**SAES-422 Multistate Research Activity Accomplishments Report**

**Project/Activity Number:** NC1180
**Project/Activity Title:** Control of Emerging and Re-emerging Poultry Respiratory Diseases in the United States
**Period Covered:** October 1st- September 30th, 2017
**Date of This Report:** December 8, 2017
**Annual Meeting Date:** October 19, 2017

**Brief summary of minutes of annual meeting**:

The annual meeting was held on Thursday, October 19, 2017, in conjunction with the USAHA annual meeting at the Town and Country Hotel, San Diego, CA, Royal Pal Salons 1-2.

Due to scheduling issue, the chair, Dr. Mary Pantin-Jackwood, was unable to attend the meeting. Dr. David Suarez chaired the meeting. He opened the meeting at 8:10 am and welcomed the station representatives and participating scientists. The list below shows the attendees.

| Name | Affiliation | Representing state |
|---|---|---|
| Mazhar Khan | University of Connecticut (UC) | CT-Rep. |
| Chang Won Lee | The Ohio State University (OSU) | OH-Rep. |
| David Suarez | Southeast Poultry Research Laboratory-USDA | Rep. |
| Haroldo Toro | Auburn University (AU) | AL-Rep |
| Rodrigo Gallardo | University of California, Davis (UC-Davis) | CA-Rep. |
| Timothy Johnson | University of Minnesota MN (UM) | MN-Rep. |
| Calvin Keeler | University of Delaware (UD) | DE-Rep. |
| Maricarmen Garcia | University of Georgia (UGA) | GA-Rep. |
| Keith Jarosinski | University of Illinois (UI) | IL-Rep. |
| Donald Reynolds | University of Nebraska (UN) | NE-Rep. |
| Robert Smith | NIFA | |
| Eric Gingerich | Diamond V | |
| Michael Abundo | OSU | OH |
| John Ngunjiri | OSU | OH |
| Mohamed El Gazzar | OSU | OH |
| Yehia Saif | OSU | OH |

The meeting minutes of the 2016 NC1180 meeting were approved by the participating station representatives and members.

Dr. Timothy Johnson was elected as new Chair and Dr. Rodrigo A. Gallardo as new Secretary. Dr. Suarez nominated Dr. Johnson to be the new chair of the committee seconded by Dr. Garcia, all voted and agreed. Dr. Suarez nominated Dr. Gallardo to be the new secretary of the committee seconded by Dr. Lee, all voted and agreed.

The meeting venue and time for annual NC1180 meeting in 2018 was discussed. Dr. Toro suggested bringing the meetings to the Universities, due to cost. Dr. Saif commented that travel would be an issue if we change to universities; historically it has been a meeting held in a central U.S. Dr. Lee suggested that NC1180 is linked to PRD-CAP and the reason why the attendance is good is because of PRD-CAP. Until PRD-CAP finishes he thinks is better to link the meeting to USAHA. Dr. Keeler suggested staying with USAHA until PRD CAP finishes, then the idea would be revisited. Dr. Saif also mentioned that the meeting was also associated with the PAC meeting, but the problem was that there is a very short time to turn in the report. The decision for 2018 was to stay in the current format linked to PRD-CAP and associated with the USAHA meeting. In 2018, the USAHA meeting will be held in Kansas City. The dates would be October 24th and 25th for PRD CAP and NC-1180 respectively.

Dr. Lee commented about the duration of the meeting. He suggested two possibilities: start early and finish at noon, or start early and finish at two with lunch and discussion. Dr. Suarez suggested doing it until 2pm this year and evaluating how it goes. Dr. Suarez suggested inviting more researchers; guests from industry are fine. Indiana representative was not present due to injury. We don't have Rep from Ark anymore. We will continue to encourage Dr. Banda from Mississippi to join the meeting. Penn State hired a new extension veterinarian Dr. Gino Lorenzoni. Dr. Lee will reach out to him so he can join the meeting. The group was encouraged to recruit more people.

Business meeting adjourned 9:15am.

The presentation of annual progress report from each participating station began immediately after the business meeting. All members were actively engaged in the discussions on surveillance, pathogenesis, new diagnostics tools, and vaccine/immunology of various poultry respiratory and immunosuppressive diseases. Research findings and new knowledge were freely communicated among the participating members.

The meeting adjourned at 2:00 pm.

**Accomplishments:**

**Objective I: Identify reservoirs of infectious respiratory disease agents in wild birds and poultry.**

- Low pathogenic avian influenza virus (LPAIV) is not viable in dry contaminated feed or distilled or chlorinated city water drinking water, and therefore does not represent a significant method in which AIV can be transmitted. In contrast, darkling beetles can harbor the virus and could serve as vector for the spread of AIV (AU).

