NC1180: CONTROL OF EMERGING AND RE-EMERGING POULTRY RESPIRATORY DISEASES IN THE UNITED STATES

DECEMBER 2013 MEETING MINUTES (SEE END OF DOCUMENT) AND NC1180'S TERMINATION REPORT

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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, which include hunter-killed or nesting waterfowl and shorebirds, starlings, and raptors and from commercial poultry flocks (live bird markets and backyard) were accomplished. The enormous data obtained from different states (AL, DE, GA, MN, OH) 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 laryngotracheitis (LT) virus and infectious bronchitis virus isolates of Arkansas, Massachusetts, and Delaware from commercial broiler chickens and Newcastle disease virus isolates from wild birds.

3. Delmarva has observed continuously 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 isolated and characterized current pathogenic respiratory viruses, bacteria, and mycoplasmas circulating within the poultry industry in Georgia. Identified at least 41 MG genotypes that are distinguishable from live vaccines and unique to individual countries or regions.

5. Using gene targeted sequencing and random amplified polymorphic DNA analysis, GA identified the circulation of field strains within complex and companies and analyzed numerous MG and MS strains.

6. SEPRL (USDA) characterized new avian paramyxovirus isolated from penguins. It was determined that the viruses corresponded to a new serotype (serotype 10).

7. SEPRL (USDA) obtained Newcastle disease viruses from Mexico, China, Pakistan, Indonesia, Malaysia, Venezuela, Pakistan, Vietnam, Belize, Dominican Republic, South Africa, Peru and from U.S. wild birds; the viruses then have been sequenced and characterized phylogenetically. The sequence data has allowed the improvement of the current diagnostic tests for NDV to ensure that the circulating viruses can be diagnosed.

8. SEPRL on their international surveillance and characterization of avian influenza H5N1 subtypes indicated that Egypt remains one of a handful of countries where the H5N1 bird flu continues to infect poultry.

9. 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.

10. SEPRL conducted a wild bird surveillance study in the Black Sea region in Ukraine to identify avian influenza viruses. A total of 3634 samples were collected from 66 different species of birds. Sixty seven viruses were isolated covering many low pathogenicity avian influenza (LPAI) virus subtypes. The LPAI viruses were isolated mostly from mallard ducks, but also from shellducks, shovelers, teals, and whitefronted geese.

11. SEPRL conducted a study of active and passive surveillance for HPAIV subtype H5N1 in Mongolia from 2005-2011, together with the results of five outbreak investigations. In total eight HPAIV outbreaks were confirmed in Mongolia during this period. Three outbreaks were recorded in the neighboring Tyva Republic of Russia on a lake that bisects the international border. No HPAIV was isolated (cultured) from 7,855 environmental fecal samples (primarily from ducks), or from 2,765 live, clinically healthy birds captured during active surveillance (primarily shelducks, geese and swans), while four HPAIVs were isolated from 141 clinically ill or dead birds located through active surveillance.

12. MN studied the matrix (M) gene of avian influenza viruses isolated from wild birds and live bird markets in the USA. Phylogenetic analysis of the M-gene showed a high degree of nucleotide sequence identity with US isolates of AIVs but not with those of Asian or European lineages.

13. DE continued subtyping the hemagglutinin (HA) and neuraminidase (N) genes of isolates previously recovered from Delmarva wild bird surveillance submissions. From 2006 - 2010, 46 low path (LP) isolates were obtained. The most commonly isolated hemagglutinin subtype was H6 (nine isolates) while the most common N subtype was N2 (nine isolates). Isolates carrying the H6NX subtype and the HXN2 subtype were isolated each sampling year.

14. DE noted an increased isolation of IBV related to the Delaware 072 (DE/072/92) genotype in 2012-2013 with 30 isolates determined to be of the Delaware 072 genotype, nine of which have been confirmed by sequence to be highly related to DMV/2392/12 (90-91% S-1 amino acid identity with reference strains Delaware 072 and Georgia 98.

15. SEPRL examined the genetic diversity of APMV-1 isolated from migratory birds sampled in Alaska, Japan, and Russia and assessed the evidence for intercontinental virus spread. Phylogenetic analysis of wild-bird isolates provided evidence for intercontinental virus spread, specifically viral lineages of APMV-1 class II genotype I sub-genotypes Ib and Ic. This result supports migratory bird movement as a possible mechanism for the redistribution of APMV-1.

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 TaqMan® real time polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) assays. Both assays were specific, sensitive, and reproducible for ILTV detection. Although the sensitivity of LAMP was lower than real time PCR, it was faster, had a lower cost, and did not require a temperature cycler.

