**Period Covered:** October 1, 2011 to September 30, 2012

**Annual Meeting Date(s):** December 2, 2012

**Participants:**

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**Accomplishments:**

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

1. Isolation and characterization of avian influenza viruses (AIV) from wild birds and commercial poultry flocks which include live bird markets and backyard flocks were accomplished. The surveillance data obtained from different states (CT, DE) were shared. No AIV activity using USDA NAHLN-approved agent detection (real time RT-PCR and antigen capture on oropharyngeal swabs) were seen in commercial flocks (CT, DE).
2. Surveillance activities on the Delmarva Peninsula have yielded infectious bronchitis viruses of Arkansas (57), Massachusetts (10), and Delaware 072 (10). Seven isolations of NDV were made during the period, of which three were concomitants of IBV isolations.
3. Delmarva has continued to observed ILT activity. The severity of LT clinical signs and lesions are mild to moderate, very similar to that seen in adverse CEO vaccine reactions. All suspect LT cases are evaluated by real time PCR and histopathology of eyelid and trachea for confirmation.
4. GA conducted Mycoplasma diagnostics/ surveillance in commercial poultry. Diagnostic tests conducted over the past year include 1076 cultures, 6884 HI tests, 3613 PCR tests and 141 sequencing reactions.
5. SEPRL on their international surveillance and characterization of avian influenza H5N1 subtypes indicated that the Egypt remains one of a handful of countries where the H5N1 bird flu continues to infect poultry
6. SEPR determined the recent H5N1 highly pathogenic avian influenza (HPAI) viruses circulating in Vietnam was evaluated in domestic ducks. One of the viruses, A/duck/Vietnam/NCVD-672/2011 (clade 2.3.2B), was highly virulent for ducks but the other virus, A/chicken/Vietnam/NCVD-675/2011 (clade 2.3.2A) was moderately pathogenic
7. SEPRL Strains of NDV obtained recently from Mexico, Indonesia, Malaysia, Venezuela, Pakistan, Vietnam, Belize, Dominican Republic, South Africa, and Peru and from wild birds from the U.S. have been sequenced and characterized genetically.

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

1. CT in collaboration with Guangxi Veterinary Institute, China developed loop-mediated isothermal amplification (LAMP) assays to detect the *Mycoplasma gallisepticum* isolates. The newly developed LAMP assay is simple, sensitive, rapid and can identify *Mycoplasma gallisepticum* isolates visually. Consequently, this assay will be very useful screening assays.

2. DE, developed an IBV Arkansas genotype-specific real-time RT-PCR primer and probe set that could be seamlessly integrated with the published IBV real-time protocol within a multiplex real-time RT-PCR reaction

3. DE sequenced a recent 2011 ILTV field isolate from the Delmarva Peninsula that has demonstrated the capacity to break through a vaccinated flock.

4. GA Develop a multiplex assay to detect avian infectious bronchitis virus types**.** Four most common IBV serotypes diagnosed in the USA; Arkansas (Ark), Connecticut (Conn), Delaware (DE).

5. GA developed and validateda quantitative method for detection of infectious laryngotracheitis virus (ILTV) in clinical and laboratory samples**.** This methodology allows for the quantitation of virus copy numbers in a given clinical sample and it also allows for elimination of cumbersome classical virology and serology methods.

6. IL genetically characterized a vaccine strain of fowlpox virus involved in outbreaks in vaccinated flocks and determined that the reticuloendotheliosis virus genome was present in the fowlpox virus genes.

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

1. MN genetically analyzed the matrix (M) gene of avian influenza viruses isolated from wild birds and from the live bird markets indicated that independent evolution of M gene in the absence of antiviral drugs will lead to mutation causing resistance.
2. MN demonstrated during surveillance program that water borne transmission of influenza A virus likely occurs.
3. OH, investigated the replication of swine and human influenza A viruses in juvenile and layer turkeys.OH, noticed an enhanced replication of swine influenza viruses in immune compromised (dexamethasone-treated) juvenile and layer turkeys.
4. OH, assessed and quantified apoptosis and T cell mediated cytotoxicity in IBDV infected chickens.
5. OH, investigated an attachment of avian and mammalian influenza viruses in the respiratory and reproductive tracts of layer turkey hens
6. OH, demonstrated persistence and tissue distribution of infectious bursal disease virus in experimentally infected SPF and commercial broiler chickens
7. OH, showed the molecular evidence for a geographically restricted population of infectious bursal disease viruses.
8. OH, demonstrated the diversity of genome segment B from infectious bursal disease viruses in the United States SEPRL,Identified genetic and biological determinants of tissue tropism and transmission of avian influenza virus in chickens.
9. SPRL has determined gross and microscopic lesions from Newcastle disease virus (NDV) infections with novel circulating NDV of different genotypes in various species of birds.