- The potential of highly pathogenic (HP)AI (H5N8) to persist for an extended time in poultry bedding (60 hours post spiking in some cases) was confirmed. On the other hand, LPAIV was able to persist only up to 24 hours in broiler bedding with one cycle of usage. This study reflects the importance of rapid depopulation of flocks infected with HPAI viruses to cut the replication cycle of the virus (UC-Davis).

- Avian influenza was not detected in poultry, commercial or non-commercial, or exhibition/show birds in Delaware in 2017. During the winter of 2016/2017, eight LPAIV H5 and one LPAIV H7 virus were detected in wild waterfowl. As in 2015 - 16, the winter of 2016-17 was mild. Nonetheless, a high rate of IBV detection was observed (UD).

- Although house finches are frequently infected by *M. gallisepticum*, one of the causative agents of chronic respiratory disease, it is unclear whether there may be spill-back from these wild birds to backyard chickens. Although *M. gallisepticum* is common among backyard chickens, finches are unlikely to be playing a major role in that exposure (UI).

- Reducing inflammatory processes in house finches limits both pathology and behavioral changes during *M. gallisepticum* infection without changing the amount of pathogen present. This helps us better understand why, mechanistically, individuals differ in their responses to infection (UI).

- Deep sequencing of H7N8 AIV from surveillance zone supports H7N8 HPAI was limited to a single outbreak farm in Indiana during the 2016 HPAI outbreak in turkeys. Genetic testing conducted determined the mild and deadly forms of H7N8 influenza were closely related, and the deadly form likely arose from the mild form on a single farm (SEPRL).

- Novel reassortant AIV (H5N8) was detected in wild aquatic birds, Russia in 2016. Sequence analysis showed the viruses were related to poultry outbreaks in the region and that this area comprises important wild aquatic bird migration routes. The re-emergence of a novel virus in wild birds is of concern for dissemination of novel reassortant virus during the coming fall migration (SEPRL).

- Reoccurrence of a H5N2 HPAIV was found in a wild mallard in Alaska, 2016. This indicates the virus has not disappeared and raises concern that the virus might return to the lower 48 states during the fall wild waterfowl migration (SEPRL).

- Newcastle Disease (ND) vaccine viruses may co-circulate between synatropic birds and poultry in North America and across the globe. These results and the ubiquitous nature of wild pigeons highlighted their potential role as indicator species for the presence of Newcastle disease virus of low virulence in the environment (SEPRL).

- Identification of previously unrecognized genetic diversity in avian paramyxovirus serotype 1 (APMV-1) of low virulence isolated from wild birds. Because more recent viruses were related to viruses circulating in North America during previous years, it was determined that diversity is likely a function of continued viral evolution in reservoir hosts (SEPRL).

- Highly virulent NDV were found in poultry and captive non-poultry avian species in Pakistan from 2011 to 2016 demonstrating that virulent viruses of the sub-genotype VIIi in vaccinated poultry are epidemiologically linked to viruses present in pets, backyard chickens and wild birds. (SEPRL).

- Birds infected with NDV were often co-infected with H9N2 low pathogenic avian influenza in Pakistan. As part of the same objective we identified and characterized new avian paramyxoviruses.

The awareness of mixed infection with multiple agents and the NDV variants will lead to more specific diagnostics and control strategies (SEPRL).

**Objective II. Develop improved diagnostic capabilities including real time PCR as well as other rapid on-farm tests for economically important respiratory diseases**.

- Methods have been developed to characterize the avian respiratory viral microbiome from tracheal samples. Both DNA viruses (herpesvirus, gyrovirus, adenovirus) and RNA viruses (coronavirus, paramyxovirus) can be identified. An assay detecting the GA08 and GA type "Folds" vaccine strains has been developed and employed (UD).

- Specific tests were developed for the Mass, Ark, DE, GA98, GA07 and GA08 genetic types of infectious bronchitis virus (IBV). Previously IBV typing was done by RT-PCR amplification of the spike gene followed by sequence analysis. At best 3 days were required unless samples needed to be propagated in embryonated eggs. Rapid identification of IBV types is important for selection of vaccines because different types do not cross protect. The test described can be done directly on clinical samples making it possible to identify IBV type in just a few hours (UGA).

- A simplified, sequence-independent single-primer amplification (SISPA) technique in combination with the MiSeq Platform to target viral genome sequences belonging to different virus families in diagnostic samples was developed. This method allowed the successful assembly of sequences into full or near full length avian influenza virus, infectious bronchitis virus, and NDV viral genomes. This application can be adaptable to other RNA viruses due to non-specific nature of the amplification technique (SEPRL).

- To address the need for rapid identification assays for variant NDV strains, a technique that allows ultra-deep sequencing of nucleic acids without using target-specific primers was developed. The method is robust and cost-effective allowing detection of unsuspected variants and co-infecting agents (SEPRL).

