

▶ AAAP Symposium  
"Maintaining Intestinal Health  
with Non-Antibiotic Compounds"

● July 9 - 11, 2024

**aaap**  
AMERICAN ASSOCIATION OF AVIAN PATHOLOGISTS

# *Proceedings*



**American Association of Avian Pathologists (AAAP)**

AAAP will be the premier organization that advances science-based knowledge, expertise, and education on poultry health, welfare, and food safety to provide member value and stakeholder trust.

**Sixty-Seventh Annual Meeting  
American Association of Avian Pathologists  
St. Louis Union Station Hotel  
July 9-11, 2024, St. Louis, MO**

# In-Person Oral Presentations

## Antimicrobial/Antibiotic Resistance

### **Efficacy of a Silane Quaternary Ammonia Compound (SiQAC) applied to feed trailers for inactivating low pathogenic avian influenza 15 days post-application**

JeJuan Bryant<sup>1</sup>, Ivan Ricardo Alvarado Ortiz<sup>2</sup>, Derald Holtkamp<sup>3</sup>, Jianqiang Zhang<sup>3</sup>, Emma Castillo<sup>2</sup>

NCSU-CVM Veterinary Student<sup>1</sup>, Merck Animal Health<sup>2</sup>, Iowa State University<sup>3</sup>

A Silane Quaternary Ammonia Compound (SiQAC) that forms an antimicrobial coating on a variety of surfaces was designed to be applied as a spray forming a transparent coating that can persist for as long as 90 days. The purpose of this study was to evaluate the efficacy of this SiQAC applied to feed delivery trailers to reduce the viral titer of low pathogenic avian influenza (LPAI) 15 days post-application. Three aluminum coupons per treatment group were adhered to the exterior of a feed truck trailer traveling between Des Moines, Iowa and Sandusky, Ohio. The treatment groups included 1: Treated with diluted (10%) SiQAC; 2: Untreated control for 10% group; 3: Treated with undiluted ready-to-use (RTU) SiQAC; 4: Untreated control for RTU group and 5: Negative control. Application of the SiQAC for groups 1 and 3 was completed using a handheld ultra fine mist sprayer at a rate of 500 square feet per gallon resulting in 1.262 mL of product per 10.2 by 15.2 centimeter coupon. Coupons were placed face-up on the feed trailer secured with double sided tape and metal cable seals and placed from the front of the trailer to the back. The order was blocked by replicate and randomly assigned using Excel. Fifteen days post-application of the SiQAC, the coupons were collected, and inoculated with LPAI in a lab hood. The titer of the LPAI virus used was  $10^8$  EID<sub>50</sub>/mL. Coupons from treatment groups 1 through 4 were contaminated with 2mL of LPAI, while treatment group 5 was sham contaminated with 2mL of BHI broth. After 3.5 hours of contact time, the surface of the coupon was eluted with 5mL BHI broth to recover the virus, and the fluid was collected for virus isolation and titration. Two aliquots of 2mL were placed in 5mL snap cap culture tubes and stored in a -80°C freezer until virus isolation and titration was conducted using embryonated chicken eggs. A Kruskal-Wallis rank sum test was performed to compare differences in the mean titers of each treatment group. It was determined that there were no statistical differences in the mean titers between the groups treated with the SiQAC and their respective untreated control groups.

### **Remediating the Poultry Litter Resistome**

John Maurer<sup>1</sup>, John J. Maurer<sup>2</sup>, Alexa Hoke<sup>3</sup>, Mark Williams<sup>4</sup>, Margie D. Lee<sup>5</sup>, Kesheva C. Das<sup>6</sup>

Virginia Tech<sup>1</sup>, School of Animal Sciences, Virginia Tech, Blacksburg, VA<sup>2</sup>, School of Animal Sciences, Virginia Tech, Blacksburg, VA<sup>3</sup>, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA<sup>4</sup>, Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA<sup>5</sup>, College of Engineering, University of Georgia, Athens, GA<sup>6</sup>

There is significant concern that agricultural use of antimicrobials leads to spill over of antimicrobial resistance (AMR) into the human population. While poultry litter contains an abundance of AMR genes and mobile genetic elements associated with its dissemination. Reduce this reservoir, and its potential to transmit AMR to pathogens is diminished. Four conditions: intermittent wet-dry, forced aeration, anaerobic, and static incubations were assessed for their impact on the community resistome of poultry litter. A litter sample was taken prior to the treatments and served as a control. qPCR was used to estimate the gene load of streptomycin-resistance, sulfonamide-resistance, and class 1 integron-integrase genes *aadA*, *sul1*, and *intI1* respectively in the poultry litter community. Streptomycin-resistance gene *aadA* was significantly reduced by the anaerobic incubation by 0.52 log<sub>10</sub> but increased with the

aerobic incubation by 0.41 log<sub>10</sub>. When levels of *aadA* were normalized to total bacterial genomes' following each treatment, the wet-dry and aerobic incubations significantly increased *aadA* copies per bacterial genome by 0.81 and 1.03 log<sub>10</sub> respectively. *sul1* copies per bacterial genome was significantly reduced by the static treatment by 0.63 log<sub>10</sub> ( $p < 0.01$ ). The anaerobic incubation had the most significant impact in reducing bacterial loads, *aadA*, and *sul1* per gram of litter. Changes observed in integron and associated antibiotic resistance gene abundance reflected fluctuations in litter microbiome composition at the family, genus and sequence variant level. While these poultry litter incubations failed to substantially reduced integron and associated antimicrobial resistance gene abundance, certain conditions did reduce pathogenic taxons. The approach to remediating resistance in poultry litter needs to be focused more on reducing bacterial pathogens than reducing the litter communities' resistome.

### **Phenotypic characterization of *Castellaniella* spp. associated with mortality events in commercial broiler breeders**

Tiffani Allen<sup>1</sup>, Yi-Chen Luo<sup>1</sup>, Jenny Nicholds<sup>1</sup>, Grazieli Maboni<sup>1</sup>

Poultry Diagnostic and Research Center, University of Georgia<sup>1</sup>

*Castellaniella* is a Gram-negative bacterium commonly isolated from soil. Before 2015, *Castellaniella* had not been associated with mortality in animals until reported as a causative agent of suppurative inflammatory disease in lagomorphs. Beginning in 2018, the Poultry Diagnostic and Research Center (PDRC), University of Georgia, repeatedly isolated *Castellaniella* from 19 cases in commercial broiler breeders. In all cases, a combination of swollen wattles, lameness, and/or mortality were observed, presenting similarly to chronic Fowl cholera caused by *Pasteurella multocida*. Despite this clinical presentation, isolates were identified as *Castellaniella* spp. via 16S rRNA gene sequencing. Prior to this study, no cases of *Castellaniella* have been documented in any poultry species. The purpose of this study is to provide a comprehensive phenotypic characterization of *Castellaniella* associated with clinical disease in chickens. Isolates were plated on Blood Agar and Lurio-Bertani (LB) agar to determine bacterial morphology. On both agar types, colonies were round, mucoid in texture, varying from small to large. Isolates were analyzed to determine the minimum inhibitory concentration (MIC) using the Sensititre™ Vet Avian AST plate. High MIC values were obtained for ceftiofur (18/18, MIC ≥4), clindamycin (18/18, MIC >4), erythromycin (18/18, MIC >4), novobiocin (18/18, MIC >4), penicillin (18/18, MIC ≥8), and tylosin (18/18, MIC >20), indicating antimicrobial resistance. Low MIC values were obtained for enrofloxacin (17/18, MIC ≤0.25), florfenicol (18/18, MIC ≤1), neomycin 18/18, MIC <2), spectinomycin (17/18, MIC ≤16), and tetracycline (18/18, MIC ≤4), indicating antimicrobial susceptibility. Biochemical analysis was conducted using VITEK 2 System GN ID Card which implements the use of carbon source utilization, inhibition and resistance, and enzymatic activities for bacterial identification. L-Proline Arylinidase (ProA), L-lactate alkalization (ILTAK), succinate alkalization (SUCT), and L-lactate assimilation (ILATa) were commonly used as carbon sources while L-MALATE assimilation (IMLTa) and Ellman (ELLM) were weakly utilized. Other studies reported biochemical analysis data of *Castellaniella* isolates using characteristics as carbon source utilization, sugar fermentation, and qualitative enzyme tests on a standard microdilution plate. ProA and IMLTa were also used carbon sources. This is the first known study documenting antimicrobial susceptibility profiles from clinical avian isolates. Based on our data, tetracyclines are recommended in treatment of clinical cases while penicillin should be avoided due to a potential mechanism of intrinsic resistance. With the emergence of drug resistant bacteria in veterinary medicine, increased pressure has arisen to decrease antimicrobial use. In conclusion, we provided data to guide veterinarians with proper, responsible selection and use of antimicrobial agents to maintain antimicrobial stewardship.

## **Avian Influenza**

### **Investigating the Prevalence of Highly Pathogenic Avian Influenza (HPAI) Virus Among Migratory Wild Shorebirds in Iowa**

Valerie Kim<sup>1</sup>, Maria Chaves<sup>1</sup>, Amro Hashish<sup>1</sup>, Mostafa Shelkamy<sup>1</sup>, Eman A. Gadu<sup>1</sup>, Yuko Sato<sup>1</sup>, Rachel Ruden<sup>1</sup>, Stephen J. Dinsmore<sup>1</sup>, Mia Kim Torchetti<sup>2</sup>, Mohamed El-Gazzar<sup>1</sup>

The role of wild migratory waterfowl as the primary source of introduction to domestic poultry is evident in this current Eurasian H5 Highly Pathogenic Avian Influenza (HPAI) outbreak. However, there is minimal available information on its prevalence in migratory wild shorebird populations that either nest in or pass through Iowa. This study aims to conduct surveillance to estimate the prevalence of HPAI in migratory wild shorebirds in Iowa. In this study, 184 birds were sampled from July 14th, 2022 through September 4th, 2023 including killdeer, ring-billed gull, herring gull, franklin's gull, pectoral sandpiper, least sandpiper, and spotted sandpiper by nest capturing and spotlighting methods. We collected a total of 909 samples; oropharyngeal (OP) and cloacal swabs, serum, fecal, and tissue (lung, trachea, intestine) samples. Swab samples were then tested for Influenza A virus (IAV) using real-time PCR (qPCR) targeting the conserved M gene. Meanwhile, the serum samples were screened for antibodies using a Multi-species Enzyme-Linked Immunosorbent Assay (ELISA). Notably, only one swab sample (1:177 = 0.5%), obtained from a pectoral sandpiper, yielded positive qPCR results (with a Cycle Threshold, CT value of 23). Additionally, one serum sample (1:131 = 0.76%) from a franklin's gull, also tested positive for ELISA. The qPCR positive sample was then submitted for subtyping using H5/H7 subtyping qPCR, however, the sample came back negative. Subsequently, cleavage site analysis confirmed it as LPAI (Low Pathogenic Avian Influenza) and Sanger sequencing classified this as a H4 subtype. Whole genome sequencing further classified the virus as H4N6 LPAI. This investigation lends support to the notion that although shorebirds may play a role in circulating and maintaining LPAI viruses, they have a minor role in transmitting HPAI and are unlikely to evolve into an endemic source of infection. However, testing more samples is still needed to confirm this finding.

