of this testing is performed at the state level; the NVSL provides reagents for the agar gel immunodiffusion (AGID) test and controls for the rRT-PCR test in addition to confirmation and characterization of positive specimens. For commercial poultry during FY2015, one detection of North American lineage H7N3 LPAI of wild bird origin (CA: turkey) was reported to the World Organisation for Animal Health (OIE). Other AIV isolated are listed by H-type in **Table 2**.

AI Antibody Subtyping. The NVSL received 299 submissions (1973 sera) for AI antibody confirmation and subtyping in FY2015 from 28 states predominantly from chickens and turkeys. Antibodies to influenza H1 and/or H3, with N1 and/or N2 antibodies were detected predominantly in turkey samples (97%) where vaccination is common; over two thirds of samples were from OH with sporadic detections from 10 other states (AZ CA GA IA MN MO NC PA OR SD). Antibody was also detected as follows: H4 (MN and KS: turkey – serologic only), H5N2 (MI: turkey); H7N9 (KS: turkey serologic only), H10N2 (TN: chickens, serologic only).

Surveillance in Wild Waterfowl. The Eurasian H5 clade 2.3.4.4 events of late 2014 prompted enhanced active surveillance from late December 2014 through June 2015 in the Pacific flyway; results for this effort are listed [here](#). An updated strategy and new Wild Bird Surveillance program was initiated on 1 July 2015 (refer to this [link](#) for the surveillance plan, and this [link](#) for detections from July 2015); with the 2014 updates to the H5/H7 molecular assays, NAHLN laboratories participating in the wild bird surveillance testing forward only H5/H7 suspects to NVSL and other influenza A positive samples are forwarded to the NAHLN laboratory at Colorado State University for the Wildlife Services repository. Other wild bird efforts such as routine mortality event testing, and characterization of H5/H7 viruses submitted by university and independent researchers was conducted. In FY2015, 977 wild bird specimens were received at NVSL from all efforts. The Eurasian H5 clade 2.3.4.4 findings are listed at links above; non-HPAI subtypes (n=260) are listed in **Table 3**.

Avian paramyxovirus serotype-1 (APMV-1). In FY2015 a total of 179 APMV-1 viruses were isolated from 23 states (AK AL CA CO CT DE FL IA ID MA MD MI MN NC NE NJ NY OR PA RI SC UT WA; includes the 90 LBM isolates mentioned above). Pathogenicity of representative APMV-1 isolates obtained from birds was determined by the intracerebral pathogenicity index (ICPI) test and/or by analysis of the deduced amino acid profile at the fusion protein cleavage site. There were no virulent viruses (vNDV) isolated in FY2015. Of the 179 isolates, 152 were characterized as low virulent NDV (loNDV) and 27 were identified as pigeon paramyxovirus type-1 (PPMV-1) from falcons, dove, racing and other pigeons in 9 states (AZ CA ID MN NY OR PA UT WY). PPMV-1 isolates were identified by the HI test with monoclonal antibodies specific for PPMV-1 and sequence analysis of fusion protein cleavage site.

Proficiency Test Panels. For AGID, 112 laboratories were invited to participate in the voluntary proficiency test (PT); 92 panels were shipped (including Chile (1) and El Salvador (2), Canada (2) and Japan (1)). A total of 68 laboratories from 39 states passed with a score of 90% or better. The NAHLN laboratories conducting surveillance testing for AI and/or ND are required to have one or more diagnosticians pass an annual PT to perform official rRT-PCR testing. In FY 2015, AI (matrix/H5/H7) PTs were distributed for 285 diagnosticians in 58 laboratories and for 250 diagnosticians in 56 laboratories for APMV-1 (Newcastle disease) rRT-PCR. Results for the 2014 international OFFLU AI Ring Trial, (coordinated, prepared, and shipped by the NVSL with assistance from the Frederick Loeffler Institute) were reported to the participating laboratories, OIE, FAO, and OFFLU organizations. The panels included 15 samples and participants conducted influenza A, H5 and H7 subtyping rRT-PCR, as well as sequence analysis for molecular pathotyping. Participants represented 20 labs from different countries, including 9 OIE/FAO Reference Centers and 11 Regional Laboratories. While the majority of labs accurately detected influenza A, subtyping by PCR continues to be challenging and demonstrates the difficulty capturing the diversity present in the H5 and H7 subtypes. Accurate detection of viruses from opposite hemispheres using a single assay presents the greatest challenge.