**Objective III. Investigate the pathogenesis and polymicrobial interactions of specific infectious agents associated with poultry respiratory diseases (this includes interactions with underlying immunosuppressive agents).**

- Genetic resistance or susceptibility to infectious diseases has been largely associated with the avian major histocompatibility complex (MHC) genes. A congenic genetic chicken line was identified as the most resistant and one as the most susceptible to disease caused by IBV. Understanding the effects of the MHC and innate immunity on infectious bronchitis virus will provide substrate for investigation in the control of this endemic pathogen (UC-Davis).

- A IBV-like strain of coronavirus detected in chickens with runting stunting syndrome, identified as Cal ent, did not increase enteric signs or pathology in an experimental setting but showed higher viral load in the small intestines and cloacal swabs compared to IBV. Cal ent is a virus close to CA variants and Ark vaccine, suggesting that is a mutant of field strains and Ark vaccine.

Understanding the IBV virus dynamics in order to plan preventative strategies accordingly (UC-Davis).

- During 2017 an increase in the amount of Coryza cases and their severity has been noticed in the State of California. Three strains of *Avibacterium paragallinarum* were sequenced by NGS to determine if the latest outbreaks were caused by variants types of the bacteria. Homologies of 100% were encountered when the isolated sequences were compared with H18 and Modesto reference isolates. These two strains are part of the inactivated vaccines used in the field (UC-Davis).

- Current vaccines against MD are relatively effective at limiting the development of lesions in affected birds, but do not block transmission of MDV and constant evolution of the virus. Two MDV-encoded viral genes, namely glycoprotein C (gC) and the conserved herpesvirus protein kinase (CHPK), were shown to be essential for transmission of MDV. Identification of MDV genes essential for transmission could have major implications in the design of vaccines that could target MDV spread in a chicken house (UI).

- Chickens were experimentally infected with infectious burdal disease virus (IBDV) variant (T1) and LPAIV (H5N2) one week after T1 infection. T1 infection induced severe bursal damage. When coinfection group (IBDV+LPAIV) were compared to the LPAI virus alone infected group, higher titers of influenza virus were shed and antibody titers were observed. These studies will help better define the role of immune suppression on respiratory disease in chickens (OSU).

- Turkeys are in general highly susceptible to waterfowl-origin influenza viruses. In addition, interspecies transmission of swine influenza viruses to turkeys occurs frequently. Furthermore, reverse zoonoses of human-to-turkey transmission had been documented with several incidences of pandemic H1N1 infection in turkeys. Recent swine influenza viruses showed limited ability to replicate and transmit in turkeys. However, infection with recent turkey-origin swine influenza viruses resulted in significant loss of egg production without any other clinical signs or sustained virus replication and shedding. Furthermore, recent seasonal human H1N1 and H3N2 viruses showed the potential to replicate and transmit in turkeys (OSU).

- The pathogenesis of the H7N8 low and highly pathogenic AIV from the Indiana 2016 outbreak varies between chickens, turkeys and mallards. The more pathogenic form of the virus did not cause disease or death in the ducks like in the chickens and turkeys, meaning ducks could easily carry the virulent form for poultry while remaining healthy themselves. This information is critical in understanding the epidemiology of avian influenza and its control (SEPRL).

- H5N2 HPAI viruses from the United States 2014-2015 outbreak have an unusually long pre-clinical period in turkeys. These results suggest that virus spread during the outbreaks may have been enhanced because turkeys were infected but not showing clinical disease, which would allow a longer period of time before quarantine measures would have been taken facilitating spread (SEPRL).

- The H5 HPAI viruses from the 2014-15 outbreak in the U.S. infected domestic ducks and geese and easily transmitted to contact birds, with geese being more susceptible to infection and disease than ducks. Most of the birds did not show clinical signs but excreted virus for several days,

representing a risk to other poultry species. These findings emphasize the need to improve active surveillance in domestic waterfowl and increase biosecurity to reduce contact between poultry and wild waterfowl in order to prevent and control avian influenza in poultry (SEPRL).

- Historically, surveillance and research efforts for the AIV in waterfowl have focused on dabbling ducks, but the role of diving ducks in avian influenza virus ecology has not been well characterized. Similar to dabbling ducks, diving ducks (Lesser Scaups and Ruddy Ducks) are susceptible to infection with H5 highly pathogenic avian influenza virus lineage (SEPRL).

- Wild aquatic birds have been associated with the intercontinental spread of Asian H5 subtype HPAIV's, but dispersion by wild waterfowl has not been implicated with spread of other highly pathogenic viruses. Mallards inoculated with one of fourteen different H5 and HPAIV's from previous outbreaks in poultry, and contact exposed ducks, became infected, with the exception with two H5 viruses. Clinical signs were only observed in ducks infected with three of the viruses. This study highlights the possible role of wild waterfowl in the spread of any HPAIV (SEPRL).