2. CT designed nine pairs of neuramidinase (NA) subtype-specific primers using Primer Hunter design tool and successfully used in real time RT-PCR with four primer-pool reactions to differentiate nine NA subtypes of AIV.

3. GA developed an indirect N1 and N2 ELISAs which were proven to be effective and rapid assay to identify exposure to challenge virus during a DIVA vaccination strategy. In addition, a species–independent competitive ELISA (cELISA) for the detection of H6, H7, H9 antibodies in several species was developed.

4. MN developed degenerate primer set for full-length amplification of four genes of influenza A viruses in a single reaction.

5. OH established the chloroform-Mag MAXTM method of viral RNA extraction followed by RRT-PCR which can be used as rapid and sensitive test to determine the titer of the viral RNA. Using this method, it was found that different commercial vaccines contain varied antigen contents.

6. SEPRL (USDA) developed two real time RT-PCR assays that allow the differentiation of North American H1N1 from pandemic H1N1. In addition, the current H7 RRT-PCR was improved to detect a broader range of H7 viruses that are found in Western hemisphere.

7. SEPRL demonstrated that NDV Matrix assay failed to detect a virulent NDV. If genotype VII virus was found in North America this assay could be used in the NALHN laboratories.

8. 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.

9. 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.

10. GA developed a multiplex detection of avian influenza HA (H5 & H7) and NA (N1 & N2) subtypes using a microsphere assay.

11. GA developed a species-independent competitive ELISA (cELISA) for the detection of influenza A antibodies directed to H6, H7, and H9.

12. IL developed a photolase gene specific PCR. Based on sequence information, avian pox viruses could be differentiated into four different groups.

13. OH developed 19-plex assay which can differentiate different HA subtypes of avian influenza viruses.

14. SEPRL developed an enzyme-linked immunospot assay which can detect avian influenza specific antibody-secreting B cells in chickens.

15. CT in collaboration with Guangxi Veterinary Institute, China developed loop-mediated isothermal amplification (LAMP) assays to detect the H3 subtype AIVs visually and rapid detection of group I avian adenoviruses and Mycoplasma gallisepticum isolates. The newly developed H3-RT and group I avian adenoviruses LAMP assays are simple, sensitive, rapid and can identify H3 subtype AIVs and group I avian adenoviruses visually. Consequently, they will be very useful screening assays.

16. GA developed a rapid multiplex microsphere assay for the simultaneous detection of all avian influenza viruses (AIV) as well as differentiation of H5, H7, N1 and N2 subtypes.

17. GA developed and validated N1 and N2 ELISAs as the assays that will be required for the implementation of a DIVA control strategy for H5N1, H5N2, H9N2, H3N2 and H1N1 poultry infections that will be required for the implementation of a DIVA control strategy for H5N1, H5N2, H9N2, H3N2 and H1N1 poultry infections.

18. GA developed 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).

19. SEPRL evaluated numerous elements of the sample collection procedure for avian influenza virus with chickens including: swab construction type, transport media, transport of dry versus wet, the effect of removing the swabs in the field versus the lab, and the number of swabs which could be placed in a single vial to be test together. Some of the alternative procedures and materials were found to improve sensitivity over current methods.

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. DE isolated 5038 IBDV isolate from commercial broiler chickens. Although unique based on VP2 sequencing and monoclonal antibody testing, may not be capable of breaking through maternal immunity in a laboratory designed trial may not be able to break through in real world progeny challenges.

2. GA identified that temperature plays a pivotal role in the survivability of LPAI virus in feces and in contact with litter. GA also identified that a percentage of chickens receiving recombinant or TCO vaccines carry a significant amount of virulent ILTV in the trachea in the absence of clinical signs after being challenged with virulent ILTV.

3. Comparative genomic analysis of IBV indicates that the replicase protein in addition to the already recognized spike gene of coronaviruses plays a key role in pathogenicity. GA have identified regions in the replicase that likely effects cleavage and assembly of the enzyme.

4. OH identified amino acids contributing to antigenic drift in the Del-E infectious bursal disease virus. The short term implication this has for the poultry industry is that diagnostic assays designed to identify the 254 and 222 amino acids will discover viruses that have antigenically important mutations.

5. OH showed that two virulent infectious bursal disease viruses (vvIBDV) from California are identical and meet all the characteristics of a vvIBDV. Because they have the potential to spread rapidly and cause high mortality in chickens, the impact of these viruses on the U.S. broiler and layer industries could be considerable.

6. OH detected low pathogenic influenza viruses in albumin of eggs using real time RT-PCR and virus isolation in embryonated chicken eggs. Swabs from egg shells were also found positive by RRT-PCR.

7. GA conducted comparative genomic analysis of IBV which indicates that the replicase protein in addition to the already recognized spike gene of coronaviruses plays a key role in pathogenicity. GA have identified regions in the replicase that likely effects cleavage and assembly of the enzyme.