AI Diagnostic Reagents Supplied by the NVSL. The following reagents were distributed for rRT-PCR testing and support of NPIP and LBM surveillance during FY 2015:

AGID Diagnostic Reagents:

- 12,124 units of AGID reagents (antigen and enhancement serum) were shipped to 67 state, university, and private laboratories in 36 states sufficient for approximately 1,454,880 AGID tests
- An additional 1005 units (120,600 tests) were shipped to 16 international laboratories (13 countries)

AIV Diagnostic Reagents:

AIV rRT-PCR Controls

- 101 vials of positive amplification control (M, H5 & H7) 20 states; 10 internationally to 1 country
- 508 vials of positive extraction control 39 states; 2 internationally to 1 country
- 572 vials of negative extraction control 39 states; 8 internationally (3 countries)

APMV-1 Diagnostic Reagents:

LaSota Antigen (inactivated)

- 80 vials (2 ml) to 9 national and 28 vials to 4 international labs

APMV-1 Antiserum

- 14 vials (2 ml) to 5 national and 94 vials to 7 international labs

APMV-1 rRT-PCR Controls

- 13 vials of positive amplification control to 9 states; 10 vials internationally (3 countries)
- 94 vials of positive extraction control to 20 states; 8 vials internationally (4 countries)

Table 1a. Samples NVSL received for outbreak testing during the 2014-2015 Eurasian H5 events by purpose.

Month	BACKYARD	COMMERCIAL	WILD BIRD	Grand Total
December-14	10	0	42	52
January-15	139	2	335	476
February-15	7	1	37	45
March-15	9	24	49	82
April-15	14	367	52	433
May-15	84	355	27	466
June-15	28	25	18	71
Grand Total	291 (56% H5+)	774 (65% H5+)	560 (30% H5+)	1625 (51% H5+)

Table 1b. Samples received for avian influenza and Newcastle disease testing during FY2013-15 by purpose.

	FY2013	FY2014	FY2015
IMPORT	4944	1562	1309
EXPORT	378	519	412
LBM-BYD	649	658	1288
COMMERCIAL	266	283	3489

Table 2. FY2015 AIV isolates from LBM, backyard, and commercial submissions by state and H-type.

Purpose	Subtype	Source	State
LBM/ backyard	H1N1	Duck, swan	NJ NC
	H2N2	Guinea, turkey, duck, chicken, quail	CT NC NY PA RI
	H3N9	Duck, goose	PA
	H11N9	Duck, goose	PA
	H5N1 LPAI	Chicken	NJ
Other Commercial	H1N2	Turkey	IA
	H3N2	Quail	CA
	H7N3 LPAI	Turkey	CA

Table 3. Influenza A isolates from wild birds by district (specific states where samples collected are listed) and by H and N-type with predominant N-type underscored (n=260; collection dates range from 2012-15). Samples are not representative of all Districts.

USDA DISTRICT	H1 (N1, N3, N9)	H3 (N1, N6, N8)	H4 (N1, N2, N4, N6, N8, 9)	H5 LP AI (N2, N9)	H6 (N1, N8)	H7 LP AI (N1, N2, N3, N4, N7, N9)	H9N2	H10 (N3, N7, N8)	H11 (N2, N3, N9)	H16N3	mixed	Total
1 (CT, MA, NH, NJ, NY, PA)	7	2	8	0	0	88	0	1	0	1	0	107
2 (GA)			1									1
3 (IL, MI, MN, OH)	0	0	5	2	1	1	0	1	3	0	0	13
4 (LA, OK, TX)	0	0	0	2	0	29	0	3	0	0	0	34
5 (ID, KS, MT)	1	6	8	4	0	6	0	0	4	0	1	30
6 (AK, CA, CO, NM, NV, OR, UT, WA)	9	5	14	0	1	23	1	13	6	0	3	75
TOTAL	17	13	36	8	2	147*	1	18	13	1	4	260

* 63% HA gene molecular confirmation only

Poultry *Salmonella*, *Mycoplasma*, and *Pasteurella* Diagnostics at NVSL

Brenda Morningstar-Shaw, USDA-NVSL-Diagnostic Bacteriology Laboratory, Ames, IA

Salmonella serotyping

The Diagnostic Bacteriology Laboratory within the National Veterinary Services Laboratories (NVSL) routinely serotypes *Salmonella* isolates submitted by private, state, and federal laboratories as well as veterinarians, researchers and other animal health officials. This report summarizes *Salmonella* serotyping submissions to NVSL from January 1 through December 31, 2014 originating from poultry. *Salmonella* isolates are identified as clinical (clinical signs of salmonellosis from primary or secondary infection) or non-clinical (herd and flock monitoring programs, environmental sources, food). Serotyping data from isolates submitted for research purposes are not included in the summary.