#### **Viral Shedding and Environmental Dispersion of Clade 2.3.4.4B H5 High Pathogenicity Avian Influenza Virus in Ducks: Implications for Environmental Sampling in HPAI Surveillance**

Jean-Luc Guerin<sup>1</sup>, Fabien Filaire<sup>1</sup>, Pierre Bessiere<sup>1</sup>, Kateri Bertran<sup>2</sup>, Nicolas Gaide<sup>1</sup>, Rosa Valle<sup>2</sup>, Aurelie SEcula<sup>1</sup>, Albert Perlas<sup>2</sup>, Charlotte Foret-Lucas<sup>1</sup>, Miquel Nofrarias<sup>2</sup>

National Veterinary School of Toulouse, INRAE, University of Toulouse, FRANCE<sup>1</sup>, IRTA, CReSA, UAB, Bellaterra, Catalonia. SPAIN<sup>2</sup>

High pathogenicity avian influenza viruses (HPAIVs) have caused major epizootics in the last years with devastating consequences for poultry and wildlife worldwide. Domestic and wild ducks can be highly susceptible to HPAIVs, and infection leads to efficient viral replication and massive shedding (i.e., high titers for an extended time), contributing to widespread viral dissemination. Importantly, ducks are known to shed high amounts of virus in the earliest phase of infection, but the dynamics and impact of environmental contamination in the epidemiology of HPAIV outbreaks is poorly understood. In an experimental study, we monitored mule ducks experimentally-infected with two H5N8 clade 2.3.4.4b HPAIVs sampled in France in the 2016-2017 and 2020-2021 epizootics. We investigated viral shedding dynamics in oropharynx, cloaca, conjunctiva, and feathers; bird-to-bird viral transmission; and the role of the environment in viral spread and as sampling strategy for early detection and surveillance. Our findings confirmed that viral shedding started before the onset of clinical signs, i.e., as early as 1 day post-inoculation (dpi) or post-contact exposure, peaked at 4 dpi, and lasted for 14 dpi. Detection of viral RNA in aerosols, dust, and water samples mirrored viral shedding dynamics, and viral isolation from these environmental samples was successful all throughout the experiment. Our results confirmed that mule ducks can shed high HPAIV titers through various excretion routes while being asymptomatic, and that environmental sampling could be a non-invasive tool for early viral RNA detection in HPAIV infected farms. There are many possible lines of research to improve the efficiency of dust sampling: one of the main drawbacks is that dust may contain organic substances that can inhibit RT-qPCR reactions. To limit this inhibition, we tested the use of bovine serum albumin (BSA), a molecule known to facilitate DNA polymerization in the presence of numerous inhibitors, including those from feces, litter or food. Dust samples were collected on 107 farms localized in areas affected by epizootics of clade 2.3.4.4b HPAIV H5N1. We used a latent class modelling approach to evaluate the effect of the addition of BSA to the RT-qPCR reaction mix, or the

dilution of template RNA on RT-qPCR detection performance. Our results indicate that the addition of BSA to the RT-qPCR reaction mix improved significantly the sensitivity of the method. Our results suggest that the use of BSA could be routinely implemented in HPAIV dust monitoring RT-qPCR protocols. Altogether, these results, combined with previous field observations, suggest that dust sampling may represent a relevant alternative to tracheal or cloacal swabbing, as it is cheap, non-invasive for animals, simpler and quicker to carry out. Further research and validation are needed to implement dust sampling in the toolbox of HPAI surveillance.

### **Enhanced process to select avian influenza vaccines for protection against diverging field viruses**

David Swayne<sup>1</sup>, Erica Spackman<sup>2</sup>, Miria Criado<sup>3</sup>

Birdflu Veterinarian, Watkinsville, Georgia, USA<sup>1</sup>, US National Poultry Research Center, Agricultural Research Service, USDA, Athens, Georgia, USA<sup>2</sup>, Department of Pathbiology, College of Veterinary Medicine, Auburn University, Auburn, Alabama, USA<sup>3</sup>

In 2023, the global spread of H5N1 2.3.4.4b highly pathogenic avian influenza viruses (HPAIVs) and resulting panzootic in poultry has challenged countries to change control strategies. Multiple countries in Latin America began targeted vaccination as a preventative strategy in long-lived chickens and France in domestic ducks. Vaccines have been used to control H5 and H7 highly pathogenic avian influenza viruses (HPAIVs) in poultry since 1995 in various countries of Asia, Africa, Europe and North America where HPAI had become endemic, especially for various clades of H5 Goose/Guangdong (Gs/GD) Eurasian lineage of HPAIV. However, vaccine resistance has developed, requiring rapid change in seed strains to maintain field effectiveness. A process was developed, increasing the use of in vitro assays, to accelerate the vaccine seed selection process or to assess the effectiveness of existing vaccines. This process utilized Next Generation Sequencing to identify changes within the hemagglutinin gene that code for antigenic epitopes of field viruses compared to consensus strains, then followed by antigenic characterization of selected unique viruses with predefined standardized antisera to vaccine and reference strains. This in vitro process fast-tracked selection of targeted field viruses for final indirect and direct efficacy determination in a reduced number of chicken challenge groups. If the difference in antibody titer between the vaccine seed strain and predominant field strain is, or becomes, greater than  $4\log_2$  (1:16), changing the vaccine should be considered. Multiple countries also accelerated licensing by using efficacy data from other countries to demonstrate protective ability of vaccines.

### **The effect of inactivated vaccines on highly pathogenic avian influenza virus shedding**

Erica Spackman<sup>1</sup>, David Swayne<sup>2</sup>

US National Poultry Research Center, USDA-ARS<sup>1</sup>, Birdflu Veterinarian, LLC<sup>2</sup>

In addition to improving survival, vaccines for highly pathogenic avian influenza virus (HPAIV) can be distinguished in how they reduce virus shedding by the oral and cloacal routes. In challenge studies quantities of virus shed by vaccinated chickens is often compared to sham vaccinated control groups. However, because of rapid mortality in sham vaccinated chickens, data are generally limited to the first 2-3 days post challenge. When developing vaccine programs that will succeed in halting virus spread, understanding how different vaccines reduce virus shed is crucial to selecting the optimal vaccine. Here data from 51 published trials, each with a different vaccine and challenge virus, and which had comparable designs and archived data were compared for virus shed reduction among the vaccinated birds. Studies were included if chickens were vaccinated once for H5 or H7 HPAIV and challenged 3-4 weeks later with a dose of  $5.5-7\log_{10}$  50% egg infectious doses. Factors that impacted shed reduction were not always clear, although challenge virus similarity to the vaccine and antibody titers (as a measure of vaccine immunogenicity) had some positive predictive value. More precision is needed to identify how distant an antigen can be and still be effective. Adjuvants appeared to have an effect, but there was insufficient data for definitive results. Further studies are needed to identify which epitopes are critical for limiting virus infection and more data are needed in poultry species other than chickens.

## **Cross-Protection Study of Inactivated H5N1 2.3.2.1c Vaccine against Novel H5N1 2.3.4.4b Virus Circulating in Indonesia**

Elly Setiawaty<sup>1</sup>, Melina Jonas<sup>1</sup>, Aprilla Sahesti<sup>1</sup>, Wahyu Haryanto<sup>1</sup>, Tini Prihartini<sup>1</sup>, Christina Arum<sup>1</sup>, Eko Hidayat<sup>1</sup>

PT Medion Farma Jaya<sup>1</sup>

Background AIV was first detected in Indonesia in 2003. The government policy is to prevent disease by vaccination. H5N1 and H9N2 are currently circulating. H5N1 2.3.2.1c were found in 2019. The new clade of H5N1 2.3.4.4b was detected in late 2022 in South Kalimantan. This study aims to compare the efficacy of the old vaccine strain (H5N1 2.3.2.1c) against the new field isolate from clade 2.3.4.4b using PD50 and cross-immunity tests. Materials & Method Fifty-five SPF chickens aged 4 weeks were divided into 11 groups. Five groups were vaccinated with H5N1 2.3.2.1c inactivated vaccine at doses 1, 1/5, 1/25, 1/125 and 1/625. Another five groups were vaccinated with H5N1 2.3.4.4 inactivated vaccine at the same dose variation. The remaining group was used as non-vaccinated control. All groups were challenged at 3 weeks post-vaccination with H5N1 2.3.4.4b. Tracheal and cloacal swabs were taken at 7 days post-challenge to detect viral shedding. Clinical symptoms and pathological changes were observed throughout the experiment. PD50 score was calculated using the Spearman-Kärber method. For the cross-immunity test, two groups containing ten SPF chickens aged 4 weeks were each vaccinated with H5N1 2.3.2.1c and 2.3.4.4 inactivated vaccines. Ten SPF chickens were used as non-vaccinated control. The HI titer was measured weekly using the antigens from H5N1 2.3.2.1c and 2.3.4.4. Result PD50 score of the H5N1 2.3.4.4b vaccine group was higher than that of the H5N1 2.3.2.1c (204 vs 11). Chickens vaccinated with H5N1 2.3.4.4b could fully protect against the H5N1 2.3.4.4b challenge until dose 1/25. Lethargy, fatigue and death were observed on a small number of chickens vaccinated at dose 1/125. Vaccination with H5N1 2.3.2.1c at 1 dose could protect against mortality but did not prevent viral shedding. More severe symptoms (loss of appetite, cyanotic comb, lethargy, fatigue and death) were observed in the H5N1 2.3.2.1c vaccine group at dose < 1/5. Pathological findings from this group also showed blood vessel dilatation, tracheal haemorrhage and swollen kidney. Chickens vaccinated with inactivated vaccines showed onset of immunity at 3 weeks post-vaccination with the HI titer of 8.2 log<sub>2</sub> for H5N1 2.3.4.4b and 7.5 log<sub>2</sub> for H5N1 2.3.2.1c. The HI titer remained high until the experiment ended at 8 weeks post-vaccination. H5N1 2.3.4.4b and 2.3.2.1c showed low cross-immunity. The difference in HI titer measured using the heterologous antigen in H5N1 2.3.4.4b vaccine group was 4.5 log<sub>2</sub> and in H5N1 2.3.2.1c vaccine group was 4 log<sub>2</sub>. Conclusion H5N1 2.3.2.1c inactivated vaccine could not provide full protection against the 2.3.4.4b challenge. There was no cross-immunity between H5N1 2.3.4.4b and 2.3.2.1c. The difference in HI titer tested using both antigens could be used in surveillance-based seroprevalence from chickens without H5N1 2.3.4.4b vaccination.

## **Application of enzyme linked lectin assay (ELLA) for differentiation of infected from vaccinated animals (DIVA) in poultry for avian influenza**

Sherif Ibrahim<sup>1</sup>, Erica Spackman<sup>1</sup>, David L Suarez<sup>1</sup>, Chang-won Lee<sup>1</sup>

South-East Poultry Research Laboratory (SEPRL), ARS-USDA<sup>1</sup>

Validation of a test for differentiating infected from vaccinated animals (DIVA) is required for use with vaccines to control H5N1 highly pathogenic avian influenza (HPAI) infection in order to differentiate vaccinated and infected from vaccinated but never infected birds. The present study aimed to develop, and bench validate an enzyme linked lectin assay (ELLA) for antibody to influenza neuraminidase subtype 1 (N1) as a DIVA test after vaccination with either vaccines only expressing the hemagglutinin or whole virus inactivated vaccines with a mismatched neuraminidase gene. Beta-propiolactone (BPL) inactivated A/flycatcher/CA/14875-1/94 (H7N1) – (FCH7N1) was used as a whole virus antigen to detect N1 antibody by ELLA (N1-ELLA) at an effective concentration of 98% neuraminidase (NA) activity when fetuin was used as a sialic acid rich substrate. To characterize the analytical specificity, less than 10% neuraminidase inhibition (NI) background was observed with influenza negative chicken, ducks, or turkey sera. Conducting the N1-ELLA with different N1 sera against A/turkey/turkey/1xPR8/2005, A/chicken/Egypt/102s-NLQP/2008, and A/AmWi/SC/22-345-001/2022, showed high linearity and precision over a wide range of sera

dilutions with high repeatability (<30% CV). Minimal cross-reactivity between the N1 antigen and sera generated against different NA subtypes was observed. The N1-ELLA using FCH7N1 was able to detect highly pathogenic H5N1 virus infection in birds vaccinated with an H5N9 inactivated whole virus vaccine or H5 recombinant vaccines. Overall, the N1-ELLA showed high specificity and sensitivity and has potential for application as a DIVA test with further validation.