- Changes in adaptation of H5N2 highly pathogenic avian influenza H5 viruses were demonstrated in chickens and mallards. Increased virus adaptation to chickens was observed with poultry H5N2 viruses; however, these viruses still easily infected mallards. Genetic changes in the viruses were also determined, which helps in understanding how avian influenza viruses change to better infect and replicate in poultry species (SEPRL).

- To assess the impact of genetic resistance of broilers and/or any age-related effects, the ability of a H5N2 HPAI virus to infect and cause disease in commercial 5-week-old, 8-week-old, and adult broilers was investigated. Age was not a determinant factor in susceptibility of broilers for H5N2 highly pathogenic avian influenza virus. This apparent lower susceptibility of broilers compared to layers to H5N2 may have accounted, at least partially, for the lack of affected broiler farms in the midwestern U.S. outbreaks (SEPRL).

- Recombination is a feature of many alphaherpesviruses including ILTV. Natural (field) ILTV recombination is widespread, including recombination between attenuated ILTV vaccine strains to create virulent viruses. These virulent recombinants have had a major impact on animal health. In the laboratory, ILTV replication and recombination were closely related, and that the recombinant viral progeny are most diverse four days after co-inoculation, at the peak of viral replication. These studies bring new insights into alphaherpesvirus recombination that can be used to inform the future development and utilization of vaccines (SEPRL).

**Objective IV. Develop new prevention and control strategies for poultry respiratory diseases.**

- Protection conferred by increasing doses of kidney-cell-adapted IBV ArkDPI-derived vaccine administered at 1 day of age showed effective protection against Ark virulent challenge. The CEK-adapted IBV ArkDPI vaccine offers improvement and refinement of current ArkDPI-derived vaccines by both eliminating emergence of vaccine-like viruses after vaccination and eliminating emergence of novel strains originating from Ark challenge (AU)

- Commercial ArkDPI-derived infectious bronchitis virus (IBV) live-attenuated vaccines contain viral subpopulations similar to virulent ArkDPI that are rapidly selected in chickens and persist in vaccinated chickens. The reduced replication of CEK-adapted Ark vaccine in chickens could be beneficial as it would reduce adverse reactions. Protection trials show that replication levels of the CEK-adapted Ark vaccine are sufficient to induce protective immunity (AU).

- The protection conferred by IBV S-ectodomain vaccination delivered via a mucosal route using chitosan nanoparticle technology was evaluated. Unexpectedly, chickens vaccinated with recombinant S-ectodomain protein loaded chitosan nanoparticles did not show statistically significant reductions of viral RNA in either tears or tracheas compared to controls vaccinated with control chitosan nanoparticles (AU).

- Because use of a recombinant S ectodomain protein representing the ArkDPI vaccine subpopulation previously designated C2 conferred effective protection against challenge, a recombinant LaSota virus expressing a codon-optimized gene expressing this protein was produced. The rescued rLS/IBV-rS virus has been confirmed by the HA test and sequencing analysis (AU).

- IBV S2 expressed from recombinant virus was used to confer protection across serotypes in Chickens. The study showed that cross protection cannot be attributed to the S2 portion of the spike (S) IBV protein (AU).

- Studies characterizing the receptor-binding domain of Ark-type IBV S1 protein confirmed that the S1 NTD (aa 19-258) of Ark serotype (ArkDPI vaccine subpopulation selected in chickens) protein comprises the RBD, is necessary and sufficient for binding to chicken tissues, including conjunctiva, nasal mucosa, nasolacrimal gland, lacrimal duct, choana, trachea, cecal tonsil, cloaca, and kidney. Understanding the interaction of the IBV S protein with host receptors will be useful for developing strategies to interfere with this interaction and thus inhibit infection (AU).

- Results of S1 IBV tissue binding assays did not demonstrate differences in binding to chicken tissues among the ectodomains representing selected vaccine subpopulations and those representing the vaccine major population. While increased binding of S1 may contribute to selection of some ArkDPI IBV vaccine subpopulations in chickens, mechanisms other than increased binding may play a predominant role in selection of other vaccine subpopulations (AU).

- Two recombinant ILT vaccines have been developed. Field and laboratory data have demonstrated that both LT recombinant vaccines provided only partial protection in older aged broilers and do not reduce the replication of the field virus, thus allowing for virus shedding, and transmission (AU).

- A transgenic plant (*Arabidopsis thaliana)* expressing the hemagglutinin (HA) gene of a LPAIV H1N1 subtype was constructed and immune response evaluated in chickens. The study demonstrated that chickens given high or low doses of the HA transgenic soluble plant protein had a higher HI antibody, enhanced cytokine responses, and higher body weight gain after challenge compared to chickens given a commercial inactivated vaccine. Plant-based vaccines can be produced in mass at a quicker rate and more economically than conventional vaccines and can be

given in the drinking water. The TPV developed has the potential for further development into a commercial vaccine against AIV (AU).