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

1. AL developed a transgenic plat vaccine against avian influenza.
2. CT generated throughput gene sequence data of IBV field isolates from the commercial poultry flocks vaccine with various IBV vaccines.
3. DE generated a new generation ILT vaccine containing deletions in essential genes.
4. GA examined the dynamics of IBV vaccination and protecting poultry against Arkansas field strains of IBV.GA developed mutant vaccine against infectious laryngotrachitis infection.
5. IN investigated the infectious bursal disease kinetics using DNA vaccine in chickens.
6. SEPRL performed vaccine efficacy studies using circulating AI viruses from Vietnam.
7. SEPRL developed new vaccine platforms to control and prevent avian influenza outbreaks.
8. SEPRL determined the AI vaccine efficacy following vaccination with recombinant herpesvirus of turkey-vectored avian influenza vaccine against highly pathogenic H5N1 challenge.

**Work Planned for Next Year**

1. Continue surveillance, screening, and characterization of respiratory pathogen from wild and domestic bird populations;
2. Continue development and refinement of diagnostic assays to detect and differentiate poultry pathogens
3. Continue development, refinement and testing of vaccine against influenza, ILT, IBDV, IBV, Mycoplasma and other respiratory pathogens of poultry.
4. The molecular basis for antigenicity, pathogenicity, and transmission of respiratory pathogens will be studied using naturally occurring viruses and reverse genetically created viruses.
5. Collaborative work will continue with a number of national and international partners.

**Impacts:**

1. Continuous surveillance and characterization of ILTV’s from poultry house environments would help in the understanding of the origin, evolution, transmission and control of present and future ILTV outbreaks. Composting litter, a through cleanout out and disinfection of a house, and possibly the use of commercial recombinant vaccines given *in ovo,* will reduce the incidence and severity of subsequent ILTV outbreaks.
2. Avian influenza subtype H5 and H7 were negative from the LBM and domestic poultry birds in New England states in Delaware and Ohio commercial farms. However wild birds do carry H5 and H7 subtypes in their population and continued surveillance is warranted.
3. Infectious laryngotracheitis virus and infectious bronchitis virtues circulating in commercial broiler chickens flocks in Alabama, Delaware, and Georgia states. Surveillance activities on the Delmarva Peninsula have yielded infectious laryngotracheitis virus (ILTV) and infectious bronchitis virus (IBV) isolates from commercial broiler chickens and an avian paramyxovirus (APMV)-4 isolate from wild birds.
4. Quasi-species phenomenon in the IBV strains occurring in the field. This identification and characterization will be helpful for designing the better vaccines against IBV infections for poultry.
5. Continuous surveillance and characterization of MG from poultry would help in the understanding of the origin, evolution, transmission and control of present and future. Development of rapid tool loop mediated isothermal polymerase to identify MG infection will be very cost effective without the use of sophisticated and expensive thermal cyclers.
6. Multiplex assay to detect avian infectious bronchitis virus serotypes. For an additional $0.21 per reaction, multiplexing a Arkansas genotype specific with the universal infectious bronchitis virus (IBV) rRT-PCR assay permitted detection of the most common genotype in Delmarva broilers without impacting test sensitivity. Monitoring infectious bronchitis viruses from commercial broiler chickens is important for evaluating the effectiveness of vaccination programs and to isolate and characterize field viruses that break through vaccine induced immunity.
7. Method of delivery of Ark vaccines fully protects broilers. This is important for control of IBV Ark type viruses in the field.
8. Both traditional and recombinant-based approaches for the construction of the next generation of infectious laryngotracheitis virus (ILTV) live vaccines. Infectious laryngotracheitis is an economic disease that also has important trade implications for the U.S. poultry industry. Vaccination using CEO and recombinant vaccines is helping control the disease but more research is warranted to develop improved vaccines and control strategies.
9. Quantitative tool to detect ILTV in birds can be used to establish the viral load in chickens, which provides valuable data for estimating transmission and control.
10. IBDV large segment gene-based DNA vaccination in inhibiting and/or eliminating infectious bursal disease virus infection as illustrated by DNA vaccination kinetics and bursal transcriptome has the great potential for practical use in the field for protection of chickens against infectious bursal disease in the poultry industry
11. PCR amplification of selected genomic fragments from DNA isolated from formalin fixed tissue sections of histologically positive cases of avianpox virus infection is convenient for genetic characterization of these viruses.
12. Rapid aptamer-based approach that will enable faster and cost-effective identification of influenza virus in animal samples.
13. Swine influenza viruses (SIVs) continue to be a threat for turkey industry and immunosuppression of the bird may enhance the transmission and adaptation of swine influenza viruses in turkeys through enhancement of virus replication, prolonged virus shedding, and possible decrease of infectious dose required to initiate infection.
14. Virus histochemistry can be applied as a useful *in vitro* screening tool to predict the *in vivo* replication of influenza virus which may help to reduce the use of live animals and research cost.
15. Studies provide new insights into the pathogenesis of IBDV and provide mechanistic evidence that the cytotoxic T cells may act through both Fas-FasL and perforin-granzyme pathways in mediating the clearance of virus-infected cells. The findings can be used to develop novel target for IBDV control.
16. IBDV RNA can be detected in thigh and breast muscles for short period of time. However, the presence of vRNA is not indicative of the presence of the infectious virus and does not necessarily correlate with virus isolation data. The first detailed report on the persistence and distribution of classic and variant strains of IBDV in different tissues of SPF and commercial chickens will be useful for risk assessment and develop prevention strategy.
17. The phylogeographic data suggest specific population of IBDV has been restricted for over 14 years to Northeast Ohio. Since commercially available classic and variant vaccines do not effectively control this population of IBDV, other alternatives are needed.
18. Molecular epidemiology study of IBDV shows the evidence of recombination events, in addition to reassortment, in creating genetic diversity both in variant and classic strains. Furthermore, the study shows importance and usefulness of analyzing genome segment B during routine molecular diagnosis of all IBDV strains.
19. Gene mutations detected in AIV in Egypt is more difficult to control outbreaks, because the vaccine is less effective against these mutant groups of AIV.
20. Serious concern for the control of H5N1 in Vietnam must consider the important role of domestic ducks in the epidemiology of H5N1 HPAI
21. Information has implications for infection through artificial insemination and shows that the AI virus can replicate in the reproductive tract, which may mean the virus can be found in or on eggs
22. An edible transgenic plant vaccine against the H5 and H7 AIV subtypes, which could be mixed in poultry feed, could be farther developed for use in controlling AIV in chickens, in 3rd world countries. This is important since these poorer countries are a constant source of AIV infections in poultry and swine populations. In addition, vaccines against animals are needed to prevent future pandemics in humans, which contain triple reassortments of AIVs from birds, humans, and swine.