### **An update on the assessment of the risk of moving washed and sanitized eggs off a highly pathogenic avian influenza (HPAI) positive premises**

Mickey Leonard<sup>1</sup>, Rosemary Marusak<sup>1</sup>, Carol Cardona<sup>1</sup>, Marie Culhane<sup>1</sup>, Peter Bonney<sup>1</sup>, Amos Ssematimba<sup>1</sup>, Kaitlyn St. Charles<sup>1</sup>, Emily Walz<sup>1</sup>, Sylvia Wanzala Martin<sup>1</sup>, Catherine Alexander<sup>1</sup>

University of Minnesota<sup>1</sup>

The University of Minnesota Secure Poultry Supply (SPS) team conducts risk assessments for continuity of business (COB) movements within and out of Control Areas during highly pathogenic avian influenza (HPAI) outbreaks. As the global poultry industry continues to grow and change, new opportunities to protect the food supply have been identified and, in tandem, new COB needs have arisen. To address these needs, the SPS team, in discussions with regulators and industry representatives, pursued assessing the risk of the movement of washed and sanitized eggs from a HPAI positive premises to an off-site location within or out of the Control Area without birds. Since COB movements off of a positive premises have historically been prohibited, we justify exploring this egg movement because: HPAI response costs are high, washed and sanitized egg movements from monitored premises are negligible risk, and destroying eggs laid prior to a positive diagnosis is removing animal protein from the food supply. In the summer of 2023, with funding from USDA National Animal Disease Preparedness and Response Program, the SPS team began work on a risk assessment of the movement of washed and sanitized eggs from an HPAI positive premises which were laid prior to a positive diagnosis and safely stored in coolers. A workgroup comprised of state and federal officials, industry representatives, academicians, and other allied industry personnel, meet regularly to assess ways that virus could contaminate clean eggs via on-farm spread, vehicles, equipment, and people, and subsequently spread virus to off-site susceptible poultry during egg product movement and delivery. While analysis is still underway, initial stages of the work have been completed. The workgroup provided background information, including general industry demographics, practices, and biosecurity pertaining to egg movement during routine operations. Then, entry (contamination) and exposure pathway components were defined. Pathways will include risk ratings for each of the following biosecurity levels: industry standard practices, Secure Egg Supply mitigations for monitored premises, and enhanced, targeted mitigations needed for a positive premises. Differentiation of these biosecurity levels accounts for variability in farm characteristics and practices across the industry. Additional considerations reviewed were temporal dynamics. On a positive premises, time plays a new role in risk of the movement as the virus load on the farm may increase until depopulation is complete, and depopulation activities could lead to cross-contamination of the eggs to be moved. Additionally, internal egg contamination is a necessary consideration when determining the safety of moving eggs. These time dynamics, along with the pathway analysis results, will be discussed with the workgroup to define the overall movement risk. Current work including biosecurity gap analysis and pathway risk likelihood ratings will be reported.

### **Walks like MSD, talks like MSD, think...?**

Kayla Niel<sup>1</sup>, Jonathan Elissa<sup>1</sup>, Megan Lighty<sup>1</sup>

Penn State University Animal Diagnostic Laboratory<sup>1</sup>

Three 28-week-old Ring-necked pheasants, one male and two females, were submitted to the diagnostic lab in December 2023, a few days prior to Christmas. The farm owner reported slightly elevated mortality over the previous four days in one flight pen out of 50 total pens on the premises. No other clinical signs were observed. The farm owner also mentioned that there had been an issue with the refrigerator that stored the marble spleen disease (MSD) vaccine that was administered to the affected birds during grow. On postmortem examination, all three birds had

adequate body condition and feather cover with crops and gizzards full of feed and feathers. Spleens were mildly to moderately enlarged with mottling and lungs were severely congested. Lung sections collected from all three birds sank in formalin. Few other significant gross lesions were observed. With the provided history, clinical signs, and gross lesions, the top differential diagnosis was MSD. MSD is a contagious disease of confinement-reared pheasants caused by an adenovirus, turkey adenovirus A (TAdV-3). It affects pheasants between 2-8 months of age and may cause mortality between 5-15% of the flock. Pheasants are typically found dead without any previous clinical signs. Tissue samples, including spleen and lung, were collected for histopathologic evaluation. Oropharyngeal swabs were also collected for avian influenza (AI) PCR per lab protocol for submissions >14 days of age with elevated mortality. AI matrix RRT-PCR came back early the next morning with a "NONNEG" result (Ct value 20.73). AI H5 HA subtype RRT-PCR and AI H5 2.3.4.4 RRT-PCR were then performed and were also "NONNEG", with Ct values of 22.93 and 19.18, respectively. These results were later confirmed by the National Veterinary Services Laboratory (NVSL). To date, there are few published reports in the literature of highly pathogenic avian influenza (HPAI) in gamebirds. The few case reports or studies available describe neurologic signs (ataxia, opisthotonos) as the predominant indicator of HPAI in pheasants, which were not reported in this case. This demonstrates the lack of current information and need for information sharing in these HPAI cases, particularly with respect to gamebirds. This case report will discuss the clinical presentation, diagnostics performed, and lessons learned.

### **Trials to evaluate safety and immunogenicity of HPAI vaccines for California condors**

David Suarez<sup>1</sup>, Henry Kariithi<sup>1</sup>

Agricultural Research Service<sup>1</sup>

The Mexican lineage H5N2 was first reported in 1994 and despite an intensive vaccination program the virus continues to circulate in Mexico and has spread into several other countries. A next generation sequencing program was developed to randomly amplify potentially any pathogen from clinical samples from FTA cards. Over a 3 year period of surveillance the H5N2 virus was detected from numerous respiratory samples. Analysis of the hemagglutinin protein demonstrated that the virus continues to evolve with two major sublineages. The different lineages were about 10% different in amino acid sequence, which suggests that a vaccine with a single antigen like would not provide good clinical protection. One lineage of H5N2 had a unique hemagglutinin cleavage with four basic amino acids, which has the potential of being highly pathogenic although increased mortality was not reported. The sequence divergence in the hemagglutinin also created issues with the original H5 real-time RT-PCR test, and new primers and probe had to be developed to reliably detect the virus. Analysis of the other genes do show some reassortment from the original introduced virus. The Mexican H5N2 lineage continues to be a concern for the poultry industry in the region. Vaccination is still commonly used, and knowledge of the circulating strains are necessary to effectively control the spread of infection.

### **Improvement of Oxford Nanopore Influenza A Whole Genome Sequencing Protocol**

Iryna Goraichuk<sup>1</sup>, Jacqueline Risalvato<sup>1</sup>, Mary Pantin-Jackwood<sup>1</sup>, David L Suarez<sup>1</sup>

USDA-ARS-USNPRC<sup>1</sup>

The high mutation rate of Influenza A, coupled with recurrent detection of avian influenza A in mammals, underscores the necessity of studying Single Nucleotide Polymorphisms (SNPs) to unravel potential markers of mammalian adaptation. Achieving a reliable SNP analysis demands obtaining complete genome sequences with a minimal threshold of read depth coverage. While the conservative termini of influenza allows simultaneous amplification of all 8 segments, the challenge lies in attaining sufficient read depth coverage, especially for the longer polymerase segments (PB2, PB1, PA). Sequencing reads distribution in these segments often exhibits higher coverage in the 5' and 3' termini but lower coverage in the middle region, possibly due to the amplification of shorter defective interfering particles (DIP). DIP, derived mainly from polymerase segments, shares conservative termini with complete segments but lacks a central part of the intermediate sequence, resulting in shorter lengths (ranging from 200 to 800 nt). In this study, we compared the established Oxford Nanopore ligation sequencing influenza



whole genome V14 protocol with an alternative set of primers and an alternative RT-PCR kit, aiming to enhance read depth coverage, particularly for polymerase segments. Additionally, we assessed four purification methods to optimize the removal of DIP and other short untargeted reads, such as dNTPs, primers, and other nonspecific products. Automation for the purification step was explored using the automated liquid handling KingFisher purification system, employing two different magnetic bead-based purification kits and comparing outcomes to manual use. The Nanopore sequencing libraries were prepared using the Native Barcoding Kit 24 V14 (SQK-NBD114.24) and sequenced on a single R10.4.1 flow cell (FLO-MIN114) using a MinION Mk1C. Sequencing was run until all pores of the flow cell were exhausted. After the sequencing run, Nanopore raw reads were basecalled, demultiplexed, and trimmed within the MinKNOW software on a MinION Mk1C instrument. Influenza genome consensus sequences were obtained by BWA-MEM mapping with reference genomes within the Galaxy platform. The refined protocol, incorporating the alternative RT-PCR kit together with the alternative set of primers, demonstrated a substantial increase not only in total influenza read sequences but, more notably, in polymerase segments. This enhancement together with the optimal purification kit, facilitates superior depth of coverage, which is crucial for accurate SNP analysis, especially in challenging polymerase regions. Furthermore, this optimized Nanopore protocol is applicable across different NGS platforms, offering flexibility in selecting the platform that best aligns with the specific experimental needs for comprehensive influenza whole-genome studies.

### **Development of optimized CRISPR/Cas13 transgenes for the control of avian influenza virus in chicken cells**

Karel Schat<sup>1</sup>, Emily JL Hann<sup>2</sup>, Debolina Majumdar<sup>2</sup>, Daniel Layton<sup>2</sup>, Mark Ziemann<sup>3</sup>, David M Cahill<sup>4</sup>, Arjun Challagulla<sup>2</sup>

Cornell University<sup>1</sup>, Australian Centre for Disease Preparedness, CSIRO Health and Biosecurity, Geelong, Vic, Australia<sup>2</sup>, Burnet Institute, Melbourne, Vic, Australia<sup>3</sup>, School of Life and Environmental Sciences, Deakin University, Geelong, Vic, Australia<sup>4</sup>

RNA-guided RNA-targeting endonucleases, such as CRISPR-Cas13, are increasingly used for transcriptome targeting and engineering, but their potential as antiviral strategies is still in its infancy. We have previously shown that CRISPR RNAs (crRNAs) against the PB1, NP and M genes of influenza A virus (IAV) and cloned in tandem to express from a single vector in chicken fibroblast DF-1 cells significantly reduced replication of WSN or PR8 IAV (Challagulla, Schat, Doran. *Methods Protoc* 4, 40. 2021). However, crRNA fidelity in view of mutations in AIV sequences and collateral activity exhibited by Cas13 warrants rigorous evaluation before it can be exploited for in vivo applications. In this study, we used a novel CRISPR/Cas13d system to target DsRed fluorescent mRNA in DF1 cells previously transfected with the DsRed gene. First, we stably introduced a Tol2 transposon vector carrying the Cas13d transgene into the genome of DF1 cells followed by analysis of DsRed knockdown by transfecting the cells with 28 nucleotides DsRed or non-specific crRNAs. Next, we designed multiple versions of DsRed crRNAs of varying lengths, introduced mismatches, and assessed their targeting efficiency. Multiple iterations of crRNA design revealed that Cas13d-targeting efficiency can vary greatly depending on the number of truncated nucleotides and the position of the mismatch introduced within the crRNA seed sequence. Finally, we confirmed collateral Cas13d activity using a two-fluorescent marker assay, in which we observed sequence-specific and collateral mRNA degradation. We are currently comparing an engineered version of Cas13d (Cas13-HF), which was shown to have reduced collateral activity compared to natural Cas13d. These data are important to develop optimized Cas13d transgenes and crRNAs to target economically important RNA pathogens, such as highly pathogenic strains of AIV.

## **Bacteriology**

### **Peeling Back the Many Layers of Competitive Exclusion**

Margie Lee<sup>1</sup>

Department of Biomedical Sciences and Pathobiology, Virginia Tech<sup>1</sup>

Baby chicks given a fecal transplant from adult chickens are resistant to Salmonella colonization by competitive pathogen exclusion. A two-pronged approach was used to investigate the mechanism of this process. First, fluorescent protein-tagged Salmonella Typhimurium was co-cultured with the competitive exclusion product Aviguard (nonpermissive community) or with cecal contents from a chicken with high Salmonella levels (permissive community) in a medium mimicking the nutrient availability of the cecal lumen. In this approach, jellyfish yellow and cyan fluorescent proteins (YFP and CFP) were used to monitor Salmonella growth rate and virulence in gene fusions with *rrn* and *hilA* operon, respectively, and total RNA was extracted after a 6-hour co-culture with either community or medium alone to investigate Salmonella transcriptomes. Second, Salmonella was orally administered to two-day-old chickens and the cecum harvested from birds up to 42 days post inoculation. DNA and RNA were extracted from the bacterial pellet obtained from the cecal contents, and qPCR of community DNA was used to estimate Salmonella abundance. In addition, community transcriptomics was performed by RNA-Seq. These experiments revealed that Salmonella growth was greatly reduced, and *hilA* expression repressed following just 6-hour co-culture with the nonpermissive community, Aviguard. Microarray analysis revealed Aviguard's repression of the SPI-1 type III secretion system, its ancillary effectors, and the *prm* operon responsible for LPS modification and resistance to defensins, while the anti-inflammatory gene *avrA* was upregulated. Propanediol utilization and associated vitamin B12 synthesis was central to Salmonella metabolism in the presence of either microbial community. There were significant metabolic disparities between Salmonella populations grown with the two different communities regarding sugar and amino acid catabolism, respiration, amino acid and nucleotide synthesis. However, no single or multiple metabolic gene knockouts severely impaired Salmonella growth in co-culture with the competitive exclusion product. Bayesian network analysis and Pearson and Spearman correlations uncovered significant differences in the stress response transcriptome between cecal communities with high and low Salmonella abundance. Antimicrobials were central to this stress response in the low Salmonella abundance community as identified in network connections and correlation coefficients for enzymes associated with polyketide synthesis or antimicrobial resistance. Overall, the mechanism of competitive exclusion is a complex community interaction that modulates pathogenic behavior and may exclude Salmonella through antagonism.