Salmonella serotyping at the NVSL is an ISO 17025 accredited test. Salmonellae are typed using polyvalent and single factor antisera to determine the O and H antigens. Approximately 60% of the sera used at the NVSL are produced in house as previously described. (Ewing) The remaining antisera are purchased from commercial vendors. All sera are subject to extensive quality control testing prior to use. *Salmonella* antigenic formulae are determined as previously described (Ewing) and interpreted via the White-Kauffmann-Le Minor scheme (Grimont). The subspecies designation precedes the antigenic formula for those serotypes other than subspecies I.

From January 1 to December 31, 2014 there were 4,688 isolates from chicken sources and 1,188 isolates from turkey sources submitted to the NVSL for *Salmonella* serotyping. The most common isolates from chickens and turkeys are listed in Tables 1 and 2 respectively.

The NVSL provided a *Salmonella* Group D proficiency test to assess the ability of laboratories to isolate *Salmonella* from environmental samples and determine the serogroup (specifically group D) of any *Salmonella* isolated. The test consisted of 10 lyophilized cultures containing various combinations of *Salmonella* and common contaminants that simulated an environmental swab. The 2014 test included *Salmonella* serotypes Enteritidis, Javiana, Anatum, Oranienburg, Heidelberg, and an *sdF* negative Enteritidis. Contaminant bacteria included *Enterobacter cloacae*, *Citrobacter sedlakii*, *Citrobacter amalonaticus*, *Citrobacter freundii*., *Pseudomonas aeruginosa*., and *Providencia rettgeri*.. Laboratories were instructed to test the samples according to the procedures used in their laboratories. The NVSL randomly retained 11% of the test kits and tested them blindly for QA purposes. The results of the proficiency test are shown in Table 3.

Additionally, the NVSL offered a *Salmonella* serotyping proficiency test to allow laboratories to assess their ability to serogroup or serotype *Salmonella*. The panel consisted of 10 pure *Salmonella* isolates, including

Salmonella serotypes Berta, Saint Paul, Montevideo, Pensacola, Idikan, Essen, Liverpool, Fresno, Lille, and Enteritidis. Participants were given the option to perform serogrouping, partial serotyping, or full serotyping of the isolates and were graded based on appropriate identification to the level of typing they performed. The NVSL randomly retained 15% of the test kits and tested them blindly for QA purposes. The results of the proficiency test are shown in Table 4.

Table 1: Most common serotypes in 2014: Chicken

Clinical		Non-Clinical	
Serotype	No. Isolates	Serotype	No. Isolates
Enteritidis	86	Senftenberg	1106
Kentucky	30	Mbandaka	473
Infantis	13	Kentucky	450
Typhimurium	11	Enteritidis	291
Senftenberg	9	Typhimurium	93
All others	71	All others	2055
Total	220	Total	4468

Table 2: Most common serotypes in 2014: Turkeys

Clinical		Non-Clinical	
Serotype	No. Isolates	Serotype	No. Isolates
Senftenberg	87	Senftenberg	271
Heidelberg	37	Anatum	96
Albany	29	Hadar	93
Ouakam	22	Muenster	74
Montevideo	16	Agona	52
All others	114	All others	247
Total	305	Total	833

Table 3: Summary of NVSL *Salmonella* Group D proficiency test

	2010	2011	2012	2013	2014
Participants	55	70	73	61	80
Mean Score	92%	97%	92%	94%	98%
Score Range	100-44%	100-85%	100%-29%	100-68%	100-80%
Below Passing	3	0	N/A*	N/A**	0

Because of the change in grading method, a pass/fail designation was not assigned.

*2012 Seven individuals scored less than 80%

**2013 Four laboratories scored less than 80%

Table 4: Summary of NVSL *Salmonella* Serotyping proficiency test

	Serogrouping 2012	Serotyping 2012	Serogrouping 2013	Serotyping 2013	Serogrouping 2014	Serotyping 2014
Participants	22	13	18	14	34	23
Mean Score	98%	92%	98%	98.50%	99%	95%
Score Range	100-90%	100-70%	100-90%	100-90%	100-80%	100-80%

Salmonella Enteritidis

The number of *Salmonella* Enteritidis (SE) isolates submitted from chickens in 2014 is shown in Table 5. The most common SE phage types are shown in Table 6.

