**NC1180 MULTISTATE PROJECT ANNUAL REPORT 2025**

**Tittle: Control of endemic, emerging, and re-emerging poultry diseases**

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**Secretary: Ramon Zegpi, The Ohio State University**

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**Period the Report Covers:** (August 08, 2024 – July 28, 2025)

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**Summary of Annual Meeting**. We held the NC1180 2025 meeting July 28 at the Hilton Downtown Portland, OR, one day before the yearly meeting of the American Association of Avian Pathologists (AAAP). Presenting stations were SEPRL, GA, IA, MD, CA, and AL. In addition, Dr. Tamer Sharafeldin presented for SD and Dr. Sofia Egana Labrin for MS. Both have expressed interest in joining the project. Presenters were given the choice to either deliver a traditional station report or talk about a specific topic in more depth; most presenters opted for the former. There were about 20 participants in person and 5 participants online. After the presentations, ideas for future meetings were discussed. These included the importance of NC1180 for AAAP and how it can be improved. AAAP should be made more aware of NC1180 activities, and a mini-symposium was mentioned as an option. There was broad agreement that next year’s meeting should be adjacent to the AAAP meeting again. Dr. Ruediger Hauck, the previous secretary, succeeded Dr. Maricarmen García as chair of the group. The group voted and appointed Dr. Ramon Zegpi as the new secretary.

**OBJECTIVE 1** - *Investigate the ecology of infectious diseases of poultry*

*Viral diseases:* **GA and IA** investigated genotypes of infectious laryngotracheitis virus (ILTV) in poultry, finding that genotype VI, not vaccine-related strains, is the dominant cause of outbreaks. Broiler breeders may act as reservoirs for this genotype. **DE** found that respiratory and enteric diseases in Delaware, Maryland, and Virginia (Delmarva) poultry are multifactorial, often involving infectious bronchitis virus (IBV), Newcastle Disease virus (NDV), and immunosuppressive agents. NDV strains were similar to vaccine strains but not identical. **AL** conducted NDV surveillance in wild and international birds, developing a new sequencing protocol to improve data quality and successfully sequencing 96 NDV isolates. **AL and SEPRL EEAVD – USDA** sequenced NDV samples from South American countries to monitor emerging variants. **DE** began establishing protocols to study low-pathogenic avian influenza virus (LPAIV) in poultry, detecting highly pathogenic avian influenza virus (HPAIV) in various bird populations across Delmarva. **DE** continued surveillance at live poultry auctions with no detections of avian influenza during the reporting period. **CA** conducted IBV surveillance, identifying new variant strains linked to vaccine use and forming a “new lineage 27,” with ongoing genome sequencing and challenge studies planned. **CA** also monitored avian reoviruses (ARV) in broilers, finding a shift in dominant genotypic clusters and suggesting autogenous vaccines may be reducing viral load and disease prevalence. **MD** characterized infectious bursal disease virus (IBDV) strains in Delmarva, identifying antigenic drift variants that may evade vaccine-induced immunity, emphasizing the need for continued surveillance. **IA** established a challenge model for avian metapneumovirus subtype A in turkeys, identifying effective doses for future vaccine testing. *Bacterial diseases*: **GA** studied Focal Ulcerative Dermatitis Syndrome (FUDS) in layer hens and found that *Staphylococcus agnetis*, not *S. hycus*, is the causative agent, highlighting the value of genome sequencing in diagnostics. **GA** analyzed *Salmonella* serovar patterns in poultry, revealing that serovar Kentucky often excludes others due to growth advantages and vaccination pressures, suggesting feed as a source for cattle-associated serovars. **IA** investigated non-pathogenic *Avibacterium paragallinarum* (npAP) isolates, confirming they are non-pathogenic and do not protect against classical strains, but may be candidates for genetically modified vaccines. **MD and IA** developed MLST schemes for *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Avibacterium paragallinarum*, creating global databases that support epidemiological studies and disease control strategies.