### **Differentiation of nonpathogenic from pathogenic *Enterococcus cecorum* isolates, identifying unique genetic characteristics providing virulence, survival, and adaptation traits**

Martha Pulido Landinez<sup>1</sup>, Marcela Arango<sup>1</sup>, Lifang Yan<sup>1</sup>, Jay Kay Thornton<sup>1</sup>, Roxana Sanchez-Ingunza<sup>2</sup>

Mississippi State University<sup>1</sup>, RSI Poultry Veterinary Consulting LLC<sup>2</sup>

Emerging pathogenic *Enterococcus cecorum* (EC) is causing important losses to the broiler industry worldwide because of the presentation of systemic disease, causing high mortality, and the increase in morbidity related mainly to leg problems leading to a bad productive performance. Also, two additional concerns have been reported: leg problems frequently associated with broilers' welfare, and reports of EC exhibiting high resistance to antibiotics. Consequently, the characterization of EC isolated from field cases is important to understand the evolution of this problem. During 2023, the Poultry Research and Diagnostic Laboratory of Mississippi State University identified a total of 348 cases of *Enterococcus* spp. Among these, 117 EC were isolated from broiler breeders, layer breeders, and broiler chickens. Phenotypical and genotypical analysis to differentiate pathogenic and commensal EC strains were performed detecting the presence of the *cpsO* gene (previously associated with pathogenicity) in 57 EC isolates. The objective of this study is to identify the presence of virulent genes performing WGS on 24 EC isolates previously identified as pathogenic, to compare to nonpathogenic and commensal EC. The criteria to select pathogenic EC isolates for WGS analysis will include a history of high mortality, identification at necropsy of severe systemic lesions in breeders and chickens, the report of high mortality, and the presence of the *cpsO* gene. Nonpathogenic and commensal EC will be selected among EC isolated from the intestine and lesions, being negative to the presence of the *cpsO* gene. *Enterococcus*' WGS will be performed following PRDL Molecular Lab procedures. The results of this study will provide insights into the differentiation of pathogenic and non-pathogenic EC and the identification of genes providing survival, virulence, and adaptation traits to EC present in the US poultry industry.

## **Genotypic characterization of *Enterococcus cecorum* isolated from field cases**

Marcela Arango<sup>1</sup>, Rebecca Mackey<sup>1</sup>, Jay Kay Thornton<sup>1</sup>, Natalie Manginsay<sup>1</sup>, Martha Pulido-Landinez<sup>1</sup>

Poultry Research and Diagnostic Laboratory (PRDL), Mississippi State University<sup>1</sup>

*Enterococcus cecorum* (EC) has been considered a commensal Gram-positive bacterium of the chicken's gastrointestinal tract. Recently, emerging pathogenic EC has been identified as a common cause of systemic disease and lameness in broilers. The presence of this EC on chicken farms negatively impacts the health and welfare of affected chickens and their productive performance, causing significant economic losses. In addition, some EC strains are reported to be highly resistant to antibiotics. Consequently, EC is currently one of the most important concerns of the poultry industry worldwide. During 2023, Mississippi State University's Poultry Research and Diagnostic Laboratory identified a total of 348 cases of *Enterococcus* spp. infection. These bacteria were isolated from broiler breeders, layer breeders, hatcheries, and broiler chicken cases. One hundred *Enterococcus cecorum* (EC) isolates were randomly selected to perform phenotypic and genotypic analyses to differentiate pathogenic and commensal strains. The presence of the *cpsO* gene (previously associated with pathogenicity) was confirmed in 57 isolates. Among these, 54 pathogenic EC isolates did not ferment mannitol. The goal of this study was to use phenotypic and genotypic methods to establish whether there are differences among pathogenic EC isolated from broiler breeder pullets, broiler breeders, and broiler chickens (n=57). Additionally, we aimed to analyze the phenotypic and genotypic characteristics of EC isolated from broilers (n=54), comparing these traits by age, site of isolation (septicemic lesions, skeletal lesions, and septicemic and skeletal lesions combined), presence of concomitant infections, and farm location, in order to establish the genetic relatedness of the studied isolates. The results of this study will provide insights into the dissemination of EC clones within and between different types of birds, lesions, and farm locations.

## ***Clostridium colinum* in quail: Battling to reduce the need for antibiotics use**

Roxana Sanchez-Ingunza<sup>1</sup>, John Tuck<sup>2</sup>

RSI Poultry Veterinary Consulting LLC<sup>1</sup>, Tuck Farms<sup>2</sup>

*Clostridium colinum* causes ulcerative enteritis (UE) in quail and have been associated with focal duodenal necrosis in egg layers. Litter removal, coccidiosis control, prevention of stress and immunosuppression are among the most important prevention procedures. The UE cases presented in this paper started post-feed change, from starter to grower feed, and up to 15 weeks of age. UE control was complicated by stressing conditions that occurred during rearing. *Coccidia* infection predisposed the birds to UE but an elevated mortality together with enteritis were not observed under a high coccidian challenge in birds raised in new houses with no previous history of UE. Historically, antibiotics have been used to reduce mortality. The use of non-antibiotics alternatives was based on the availability of products claimed to be effective against *Clostridium perfringens* in chickens. The potential use of specific probiotics/nutritional products in order to delay the presentation of the disease by maintaining gut health was investigated. The success in preventing the disease by this approach may require a continuous or pulse applications together with the concurrent application of more specific products targeting *C. colinum*. In conclusion, at the current time, a single tool to prevent/control UE is not available. Good husbandry and in-feed or water application of non-antibiotics products with intestinal and systemic effect against *C. colinum* are required. Additionally, the recovery of *C. perfringens* and *C. colinum* from intestinal lesions and its role in the clinical presentation and effectiveness of the prevention measures need to be rule-out.

## **Exploring Focal Duodenal Necrosis: Two Challenge Experiments Investigating Disease Replication in Layers**

Yuyang Tsai<sup>1</sup>, Julia Ienes Lima<sup>1</sup>, Klao Runcharoon<sup>1</sup>, Catherine M. Logue<sup>1</sup>, Daniel Antonio Sebastian Pineda<sup>1</sup>, Nicole Gottdenker<sup>1</sup>

Department of Population Health, College of Veterinary Medicine, The University of Georgia<sup>1</sup>

Focal duodenal necrosis (FDN) is an intestinal disease of table egg layers, which is considered one of the top five concerning diseases of the table egg layer industry. This challenge experiment attempts to reproduce the FDN disease and lesions in layers. The challenge strains include *E. coli* (EC), *Clostridium perfringens* (CP), and *Clostridium colinum* (CC). Sixty-six laying hens (Hy-line W-36) at 26 weeks of age and randomly divided into 5 groups as follow: treatment group (1) CP, (2) EC+CC and (3) CP+EC+CC; control group (1) PBS+customized feed and (2) PBS+commercial feed. All treatment groups and control group 1 received a corn-soy diet containing 10% DDGS, 5% protein meal, with 20% fine and 80% coarse limestone particles. The bacterial cocktails formulated at a concentration of 10<sup>8</sup>-10<sup>9</sup> cfu/mL. This daily oral challenge continued for 21 days. At day 4, 8, 11, 15, 18 and 22, three birds were selected then were euthanized and examined for any potential lesions present in the duodenum. Furthermore, the mucosal surface of the duodenum was subjected to aerobic and anaerobic culturing to detect the presence of potential pathogens. Duodenal samples were evaluated for the presence of gross lesions including mucosal hyperemia, red foci/patches, and mucosal erosion. Each of these lesions was scored 0 = not present, 1 = present. For histopathological lesion scores, duodenal samples were examined and scored by a pathologist using a blinding mechanism where no indication of challenge was indicated. Intestinal samples were evaluated for the presence of lymphoplasmacytic inflammation, heterophilic inflammation, hemorrhage, necrosis of enterocytes, cystic crypts and/or crypt necrosis, and inflammatory infiltrate in the lumen. Three gross lesions for the scoring system (mucosal hyperemia, red foci/patches, and mucosal erosion) were recorded in the challenged groups. The result of gross lesion scores indicated that treatment group 3 (*E. coli*, *Clostridium perfringens*, and *Clostridium colinum*) had the highest lesion score (1.67 pts) on average. Histopathological changes include mild to moderate number of lymphocytes and scant plasma cells, expansion of the lymphoid-associated tissue and lymphoid tissue hyperplasia. The lamina propria of some evaluated intestines showed mild infiltration by a number of lymphocytes and plasma cells, and some enterocytes were hypereosinophilic, with vacuolated cytoplasm and were exfoliated into the intestinal lumen. Lesion score for histopathology is still in the process. The microscopic lesions did not show characteristic FDN lesions suggesting that other predisposing factors may be involved in lesion development or other pathogens that still need to be determined to successfully reproduce the specific lesion for FDN.

#### **Assessment of water-based interventions for the control of *Campylobacter hepaticus* in laying hens.**

Catherine Logue<sup>1</sup>, Roel Becerra<sup>1</sup>, Charlize S. Nakatsu<sup>1</sup>

University of Georgia<sup>1</sup>

Spotty liver disease (SLD) caused by *Campylobacter hepaticus* has emerged as an important cause of disease and loss to the table egg industry in the United States. SLD results in focal liver lesions, reduced egg production, and mortality. Currently, there are no approved treatments, or commercial vaccine available for *C. hepaticus* control and limited research available on approaches to control this pathogen. This study assessed the effect of water delivered treatments (oregano, apple cider vinegar and citric acid) for the control of *C. hepaticus* in challenged Specific Pathogen Free (SPF) chickens and potential transmission of the organism to naïve birds. 148 SPF chickens, 17 weeks of age, were allocated into 5 groups where 4 groups (with 51% of the birds in each group orally challenged and 49% left naïve) and a negative control group (n=8) were orally challenged with *C. hepaticus* at a dose of 10<sup>7</sup> cfu/mL on days 1, 4, 7. At day 10 post challenge, 3 challenged groups were treated for 5 days via water with oregano, citric or apple cider vinegar. One challenged, non-treated group and the negative control group received water. Bile and liver samples from challenged and non-challenged, naïve exposed birds were collected at days 9, 15, 20, and 27 post challenge to evaluate the presence of *C. hepaticus* by culture and polymerase chain reaction (PCR). *C. hepaticus* was detected in challenged birds in each group (range 11-33% of samples were culture positive) compared to PCR analysis of liver samples where a greater number of positive samples were found (range 29-88% positive). The treatments failed to clear *C. hepaticus* from challenged birds. Greatest reductions in *C. hepaticus* prevalence were observed with oregano (33-44% of challenged birds were positive post treatment) compared to the positive control group (64-88%) or birds treated with apple cider vinegar (59-83%) or citric acid (65-88%). >29% of naïve birds tested positive for *C. hepaticus* demonstrating horizontal transmission. Further investigation of treatments for *C. hepaticus* are warranted to control this emerging disease.

## **Impact of Avibacterium paragallinarum infection on the Upper Respiratory Tract Microbiome of Chickens**

Mostafa Ghanem<sup>1</sup> Dhiraj Chundru<sup>1</sup>, Madhusudan Timilsina<sup>1</sup>

Department of Veterinary Medicine, University of Maryland, College Park, MD, USA<sup>1</sup>

Avibacterium paragallinarum (AP), is the causative agent of infectious coryza, an upper respiratory tract (URT) disease of chickens that leads to decreased egg production in layers and early marketing of broilers. Pathogens could influence the microbiome of the chicken's respiratory tract leading to more severe diseases in chickens. The impact of AP infection on the microbiome of URT of chicken is unknown and whether different AP strains influence the microbiome differently is also unclear. This study aimed to investigate the impact of AP infection on the URT microbiome and to reveal if specific strains of AP influence the URT microbiome differently. In this study, four different field strains of Avibacterium paragallinarum were used to infect four groups of specific-pathogen-free chickens at four weeks of age, a fifth group was used as control. Pooled choanal swabs were collected from infected chickens and the control group at various time points post-challenge, and DNA extraction was performed and used for microbial 16srRNA gene sequencing. The results showed that the microbiome of the chicken's URT was primarily composed of Firmicutes, followed by Proteobacteria, Actinobacteria, Bacteroidota, and other phyla. Following AP infection, there was a significant decrease in the alpha-diversity of the URT microbiome indicating a decline in microbial diversity. Additionally, beta-diversity analysis revealed significant differences between the infected and non-infected groups, indicating a distinct microbial composition in response to AP infection. The microbial diversity reached the lowest level on the 4th-day post-infection (DPI) and returned to normal level on the 9th DPI. While there was no significant difference in the alpha diversity between groups infected with different AP strains, the beta diversity analysis indicated distinct microbiome compositions based on the infecting strain. These findings demonstrate that AP infection significantly impacts the URT microbiome composition and diversity in chickens, with variations depending on the specific AP strain. Further research is crucial to understand how respiratory pathogens like AP influence the URT microbiome and its potential role in disease severity and poultry health management.