8. 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.

9. 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.

10. OH studied the persistence of classical (STC) and variant (IN) IBDVs and the two strains were detected much longer in bursal tissues (up to 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.

11. SEPRL demonstrated 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.

12. 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 showed 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.

13. AL investigated venereal transmission of IBV by artificially inseminating old hens either with semen from IBV infected roosters or with IBV suspended in naïve semen. IBV RNA was detected in the trachea of all hens inseminated with IBV-spiked semen and in 50% of hens inseminated with semen from IBV-infected males. These results provide experimental evidence for IBV venereal transmission.

14. AL investigated that the dominant genotype of the vaccine strain of IBV was rapidly negatively selected in all chicken groups [CAV, IBDV, CAV+IBDV, and immunocompetent]. These results suggest that the generation of genetic diversity in IBV is constrained. This finding constitutes further evidence for phenotypic drift occurring mainly as a result of selection.

15. OH studied maternal immunity in limiting the spread or reducing the severity of the clinical disease caused by very virulent infectious bursal disease virus (vvIBDV).

16. OH investigated Fas/FasL and perforin systems as important mechanisms of T cell-mediated cytotoxicity in infectious bursal disease virus infected chickens.

17. 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.

18. 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.

19. OH demonstrated persistence and tissue distribution of infectious bursal disease virus in experimentally infected SPF and commercial broiler chickens

20. OH showed the molecular evidence for a geographically restricted population of infectious bursal disease viruses.

21. OH demonstrated the diversity of genome segment B from infectious bursal disease viruses in the United States.

22. SEPRL identified genetic and biological determinants of tissue tropism and transmission of avian influenza virus in chickens.

23. SEPRL studied a new avian influenza virus, H7N9 that was identified as causing human infections and based on sequence information the virus was suspected to have come from a poultry source. In laboratory experiments, chickens, quail, muscovy ducks, Pekin ducks, Embed geese, and pigeons were challenged with the H7N9 virus. Birds from each group challenged became infected although none became ill, but quail and chickens shed large amounts of virus.

24. SEPRL the pathologic and immune characteristics of chicken dendritic cells (DC) following infection with high and low pathogenic avian influenza viruses were determined. Chicken DCs were determined to take up avian influenza virus, and supported replication of both high and low pathogenic influenza viruses. The DCs mounted a robust antiviral immune response, including interferon alpha.

25. SEPRL determined that of interferon gamma (IFN- γ) expression during Newcastle disease virus (NDV) infection results in a marked decrease of pathogenicity in 4-week-old chickens, as evidenced by lack of mortality, decreased disease severity, virus shedding, and antigen distribution.

26. OH conducted an antigenic analysis of 18 H5N1 isolates from 2006 to 2012 that represent different clusters using hemagglutination inhibition (HI) and virus neutralization (VN) assays. Antigenic relatedness of ancestral Egyptian H5N1 isolate (459-3/06) with other isolates ranged from 30.7% to 79.1% indicating significant antigenic drift of the H5N1 viruses from the ancestral strains.

27. OH evaluated the pathogenicity induced by co-challenge with the rB strain of very virulent infectious bursal disease virus (vvIBDV) and IBDV pathotypes endemic in the United States in specific pathogenic free (SPF)chickens. Co-challenge with rB and the antigenically similar STC strain did not result in a significant decrease in mortality compared to challenge with the pathogenic rB strain at 4 weeks of age but a significant reduction in the mean bursa lesion score was observed.

28. OH studied the antigen persistence and cytotoxic T cells response in infectious bursal disease virus infected SPF chickens. Gradual reduction in viral RNA load but persistent detection of IBDV-STC was confirmed up to 56 DPI by real-time quantitative RT-PCR.

29. MN determined the effect of avian influenza virus NS1 allele on virus replication and innate gene expression in avian cells. Replication of two reassortant viruses demonstrated that the B allele virus replicates more and to higher titers than the A allele virus in duck cells; however, the A allele virus replicates more in the cells from chickens and turkeys.

30. MN studied the expression profiles for genes in the turkey major histocompatibility complex B-locus. Most MHC-B genes were broadly expressed across tissues. Previously undescribed splice variants were also detected and sequenced from 3 genes.

31. MN determined the role of enteric viruses in Light Turkey Syndrome (LTS), which is characterized by lower weight in market age turkeys than their standard breed character. In the surveillance study astrovirus, rotavirus, and reovirus were detected alone or in various combinations in both LTS and non-LTS flocks.

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

1. AL developed two recombinant vaccines against H1N1 AIV (one DNA and the other in yeast) and found to induce a measurable immune response in young chickens. The DNA vaccine was given by injection and the yeast vaccine in the drinking water.