Table 5: Number of chickens *Salmonella* Enteritidis isolates per calendar year at the NVSL

	2010	2011	2012	2013	2014
No. chicken isolates	4987	3940	3502	3912	4688
No. chicken SE isolates	1500	776	507	400	377
SE percent of all isolates	30.1%	19.7%	14.5%	10.2%	8.4%

Table 6: Most common *Salmonella* Enteritidis phage types from chicken sources per calendar year

Rank	2010	2011	2012	2013	2014
1	8	8	8	8	8
2	13	13a	13	13	RDNC
3	13a	13	RDNC	13a	2
4	RDNC	RDNC	13a	RDNC	13a
5	23	23	23	23	13

RDNC = reacts, does not conform

Salmonella Pullorum and Gallinarum

The NVSL provided 2,570 ml of *S. Pullorum* tube antigen, 158 ml of *S. Pullorum* stained microtiter antigen, and 478 ml of antisera to testing laboratories between January 1 and December 31, 2014. The NVSL conducted 437 *S. Pullorum* microtiter tests in 2014. The NVSL did not identify any *Salmonella Pullorum* isolates in 2014.

Pasteurella and Mycoplasma

The NVSL received 128 isolates for somatic typing in 2014. The NVSL also supplied 106 ml of *P. multocida* typing sera. The amount of *Mycoplasma* reagents provided are shown in Tables 7 and 8.

Table 6: *Pasteurella multocida* somatic typing. Table shows number of isolates per fiscal year for each type.

	2010	2011	2012	2013	2014
Type 3	38	25	38	28	18
Type 3,4	27	12	33	17	36
Type 1	25	17	10	10	10
All other	70	52	100	90	62
TOTAL	160	106	181	145	126

Table 7: *Mycoplasma* antisera (ml) provided by NVSL per fiscal year

Antisera	2010	2011	2012	2013	2014
<i>M. gallisepticum</i>	256	306	274	532	246
<i>M. meleagridis</i>	32	54	40	108	34
<i>M. synoviae</i>	256	326	342	672	212
Negative	222	150	175	344	156
Total	766	836	831	1656	648

Table 8: *Mycoplasma* antigen (ml) provided by NVSL per fiscal year

Antigen	2010	2011	2012	2013	2014
<i>M. gallisepticum</i>	150	195	175	245	170
<i>M. meleagridis</i>	75	95	80	40	85
<i>M. synoviae</i>	215	220	245	290	230
Total	440	510	500	555	485

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Committee on Salmonella Report

Doug Waltman, PhD, Georgia Poultry Laboratory, Oakwood, GA

The Committee on Salmonella met on Tuesday, October 27, 2015 and heard updates and research findings from a series of speakers. Dr. Megin Nichols, the Enteric Zoonoses Activity Lead for the Outbreak Response and Prevention Branch of the Division of Foodborne, Waterborne and Environmental Diseases at CDC, spoke on “2015 Enteric Zoonoses Outbreaks: Public Health Impacts and Challenges. She overviewed several outbreaks that have occurred in 2015, and then focused on the pork outbreak in Washington.

Dr. Karen Becker, the Director of the Applied Epidemiology Staff within FSIS’s Office of Public Health Science, spoke on “An FSIS Update on Policy and Action to Prevent and Control Foodborne Disease Associated with *Salmonella*. She first added additional processing plant information to the Pork outbreak that was introduced by Dr. Nichols. She then shared an update on FSIS activities on controlling *Salmonella* including their standards, including upcoming directions.

Brenda Morningstar-Shaw, went over *Salmonella* serotyping results from the NVSL Diagnostic Bacteriology Laboratory with her presentation of the annual NVSL *Salmonella* Report.

Dr. June deGraft Hanson, a member of the Office of Food Safety, Division of Dairy, Egg, and Meat Products of FDA, spoke on “the Egg Safety Rule : Progress and Update”. She overviewed the Egg Rule and then provided the results of the program activities and testing.

Dr. Jean Guard of the U.S. National Poultry Research Center presented “An Approach to Serotyping *Salmonella enterica* that Facilitates Independent Analysis of Farm Ecology by Producers”. She shared her work on and research with intergenic sequence ribotyping (ISR) for serotyping *Salmonella*. And finally Dr. Doug Waltman gave a presentation titled “Just when you think you have *Salmonella* figured out ...” His presentation reviewed a retrospective study of the *Salmonella* isolated from individual houses from flocks on farms from one breeding company.

Dr. Doug Waltman and Dr. Richard Sellers, chair and vice-chair, respectfully, are rolling off of the Committee on Salmonella. Dr. Donna Kelley of the University of Pennsylvania has volunteered and has been recommended to the Executive Committee to take the Committee Chair.