## **The plasmid-encoded serine protease autotransporters Tsh and Sha contribute to avian pathogenic Escherichia coli colonization of the lungs in turkey**

Charles M. Dozois<sup>1</sup>, Sabin Dhakal<sup>1</sup>, Pravit Pokharel<sup>1</sup>, S ébastien Houle<sup>1</sup>

INRS-Centre Armand-Frappier<sup>1</sup>

Escherichia coli is an important bacterial pathogen of poultry and produces multiple virulence factors that can contribute to avian colibacillosis. Serine protease autotransporters of Enterobacteriaceae (SPATEs) are high molecular weight secreted proteins that contribute to virulence and function as proteases, toxins, adhesins, and/or immunomodulators. These virulence factors can play an important role in the different stages of bacterial infection of different host species. An extra-intestinal pathogenic E. coli (ExPEC) O1:K1 strain, QT598, originally isolated from a turkey, was shown to produce five distinct types of SPATE proteins: Tsh, Sha, Vat, TagB, and TagC. Herein, we investigated the cumulative and specific roles of SPATEs in a turkey respiratory infection model. In order to do this, different combinations of SPATE-encoding genes were re-introduced into the genome of a mutant derivative of QT598 lacking all 5 SPATEs genes ( $\Delta 5$  SPATEs) in the turkey model of infection. Loss of all 5 SPATEs resulted in a significant reduction in colonization in extraintestinal tissues including lung and liver. Complementation of the  $\Delta 5$  SPATEs mutant with all 5 SPATE-encoding genes provided a regain to bacterial numbers similar to the wild-type parent QT598. Interestingly, complementation with only the tsh and sha genes was sufficient to restore levels of infection in the lungs to wild-type levels. By contrast, Further, all five SPATE genes were found to be expressed in vivo in infected turkey lungs and air sacs, however expression of tsh and sha genes was markedly higher than expression of the other SPATEs in these respiratory tissues. Taken together, results demonstrate an important role for SPATEs for E. coli infection of turkey tissues, Further, the tsh and sha genes encoded on a ColV-type virulence plasmid pEC598 play a predominant role in infection of the turkey lungs.

## **Mycoplasma gallisepticum Live attenuated Vaccine Reversion to Virulence - What Changed**

Eniope Oluwayinka<sup>1</sup>, Naola Ferguson-Noel<sup>1</sup>

Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens<sup>1</sup>

*Mycoplasma gallisepticum* infection causes significant morbidity and mortality leading to huge economic losses in poultry production. Live attenuated vaccines are commonly used for control and unfortunately there have been reports of reversion to virulence. The objective of this study was to identify genome differences among virulent revertant vaccine strains isolated from commercial flocks in the USA; and based on these differences, predict virulence other revertants among isolated vaccine strains. Fourteen *M. gallisepticum* vaccine strains (ts-11) isolated from poultry farms, including seven previously identified by in vivo studies as virulent (n=3), avirulent (n=3) and vertically transmitted (n=1), were selected and genomic DNA isolated using conventional methods. Full genome libraries were generated using Illumina technology and contigs for each strain were annotated using the fully annotated *M. gallisepticum* reference genome. Proteome comparison and variant analysis between *M. gallisepticum* ts-11 reference genome and the isolates were done using tools in PATRIC. Analyses revealed nine common genetic differences, including four high and five modifier single nucleotide polymorphism (SNP) effect impacts, among all the isolates that differentiated them from the vaccine strain. The virulent revertants had differences in common (a non-synonymous mutation of the ABC transporter, substrate - binding protein and a hypothetical protein) and these mutations were present in three other isolates. This study suggests that some genetic mutations in the vaccine strain may be responsible for reversion to virulence. Further studies will involve in vivo studies on isolates with similar mutations found in the virulent revertants to confirm their pathogenicity.

## Case Reports

### I SPY HPAI - Gross lesions highly suggestive of HPAI

Yuko Sato<sup>1</sup>, Cheng-Shun Hsueh<sup>1</sup>, Maria Chaves<sup>2</sup>, Rachel Ruden<sup>3</sup>, Mohamed El-Gazzar<sup>2</sup>

Iowa State University, Department of Veterinary Pathology, College of Vet Med<sup>1</sup>, Iowa State University, Department of Diagnostic and Production Animal Medicine, College of Vet Med<sup>2</sup>, Wildlife Bureau, Iowa Department of Natural Resources<sup>3</sup>

During the spring of 2022 through spring of 2024, several commercial, backyard, and wild bird specimens either euthanized or found dead were submitted to Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). As we continued to receive cases throughout the highly pathogenic avian influenza (HPAI) outbreak, we started to notice gross lesions that are highly suggestive of HPAI. The most common gross lesion observed throughout all bird species was severe congestion and/or hemorrhage of the brain. This finding was supported by qPCR results with low T values for Influenza A virus (IAV) of brain tissues in comparison to other tissue types. Additionally, significant IAV nucleoprotein (NP) (clone HB65, at 1:500, Iowa State University Hybridoma Facility, Ames, IA) was detected by immunohistochemistry (IHC) of those brain sections, irrespective of the presence or absence of microscopic lesions. Especially coupled with a lack of additional diagnostic gross findings, gross observation of brain lesions helped tip off diagnosticians of the likelihood of HPAI as a top differential. Other suggestive necropsy findings included full crops/gizzards with no loss in body condition (suggestive of acute death), pulmonary congestion, splenomegaly, enteritis, mild ascites, pancreatic necrosis, cyanosis and petechial hemorrhages in subcutaneous adipose tissue. This presentation will go through a series of cases with representative photographs, where HPAI was confirmed.

### Layers Don't get Bacterial Chondronecrosis and Osteomyelitis, Right?

Geoffrey Lossie<sup>1</sup>

Purdue University/Indiana Animal Disease Diagnostic Lab<sup>1</sup>

A group of 66-day old Hy-Line Browns were submitted to the Indiana ADDL for necropsy. The flock had a 2.5 week history of increased culling for "lameness." The previous flock placed at this location also had a period of elevated

lameness occurring at 33 days of age. Diagnostics completed on the previous flock noted: splenomegaly, brittle/fragile bones, litter eating, coccidiosis, and fibrinous pericarditis. 2/3 birds had bilateral disassociation of the articular cartilage from the underlying growth plate (femoral head erosion). This separation yielded dark red, rough, and pitted physes. Growth plates appeared within normal limits, as was bone strength. The thigh musculature had prominent tan to pale yellow, mottling/discoloration with injected vasculature. 3/3 birds had slight splenomegaly, and 2/3 birds had moderate fibrinous pericarditis. Aerobic culture of the heart isolated few *Enterococcus cecorum*, while aerobic culture of the bone marrow of the femur yielded many: *Enterococcus cecorum*, *Streptococcus alactolyticus*, and *Escherichia coli*. Histopathologic examination noted fibrinous/lymphocytic epicarditis and necrotizing/fibrinous splenitis. Skeletal muscle exhibited necrotizing/granulomatous myositis with intralesional bacteria. Examination of the femoral heads, noted a roughly linear expanse of necrosis extending along the entire length of the physis at the distal aspect of the zone of hypertrophy. A single section of bone showed evidence of cortical thinning and fibrosis with rare fractured lamellar bone. Collectively, lesions are highly suggestive of bacterial chondronecrosis and osteomyelitis (BCO). BCO is associated with "meat type birds" i.e. broilers and turkeys, and is not described as affecting layers. The current overall accepted pathogenesis of BCO is that there is a transient period of bacteremia or septicemia, thought to often be secondary to break down of the gastrointestinal barrier allowing translocation of bacteria, with bacteria settling out in the developing growth plates. While a coccidiosis break was not diagnosed within this flock, a subclinical infection may have led to the breakdown of the gastrointestinal barrier. It is suspected that coccidiosis may have also led to a case of "field rickets." A previous bout of rickets is supported by the bony remodeling (thinning and fibrosis) noted in the cortical bone of the femoral head, however there were no indications of active rickets. These findings represent a unique case, as to the author's knowledge, there has never been a report of BCO in laying hens. This may represent an emerging disease process in a novel species group, and may be an indicator that BCO should be considered a differential when working up cases of lameness in laying hens.

### **High Pitched Layers - Case Report**

Gigi Wing Lin<sup>1</sup>

BC Ministry of Agriculture and Food<sup>1</sup>

*Mycoplasma gallisepticum* (MG), a bacterium in the class Mollicutes, causes chronic respiratory disease in chickens and infectious sinusitis in turkeys. Over the period spanning from 2008 to 2021, a total of 9 cases of MG were officially reported in commercial turkey flocks in British Columbia (B.C.). On the contrary, data obtained from the provincial veterinary laboratory, the Animal Health Center, reveals that no cases of MG have been diagnosed in other commercial poultry commodities from 2013 to 2020. The case report detailed the first case of MG diagnosed in commercial table-egg layers since the provincial data became available in 2013. On February 24th, 2021, a flock (approximate flock size: 14,000) of 32-week-old aviary table-egg layers was presented with change in vocalization and suboptimal egg production. The egg production levels were fluctuating between 81.1 to 95.5% in the last three weeks with no reported changes in egg quality. The other flocks on the same premise including two pullet flocks and one 52-week-old layer flock, did not show any clinical symptoms. Diagnostic findings confirmed MG positive through PCR and ELISA. Due to the extended egg withdrawal time, cost of medication, and the flock's performance, antimicrobial treatment was not initiated. The option of revising the vaccination program was not considered due to the lack of commercial MG vaccine in Canada. The disease management plan involves enhanced biosecurity measures, reduction of management stressors, and routine serological surveillance. The farm manager assigned one designated worker to manage the MG positive flock. Enhanced cleaning and sanitation protocols were implemented throughout all the common areas. A hydrogen-based water line cleaner was administered to the water system intermittently. To minimize irritation to the respiratory tract from dust, the light intensity levels were lowered to reduce birds' activity. The set temperature was also increased slightly by 1 degree Celsius to reduce chilling. Active screening for MG with ELISA in other flocks and new replacement flocks was also implemented. Despite the relatively mild clinical signs presented, the flock experienced suboptimal egg production until the end of flock life, causing significant economic impact. Additional costs were attributed to enhanced biosecurity and disease surveillance. Fortunately, MG did not spread to other flocks on the premise. All the existing and replacement flocks

continued to show negative titers for over one year after the initial diagnosis. The presented case study demonstrated the clinical impact of MG infection in a commercial layer flock without antimicrobial treatment and highlighted the success of limiting MG from spreading to existing and subsequent flocks on the same premise through enhanced biosecurity, robust surveillance protocols, and improvements made in management practices.

### **It's Hard To Be Positive When You're B Positive**

Molly Parker<sup>1</sup>

Select Genetics<sup>1</sup>

This presentation will illustrate reproductive, respiratory and neurologic lesions associated with avian metapneumovirus (aMPV) type B infection in turkey breeders. Decreases in egg production and morbidity were reported in laying hens in production, followed by the development of central nervous symptoms in some birds. Initial testing was inconclusive with an absence of brain lesions, both grossly and on histopathology. Histopathology of whole heads demonstrated a cranial osteomyelitis of the inner and middle ear explaining the neurologic symptoms, as well as indicated a non-specific viral etiologic agent for the respiratory and reproductive symptoms. Convalescent sera identified aMPV as the likely etiologic agent, based on seroconversion. Subsequent outbreaks presented similarly and also reported seroconversion. However, samples tested on an aMPV RT-PCR consistently returned negative results. After utilizing an aMPV type B specific primer, positive RT-PCR results were obtained. While it is well established that chickens infected with aMPV can demonstrate central nervous symptoms, there have been no cases reported in the literature of neurologic symptoms in turkeys infected with avian metapneumovirus. This case is also the first report of aMPV type B in the United States.