2. CT tested in ovo vaccination of recombinant DNA plasmid containing IBV spike gene with interferon-a which showed over 98% of protection rate against M41 field isolate challenge.

3. DE developed a second generation escape resistant RNAi constructs against avian influenza virus and found that avian-specific RNAi constructs against avian influenza virus did not increase the efficiency of RNAi inhibition.

4. IN demonstrated that IBDV large segment gene-based DNA can elicit specific immune response and provide protection of broiler chickens with maternally derived antibody against infection challenge.

5. OH developed NA- and NS-based DIVA vaccine strains using traditional reassortment as well as reverse genetics methods against H3N2 influenza in turkeys. The reassortant DIVA vaccines significantly reduced challenge virus shedding in the oviduct of breeder turkeys as well as trachea and cloaca of both young and old breeder turkeys, suggesting that proper vaccination could effectively prevent egg production drop and potential viral contamination of eggs in infected turkeys.

6. SERPL demonstrated that H7 AI vaccine may not protect against intercontinental H7 field viruses and vaccine may need to be from the same H7 lineage as field viruses to provide protection. In addition, turkeys vaccinated with commercial H1N1 vaccine have a low chance of being protected against swine-origin H1N1 infection.

7. SEPRL developed a model system for NDV vaccination which mimic egg production losses seen in Asia and Mexico in vaccinated poultry were developed and this system will allow the comparison of vaccines.

8. DE are utilizing both traditional and recombinant-based approaches for the construction of the next generation of ILTV live vaccines.

9. OH used in vitro analysis of virus particle subpopulations in candidate live-attenuated influenza vaccines which could distinguish effective from ineffective vaccines.

10. SEPRL showed that intranasal administration of alpha interferon reduced morbidity associated with low pathogenic avian influenza virus infection.

11. SEPRL demonstrated that commercial influenza vaccines have variable efficacy for protecting chickens and ducks against H5N1 highly pathogenic avian influenza (HPAI) viruses.

12. 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.

13. CT developed nanoparticle based vaccines carrying M2e of influenza virus and demonstrated the immunogenicity and protection induced by M2e-based vaccine by challenge studies.

14. 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.

15. IN showed that DNA vaccination confers protection against IBDV challenge by delayed appearance and rapid clearance of the invading viruses.

16. 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.

17. 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.

18. AL evaluated protection conferred by mucosal vaccination with replication competent adenovirus (RCA)-free recombinant adenovirus expressing a codon-optimized avian influenza (AI) H5 gene from A/turkey/WI/68 (AdTW68.H5ck).

19. AL developed a DNA vaccine consisted of the entire HA gene of an AIV H1N1 subtype (A/bluewinged teal/ AL/167/2007) cloned into the eukaryotic expression vector. The immunological responses induced by DNA vaccine against AIV were also investigated.

20. AR made comparison of ILTV genome sequences of two US CEO vaccines.

21. CT evaluated the level of protection of M2e-nanopartle based vaccine using quantitative real time PCR at 4, 6, and 8 days post-challenge with H5N2 LPAI by measuring virus shedding from trachea and cloaca.

22. IN conducted studies to determine if the combination of chicken calreticulin (CRT) gene and infectious bursal disease virus (IBDV) large segment (VP243) gene in a fusion gene or a chimeric DNA could enhance protection against IBD by DNA vaccination.

23. MN Correlated between virulence and MDR in avian E. coli and characterized the biology of the emergent IncA/C plasmid group.

24. OH in collaboration with the University of Cincinnati utilized flexible norovirus P particle as a novel influenza vaccine platform in vitro analysis of virus particle subpopulations in candidate live-attenuated influenza vaccines which could distinguish effective from ineffective vaccines.

25. SEPRL showed that a single vaccination can protect ducks and geese from avian influenza virus if the virus and vaccine are related. Reduction of pandemic H1N1 avian influenza growth with use of chicken interferon was investigated.

26. SEPRL generated and evaluated a bivalent vaccine against avian metapneumovirus and Newcastle disease viral diseases.

27. AL developed a transgenic plant vaccine against avian influenza.

28. CT generated throughput gene sequence data of IBV field isolates from the commercial poultry flocks vaccine with various IBV vaccines.

29. DE generated a new generation ILT vaccine containing deletions in essential genes.

30. GA examined the dynamics of IBV vaccination and protecting poultry against Arkansas field strains of IBV. GA developed mutant vaccine against infectious laryngotracheitis infection.