- In a study done to test DE as adjuvant in an ArkDPI live IBV (IBV) vaccine after ocular or spray application showed that DE had no detrimental effect on the vaccine virus. However, no improvements on vaccine results were observed when using DE (UC-Davis).

- One poultry producer is continuing to successfully utilize a vaccine developed under the regulation 9CFR PART 107.7(b) to control an avian health issue using a homologous genotype IBV variant vaccine that was not available from biologics manufacturers. No adverse reactions have been reported by any company growing chickens in Delaware, Maryland and/or Virginia. Protection against variant IBV PA/1220/98 and DMV/1639/11 was not improved by the addition of the 4/91 vaccine (UD).

- The efficacy of three commercial disinfectants and three common chemicals to inactivate IBV was evaluated. Manure tea increased the organic load sufficiently to reduce the ability of the disinfectants and chemical to inactivate the virus. This raises major concern for cleaning and disinfection of vehicles under high organic loads (UD).

- Protection conferred by replication deficient alphavirus RNA particle vaccine containing AIV HA gene was superior to an inactivated AIV oil emulsion vaccine, not only protecting layers from mortality but significantly decreasing viral shed from the respiratory and digestive tracts. Modern vaccines may represent efficacious tools for use in conjunction with current approaches taken to eradicate and control avian influenza (UD).

- A Poultry Respiratory Health Seminar, an Emergency Poultry Disease Response Course, and multiple regional respiratory disease workshops were conducted during 2016-2017. Multiple regional respiratory disease workshops or sessions within existing workshops were held. The largest impact was achieved through the sponsorship of a session at Delaware Ag Week (258 commercial poultry growers, 51 small flock producers)(UD).

- Measurement of the rate of recombination of two virulent US ILTV strains (Genotype IV and Genotype VI) in non-vaccinated and vaccinated chickens identified the location of recombination breakpoints in a selection of the recombinant progeny. Furthermore, full genome sequences of field isolates from different geographical regions, as compared to in vivo obtained recombinant progeny, identified conserved recombination hot-spots in the ILTV genome. These results are relevant in the future development of live attenuated ILTV vaccines because “recombination hot spots” once identified can be modified to decrease the chances of virus recombination, subsequently this will result in much more stable live ILTV vaccines (UGA).

- A single locus PCR-based diagnostic assay to differentiate (ILTV) strains from commercial and backyard flocks from the US allows the rapid and accurate genotyping of vaccine strain field isolates in commercial poultry, and backyard flock isolates and is a much simple, one target PCR/sequencing assay than previous multi locus PCR sequencing assays (UGA).

- Currently there are at least eight commercial HVT vector vaccines against different avian viruses. Therefore, it has been a common practice for companies to co-administer recombinant HVT products. Chickens that received combinations of HVT vectors showed significant increase in clinical signs of LT as compared to the non-vaccinated/non-challenge group of chickens. Therefore, the protection against LT was compromised. Results from this experiment shed light on how to optimize vaccination programs that uses combinations of HVT vector vaccines (UGA).

- *Mycoplasma synoviae* (MS), and ILTV can cause tremendous economic losses due to mortality, condemnations, cost of treatment and vaccination, and cost of control and monitoring to the commercial broiler industry. The impact of MS infection on ILTV CEO vaccine replication kinetics and protection following virulent ILTV challenge was investigated. MS-infected birds had more severe clinical signs and higher mortality after ILTV challenge than MS-free broilers. These results inform the poultry industry as to the increased risk of severe vaccine reactions and exacerbation of respiratory disesase (including airsacculitis) when MS postive broilers are infected with ILTV. MS infection may also affect CEO vaccine efficacy (UGA).

- Vaccination schedules can affect the development and longevity of immunity when multiple live attenuated vaccines are given either simultaneously or sequentially to pullets, and that co-infections with LPAIV, IBV variants, NDV, ILTV or mycoplasma at the time of vaccination can compromise protection. Vaccinated birds challenged with either IBV, ILTV or NDV had statistically lower or undetectable viral loads compared to non-vaccinated challenged birds (UGA).

- The effects of air quality on the onset (infection), transmission and severity of respiratory disease caused by IBV was evaluated. Interestingly high ammonia exposure did not affect clinical signs, ciliostasis, viral load or post-challenge antibody levels. High ammonia exposure was associated with decreased body weights and increased airsacculitis in non-vaccinated challenged birds (UGA).

- A study examining the onset of local immune responses triggered by infectious laryngotracheitis virus (ILTV) infection allowed to identify subtle leukocyte shifts characteristic of CEO vaccination as compared to high depletion of leukocytes cause by infection with virulent isolate. These changes maybe the first indication of the onset of vaccine protective immunity. Understanding local avian host response to ILTV can aid in the development of new vaccines and treatment strategies (UGA).