### **Torticollis in Turkey Poults**

Laura Tensa<sup>1</sup>, Brian Wooming<sup>1</sup>

Cargill<sup>1</sup>

Reovirus is a ubiquitous virus in turkeys and has been found in association with many disease presentations including tenosynovitis, enteritis, and hepatitis. In the Shenandoah Valley of Virginia, there has been a rash of poults displaying neurological symptoms that tested negative for the common causes viral and bacterial causes of torticollis. On histopathology, lesions were consistent with a viral challenge. Further diagnostics isolated reovirus from the brains of symptomatic poults between 3 and 5 weeks old, and affected flocks would later develop clinical signs associated with classical reoviral tenosynovitis. In the summer of 2023, a cluster of torticollis cases were diagnosed in one production complex over the span of approximately three months. The incidence of affected poults ranged between 0.25% and 1% of the flock. Torticollis was seen in both breeder and commercial poults. Traceback of affected flocks did not find any single common breeder flock, and multiple poult sources (hatcheries) were associated with these flocks. Reovirus was isolated out of the brain of one case, out of the vertebra in one case, and out of the legs of multiple affected flocks. After this time period, the number of poults with torticollis decreased back to normal levels for the complex.

### **Aspergillus flavus and Penicillium spp Infection in a Five Week Old Broiler Breeder Pullet Flock**

Hollyn Maloney<sup>1</sup>

Prairie Livestock Veterinarians<sup>1</sup>

A five week old broiler breeder pullet flock experienced acute morbidity and mortality. Daily mortality spiked to 0.24% on day 32 and peaked at 0.49% on day 43. Total weekly mortality for weeks five, six, and seven were 0.44%, 1.7%, and 1.6% respectively. The male replacement flock was housed in an adjoining barn and was unaffected by this outbreak. Affected birds showed severe neurological signs including torticollis, ataxia, paresis, paddling, backwards walking, dog sitting, and tremors. Feed consumption was reduced but water consumption remained normal. Post-mortem examination on day 36 found 0.3 to 0.7 cm tan nodules throughout the lungs and cranial air



sacs. Two birds had a single nodule each on the liver that extended into the liver parenchyma. Histopathology on lung tissue found chronic, granulomatous, necrotizing pneumonia with multifocal moderate to severe intralobular fungal hyphae. Histopathology on brain tissue found chronic granulomatous meningoencephalitis and vasculitis with focally extensive to multifocal intralobular fungal hyphae. Periodic acid-Schiff (PAS) stained lungs and brain tissue showed fungal hyphae that were septate parallel to bulbous and sometimes branched. Staining was inconclusive for species identification. Fungal culture identified *Aspergillus flavus* and *Penicillium* spp. The distribution of fungal granulomas in the lungs and air sacs was consistent with inhalation of spores from the environment. Feed was negative for mycotoxins. The litter was sprayed twice a week with copper sulfate. Feed bins were inspected for any feed that may have been caught in them and molded. Disinfectant, anti-fungal powder was blown throughout the barn using the recirculation fans and blown into the feed bins to coat the inside of the bins. The waterlines were cleaned, disinfected, and flushed. Mortality remained elevated at a daily mortality of 0.32% on day 50. Post-mortems on that day found fungal granulomas throughout the lungs, air-sacs, base of the heart, liver, and kidneys in half of the birds. The other half of the birds had evidence of femoral head necrosis, occasional septic arthritis, splenomegaly, and litter eating. Histology on bones did not find evidence of fungal or bacterial infection. Bone culture was negative for bacteria. Histology on bursas found bursal atrophy with lymphocyte apoptosis. Bursal samples were negative for Infectious Bursal Disease Virus and Chicken Anemia Virus. *A. flavus* has been reported to cause immunosuppression so it is likely that the immunosuppression seen at day 50 is caused by the fungal infection and elevated stress levels and is not a primary cause of the initial infection. Daily mortality dropped to less than 0.1% at the end of week eight. While the exact source of the mold was not identified, treatment of the environment appeared to reduce new cases and no further birds with neurological signs were reported by the end of week nine.

### **Where my eggs at doc?**

Reginald Onyema<sup>1</sup>

Aviagen North America<sup>1</sup>

This is a case report for a male-line grandparent flock that presented with zero egg production and poor sexual maturity at 27 weeks-of-age. Initial investigations revealed no health or management issues in the breeder house. There were no obvious clinical signs and no obvious lesions on necropsy that explained the absence of sexual maturation. Diagnostic results from histology, PCR and serology suggested a previous, mild DMV 1639 challenge, but this was not likely the main driving force for their production woes. Investigations from the pullet house showed that the likely cause for no production was photo-desensitization in the pullet house, causing adult photorefractory in the breeder house. The flock underwent a modified lighting, feed and water program to bring them into production.

### **Economic Impact, and Molecular characterization of CAstVs obtained from outbreaks of White Chicken Syndrome (WCS) in Western Canada**

Victor Palomino-Tapia<sup>1</sup>, Carl A. Gagnon<sup>2</sup>, Emily Martin<sup>3</sup>, Davor Ojkić<sup>3</sup>

Maple Leaf Foods<sup>1</sup>, Swine and Poultry Infectious Diseases Research Center (CRIPA), University of Montreal, QC, Canada<sup>2</sup>, Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada<sup>3</sup>

In the last 10 years, Chicken Astrovirus (CAstV), the causative agent of a condition known as White Chicken Syndrome (WCS), has emerged as an economically important disease across western Canada. Cases submitted had a history of drop of production in the breeder flock at time of lay, very poor hatchability and poor viability of hatched chicks, which are characterized by chicks very white in colour with green livers. Livers from affected birds were collected and submitted to the Animal Health Laboratory for diagnostic confirmation and to the Swine and Poultry Infectious Diseases Research Center (CRIPA) for Next Generation Sequencing (NGS). Clinical signs, hatchery production and economic impact, molecular studies and histology data will be discussed in detail at the conference.

### **Field Experiences with Avian Metapneumovirus in U.S. Broilers and Broiler Breeders**

Andy Bishop<sup>1</sup>, Chelsea Phillips<sup>2</sup>, Aaron Cowieson<sup>3</sup>

Amick<sup>1</sup>, Thomas Frost<sup>2</sup>

### **Enterococcus fecalis Infection In Laying Hens**

Mike Petrik<sup>1</sup>

McKinley Hatchery<sup>1</sup>

Description of an investigation of the clinical signs and decision tree involved in an *Enterococcus fecalis* infection in laying hens in Canada. This infection resulted in runting, stunting, lameness and amyloid arthritis in leghorn chicken in one of the first identified cases of *E. fecalis* in leghorns in North America. The presentation of *E. fecalis* infection in leghorns is significantly different than the disease presentation of *E. fecalis* in broiler chickens.

### **A Deadly Case of Stud Tom Torticollis**

Jake Carlson<sup>1</sup>, Dr. Ben Wileman<sup>1</sup>, Dr. Marissa Studniski<sup>1</sup>, Dr. Molly Parker<sup>1</sup>

Select Genetics<sup>1</sup>

In late August, a flock of mature turkey breeder toms began to experience increased levels of mortality. Many of the birds were exhibiting neurologic signs that progressed to recumbency, torticollis and eventually death. Tissue samples and trachea swabs were collected from the affected birds and the flock tested negative for influenza, MG/MS and New Castle via PCR. Bacteriology of lungs and livers yielded only *E. coli*. Further testing was pursued to rule out the potential for a toxic compound. Liquid chromatography mass spectrometry was utilized to test feed, water and livers from the affected birds. The liver samples were positive for tolycaine, an amidobenzoic acid which has been shown to cause similar clinical signs leading to death in rats. This presentation follows the progression and resolution of clinical signs and outlines the clinical reasoning and sample collection methods used in this case. The presentation also covers a clinical trial performed, with birds that did not enter the food supply, where different doses of tolycaine were administered to breeder turkeys via intramuscular injection. The goal of the trial was to monitor birds for clinical signs post administration of tolycaine and then to perform gross necropsy and sample collection with tissues submitted for mass spectrometry to find the detectable level threshold of tolycaine. This case is still under investigation and all findings will be reported.

### **Swollen Heads with an acute elevated mortality in Commercial Turkeys.**

Claude Hebron<sup>1</sup>, Dallas Clontz<sup>2</sup>

Prestage Farms<sup>1</sup>, Veterinary Diagnostic Pathology, LLC<sup>2</sup>

A dramatic increase in sinusitis, swollen heads and acute mortality have been noted in a commercial turkey operation in Eastern North Carolina that began in December 2023. This increase has occurred primarily in birds two to seven weeks of age. Nasal turbinate tissue had been collected for qPCR from a flock of four week old turkey poults and tested positive for Avian Metapneumovirus Type B. Histopathologic sections revealed lesions consistent with primary avian metapneumovirus infection complicated by secondary bacterial coinfection. A number of factors have been identified as possible contributors to this outbreak which will be discussed.

## **Coccidiosis**

### **Practical Guide to Successful Coccidiosis Vaccination in Broilers**

Sara Throne<sup>1</sup>

Simmons Foods<sup>1</sup>

Successful rotations of coccidiosis vaccine in broilers requires a lot of preparation and work before the vaccine ever makes it to the hatchery. It starts with developing a coccidiosis plan with your team 12-24 months in advance taking into consideration past product usage, costs, as well as PVP program designations. This allows you to know your plan and work your plan. The cycles leading into the coccidiosis vaccine rotation is important especially as it comes to litter management. We try to avoid fresh litter on our 1st cycle of vaccine so working with growers to plan clean-outs is important. Preparing our service tech team and growers is also a critical step. It is important to spend time educating your techs on coccidiosis, fecal and cecal droppings, and coccidiosis lesions. While always important, the environment our birds are in plays a critical role in coccidiosis vaccine implementation and success. Additionally, we emphasize the importance of the basics-feed, water, lights, temperature, litter, and air quality. We also stress the importance of brooding management. And yes, lastly, we have to ensure that all things are done correctly in the hatchery to ensure that viable vaccine makes it to the hatchery. That means handling it correctly to keep oocysts viable-from receiving, storing and mixing. And importantly it includes making sure that birds ingest and are able to replicate the vaccine. By building and executing a solid plan, as a team, we have been able to successfully perform with a coccidiosis vaccination program as a key component of our health and welfare program in our broilers. The goal of this presentation is that the audience will be able to walk away with practical actions they can make to build or improve their coccidiosis vaccination program.

### **Practical ways to assess coccidiosis vaccination in commercial broilers.**

Matilde Alfonso<sup>1</sup>

Ceva Animal Health<sup>1</sup>

Coccidiosis is a protozoal disease causing enteritis and poor performance, and can be fatal in chickens. It is of great economic significance in commercial broilers. Control of the disease can be achieved with anticoccidial drugs or through immunization. Coccidiosis vaccines including live sporulated oocysts of different *Eimeria* species are used in commercial broilers. Vaccines are mass-applied in the hatchery using different delivery systems: in ovo injection, water spray, and gel droplets. The goal of these vaccination systems is to deliver an adequate dose of vaccine, uniformly, to all the birds. There are many factors that can impact the immunization process: vaccine (viability of the oocysts, number of oocysts per dose, attenuation level), vaccine mixing (diluent, dosing, distribution of oocysts), vaccine application (coverage, volume, timing), vaccine ingestion by the birds (chick comfort, chick preening activity), and vaccine replication in the birds. Although coccidiosis vaccines have been available in the USA since the 1950's, and its use has become more common in the last 15 years, there is limited research on how these factors can impact vaccination success. The broiler industry is currently lacking a practical way to assess coccidiosis vaccination. This presentation will describe practical ways to assess multiple steps of the coccidiosis vaccination process in the hatchery including vaccine mixing, vaccine application and dosing, vaccine intake, and vaccine replication. Details on the procedures utilized, equipment needed, and results will be presented from a field veterinarian perspective.

