NC1180 MULTISTATE PROJECT ANNUAL REPORT 2023

Tittle: Control of endemic, emerging, and re-emerging poultry respiratory diseases
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Period the Report Covers: (November 1st, 2022 – August 1^{4th}, 2023)
Reporting stations: AL, CA, GA, DE, IA, IL, MD, MN, NE, and SEPRL (USDA)
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Brief summary of minutes of annual meeting: It should be noted that the annual meeting was held earlier than previous years due to the impending project renewal. The meeting began with a Zoom meeting whereby, recognized experts from the poultry sectors of commercial broiler chickens, laying chickens and turkeys provided valuable information on the most critical research priorities in their respective poultry fields. This was the first time the NC1180 group has solicited stakeholder input at their annual meeting and this event proved invaluable for developing the project renewal. Following the stakeholder input meeting there was discussion on the project renewal process followed by station reports.

ACCOMPLISHMENTS:

OBJECTIVE 1 - *Investigate the ecology of poultry respiratory diseases and their role in poultry flocks.*

Bacterial diseases- **CA** in collaboration with **IA** and **MD** have established a genotyping assay for *Avibacterium paragallinarum (A.P.),* the causative agent of infectious coryza (IC), different genotypes were established along the three main serotypes (A, B and C). Results indicated that current strains causing outbreaks of the disease in the U.S. include a genotype cluster within serotype C strains and few serotype C strains that share genotype sequences with serotype B strains. **IA** has conducted passive surveillance of layer flocks for A.P. using qPCR and found positive A.P. samples that on culture the bacteria presented nontypical (nt) colonies. Full genome sequence of the nt A.P. isolates indicated the absence of some virulence factors. **MN** studied the ecology of avian colibacillosis and found that strains of avian *E. coli* colonizing the gut are not representative of those found dominating clinical disease. **MD** gathered antimicrobial resistant (AMR) profiles and full genome sequences of *Ornithobacterium rhinotracheale* (ORT) isolates from commercial poultry in the U.S. This study revealed a significant rise in ORT antimicrobial-resistant, and ORT AMR profiles not necessarily correlate with genotypes. Viral diseases- **CA** conducted prevalence studies of avian hepatitis E virus (aHEV) and found that the spread of the virus is facilitated by the stress of production. Currently circulating aHEV genotypes include classical strains and viral genotypes not previously detected in the U. S. **GA and DE** have conducted active surveillance for IBV. **GA** IBV surveillance revealed wide differences in the level of infection and replication of the vaccine strains among vaccinated flocks and differences in protection against circulating non-vaccine origin strains in vaccinated flocks. In layer and breeder flocks' new viral genotypes were frequently found. **DE** reported a high number of ILTV cases from February to early July of 2023. A total of 450 positive ILTV cases were detected by PCR and 44 were confirmed by histopathology. Outbreak related

viruses were not genotyped. Other infections were reported together with ILTV such as IBV, ORT and E. coli. **SEPRL (EEAVD)** conducted sequence analysis of the North American (NA) H7 lineage viruses from wild birds and compared the HA sequences to H7 sequences from poultry viruses. Compared to wild birds H7 viruses, the H7 poultry viruses tended to contain minor genetic groups, suggesting either a biased sampling for AIVs in wild birds or a tendency of having reassortment with minor genetic groups prior to the virus's introduction to poultry.

OBJECTIVE 2- *Develop new and improved diagnostic tools for poultry respiratory diseases.*

AL is developing a protein histochemistry assay to determine changes in the tropism and virulence of newly emerging NDV strains. **GA** has developed new multiplex amplification and Nanopore sequencing genotyping assays for ILTV and IBV. The novel ILTV assay provided more accurate discrimination among ILTV genotypes of currently circulating viruses. The IBV new genotyping assay allowed the detection of multiple IBVs in a single sample, including IBVs of previously unknown genotype. **SEPRL (EEAD)** has developed a platform to sequence total RNA using a random sequencing approach with rapid and precise characterization of pathogens to facilitate the identification of multiple pathogens in multifactorial respiratory diseases cases. **IA** is in the process to optimize and validate a one-step Nanopore sequencing assay for the rapid identification and genetic characterization of poultry viral agents IBV, AI and virulent NDV directly from clinical samples. Utilizing a combination of plasmid and clone-specific markers **MN** has developed an avian pathogenic *Escherichia coli* (APEC) typing scheme which effectively differentiate between strains of higher and lower virulence. **IL** has developed several monoclonal antibodies that target chicken complement receptors which will facilitate the study of virus interactions with the complement system. **NE** has developed a whole blood assay for assessing innate immune response based on the level of the IL6 protein expression after LPS stimulation for rapid evaluation of vaccines, vaccine adjuvants, and vaccine programs responses.