- Current Marek's Disease (MD) vaccines do not spread well. Studies are being conducted to improve the current vaccines in order to generate vaccines that could be spread and provide protection to the whole flock. Vaccines that can spread from bird-to-bird would be beneficial to the poultry industry in order to protect chickens that were missed during the vaccination process (UI).

- Infectious bursal disease virus (IBDV) has been established as a replication-competent viral vector capable of carrying an epitope at multiple loci in the genome. Studies were conducted to enhance the safety and increase the insertion capacity of IBDV and develop a replication-incompetent IBDV vector. GFP-IBDV developed in the present study is a replication-incompetent IBDV vector which expresses a foreign protein in infected cells without the capability to produce viral progeny (IN).

- Live attenuated influenza vaccine (LAIV) provide numerous advantages, such as eliciting broader range of immune response, ease of administration and broad reactivity to diverse strains. A pc4-LAIV vaccine elicits higher innate signaling sensitization, mucosal antibody response and better clearance of heterologous challenge virus in day old chicks than inactivated vaccine (IV). The study demonstrated that pc4-LAIV can provide good protection in young chickens and the live-priming and IV-boosting can enhance antibody response and protection efficacy (OSU).

- Three vaccines, developed based on updating existing registered vaccines or currently licensed technologies, were evaluated for possible use for protection of chickens by U.S. as emergency H5 vaccines against H5N2 high pathogenicity avian influenza virus. A reverse genetics avian influenza inactivated vaccine (rgH5N1), a recombinant herpesvirus turkey vectored vaccine (rHVT-H5), and an RNA particle vaccine (RP-H5) were evaluated. Single vaccination with either rgH5N1 or RP-H5 vaccines provided clinical protection in adult chickens and significantly reduced virus shedding. Double rgH5N1 vaccination protected adult chickens from clinical signs and mortality when challenged 20 weeks post-boost, with high levels of long-lasting protective immunity and significantly reduced virus shedding. These studies support the use of genetically related vaccines for emergency vaccination programs against H5 highly pathogenic avian influenza virus in young and adult layers (SEPRL).

- Virus-like particle vaccines were developed for avian influenza H5/H7/H9 antigens derived from the H5N1, H7N3 and H9N2 AI subtypes. All vaccinated birds survived challenge with H5N2 and H7N3 highly pathogenic avian influenza viruses. For low pathogenic avian influenza H9N2 challenge, no mortality was observed following challenge; however, immune responses to the vaccine were detected. Thus, triple-subtype H5/H7/H9 virus-like particle vaccines induced protection against multiple AI challenges (SEPRL).

- There has been a general concern in the poultry industry that the maternally derived antibodies against Newcastle disease virus (NDV) may interfere with the NDV vectored vaccine protections against NDV and the targeted avian pathogen. The presence of maternal antibodies to NDV and ILTV did not significantly interfere with the ability of the NDV LaSota strain-vectored ILTV gB and gD vaccine candidates to elicit protective immunity against infectious laryngotracheitis and Newcastle disease (SEPRL).

- It was demonstrated that vaccine failures in the field are not caused by multiple successive challenges with virulent virus or by high challenge doses that may be capable of overwhelming the immune system. Repeated challenges with high doses of virulent NDV were done in chickens and it was shown that it not decrease the efficacy of currently used vaccines. The work is important because it demonstrates that additional factors are yet to be identified as being responsible for field vaccine failures while re-assuring the producer on the efficacy of current vaccines (SEPRL).

- To develop predictive biology strategies for risk assessment of virus evolution codon usage and codon adaptation indexes was compared among different NDV's. Distinctive codon usage for the two APMV-1 classes and for different transcriptional regions was found. The data suggested that codon usage has changed significantly since the two APMV-1 classes diverged, however codon adaptation in APMV-1 occurs through a slow evolutionary process and is not likely to have significant role in the increased virulence of new genotypes. The identification of genomic regions

with faster rates of codon usage adaptation may lead to the development of more focused predicted studies (SEPRL).

- To address the need to develop new technologies that may lead to the production of improved NDV vaccines a chicken induced pluripotent stem cells that are tolerant to Newcastle disease virus and therefore more likely to produce higher titers of vaccine viruses was created. These new technologies may facilitate the production of high titer vaccines in cell culture (SEPRL).

**Impacts:**

**Objective I**

- AIV is not viable in dry contaminated feed or distilled or chlorinated city water drinking water, and therefore does not represent a significant method in which AIV can be transmitted. In contrast, darkling beetles can harbor the virus and could serve as vector for the spread of AIV.

- Finches are unlikely to be playing a major role in that exposure to *M. gallisepticum,* a common among backyard chickens,.

- Deep sequencing of H7N8 avian influenza viruses from surveillance zone supports H7N8 highly pathogenic avian influenza was limited to a single outbreak farm in Indiana during the 2016 HPAI outbreak in turkeys.