### **Utilizing Oocyst Per Gram (OPG) surveillance to monitor coccidiosis management in commercial cage-free, floor-raised leghorn pullets**

John Schleifer<sup>1</sup>

Devenish Nutrition, Inc.<sup>1</sup>

The increased demand for cage-free, pasture-raised eggs by the US consumer has resulted in a dramatic increase in the percentage of leghorn pullets being raised in floor-rearing settings. This change in management practices over the last 5-7 years presents several unique dynamics in coccidiosis control management compared to broilers and caged pullets. An added aspect of this market change is that approximately greater than 50% of these pullets are raised under organic specifications. All of these management practices, significantly limit coccidiosis control options. This case report will document and discuss the utilization of routine OPG analysis of fecal samples from commercial cage-free, floor-raised pullet production facilities during the calendar year of 2023. Aspects of

coccidiosis OPG differentiation will be presented based on geographic regionality, seasonal differences and coccidia speciation. The challenges of utilizing this analysis in commercial production systems will be discussed. The advantages and disadvantages of utilizing OPG analysis as a coccidiosis monitoring practice in cage-free, floor-raised production systems will be presented.

### **Rotating Chemical Coccidiostats Following Prolonged Use in Broilers**

Nicholas Brown<sup>1</sup>

Huvepharma, Inc<sup>1</sup>

Prolonged use of a single chemical coccidiostat is associated with the development of resistance in coccidia strains. However, the nature and timeline of resistance development is not well characterized for all anticoccidials. In addition, mechanisms of action and resistance development are relatively unknown, which leads to uncertainty regarding the efficacy of transitioning from one anticoccidial to another after extended exposure. The purpose of this study was to assess the variance in zootechnical performance following rotation to a new anticoccidial versus continued use of the same anticoccidial after prolonged use. In this experiment 20 floor pens were maintained with either zoalene 113.5 g/ton or clopidol 113.5 g/ton as the in-feed anticoccidial for 7 consecutive grow-out cycles. After this, half (5 pens) of each treatment were rotated to the other anticoccidial. Performance variance was evaluated based on FCR, body weights, and mortality by feeding phase. In addition, Johnson and Reid coccidiosis lesions were evaluated at 21 and 28 days. The results of this study are pending.

### **Evaluation of Performance and Level of Coccidiosis Immunity of Coccidia Vaccinated Broiler Chickens Fed Various Feed Additives in a Coccidiosis Pen Challenge**

Brandon Doss<sup>1</sup>, Brett Lumpkins<sup>2</sup>, Greg Mathis<sup>2</sup>, David D. Smith<sup>1</sup>

Huvepharma, Inc.<sup>1</sup>, Southern Poultry Feed and Research, Inc.<sup>2</sup>

Coccidiosis is an intestinal disease of chickens that has a devastating economic impact on broiler production due to impaired nutrient absorption, poor performance, and predisposition to necrotic enteritis. This study evaluated the performance and coccidiosis immunity effects of saponin products and an anticoccidial drug when used with coccidia vaccine in a pen challenge. A total of 2,450 male Cobb 500 chicks were randomly assigned to seven treatments (7 pens/treatment; 50 birds/pen) as follows: T1) Unvaccinated, no additive; T2) Coccidia vaccinated + no additive; T3) Coccidia vaccinated + Quillaja saponin D0 – D42; T4) Coccidia vaccinated + Quillaja saponin D14 – 42; T5) Coccidia vaccinated + Quillaja/Yucca saponin D14 – 42; T6) Coccidia vaccinated + Yucca saponin D14 - 42; T7) Coccidia vaccinated + zoalene (125 ppm) D14 - 42. Performance parameters were measured on D14, 21, 28, 35, and 42. On D21 and 27, ileum samples were collected from one representative bird per pen for villi height and crypt depth analysis. On D21 a mixed coccidia inoculum of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* (100,000, 50,000 and 75,000 oocysts/ bird respectively) was administered to all groups via feed. On D27, four representative birds were removed from each pen, sacrificed, and gross coccidia lesion scores determined for *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* using Johnson and Reid (1970). Microscopic *E. maxima* scoring was also determined on D27. Data were analyzed according to the SPFR standard operating procedures for data analysis. The raw data were analyzed using STATIX program LSD test. P value 0.05 was used to separate means when ANOVA F values were significant ( $p \leq 0.05$ ). At D21, the mortality adjusted feed conversion (maFCR) of all treatments was decreased compared to T2, and T7 demonstrated increased body weight gain (BWG) compared to T1. At D28, all treatment groups had improved maFCR and BWG compared to T1, and these improvements continued through D35. At D28 T3, T4, T5, and T7 had lower maFCR compared to T2, while T7 had increased BWG compared to T2. At D35, T3, T4, and T7 had decreased maFCR compared to T2, while T7 had increased BWG compared to T2. At D42, T3, T4, and T7 had decreased maFCR compared to T2. The most significant improvements in performance were observed in T7, with T3 demonstrating improved maFCR similar to T7 at D42. All treatment groups demonstrated improved gross *Eimeria maxima* scores compared to T1. Only T7 demonstrated decreased microscopic *Eimeria maxima* scores compared to T1. Based on zootechnical performance, a saponin feed additive proved to be effective as

support for coccidiosis vaccination in this model. Saponin feed additives may be used as an alternative tool to support coccidiosis vaccination and maintain broiler performance. Keywords: Coccidiosis, Saponins, Broiler, Performance, Coccidiosis Immunity

## Diagnosics

### **A molecular beacon with RT-LAMP can detect AOAV-1 and differentiate low virulence from virulent virus strains**

Megan Mears<sup>1</sup>, Timothy L. Olivier<sup>1</sup>, Dawn T. Williams-Coplin<sup>1</sup>, Edna Espinoza<sup>1</sup>, Abhijeet A. Bakre<sup>1</sup>

Exotic and Emerging Avian Viral Diseases Research Unit, USDA<sup>1</sup>

Newcastle disease (ND), an economically important disease in poultry, is caused by virulent strains of the genetically diverse virus, Avian orthoavulavirus 1 (AOAV-1). Veterinary diagnostic laboratories rely on qRT-PCRs targeting the matrix gene to detect AOAV-1 and the sequence encoding the fusion protein cleavage site to differentiate between AOAV-1 pathotypes. This study sought to develop a single assay targeting the fusion cleavage site sequence for detection of all AOAV-1 class II virus genotypes, and to differentiate lentogenic and mesogenic/velogenic strains of AOAV-1 using a molecular beacon with reverse transcription loop mediated isothermal amplification (MB-RT-LAMP). The MB-RT-LAMP was tested with AOAV-1 fusion gene plasmids, in vitro transcribed RNA, and a rapid lysis method for allantoic fluid virus stocks and oropharyngeal swabs from experimentally infected birds. Data show that the assay can rapidly detect all AOAV-1 genotypes, approaches the sensitivity of the current fusion qRT-PCR assay ( $10^4$  copies), exhibits a high degree of specificity for AOAV-1, and the molecular beacon can differentiate mesogenic/velogenic sequences from lentogenic sequences. Additionally, a rapid lysis protocol compatible with the MB-RT-LAMP for clinical samples was developed, omitting the need for RNA isolation prior to completing the assay. This allowed for the detection and differentiation of AOAV-1 RNA from samples containing oropharyngeal swab material, and for the direct detection of LaSota viral RNA from oropharyngeal swabs of infected birds. As the MB-RT-LAMP assay can rapidly detect and discriminate between lentogenic and mesogenic/velogenic sequences of AOAV-1 within one assay without the need for RNA isolation, and is adaptable to existing veterinary diagnostic laboratories without additional equipment, this provides a promising alternative to the complex and expensive qRT-PCR method currently used.

### **Improved Efficiency by multiplexing Infectious Bronchitis Virus (IBV) Singleplex Assays**

Ramhari Thapa<sup>1</sup>, Sean Brimer<sup>1</sup>, Robert Beckstead<sup>1</sup>

Ceva Animal Health<sup>1</sup>

Infectious bronchitis (IB) is a highly contagious respiratory disease in chickens, causing significant economic losses in the global poultry industry. IBV is an enveloped, positive-sense, single-stranded RNA virus which exists in a diverse array of antigenically and genetically distinct viral strains. While the common IBV serotypes in the USA include Arkansas (Ark), Connecticut (Conn), Delaware/Georgia 98 (DE072/GA98), Delmarva1639 (DMV1639), Georgia 08 (GA08), Georgia 13 (GA13), and Massachusetts (Mass), genetic drift and/or mutations in these types can lead to variation deviating from the original serotype. Spike glycoprotein (S1) genomic region based quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR) assays are available for a quick and early diagnosis; however, their simplicity and specificity are counterbalanced by a limitation in throughput for laboratory workflows dealing with a considerable number of samples. Multiplexing of RT-qPCR assays allows simultaneous amplification of two or more target genes in a single reaction making it a cost-effective, time-saving, and precise technique that yields more information with a smaller sample volume. In this study, we considered published, modified, and novel RT-qPCR singleplex assays and multiplexed them for the rapid detection of eight IBV field and/or vaccine strains which are relevant to commercial poultry in the USA. We multiplexed nine singleplex RT-qPCR IBV assays into three triplex diagnostic assays, i.e., i) 5'UTR-Mass-vGA08 triplex assay detecting general IBV (5'UTR), Mass, and variant Georgia 08 (vGA08), ii) DMV1639-GA08-GA13 triplex assay detecting DMV1639, Georgia 08 (GA08) and Georgia 13 (GA13), and iii) Ark-Conn-DE072/GA98 triplex assay detecting Ark, Conn, and DE072/GA98 strains. The results showed that

the RT-qPCR assay validating parameters of all the multiplex RT-qPCR reactions were comparable and within the acceptable range. The limits of detection of the assays were comparable and in the range of 10-100 RNA copies/run. No competitive inhibition of the multiplexed targets or cross-reactivity with other tested IBV strains was observed. Using 294 field samples the triplex assays were evaluated and validated against current in use singleplex RT-qPCR assays. All diagnosed samples by the multiplex RT-qPCR assays proved to be highly concordant with the results of the singleplex assays, with most results within the 95% limits of agreements in Bland-Altman plots indicating relatively small bias and high degree of agreement between the two approaches. These results showed that the multiplexed IBV assays exhibit sensitivity and specificity that are highly comparable to their respective singleplex assays and will be effective in routine laboratory tests, enabling rapid and specific detection as well as quantification of IBV field and/or vaccine strains.

### **Development and Validation of New Differential qPCR Assays for Improved Diagnosis of Avibacterium paragallinarum in the Field**

Mostafa Shelkamy<sup>1</sup>, Amro Hashish<sup>1</sup>, Maria Chaves<sup>1</sup>, Mariela E. Srednik<sup>1</sup>, Nubia R. Macedo<sup>1</sup>, Eman Gadu<sup>1</sup>, Yuko Sato<sup>1</sup>, Stephan Schmitz-Esser<sup>1</sup>, Qijing Zhang<sup>1</sup>, Mohamed El-Gazzar<sup>1</sup>

Department of Veterinary Diagnostic and Production Animal Medicine, ISU, Ames, IA, USA<sup>1</sup>

Avibacterium paragallinarum (AP) is a bacterial primary pathogen that affects chickens and predominantly induces respiratory symptoms causing a disease known as Infectious Coryza (IC). Recently, several clinically normal layer flocks, which had no history of IC infection or vaccination, tested positive using the current IC real-time PCR (qPCR) assay (recN assay). Furthermore, multiple AP isolates were isolated from these normal flocks. These isolates were dubbed “non-pathogenic Avibacterium paragallinarum” (npAP) since genomic characterization of these isolates revealed significant differences from typical pathogenic AP. The circulation of npAP in layer flocks, with no diagnostic tool able to differentiate them from pathogenic AP, challenges the current IC diagnostics and complicate the effort toward the control of this disease. Therefore, the aim of this study is to develop a differential duplex qPCRs able to distinguish between the two populations to eliminate the existing diagnostic confusion. Comparative genomics between 7 npAP and 48 pathogenic AP genomes was performed. Analysis revealed two consistent features. First, the capsular polysaccharide loci is present only in the pathogenic AP, and absent in npAP. Second, HMTp210 (serotyping) gene contains long unique insertions only in the npAP. For the development of differential duplex qPCR, the HMTp210 insertion was selected as PCR target to identify the npAP. Additionally, hctA (a capsular export gene within the capsular polysaccharide loci) was selected to identify the pathogenic AP. During the validation process, 28 isolates and 10 oropharyngeal (OP) swab pools representing the pathogenic AP, as well as 7 isolates and 86 OP pools of npAP, for a total of 131 samples (35 isolates, 96 OP pools) were tested. Additionally, a wide panel of respiratory, bacterial and viral, pathogens were included in the testing. The newly developed qPCR assays showed efficiencies of 94.62% and 93.86%, with a limit of detection (LOD) of 1 copy/ $\mu$ L and 2.5 copies/ $\mu$ L, for the hctA and np-HMTp210 assays respectively. The validation process revealed 100% diagnostic specificity and sensitivity for the assays. Both assays showed high dynamic range with Ct cut-off value of 37.17 and 35.08. During a surveillance study, an additional population of npAP was discovered that possess both the HMTp210 unique insertion and the capsular polysaccharide loci (including the hctA). These recent findings push us to look for another characteristic target for the pathogenic AP as well as the necessity of investigating the role of the capsule in AP virulence.