31. IN investigated the infectious bursal disease kinetics using DNA vaccine in chickens.

32. SEPRL performed vaccine efficacy studies using circulating AI viruses from Vietnam.

33. SEPRL developed new vaccine platforms to control and prevent avian influenza outbreaks.

34. SEPRL determined the AI vaccine efficacy following vaccination with recombinant herpesvirus of turkey-vectored avian influenza vaccine against highly pathogenic H5N1 challenge.

35. IN characterized the role of the chicken melanoma differentiation-associated gene 5 in innate immune response to IBDV infection.

36. OH developed an universal flu vaccine using influenza matrix protein 2 (M2e-P) particle platform in chicken.

37. OH showed that multivalent virus-like particle vaccine protects against classic and variant IBDV infections.

38. DE performed vaccination-challenge trial to evaluate the efficacy of current vaccines against infectious bronchitis virus challenge of DMV/2392/12 and found that Shore-Bron-D and Mildvac-GA-98 provided the best protection.

39. SEPRL studied vaccine protection of poultry against the 2012 H7N3 highly pathogenic avian influenza virus currently circulating in Mexico and demonstrated that several available vaccine viruses can provide complete protection of poultry from clinical disease but not infection.

40. SEPRL showed that maternal antibody to avian influenza virus suppresses the immune response to viral vectored vaccines to avian influenza virus.

41. SEPRL examined the level of cross reactive immunity in a live recombinant avian influenza vaccine (H5 subtype) against the heterologous H7N3 HPAI from Mexico. Following challenge with a lethal dose of H7N3 HPAI all birds vaccinated with the recombinant H5 vaccine died. However, cross reactive cellular immunity was observed in H5 vaccinated birds.

42. SEPRL studied the effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses and revealed that it was possible to significantly decrease viral replication and shedding with high levels of antibodies and those levels could be more easily reached with vaccines formulated with NDV of the same genotype as the challenge viruses.

IMPACT STATEMENTS

1/1. 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.

1/2. 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

1/3. 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.

1/4. 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.

1/5. Avian influenza subtype H5 and H7 were negative from the LBM and domestic poultry birds in New England states and in Delaware commercial farms. However wild birds do carry H5 subtypes in their population.

1/6. Infectious laryngotracheitis virus and infectious bronchitis viruses circulating in commercial broiler chickens flocks in Delaware.

1/7. 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.

1/8. 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.

1/9. 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.

1/10. Independent evolution of M-gene of avian influenza in the absence of any antiviral drugs leading to mutations causing resistance indicating the need for continued active surveillance of AIVs.

2/1. 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.

2/2. 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.

2/3. A new diagnostic tests developed for ILTV, AIV and avian adenoviruses using loopmediated isothermal amplification (LAMP) techniques will be faster, specific, sensitive and cost effective will not require sophisticated equipment.

2/4. Multiplex microsphere assay for detection of avian influenza viruses provides a rapid tool to identify multiple avian influenza types in the same sample.

2/5. Development of faster high-throughput serological assays for avian influenza (AI) that can complement a vaccination strategy to allow the rapid identification of infected flocks within large populations of vaccinated poultry. Identification of infected flocks is critical for control of AI outbreaks especially when vaccines are used.

2/6. Successfully developed 19-plex assay which can differentiate different HA subtypes of avian influenza viruses. With the multiplex capacity and feasibility of the assay, the multiplex branched DNA assay has a great potential in influenza research in addition to rapid diagnosis.

2/7. Study validated that the use of glycoprotein specific ELISAs as a tool to discriminate ILTV sero-conversion due to vaccination from infection. This work involves the serological differentiation of vaccinated and field virus exposed chickens which is critical for controlling ILTV epidemics.

2/8. 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.

2/9. 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.

2/10. Next generation sequencing technologies permit the relatively rapid determination of the primary sequence of the ILTV genome from egg-passaged material.

3/1. 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.

3/2. Molecular epidemiology reinforces the importance of surveillance for MG and MS isolates in poultry for the control of avian mycoplasmas.

3/3. The sequence data has allowed the improvement of the current diagnostic tests for NDV to ensure that the circulating viruses can be diagnosed.

3/4. Utilization of next generation sequencing technologies now permits the relatively rapid determination of the primary sequence of the ILTV genome.

3/5. The egg internal and external quality was negatively affected in hens inseminated with semen containing IBV. These results provide experimental evidence for IBV venereal transmission.

3/6. Chickens infected with IBV and co-infected with CAV+IBDV will generate genetic diversity in IBV. This finding constitutes further evidence for phenotypic drift occurring mainly as a result of selection.

3/7. Examine and compare gammacorona virus genomes for recombination, comparison data indicate that reticulate evolutionary change due to recombination in IBV, likely plays a major role in the origin and adaptation of the virus leading to new genetic types and strains of the virus. These

data constitute a significant step forward in identifying pathogenicity genes in avian coronavirus infectious bronchitis.