- A novel reassortant avian influenza (H5N8) virus was detected in wild aquatic birds, Russia, 2016. The re-emergence of a novel virus in wild birds is of concern for dissemination of novel reassortant virus during the coming fall migration.

- Reoccurrence of a H5N2 HPAIV in a wild mallard in Alaska, 2016 indicates the virus has not disappeared and raises concern that the virus might return to the lower 48 states during the fall wild waterfowl migration.

- Identification of previously unrecognized genetic diversity in avian paramyxovirus serotype 1 (APMV-1) of low virulence isolated from wild birds. Diversity is likely a function of continued viral evolution in reservoir hosts. No support was found for the emergence or maintenance of APMV-1 strains predicted to be pathogenic to poultry in wild birds of North America outside of the order Suliformes (i.e., cormorants) and Columbiformes (pigeons).

**Objective II**

- Methods have been developed which can be used to characterize the avian respiratory viral microbiome from tracheal samples. Both DNA viruses (herpesvirus, gyrovirus, adenovirus) and RNA viruses (coronavirus, paramyxovirus) can be identified.

- Specific tests were developed for the Mass, Ark, DE, GA98, GA07 and GA08 genetic types of infectious bronchitis virus (IBV). Rapid identification of IBV types is important for selection of

vaccines because different types do not cross protect. The test described can be done directly on clinical samples making it possible to identify IBV type in just a few hours.

- A simplified, sequence-independent single-primer amplification (SISPA) technique in combination with the MiSeq Platform to target viral genome sequences belonging to different virus families in diagnostic samples was developed. This application can be adaptable to other RNA viruses due to non-specific nature of the amplification technique.

- To address the need for rapid identification assays for variant NDV strains, a technique that allows ultra-deep sequencing of nucleic acids without using target-specific primers was developed. Development of robust cost and effective NGS identification assays for variant NDV strains will lead to more specific diagnosis of causes of vaccine failures and to the development of more specific diagnostic agents.

**Objective III**

- Understanding the effects of the MHC and innate immunity on IBV infection in congenic genetic lines of chickens provided insight for the control of this endemic pathogen**.**

- IBV-like strain of coronavirus detected in chickens with runting stunting syndrome did not increase enteric signs or pathology. The virus is close to CA variants and Ark vaccine, suggesting that is a mutant of field strains and Ark vaccine helps understand IBV virus dynamics in order to plan preventative strategies.

- During 2017 an increase in the amount of Coryza cases and their severity has been noticed in the State of California. *Avibacterium paragallinarum* isolates sequences found homologies of 100% with H18 and Modesto reference isolates. These two strains are part of the inactivated vaccines used in the field.

- Identification of MDV genes essential for transmission could have major implications in the design of vaccines that could target MDV spread in a chicken house.

- Information determining the role of IBDV induced immune suppression on LPAIV infection provided practical information on when and how IBD should be controlled to prevent respiratory disease in chickens.

- Recent swine influenza viruses showed limited ability to replicate and transmit in turkeys. However, infection with recent turkey-origin swine influenza viruses resulted in significant loss of egg production. Furthermore, recent seasonal human H1N1 and H3N2 viruses showed the potential to replicate and transmit in turkeys.

- H7N8 HPAIV viruses from the Indiana 2016 outbreak did not cause disease or death in mallards like in the chickens and turkeys, meaning they could easily carry the virulent form for poultry while remaining healthy themselves.

- H5N2 HPAIV viruses from the United States 2014-2015 outbreak have an unusually long pre-clinical period in turkeys suggesting that virus spread during the outbreaks may have been enhanced because turkeys were infected but not showing clinical disease, which would allow a longer period of time before quarantine measures would have been taken facilitating spread.

- The H5 HPAI viruses from the 2014-15 outbreak in the U.S. infected domestic ducks and geese and easily transmitted to contact birds, with geese being more susceptible to infection and disease than ducks, emphasizing the need to improve surveillance in domestic waterfowl and increase biosecurity to reduce direct and indirect contact between poultry and wild waterfowl.

- Similar to dabbling ducks, diving ducks (Lesser Scaups and Ruddy Ducks) are susceptible to infection with H5 HPAI virus lineage. This information is critical in understanding the ecology of avian influenza in reservoir species.

- Most mallards inoculated with one of fourteen different H5 and H7 HPAI viruses from previous outbreaks in poultry, and contact exposed ducks, became infected, highlighting the possible role of wild waterfowl in the spread of any highly pathogenic avian influenza viruses.

- Increased virus adaptation to chickens was observed with poultry H5N2 HPAI viruses; however, these viruses still easily infected mallards. Genetic changes in the viruses were also determined, which helps in understanding how avian influenza viruses change to better infect and replicate in poultry species.

- Age was not a determinant factor in susceptibility of broilers for H5N2 highly pathogenic avian influenza virus.

