NC1180 MULTISTATE PROJECT ANNUAL REPORT 2022

Multistate Project NC-1180
Control of endemic, emerging and re-emerging poultry respiratory diseases
Period the Report Covers: (September 30th 2021 – October 1st, 2022)
Reporting stations: AL, CA, CT, GA, DE, IA, IL, MD, NE, and SEPRL (USDA)

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Accomplishments

OBJECTIVE 1 - *INVESTIGATE THE ECOLOGY OF POULTRY RESPIRATORY DISEASES AND THEIR ROLE IN POULTRY FLOCKS.*

Infectious bronchitis virus (IBV). Multiple stations (CA, GA, DE) are actively surveilling IBV in broiler, broiler-breeder, and layer flocks in all major poultry producing regions of the country using real-time RT-PCR or traditional RT-PCR with sequencing. As in previous years, vaccine strains were commonly detected, and the DMV/1639 strain was the predominant variant detected in the East. Cal/1737 and Cal3099 were the most common variants detected in California. CA reported analyzing full genome sequences of DMV/1639 type IBV in comparison to the QX type IBV which has also spread rapidly across the world.
Avian Influenza. CT, DE, MN, and IA reported on their surveillance efforts on AI in backyard, auction, wild, and commercial birds in their regions and globally. HPAI was detected in all populations tested at differing frequencies. SEPRL reported on transmissibility studies of LPAI in live bird markets over a 7-year period (2013-2019).
Newcastle disease virus. SEPRL reported detecting a VG/GA like lentogenic virus in a backyard chicken that had a unique F gene sequence that resembled a virulent NDV F. This finding highlighted the potential for false positives on F-gene real-time RT-PCR test designed to only detect virulent viruses.
Infectious laryngotracheitis virus. Diagnostic numbers were shared by DE, emphasis was given in combined detection of respiratory pathogens.
Mycoplasma. MD reported their characterization of MG and MS isolates using multi-locus sequencing tools (MLST). In total, almost 700 isolates and 600 alleles were sequenced for database deposition. The isolates were global in origin.
Bacterial pathogens. MN reported work to understand the ecology of avian *E. coli* in response to using a commercial *E. coli* vaccine. IA reported on atypical infectious coryza presentations and a potentially different *Avibacterium paragallinarum* lacking *Hmtp210* and *CTR*d genes. The same group is also researching the role of ORT in respiratory problems in Turkeys, undertaking a space-time study to evaluate the overall prevalence of ORT. MD and IA have been collaborating in genotyping strategies for mycoplasmas MG and MS using MLST.

IMPACT OBJECTIVE 1: Understanding the epidemiology of respiratory diseases in the US, through surveillance, mapping, and genetic characterization strategies is crucial to establish successful prevention and control strategies including vaccination, management, and biosecurity.

OBJECTIVE 2- *DEVELOP NEW AND IMPROVED DIAGNOSTIC TOOLS FOR POULTRY RESPIRATORY DISEASES.*

Bacteriology. CA reported developing a novel molecular genotyping assay for *Avibacterium paragallinarum* targeting two portions of the HMTp210 gene, the first of which classifies all strains into A, B, C-1, C-2, C-3, and C-4, and a second portion that classifies the serogroup A strains into serovars A-1, A-2, A-3, and A-4. MN reported revising the scheme for the identification of high-risk avian pathogenic *E. coli* (APEC) clones. MD in collaboration with OH, DE, and IA have developed MLST strategies to type *Avibacterium paragallinarum*, *Pasteurella multocida*, and *Ornithobacterium rhinotracheale*.
Virology. SEPRL and GA have been working on a new sampling strategy for multiple housing types after foreign animal disease outbreaks using cotton gauze instead of swabs, and sampling at bird level and on high-touch surfaces. GA is developing a novel ILTV genotyping assay covering the entire Unique Short (US) region of the ILTV genome using a multiplex PCR protocol followed by amplicon sequencing using the Nanopore platform. IA reported on studies to develop a single sequencing reaction to identify common poultry respiratory pathogens (IBV, AI, NDV) using the Nanopore system.
Immunology. IL reported on cloning and characterization of chicken complement and complement receptors, which are used in viral binding to respiratory tissues, and are analyzing these from different lines of chickens. NE reported developing a whole blood method for analyzing immune responses in chickens by measuring IL-6 in LPS stimulated whole blood but noted that several more factors need to be assessed before the assay would be ready for routine diagnostic use.
IMPACT OBJECTIVE 2: Laboratories across the U.S. are researching new approaches to detect and type bacterial and viral pathogens affecting poultry, as well as analyze the immune response to the pathogens in infected birds. The new tests are streamlining diagnostics and simplifying research. They also allow better understanding of the acting pathogens to create better prevention and controlled strategies.