3/8. In vitro expression of avian pathogenic Escherichia coli (APEC) genes. This genome-wide analysis provides novel insight into processes that are important to the pathogenesis of APEC O1. Overall, these results indicate that a number of novel APEC virulence factors exist in APEC O1 that mediate systemic infection in the chicken host.

3/9. It was confirmed the susceptibility of both juvenile and layer turkeys to swine influenza viruses (SIVs) while the viruses replicated more efficiently in the reproductive tract of turkey hens compared to respiratory or digestive tracts.

3/10. An increase in pathogenicity of AI in ducks observed with H5N1 HPAI viruses has implications for the control of the disease since vaccinated ducks infected with highly virulent strains shed more viruses and for longer periods of time, perpetuating the virus in the environment and increasing the possibility of transmission to susceptible birds.

3/11. Determining the unique sequences for chicken embryo origin (CEO) vaccines will enhance our ability to control the re-emerging epidemics ILTV in commercial chickens caused by CEO-related vaccines.

3/12. Evidence is mounting that IncA/C plasmids are widespread among enteric bacteria of production animals and these emergent plasmids have flexibility in their acquisition of MDR-encoding modules, necessitating further study to understand the evolutionary mechanisms involved in their dissemination and stability in bacterial populations.

3/13. 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.

3/14. 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.

3/15. 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.

3/16. 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.

3/17. 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.

3/18. 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.

3/19. 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.

3/20. 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.

3/21. 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).

3/22. The cross reactivity between the co-circulating H5N1 strains may not be adequate for protection against each other and it is recommended to test vaccines that contain isolates from different antigenic groups in experimental infection trials for the selection of vaccine seed strain.
3/23. vvIBDV can be present but unrecognized in commercial poultry flocks for prolonged

periods. These factors emphasize the need for continued active surveillance in the field.

3/24. Persistence of IBDV antigen causing a persistent infection in bursal tissues of SPF chicken is highly significant in the elucidation of virus pathogenesis, immunology and epidemiology and it raises questions that need to be answered in the future studies.

3/25. Chickens and quail likely played a critical role of virus spread from poultry to humans in the Chinese H7N9 avian influenza outbreak and supported the control program in poultry to eliminate the human health risk.

3/26. Early expression of IFN- γ had a significant protective role against the effects of highly virulent NDV infection in chickens, and further suggests that the level and timing of expression of this cytokine may be critical for the disease outcome.

4/1. 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.

4/2. A high titer of ILTV vaccine is required for a prompt neutralizing immune response. Thus, vaccine fractionation would seem counterproductive.

4/3. Monitoring the ability of infectious bursal disease virus (IBDV) to break through maternal immunity in young broiler chickens is important to assess the immunosuppressive potential of the viruses.

4/4. 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.

4/5. 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.

4/6. 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.

4/7. 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.

4/8. A high titer of ILTV vaccine is required for a prompt neutralizing immune response. Thus, vaccine fractionation would seem counterproductive.

4/9. Poor vaccination against IBV infection contributes to the emergence of new IBV strains via mutation and/or selection. Under these conditions only IBV virus populations identical to the challenge virus was identified. From a broad perspective it indicates that selection is an important force driving IBV evolution.

4/10. Studies indicate the ability of vvIBDV to infect chickens is not affected by maternal immunity to IBDV strains typically found in commercial U.S. chickens. However maternal immunity did reduce the severity of the clinical signs and macroscopic lesions. These data suggest vvIBDV might be infecting chickens in California and other regions of the U.S. but they are going unnoticed because maternal immunity affects the clinical picture which does not include mortality and macroscopic lesions typical of a vvIBDV infection.

4/11. Data indicated that activated T cells may be involved in antiviral immunity and mediation of virus clearance from the bursa and spleen of IBDV-infected chickens. The findings of this study will help understanding the role of T cells in the pathogenesis of IBDV and designing effective control strategies against this immunosuppressive viral disease of chickens.

4/12. Further comparison of US CEO vaccines to several ILTV genome sequences revealed that US CEO vaccines are genetically distinct from the two Australian-origin CEO vaccines, SA2 and A20, which showed close similarity. This information can be used to discriminate between vaccine ILTV strains and further, to identify newly emerging mutant strains of field isolates.

4/13. Preliminary studies suggest that the self-assembling polypeptide nanoparticle shows promise as a potential platform for a development of a universal vaccine against avian influenza type A.

4/14. It was shown that recombinant vaccines against ILTV provide some protection but do not prevent shedding, which can lead to continued spread of the virus, whereas the chicken embryo origin vaccine protected against both disease and virus shedding. This study is extremely important in the control of ILTV especially in the face of an outbreak.

