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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 enormous data obtained from different states (AL, CT, DE, MN) were shared.
2. Surveillance activities on the Delmarva Peninsula have yielded infectious laryngotracheitis (LT) virus and infectious bronchitis virus isolates from commercial broiler chickens and Newcastle disease virus isolates from wild birds.
3. In DE, the incidence of LT vaccine reaction and vaccinatl LT clinical cases increased in 2010 compared to 2009. NO new IBV variants were found during the period.
4. Using gene targeted sequencing and random amplified polymorphic DNA analsysis, GA identified the circulation of field strains within complex and companies and analyzed 170 MG and MS strains in 2010.
5. SEPRL (USDA) characterized new avian paramyxovirus isolated from penguins. It was determined that the viruses corresponded to a new serotype (serotype 10).

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

1. AL developed a method to detect CEO ILTV vaccines in drinking water lines which detects ILTV DNA in the biofilm collected from the water system by real-time PCR.
2. AK and DE used next generation sequencing technologies (Illumina) which permit the relatively rapid determination of the primary sequence of the ILTV genome. AK determined genomes of one wild type and two vaccine ILTV strains.
3. GA developed a multiplex detection of avian influenza HA (H5 & H7) and NA (N1 & N2) subtypes using a microsphere assay.
4. GA developed a species–independent competitive ELISA (cELISA) for the detection of influenza A antibodies directed to H6, H7, and H9.
5. GA develops H9 specific monoclonal antibodies and further developed H9 subtype specific ELISA systems.
6. IL developed a photolase gene specific PCR. Based on sequence information, avian pox viruses could be differentiated into four different groups.
7. OH developed 19-plex assay which can differentiate different HA subtypes of avian influenza viruses.
8. SEPRL developed an enzyme-linked immunospot assay which can detect avian influenza specific antibody-secreting B cells in chickens.
9. SEPRL identified that the optimal detection methods for avian influenza virus from wild birds depend on the prevalence of virus.

**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. AL investigated effects of immunosuppressive viruses, chicken anemia virus (CAV) and/or infectious bursal disease virus (IBDV), on evolution of infectious bronchitis virus (IBV).
2. GA conducted comparative genomic analysis of IBV which indicates that the replicase protein in addition to the already recognized spike gene of coronaviuses plays a key role in pathogenicity. GA have identified regions in the replicase that likely effects cleavage and assembly of the enzyme.
3. MN studied the host:pathogen interactions during *E. coli* infection in the broiler chicken. The genes differentially expressed in air sac tissue did not involve any of the typical APEC virulence factors, and instead involved a large number of chromosome-encoded transport system genes and genes of unknown function.
4. OH studied two isolates of vvIBDV from California which were identified to contain a vvIBDV genome segment A but instead of a serotype 1 vvIBDV genome segment B, their genome segment B was most closely related to a serotype 2 IBDV.
5. OH studied the persistence of classical (STC) and variant (IN) IBDVs and the two strains were detected much longer in bursal tissues (upto 8 weeks) followed by spleen, thymus and bone marrow. In non-lymphoid tissues both of the strains persisted longer in cecum followed by liver, kidney, pancreas, lungs, thigh and breast muscles.
6. SEPRL demonstrate that the pandemic H1N1 influenza virus does not easily infect young poultry. However, laying turkey hens were susceptible to pandemic H1N1 virus by reproductive tract exposure.
7. SEPRL demonstrated that aMPV-C wild bird isolates induced typical aMPV/C disease in the domestic turkeys. This result suggests that the wild birds may play a role in the spread of the aMPV-C virus. They also showe that the M2-2 gene is not essential for virus replication in cell culture, but required for efficient virus replication in turkeys to counteract the host’s natural defenses and immunity.