**OBJECTIVE 2** -*Develop new and improved diagnostic tools for infectious diseases in poultry*

*Viral diseases*: **IL and MD** cloned and characterized chicken complement proteins and receptors, identifying potential interactions with Marek’s disease virus glycoprotein C. This work may improve understanding of viral transmission and inform vaccine development. **IA and** **SEPRL EEAVD – USDA** developed and validated Oxford Nanopore Technology sequencing as a point-of-care diagnostic tool for HPAI and virulent NDV (vNDV) directly from clinical samples, improving extraction protocols and creating real-time analysis software to enhance outbreak response. **DE** detected vaccine-related GA08 strains of IBV but no novel variants, indicating minor evolution post-vaccine use. **DE** also observed a declining ILTV prevalence, likely due to widespread vaccination, with recent detections linked to past outbreaks. In addition, **DE** monitored avian metapneumovirus subtypes A, B, and C, finding no new detections and confirming genetic similarity to previously reported strains. **CA** investigated avian hepatitis E outbreaks in layer flocks, identifying genotypes 2 and 3 and fulfilling Koch’s postulates, advancing understanding of its pathobiology and diagnostics. **MD** developed a structural comparison tool for IBDV capsid proteins, showing that structural distance correlates better with antigenic relationships than genetic distance, improving vaccine antigen selection. *Bacterial diseases*: **IA** conducted comparative genomics of pathogenic and non-pathogenic *Avibacterium paragallinarum*, identifying genetic differences that may explain reduced virulence and inform development of modified live vaccines. **CA** developed a simplified genotyping method for *Avibacterium paragallinarum* based on the HMTp210 gene, improving diagnostics and vaccine strain selection for infectious coryza. **MD** benchmarked metagenomic tools for detecting foodborne pathogens, recommending Kraken2/Bracken for broad-spectrum detection and highlighting tool-specific strengths for different food matrices. *Parasitic diseases*: **AL** used multilocus sequence typing to differentiate *Eimeria maxima* field isolates from vaccine strains, finding that vaccines do not always replace field variants, which has implications for coccidia vaccine strategies.

**OBJECTIVE 3** - *Elucidate host pathogen interactions of infectious diseases in poultry*

*Viral diseases*: **GA and SEPRL ENAVD – USDA** discovered a viral interleukin-4 homolog in ILTV that contributes to virulence. Deleting this gene reduced pathogenicity, suggesting potential for vaccine improvement. **IL and GA** investigated glycoprotein C’s role in avian herpesvirus transmission, showing it is essential for HVT transmission in turkeys and that Marek’s disease virus gC can substitute for vaccine gC, potentially enhancing immunity. SEPRL ENAVD – USDA used comparative RNA sequencing to show distinct immune responses in resistant vs. susceptible chickens during latent MDV infection. **SEPRL ENAVD – USDA** also identified allele-specific gene expression in immune cells from Marek’s disease-resistant and susceptible chickens, revealing genetic mechanisms linked to resistance. **SEPRL ENAVD-USDA** studied the tumor suppressor gene Ikaros in Marek’s disease virus, showing that wild-type Ikaros reduces tumor formation while mutated forms increase virulence, though both still cause immunosuppression. **CA** found that maternal antibodies protect against false layer syndrome (FLS) caused by IBV, and early vaccination without maternal antibodies may induce reproductive issues. **MD** studied virus factories (VFs) in IBDV replication, discovering that VP3 protein drives liquid-liquid phase separation, which may be modulated to attenuate the virus for vaccine development. **IN** showed that IBDV infection enhances inflammatory cytokine expression in chickens co-infected with *Avibacterium paragallinarum*, suggesting increased severity of infectious coryza. **MD** used chicken and turkey intestinal organoids to study ARV pathogenesis, showing strain-specific effects on gut cells despite similar replication levels. **MD** found that ARV induces lipid droplet formation and oxidative stress, possibly hijacking lipid metabolism to prolong cell life and enhance replication. **AL** studied transcriptomic responses to ARV in chicken embryos, finding organ-specific antiviral responses and identifying key immune pathways. **AL** also performed metabolomic profiling of ARV-infected embryos, identifying altered metabolic pathways consistent with transcriptomic findings. **AL and SEPRL EEAVD – USDA** investigated how the immune system influences susceptibility or resistance to low pathogenicity avian influenza virus (LPAIV) by studying H9 subtype strains in various primary chicken cell cultures. The findings revealed strain- and tissue-specific replication patterns, with turkey-derived H9TK showing the highest replication and immune activation, particularly in tracheal cells, while IL-8 emerged as a key positive immune marker and TNF-α showed tissue-dependent effects, offering insights into host–pathogen interactions and potential immune predictors. **AL** analyzed gene expression in chicks vaccinated with NDV LaSota, V4 and Hitchner B1, identifying common and unique immune-related differentially expressed genes (DEGs) across organs, which may serve as biomarkers for vaccine efficacy. **AL and SEPRL EEAVD – USDA** developed a histochemistry assay using recombinant HN protein to predict the pathogenic potential of NDV, improving the understanding of tissue binding and virulence. *Bacterial diseases*: **GA** evaluated *Mycoplasma synoviae* outbreaks in NE Georgia, finding increased virulence and confirmed vertical and horizontal transmission, suggesting broilers contribute to spread and require enhanced surveillance.