OBJECTIVE 3– *Elucidate the pathogenesis of poultry respiratory diseases.* **AL** did a comprehensive study to show that IBV ARK-type viruses associated with outbreaks of the disease in broilers emerged through the selection of vaccine subpopulations and/or naturally occurring recombination events. Moreover, this study showed that although variant strains emerged from ARKDPI vaccination, this vaccine no longer induced effective protection against these variants. **AL** performed comparative whole genome analysis of specific pathogen free (SPF) chickens with high or low viral load of ARK-type virulent virus. Two genes in chicken chromosome 18 appear to be associated with IBV genome load differences. The role of these two genes will be further studied. **AL** is evaluating the expression of innate immune genes, the antibody responses in serum (systemic) and tears (local), and cell responses in the Harderian gland (HG) induced by vaccination with La Sota NDV vaccine. Similarly, **AL** is pursuing to identify the immune mechanisms behind AIV vaccines that elicit a rapid onset and broadly protective immunity. **CA** is studying the role of passive immunity (maternal antibodies) in preventing the development of chronic microscopic lesions in kidneys and oviducts of mature layers caused by the early exposure to IBV. Findings indicate that without adequate maternal antibodies, early IBV vaccination or challenge will cause microscopic lesions that could increase the incidence of disease throughout the life of the bird. **GA** is studying the expression of interferons (IFNs) and interferon stimulated genes (ISGs) induced by ILTV live vaccines and virulent to determine how viral strains with different degrees of virulence modulate IFN responses. **IL** has identified that the glycoprotein C (gC) gene of MDV is essential for viral transmission and that gC of a virulent MDV strain can compensate for gC function on MD vaccine. Swapping MDV vaccine gC for MD gC may enhance MDV-specific immunity without affecting vaccine replication and spread. **IL** obtained expression levels of MDV genes at the transcript and protein levels in the skin of infected chickens. Future work will resolve with genes are relevant for MDV transmission. **MD** studied the effect that IBDV infection plays in the adaptation and shedding of a mallard AI virus when inoculated into groups of chickens infected and non-infected with IBDV. IBDV exposure prolonged the shedding of the mallard AIV in chickens and increased the population diversity of the mallard AIV but did not contribute to the virus adaptation to the chicken host. **SEPRL (EEAVD)** conducted a study to determine the potential role of mallards as efficient reservoirs to amplify and disseminate the H5N1 clade 2.3.4.4b highly pathogenic avian influenza viruses (HPAIVs). Infection appeared to be subclinical in most ducks, but high virus infectivity and transmission were observed which highlights the potential role of mallards as efficient reservoirs to amplify and disseminate H5N1 viruses. **SEPRL (ENAVD)** had identified novel ILTV transcripts encoding viral immuno-modulators. The plan is to generate

knock-out mutants which lacks these homologous viral proteins and to study their pathogenesis in chickens.

OBJECTIVE 4- *Develop new prevention and control strategies for poultry respiratory diseases.* Viral Vaccines- **AL** developed a vaccination strategy for IBV were a recombinant NDV La Sota co-expressing the trimeric spike ectodomain (SE) of the ARK-DPI and the chicken granulocyte-macrophage colony-stimulating factor (GMCSF) (rLS/Ark.Se.GMCSF) was co-administered with the Massachusetts (Mass) live vaccine. The vaccine combination had cross-protective ability against heterologous challenge. **SEPRL (ENAVD)** are evaluating three vaccine platforms for *in ovo* delivery. One platform utilizes NDV La Sota strain as a vector. This construct can be safely administered in ovo and it was shown to induced significant protection against MDV when expressing MDV gB. The second vaccine platform utilize MDV serotype II strain as a vector to share expression of multiple antigens when co-administered with HVT. The third vaccine platform are mRNA-based vaccines expressing immunogens of ILTV and IBV. **SEPRL (EEAVD)** tested the protection efficacy of four available AIV H5N1 vaccines, three inactivated and one an RNA particle vaccine, against highly pathogenic Clade 2.3.4.4b virus in chickens. This study confirmed the efficacy of currently available H5N1 vaccines and assessed the potential application of serologic test to differentiate infected (after vaccination) from non-infected or vaccinated animals. **SEPRL (EEAVD)** tested the adjuvant potential of ODN-1826 and Imiquimod, agonist of pathogen recognition receptors, to increase efficacy of Newcastle disease virus vaccines. Vaccinated + adjuvant groups of chickens had higher and more uniform antibody titers and challenge virus shedding was reduced indicating that agonist of pathogen recognition receptors are effective mucosal vaccine enhancers. Bacterial Vaccines**- CA** assessed the efficacy of a commercially available trivalent inactivated vaccine against infectious coryza (IC) that contains three A.P. serovars. Chickens were protected against clinical signs and reduction of bacteria shedding was detected. Treatments- **CA** studied the protective effects of IBV passive immunity by investigating various methods to administered (spray, in-ovo injection) IBV hyperimmune serum to day-of-age chickens. Administration of antibodies via spray at day of age was effective in reducing clinical signs and viral shedding post challenge. This treatment may prevent long-term reproductive effects of early IBV infection. Biosecurity-Education-Outreach programs. **NE** continue the use of the "Big Red Biosecurity Program" outreach efforts provided information on how to improve biosecurity to avoid the introduction of Avian Influenza. **MD** has established an extension program to facilitate passive and active control of HPAI outbreaks. The group perform biosecurity compliance audits and implement risk-based planning to improve outbreak responses.

IMPACTS: Understanding the epidemiology of respiratory diseases in the U.S., through surveillance, mapping, and genetic characterization strategies is crucial to establish successful prevention and control strategies including vaccination, disease management, and biosecurity. The research accomplished in this project has allowed laboratories across the U.S. to implement new approaches to detect and type bacterial and viral pathogens affecting poultry and to establish novel approaches to study immune responses to these pathogens. These new assays are streamlining diagnostics procedures and facilitating research by providing a better understanding of the host-pathogen interaction. Understanding how vaccines allow emergence of new variants, how maternal antibodies can ameliorate chronic disease, how immunosuppressive diseases of poultry amplify H5N1 HPAI infections, and the identification of viral genes that contribute to viral transmission or immune evasion are mayor contributions of this project in the control of respiratory diseases of poultry. Knowledge on the nature of early antiviral and adaptive protective immune responses induced by vaccination is fundamental for the future design of vaccines against these diseases. Prevention and control of respiratory diseases of poultry require simultaneous strategies including robust biosecurity, effective education programs, development of safer more effective novel vaccines and optimal use of current vaccines. The NC1180 project contributes with innovative solutions in these areas that have a strong impact in the health and production of poultry.

PUBLICATIONS

** Names in bold denote collaborations between members of the NC1180 group.

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