4/15. IBDV large segment gene-based DNA can elicit specific immune response and provide protection of specific-pathogen-free and broiler chickens against infection challenge. The impact is that IBDV large segment gene-based DNA vaccine has the potential for practical application in providing protection of chickens against IBD in the poultry industry.

4/16. Studies demonstrate that chicken interferon is biologically active against the pandemic H1N1 virus, is active in other avian species, and may be useful as therapy against avian influenza infection.

4/17. Potential bivalent recombinant vaccine candidate for NDV and aMPV was safe, stable and provided a complete protection against virulent NDV challenge and decreased the aMPV disease severity following experimental aMPV-C infection in turkeys.

4/18. Method of delivery of Ark vaccines fully protects broilers. This is important for control of IBV Ark type viruses in the field.

4/19. 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.

4/20. 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

4/21. Serious concern for the control of H5N1 in Vietnam must consider the important role of domestic ducks in the epidemiology of H5N1 HPAI

4/22. 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.

4/23. 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.

4/24. Chicken MDA5-related innate immunity has the potential for practical application to combat IBDV infection in the poultry industry by its antiviral activity and amplification of adaptive immunity.

4/25. Multivalent virus-like proteins expressed in baculovirus maintained the antigenic integrity of the variant and classic viruses and have the potential to serve as a multivalent vaccine for use in breeder flock vaccination programs.

4/26. The fact that maternal antibody to avian influenza virus suppresses the immune response to viral vectored vaccines to avian influenza virus shows that the new generation of vaccines does not resolve the vaccine suppression issue.

4/27. Effective levels of humoral antibodies against NDV could be increased by (1) increasing the homology of the vaccine to the challenge virus, or (2) allowing optimal time for the development of the immune response.

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118. Ibrahim M, Eladl A, Sultan H, Arafa A, Rahman S, El-Azm K, Gaballah A, Saif YM, Lee CW. Antigenic Analysis of Different Sublineages of Avian Influenza H5N1 Viruses Circulating in Egypt. 150th AVMA Annual Convention. July 20–23, 2013. St. Louis, Chicago.

119. Rauf A, Vlasova A, Murgia M, Jung K, and Saif YM. Transition from acute to persistent infection: antigen persistence and cytotoxic T cells response in infectious bursal disease virus infected SPF chickens. Journal of Immunology, 2013, 190, 173.18. The American Association of Immunologists Annual meeting Honolulu, Hawaii, May 3-7, 2013.

120. Rauf A, Murgia M, Rodriguez-Palacios A, Lee CW, Saif YM. Persistence and distribution of Infectious bursal disease virus antigen in chickens.). 64th North Central Avian Disease Conference. March 11-12. 2013. St. Paul, Minnesota.

121. Jackwood DJ and Stoute ST. The antigenic characteristics of very virulent infectious bursal disease viruses. Southern Conference on Avian Diseases. Abstr T90. 2013.

FUNDING

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.

 Mazhar Khan (CoPI) with Ion Mandiou (PI), Racheal ONeal (CoPI) University of Connecticut,: Alex (CoPI) Gerogia Tech. USDA- NIFA-Bioinformatic. (\$425,000), 2010- 2012.
 Joe Giambrone (PI) \$20,000. Development of an edible transgenic plant vaccine against avian influenza virus. Alabama Agriculture Experiment Station Initiative grant

PATENTS

Golovan, S. P., Abrahamyan A., E. Nagy, Bedecarrats, G. Design of conservative and highly efficient anti-influenza short hairpin RNAs and microRNAs functional in avian and mammalian cells. Preliminary submission 2009. Patent application filed.

December 8, 2013 Meeting Minutes:

2013 ANNUAL NC1180, "CONTROL OF EMERGING AND RE-EMERGING POULTRY RESPIRATORY DISEASES IN THE UNITED STATES" MEETING MINUTES, DECEMBER 8, 2013

MEETING CALLED TO ORDER AT 8:00AM.

THE ANNUAL NC 1180 BUSINESS MEETING WAS HELD ON SUNDAY, DECEMBER 8, 2013 AT MARRIOT HOTEL, CHICAGO, IL. DR. LASZLO ZSAK, CHAIR OF NC 1180 OPENED THE MEETING AT 8:00 AM. HE WELCOMED THE STATION REPRESENTATIVES, PARTICIPATING SCIENTISTS. THE NAMES LISTED BELOW ARE THE STATION ATTENDEES.