- Recombination is a feature of many alphaherpesviruses including infectious laryngotracheitis virus (ILTV). New insights into alphaherpesvirus recombination can be used to inform the future development and utilization of ILTV vaccines.

**Objective IV**

- A CEK-adapted IBV ArkDPI vaccine offers improvement and refinement of current ArkDPI-derived vaccines by both eliminating emergence of vaccine-like viruses after vaccination and eliminating emergence of novel strains originating from Ark challenge.

- The reduced replication of CEK-adapted IBV Ark vaccine in chickens could be beneficial as it would reduce adverse reactions. Protection trials show that replication levels of the CEK-adapted Ark vaccine are sufficient to induce protective immunity.

- IBV S2 expressed from recombinant virus was used to confer protection across serotypes in chickens. It was shown that cross protection cannot be attributed to the S2 portion of the spike (S) IBV protein.

- Studies characterizing the receptor-binding domain of IBV Ark-type infectious bronchitis virus S1 protein confirmed that the S1 is necessary and sufficient for binding to chicken tissues.

Understanding the interaction of the IBV S protein with host receptors will be useful for developing strategies to interfere with this interaction and thus inhibit infection.

- Two recombinant ILT vaccines have been developed. Field and laboratory data have demonstrated that both LT recombinant vaccines provided only partial protection in older aged broilers and do not reduce the replication of the field virus, thus allowing for virus shedding, and transmission.

- A transgenic plant (*Arabidopsis thaliana)* expressing the hemagglutinin (HA) gene of the LPAIV H1N1 subtype protected better chickens than a commercial inactivated vaccine when given at high doses. Plant-based vaccines can be produced in mass at a quicker rate and more economically than conventional vaccines and can be given in the drinking water.

- One poultry producer is continuing to successfully utilize a vaccine developed under the regulation 9CFR PART 107.7(b) to control an avian health issue using a homologous genotype IBV variant vaccine that was not available from biologics manufacturers.

- Manure tea increased the organic load sufficiently to reduce the ability of disinfectants and chemical to inactivate IBV. This raises major concern for cleaning and disinfection of vehicles under high organic loads.

- Protection conferred by replication deficient alphavirus RNA particle vaccine containing AIV HA gene was superior to an inactivated AIV oil emulsion vaccine.

- A Poultry Respiratory Health Seminar, an Emergency Poultry Disease Response Course, and multiple regional respiratory disease workshops were conducted during 2016-2017. The largest impact was achieved through the sponsorship of a session at Delaware Ag Week (258 commercial poultry growers, 51 small flock producers).

- Measurement of the rate of recombination of two virulent US ILTV strains in non-vaccinated and vaccinated chickens identified the location of recombination breakpoints. These results are relevant in the future development of live attenuated ILTV vaccines because “recombination hot spots” once identified can be modified to decrease the chances of virus recombination, subsequently this will result in much more stable live ILTV vaccines.

- A single locus PCR-based diagnostic assay to differentiate ILTV strains from commercial and backyard flocks from the US was developed allowing the rapid and accurate genotyping of vaccine strain field isolates in poultry.

- Chickens that received combinations of HVT vectors showed significant increase in clinical signs of LT. Therefore, the protection against LT was compromised. Results from this experiment shed light on how to optimize vaccination programs that uses combinations of HVT vector vaccines.

- *Mycoplasma synoviae* (MS)-infected birds had more severe clinical signs and higher mortality after ILTV challenge than MS-free broilers. These results inform the poultry industry as to the

increased risk of severe vaccine reactions and exacerbation of respiratory disesase (including airsacculitis) when MS postive broilers are infected with ILTV.

- Vaccination of long–lived birds with live attenuated vaccines against multiple pathogens provides adequate protection against each pathogen up to 36 weeks of age indicating that long-lived birds can be protected against respiratory disease.

- Although it is important to manage low levels of ammonia in poultry houses, it appears that ammonia has little impact on development of immunity against respiratory disease in broilers, whereas production (body weight) and losses at processing (airsacculitis) were impacted by ammonia.

- Live attenuated influenza vaccine (LAIV) provide numerous advantages, such as eliciting broader range of immune response, ease of administration and broad reactivity to diverse strains. A pc4-LAIV vaccine can provide good protection in young chickens and the live-priming and IV-boosting can enhance antibody response and protection efficacy.

- Vaccine studies supported the use of genetically related vaccines for emergency vaccination programs against H5 highly pathogenic avian influenza virus in young and adult layers.

- Triple-subtype H5/H7/H9 virus-like particle vaccines induced protection against multiple avian influenza challenges.

- The presence of maternal antibodies (MDA) to NDV and ILTV did not significantly interfere with the ability of the NDV LaSota strain-vectored ILTV vaccines to elicit protective immunity against ILT and ND, suggesting that these vaccines can be applied to commercial chickens in the presence of NDV and ILTV MDA as dual vaccines.

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