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

1. DE are utilizing both traditional and recombinant-based approaches for the construction of the next generation of ILTV live vaccines.
2. *OH used in vitro analysis of virus particle subpopulations in candidate live-attenuated influenza vaccines which could distinguish effective from ineffective vaccines.*
3. SEPRL showed that intranasal administration of alpha interferon reduced morbidity associated with low pathogenic avian influenza virus infection.
4. SEPRL demonstrated that commercial influenza vaccines have variable efficacy for protecting chickens and ducks against H5N1 highly pathogenic avian influenza (HPAI) viruses.
5. AL showed for the first time that a DNA vaccine containing an HA gene of an AIV produced cellular immune responses in chickens with a T-helper 1 (Th1) preference. AL also developed an H1 vaccine in transgenic *Arabidopsis thallenia. Arabidopsis* is a commonly used small weed, whose genome has been sequenced.
6. CT developed nanoparticle based vaccines carrying M2e of influenza virus and demonstrated the immunogenicity and protection induced by M2e-based vaccine by challenge studies.
7. IN showed that a prime-boost approach for protection of broiler chickens with maternally derived antibody against IBDV infection by DNA vaccination can be achieved by priming with a high dose of DNA carrying IBDV large segment gene and boosting with a single dose of killed IBD vaccine.
8. IN showed that DNA vaccination confers protection against IBDV challenge by delayed appearance and rapid clearance of the invading viruses.
9. GA determined the baseline coverage of four different commercial IBV vaccines (Ark, Mass, GA98 and Mass/Conn) tested at a full dose in 1-day old broilers.
10. GA studied aerosol delivery of a virus-like-particle (VLP) vaccine against H5N1 avian influenza in Poultry which showed for the first time that non-replicating influenza VLPs might be used for mass aerosol vaccination in chickens.

**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 to study polymicrobial infection in poultry using a co-infection model; and continue to study *E. coli* as a primary or secondary pathogen of poultry;
4. Continue development, refinement and testing of vaccine against influenza, ILT, IBDV, ORT, *E. coli*, and other respiratory pathogens of poultry.
5. The molecular basis for antigenicity, pathogenicity, and transmission of respiratory pathogens will be studied using naturally occurring viruses and reverse genetically created viruses.
6. Collaborative work will continue with a number of national and international partners.

**Impacts:**

1. Wild birds are a reservoir of AIVs and some species may serve as potential intermediate host. Viral detection should be done by passage of fecal swab material in embryos first then by RRT-PCR and should exclude AC-ELISA.
2. Composting of AIV infected eggs for as early as 24 hours and late as 52 hours can inactivate AIV. The internal temperature of the pile must reach 560 F for the inactivation to occur. The temperature is a function of the amount of pile turning and moisture. Presently, 7 days are used in the industry to perform this function.
3. Two real time RT-PCR assays that allow the differentiation of North American H1N1 from pandemic H1N1 were developed. The National Animal Health Laboratory Network adopted these tests.
4. Low pathogenic influenza viruses were detected from internal egg contents following experimental infection in turkeys. The possibility of hatchery contamination by egg borne influenza viruses and spread of virus during movement of contaminated cracked eggs and egg flats pose concerns regarding influenza viral dissemination
5. ILTV is present in commercial poultry houses causing mild outbreaks. The viruses were found in the dust, litter, beetles, water, and rats. Heating of the house to 1000 F for 100 hours, composting of the litter for 3 days, improved beetle control, treatment of the drinking water system with commercial biofilm removers, and rodent control will reduce the amount of virus in the house.
6. Factors hindering control of ILT may be suboptimal immunization against ILT resulting from multivalent vaccinations. Reducing the number and diversity of live virus vaccines given concomitantly with ILT vaccines may optimize protection against ILTV and possibly against other viral respiratory diseases.
7. A high titer of ILTV vaccine is required for a prompt neutralizing immune response. Thus, vaccine fractionation would seem counterproductive.
8. Monitoring the ability of infectious bursal disease virus (IBDV) to break through maternal immunity in young broiler chickens is important to assess the immunosupproessive potential of the viruses.
9. IBDV large segment gene-based DNA vaccine has the potential for practical application to confer protection of chickens with maternal antibodies against IBD in the poultry industry.
10. Monitoring infectious bronchitis viruses from commercial broiler chickens is important for monitoring the effectiveness of vaccination programs and to isolate and characterize field viruses that break through vaccine induced immunity.
11. In-ovo DNA immunization may become one of the most important innovation in the DNA vaccination of poultry against IBV, allowing it to be used in commercial in-ovo vaccination as a much safer vaccine than the attenuating live IBV vaccines used currently.
12. Genomic characterization of fowlpox virus and other avianpox viruses for specific virulence markers e.g. full length REV can be done by PCR amplification of the genetic fragments with specific primers. In this regard, DNA isolated from formalin fixed paraffin-embedded tissue sections can be used effectively.

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**Abstracts, Presentations, etc:**

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