**OBJECTIVE 4** - *Develop control and prevention strategies for infectious diseases of poultry*

*Viral diseases*: **GA** developed alphavirus-based replicon vaccines for avian influenza, aiming to reduce production costs while maintaining efficacy. These vaccines amplify mRNA within host cells, allowing lower doses and sustained antigen expression. **GA and SEPRL EEAVD – USDA** created a reassortment-impaired, non-transmissible modified live vaccine for H9N2 avian influenza, incorporating a unique peptide marker and chicken IL-18. It showed strong immunogenicity and safety in chickens. **MD** created a non-replicative adenovirus-vectored vaccine H5 that induced broad cross-clade immunity in chickens. **SEPRL ENAVD-USDA** evaluated two recombinant herpesvirus of turkeys (HVT) vaccines against H5N1 HPAI, finding both highly effective, with COBRA-HVT offering slightly better virus shedding reduction than the 2.2-HVT vaccine. The ELLA-NI test showed promise for distinguishing infected from vaccinated birds and was more sensitive than ELISA. **CA** tested delivery hyperimmune serum against IBV to chicks to mimic maternal antibodies. Spray application reduced clinical signs and pathology, though viral load remained unaffected. **AL** showed that maternally derived antibodies against NDV interfere with systemic but not mucosal immune responses in birds vaccinated at one day of age. **CA** showed that adjustments to NDV vaccination programs to live + killed or live + recombinant strategies outperform live-only approaches. **AL** tested four APMV isolates for adaptation and immune response in chickens. One isolate matched NDV LaSota in immunogenicity. *Bacterial diseases*: **GA** investigated the emerging disease Spotty Liver Disease (SLD) in laying hens, caused by *Campylobacter hepaticus*. They successfully sequenced the pathogen’s genome, developed diagnostic tools, and replicated the disease in challenged birds. Despite testing various interventions, only vaccination showed consistent protection. **MD** employed reverse vaccinology for poultry bacterial diseases. Multi-epitope vaccines for *Clostridium perfringens* and *Salmonella* Infantis were designed using computational tools. *Across different pathogens*: **GA** evaluated how turning angles during egg incubation affect hatchability, chick quality, and maternal antibody transfer. Optimal angles improved all metrics, especially ARV antibody transfer. **MD** developed a web-based biosecurity compliance audits tool to assess biosecurity compliance and support outbreak response.

**IMPACT**

The present report of NC1180 with the title "Control of endemic, emerging, and re-emerging poultry diseases" documents the broad efforts of the group in disease surveillance, development of diagnostic assays, molecular characterization of pathogenesis, and development of new vaccines. Not surprisingly, avian influenza (AI) was one of the most investigated diseases. Especially worth mentioning is that AI vaccine candidates were developed from three different stations, but there were also several surveillance projects development of improved diagnostic tools for AI. The inclusion of many non-respiratory diseases and pathogens like Marek’s disease, ARV and IBDV show that the group was successful in expanding the scope of the project and that the group has become an important place to discuss current basic and applied research about poultry diseases. In nine of the projects reported more than one station was involved, demonstrating the importance of the group for fostering collaborations. Finally, the long list of industry collaborations further shows the impact the work of the group has on poultry health.

**PUBLICATIONS**

**(in peer reviewed journals only)**

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