STATE	STATION REPRES	ENTATIVES	PARTICIPATING
<u>SCIENTISTS</u>			
CONNECTICUT	MAZHAR KHAN		
DELAWARE		CALVIN KE	EELER, ERIN BRANNICK
INDIANA	TSANG LONG LIN		
ILLINOIS	ELIZABETH DRIS	KELL	
MINNESOTA	TIMOTHY JOHNSON		
OHIO	CHANG-WON LEE	MO SAIF	
SEPRL-USDA	LASZLO ZSAK	DAV	ID SUAREZ
IOWA	DARRELL TRAMPEL		

DISCUSSION 1. MEETING LOCATIONS.

FUTURE LOCATIONS FOR THE NC-1180 MEETING WERE DISCUSSED. MAJOR CONCERNS PRESENTED WERE ACCOMMODATING FOR PARTICIPANTS IN THE SOUTHERN US AND THE GENERAL FORMAT OF THE MEETING. SEVERAL IDEAS FOR LOCATION WERE PROPOSED, AND NARROWED TO 1) HOLDING THE MEETING DURING THE USAHA CONFERENCE EACH YEAR, WHICH ALTERNATES LOCATIONS AND WILL BE HELD IN KANSAS CITY NEXT YEAR; 2) HOLDING THE MEETING ANNUALLY IN ATHENS OR ATLANTA, GA; AND 3) ROTATING THE MEETING AMONG PARTICIPATING INSTITUTION SITES.

IT WAS MENTIONED THAT OPTION #3 MIGHT BE DIFFICULT BECAUSE OF THE REMOTE LOCATION OF MANY INSTITUTIONS AND DIFFICULTIES WITH TRAVEL. OPTIONS #1 AND #2 WERE BOTH CONSIDERED, AND THE CONSENSUS AT THE MEETING WAS THAT OPTION #1 WAS THE BEST CHOICE. IT WAS AGREED TO CONSULT WITH MEMBERS NOT PRESENT TO DETERMINE THE BEST FUTURE OPTIONS. HOLDING THE MEETING CONCURRENT TO ACVP WAS ALSO RETAINED AS A POSSIBLE OPTION. THE USAHA WAS STILL CONSIDERED DESIRABLE BECAUSE IT HAS A DEDICATED POULTRY SECTION IN THE MEETING ITSELF.

MEETING FORMAT WAS ALSO DISCUSSED. IT WAS AGREED THAT FUTURE MEETINGS SHOULD BE MORE INTERACTIVE IN NATURE AND INVOLVE BRIEF POWERPOINT PRESENTATIONS BY EACH PARTICIPATING GROUP INVOLVING RESEARCH DATA. THIS WILL FACILITATE BETTER INTERACTIVE DISCUSSION. ALSO, PROVIDING SKYPE OR TELECONFERENCE CAPABILITY FOR THOSE UNABLE TO ATTEND WAS PROPOSED.

DISCUSSION 2. NC-1180 RENEWAL. THE RENEWAL WAS SUBMITTED BEFORE DEC. 1ST, IT IS CURRENTLY UNDER ADMINISTRATIVE REVIEW.

DISCUSSION 3. ANNUAL REPORT WITHIN 60 DAYS OF MEETING. BECAUSE THIS IS OUR FINAL PROJECT YEAR, THE ANNUAL REPORT CAN BE COMBINED AS THE CUMULATIVE 5-YEAR REPORT. EACH PARTICIPANT IS REQUESTED TO SUBMIT 1-2 SENTENCE OUTCOMES AND 1-2 SENTENCE IMPACTS FOR THE 5 YEARS OF THE PROJECT. PEER-REVIEWED PAPERS SHOULD ALSO BE INCLUDED WITH NO SPACE LIMIT. WE WILL SEND AN EMAIL TO PARTICIPANTS REQUESTING THIS DOCUMENT. IT IS CRITICAL TO KNOW IMPACT, PUBLICATIONS, COLLABORATIVE WORK, AND FUNDING FOR THE GROUP.

DISCUSSION 4. STATION REPORTS.

THE ANNUAL PROGRESS REPORT FROM ALL STATIONS BEGAN IMMEDIATELY AFTER THE BUSINESS MEETING. THE MEMBERS WERE ACTIVELY ENGAGED IN THE DISCUSSIONS ON SURVEILLANCE, PATHOGENESIS, NEW DIAGNOSTICS TOOLS AND VACCINE/IMMUNOLOGY OF VARIOUS POULTRY RESPIRATORY AND IMMUNOSUPPRESSIVE DISEASES AND INFORMATION AND IDEAS WERE FREELY COMMUNICATED AND EXCHANGED.

THE ANNUAL PROGRESS MEETING ADJOURNED AT 4:00 PM, DECEMBER 8, 2013.

RESPECTFULLY SUBMITTED,

TIMOTHY JOHNSON, PHD SECRETARY, NATIONAL COMMITTEE 1180

DECEMBER 8, 2013