OBJECTIVE 3– *ELUCIDATE THE PATHOGENESIS OF POULTRY RESPIRATORY DISEASES.*

Infectious bronchitis virus (IBV): AL found very distinct pathogenicity patterns between the ArkDPI vaccine, vaccine subpopulations, and ArkDPI/Mass natural recombinant virus. AL studied the resistance of specific-pathogen-free (SPF) white leghorn chickens to IBV and found that instead of a Gaussian distribution the data accumulated towards higher resistance, also it was found that viral load was significant higher in males than female, but whole genome sequencing of the host suggested limited genetic basis for the wide variation of IBV genome load in SPF white leghorn chickens. GA traced IBV spike protein (S1) gene mutations of new serotype to obtain an understanding of the S1 protein architecture which will help to identify potential cross-protective epitopes for development of more universal IBV vaccines. NE obtained evidence of antibody dependence enhancement (ADE) occurring for IBV infection in an egg embryo model. CA found that IBV maternal antibodies delay respiratory signs and reduce the incidence of oviductal atrophy associated with False lay syndrome (FLS) and found a significant association between IBV infection and male chicken infertility.
Avian Influenza virus (AIV): SEPRL confirmed the high susceptibility of turkeys to both low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI). Also, confirmed that different to Gulls, transmission of AIV in Mallards is through fecal-oral shedding which underscore contamination of water sources in the introduction of AIV to poultry. Finally, transition of H7N9 virus from LPAI to HPAI required mutations in multiple gene segments. MD evaluated the contribution of infectious bursal disease virus (IBDV)-mediated immunosuppression on the replication, shedding and pathogenicity of a mallard LPAI virus in chickens and found that IBDV infection prolonged the shedding of the mallard LPAIV suggesting that immunocompromised flocks can be a factor that contributes to the spread of AIV in poultry.

Avian herpesviruses: Marek's Disease virus (MDV), Herpesvirus of turkeys (HVT) & Infectious laryngotracheitis virus (ILTV). IL investigated the importance of the viral glycoprotein C (gC) gene of different avian herpesvirus (HVT & ILTV) in transmission of MDV and found that the MDV gC can be replaced by the HVT gC and reduce MDV shedding of MDV from the feather follicle cells, but the ILTV gC did not compensated for HVT or MDV gC virus transmission function. IL also discover that MDV conserved herpesvirus protein kinase (CHPK) is important for virus transmission. Therefore, this viral protein can be target for future MDV vaccine design. GA found that ILTV CEO vaccine and virulent strains downregulate the expression of Type I interferon (IFN) in the conjunctiva after eye drop inoculation. Future identification and modification of viral genes that antagonize the type I IFN response will allow to develop better attenuated ILTV vaccines.

IMPACT OBJECTIVE 3. Substantial advances were made to better understand the emergence and host resistance aspects of IBV variants. The adaptation process of waterfowl avian influenza to poultry and effects of viral immunosuppression on AIV shedding. Overall, these new findings provide novel information on how to better control these diseases that are a continuous threat for the poultry industry.

OBJECTIVE 4- *DEVELOP NEW PREVENTION AND CONTROL STRATEGIES FOR POULTRY RESPIRATORY DISEASES.*

Novel vaccines for poultry- AL developed a vaccination strategy regime 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 protected against regionally circulating IBV variant strains. CT developed a mRNA vaccine of the IBV S1 protein formulated with cationic BSA-polyamine nanocomplex. SEPRL developed a NDV La Sota vector vaccine expressing the MDV glycoprotein B (gB) that induced significant protection against MDV-induced tumors. The rLS/MDV-gB vaccine is a cell free MDV vaccine that does not need liquid nitrogen storage. SEPRL & GA also started the production of methodology to generate capped polyadenylated transcripts of ILTV gB and IBV S1 protein to develop mRNA-based vaccines.

Evaluation of commercially available poultry vaccines- IA evaluated a vaccination program against *Mycoplasma gallisepticum* (MG) in layers combining the recombinant fowl pox MG vaccine (FP-MG; Ceva Animal Health) and the live F strain MG vaccine (Elanco). GA found that IBV vaccination together with maternally derived antibodies were the most effective tools to prevent false layer syndrome.

Treatments against respiratory diseases of poultry- NE demonstrated that NDV serum and egg yolk antibodies can be nebulized without losing their neutralization function. Nebulization of antibodies may be a feasible affordable treatment for several respiratory diseases of poultry.

Biosecurity-Education-Outreach programs. NE continue the use of the "Big Red Biosecurity Program" website that provides information and training modules for poultry producers. This year the web site and outreach efforts of NE were very active providing information on how to improve biosecurity to avoid the introduction of Avian Influenza. CA developed a game fowl wellness program with the goal to educate game fowl owners on disease prevention, biosecurity, management, nutrition, and vaccination to prevent outbreaks of NDV and HPAI.

IMPACT OBJECTIVE 4. Prevention and control of respiratory diseases of poultry require to be tackle simultaneously with robust biosecurity, effective education programs, development of safer more effective novel vaccines and optimal use of current vaccines. The NC1180 project contribute with innovative solutions in these areas that have a strong impact in the health and production of poultry.

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