Recombinant vaccine can be used as an aid during AI eradication efforts in turkey species.

1. Proper identification of the disease signs, which are crucial to quickly preventing the spread NDV. The virulent NDV that are found in the U.S. in pigeons (genotype VIb) and cormorants (genotype V) and the virulent NDV (genotype V) from the last 2002 U.S. outbreak also produces few gross lesions upon infection of poultry, unlike what is seen world-wide from other virulent NDV (genotypes VII-X111).
2. New vaccine candidates are being evaluated by a vaccine company for distribution worldwide to improve NDV control. The benefit of these vaccines is their ability to decrease the amount of virus put into the environment by vaccinated birds infected with virulent NDV.

**Cooperative projects / Collaboration:**

1. OSU and SEPRL conduct pathogenesis study of swine and human influenza viruses in turkeys. Lee and Pantin-Jackwood communicate and share information regarding the study to facilitate the progress.
2. Dr. Jackwood (OSU) and California Animal Health and Food Safety Laboratory System continue to collaborate on surveillance and characterization of vvIBDVs. OSU (Dr. Jackwood) and DE (Dr. Gelb) collaborate on studies to evaluate the efficacy of recombinant HVT-IBD vaccines.
3. OSu (Dr.Lee) continues to provide experimental samples and viruses to GA (Dr. Garcia) for the development and validation of DIVA diagnostic assay for avian influenza virus.
4. *Delaware, Collaborators:* Dr. Maricarmen Garcia (University of Georgia) and Dr. Stephen Spatz (USDA ARS SEPRL).
5. Dr. Khan (CT) collaborates with Jack Gelb, Jr. (DE) on studies for the development of Peptide nanoparticles based avian influenza vaccines.

**Grant supports.**

1. Y.M Saif & C.W. Lee. USDA CSREES NRI Integrated Research (AI-CAP 20085520418863). 05/01/08 – 02/01/13. Molecular determinants of interspecies transmission of H3N2 triple reassortant influenza A viruses.
2. Jack Gelb, Jr. (Co-PI) with M. Khan (PI) and Peter Burkhard (Co-PI) University of Connecticut’ Peptide nanoparticles a noval immunogens: Design and analysis of avian influenza vaccines. USDA-AFRI sub award to Delaware ($140,000) 12-1-11 to 11-30-14.
3. Jack Gelb, Jr. with Daral Jackwood (Ohio State University), Brian Ladman and Erin Brannick. “Studies on the efficacy of recombinant HVT-IBD vector vaccines”. U.S. Poultry and Egg Assn. ($70,740) 8-1-11 to 7-31-13.
4. Mazhar Khan (CoPI) with Ion Mandiou (PI), Racheal ONeal (CoPI) University of Connecticut,: Alex (CoPI) Gerogia Tech. USDA- NIFA-Bioinformatic. ($425,000), 2010- 2012.
5. Joe Giambrone (PI) $20,000. Development of an edible transgenic plant vaccine against avian influenza virus. Alabama Agriculture Experiment Station Initiative grant

**Publication in Journala**

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