### **Development of an Enzyme-Linked Immunosorbent Assay (ELISA) for detection and differentiation of Avibacterium paragallinarum infections**

Mariela Srednik<sup>1</sup>, Brandon Ruddell<sup>1</sup>, Muslum Ilgu<sup>1</sup>, Amro Hashish<sup>1</sup>, Yuko Sato<sup>1</sup>, Mohamed El-Gazzar<sup>1</sup>, Orhan Sahin<sup>1</sup>, Qijing Zhang<sup>1</sup>

Iowa State University<sup>1</sup>

Infectious coryza (IC) is an economically important respiratory disease in chickens and is caused by Avibacterium paragallinarum (AP), a Gram-negative pathogen. Recently, the Veterinary Diagnostic Laboratory at Iowa State

University proved the circulation of new strain of AP in normal layer flocks with no history of coryza infection or vaccination, suggesting that these strains are non-pathogenic. Therefore, these isolates were dubbed "non-pathogenic *Avibacterium paragallinarum*" (npAP). Current diagnostics cannot differentiate between the pathogenic and npAP strains. The objective of this study is to develop an Enzyme-Linked Immunosorbent Assay (ELISA) for detection and differentiation of the two Ap strains. Whole genome sequence analysis revealed a key difference between the new npAP and pathogenic strains of AP in the gene locus encoding HMTp210, an outer membrane protein and a known virulence factor involved in hemagglutination, cell adherence and in vivo colonization. Remarkably, the HMTp210 gene is three times larger in npAP than in the typical pathogenic strains. Based on the sequence analysis, we selected two regions in the HMTp210 gene, with one conserved between AP and ntAP, and the other only present in the ntAP strains. We successfully generated recombinant proteins of the HMTp210 fragments in *E. coli*. Each recombinant protein has a size of 200 AA and is located in the stalk of the HMTp210 protein. The recombinant proteins were purified, and SDS-PAGE analysis verified their purity. Subsequently, the purified recombinant proteins were used as antigens to develop an ELISA to detect anti-AP antibodies in chicken serum samples. At present, the assay successfully differentiated serum samples from AP-vaccinated chickens and from control (SPF) chickens. Testing of flocks with different AP statuses is currently being performed. These findings indicate the potential of the ELISA assay for diagnosis, differentiation, and vaccine efficacy evaluation of *Avibacterium paragallinarum*.

### **Development and Validation of New TaqMan Real-Time PCR assays for enhancing diagnosis of Spotty Liver Disease**

Eman Gadu<sup>1</sup>, Amro Hashish<sup>1</sup>, Maria Chaves<sup>1</sup>, Mohamed El-Gazzar<sup>1</sup>, Mostafa Shelkamy<sup>1</sup>, Yuko Sato<sup>1</sup>

Iowa State University, Department of Veterinary Diagnostic and Production Animal Medicine, Ames, IA.<sup>1</sup>

Spotty liver disease (SLD) is a reemerging infectious disease caused by *Campylobacter* species and primarily affects cage-free commercial layer hens, leading to sharp increases in mortality and drops in egg production. Two *Campylobacter* species have been associated with the disease, *C. hepaticus* and *C. bilis*. These two bacterial species are fastidious and very difficult to grow. Molecular identification of *Campylobacter* species from clinical samples enhances the diagnostic process by providing more accurate and rapid results, thus improving the overall efficiency of microbial identification in clinical samples. Currently, there are no probe-based real time quantitative PCR (qPCR) assays for the rapid and sensitive identification of these two species in clinical samples which limits their usefulness as a diagnostic tool. In the present study, two probe-based qPCR assays were developed to address this gap. The first qPCR assay serves as a screening test for SLD (*C. hepaticus* & *C. bilis*), targeting glycerol kinase gene, which is absent in most *Campylobacter* species other than *C. hepaticus* and *C. bilis*. A second qPCR assay can differentiate between *C. hepaticus* and *C. bilis*. A comprehensive in silico and wet lab validation for both assays demonstrated high specificity and sensitivity for the identification of *Campylobacter* from known positive clinical samples. In conclusion, these newly developed assays represent an improved diagnostic tool for the sensitive and efficient diagnosis of SLD from clinical samples.

### **Field outbreak Investigation of Spotty Liver with Artificial Intelligence (AI) assisted Histology**

Chaitanya Gottapu<sup>1</sup>, Roshen Neelawala<sup>1</sup>, Daniel Verdugo<sup>1</sup>, Subhashinie Kariyawasam<sup>1</sup>, Gary Butcher<sup>1</sup>, John Roberts<sup>1</sup>

College of Veterinary Medicine, University of Florida<sup>1</sup>

Spotty liver disease (SLD) caused by *Campylobacter hepaticus* is an emerging cause of morbidity, mortality, and loss of production in commercial-layer chickens. In recent years, there have been several outbreaks reported in the United States and 2023. The 25-week-old hens at layer farm in central Florida experienced lower feed consumption, reduction in egg production and egg size, and higher mortality. Age matched hens from a neighboring unaffected flock were collected as controls. Diseased hens had livers of 10% greater weight with serosal surface pinpoint white foci and friable red parenchyma. Faint bacterial colonies grew on *Brucella* agar supplemented with blood and were confirmed to be *C. hepaticus* with PCR. Microscopically multifocal 200 to 1000 µm diameter areas of necrosis were

surrounded by macrophage infiltration and ringed by congestion. An artificial intelligence (AI) tissue classification program (Halo, Indica Labs) was trained to classify vascular congestion, inflammation, necrosis, hepatic structures and variance in autolysis. Areas of necrosis typical of *C. hepaticus* infection were quantified and compared with non-infected controls validating that AI histopathologic classification applications show promise for diagnosing and scoring liver disease in commercial layers.

### **Development of a Deep-Learning Artificial Intelligence Diagnostic Support Tool for Chicken Renal Histopathology**

Adrea Mueller Slay<sup>1</sup>, Brenda Ozeias Santos<sup>2</sup>, Aoise Lord Bissett<sup>2</sup>, Jogile Kuklyte<sup>2</sup>, Silvia Carnaccini<sup>5</sup>, Tim Carlson<sup>6</sup>, Daniel Rudmann<sup>6</sup>

Charles River Laboratories; University of Georgia<sup>1</sup>, Deciphex, Dublin, Ireland<sup>2</sup>, University of Georgia, College of Veterinary Medicine, Athens, GA<sup>5</sup>, Charles River Laboratories, Spencerville, OH<sup>6</sup>

The United States poultry industry has a substantial economic impact, an estimated \$555.5 billion in 2022 alone. It is estimated that approximately 30% of all disease conditions in poultry involve the kidney, leading to significant economic loss. Swift and accurate diagnosis of diseases can help mitigate loss. This study aimed to develop a deep-learning artificial intelligence segmentation classifier for use as a diagnostic support tool in the chicken kidney. A segmentation classifier, separating image components into categories, was developed based on common histopathological changes seen in the chicken kidney, including tubular changes, inflammatory infiltration, urate/tophi deposits, and lymphoma. Categories for normal anatomical structure included cortex/medulla, glomeruli, and embryonic renal tissue. This classifier was trained on kidney slides from diagnostic and research cases with confirmed renal disease. These cases include both male and female, chick and adult, and broiler and layer samples. After initial annotations, the classifier was applied to additional slides with similar diversity. Performance was evaluated qualitatively by the trainer and quantitatively using F1 scores (harmonic mean of precision and accuracy) using hematoxylin and eosin-stained slides from the training and testing slide sets. Classifier improvements were accomplished using corrective annotations (negative mining) and additional training slides. Preliminary results indicated that a chicken kidney segmentation classifier using deep learning artificial intelligence may be an effective diagnostic support tool in digital pathology that can differentiate common renal lesions. To our knowledge, this is the first documentation of the use of a deep-learning artificial intelligence as a diagnostic support tool for histological analysis of the chicken kidney.

## **Enteric Health**

### **Characterization of the predicted metagenome function of "normal" microbiota in chicken intestines**

Ruediger Hauck<sup>1</sup>, Matheus Santini<sup>1</sup>, Andrea Pietruska<sup>1</sup>, Zubair Khalid<sup>1</sup>

Auburn University<sup>1</sup>

Research in humans has shown that while the taxonomic composition of the intestinal microbiota varies widely between individuals, its metabolic function is relatively stable. In research on poultry diseases, characterization of the intestinal microbiota by 16S rRNA gene sequencing has become routine. However, to the wide variation in taxonomic composition, interpretation of the results is effectively limited to comparing groups within an experiment. In addition, observed changes in the relative abundance of taxa might be compared with other trials, but more often than a taxon that has been identified as of interest in one experiment has not even been detected in another experiment. In consequence, there is no way to look at a microbiota and assess if it originated from a healthy or diseased bird. The purpose of this study was to characterize the predicted metagenome function of "normal" chickens. Available raw data of 16S rRNA gene Illumina sequencing of the intestinal microbiota of untreated control groups in published experiments were downloaded from the NCBI Sequence Read Archive and analyzed using a common workflow including denoising with DADA2 and predicting the metagenome function with Picrust2. It was tested if and how geographic location, type, and age of birds change the predicted metagenome function of the



microbiota and if predicted metagenome function is more consistent than the observed wide variability of the taxonomic composition. The results will be presented and discussed.

### **Transcriptome analysis of the jejunal mucosa of broiler chickens with subclinical necrotic enteritis fed diets containing varying calcium concentrations and limestone particle sizes**

Rana Waqar Tabish<sup>1</sup>, Joseph P. Gulizia<sup>1</sup>, Jose I. Vargas<sup>1</sup>, Jose R. Hernandez<sup>1</sup>, Wilmer J. Pacheco<sup>1</sup>, Samuel J. Rochell<sup>1</sup>, Matthew A. Bailey<sup>1</sup>, William A. Dozier<sup>8</sup>, Klint W. McCafferty<sup>2</sup>, Ruediger Hauck<sup>1</sup>

Department of Poultry Science, Auburn University<sup>1</sup>, USDA-ARS Poultry Research Unit, Mississippi State<sup>2</sup>

Calcium is a crucial macronutrient for normal growth and participates in cellular physiology, signal transduction, and bone mineralization. However, elevated levels of readily soluble calcium in poultry diets can heighten the risk of necrotic enteritis (NE) by promoting *Clostridium perfringens*' growth. In this study, we analyzed the transcriptome in the jejunal mucosa of broiler chickens fed diets with varying calcium concentrations and limestone particle sizes by RNAseq. The study employed a 3 × 2 + 1 factorial design with 6 treatments subjected to a subclinical NE challenge and one unchallenged control. Each treatment consisted of 10 pens. The main factors included calcium concentrations representing adequate, reduced, and low levels in the starter, grower, and finisher diets. Additionally, different particle sizes of limestone categorized as coarse and small were included. The unchallenged control group received a diet with standard calcium concentrations and small limestone particle size. The challenged treatments involved gavaging with a tenfold dose of a live coccidia vaccine at 14 days of age (DOA) and an inoculation of 10<sup>8</sup> CFU of *Clostridium perfringens* at 18 DOA. Jejunal mucosa samples were collected from 10 birds per treatment at 21 DOA, and total RNA was extracted. Eukaryotic mRNA was sequenced, and the reads were aligned to the chicken reference genome using TopHat. Gene expression was quantified through HTSeq. Differentially expressed genes (DEGs) across treatments were identified using edgeR, followed by pathway enrichment analysis and in silico protein-protein interaction analysis. The results will be presented at the meeting.

### **Identifying chicken *Lactobacillus* strains possessing immunomodulatory and anti-*Clostridium perfringens* properties**

Ravi Kulkarni<sup>1</sup>, Carissa Gaghan<sup>1</sup>, Hosni Hassan<sup>1</sup>

NC state University<sup>1</sup>

Virulent strains of *Clostridium perfringens* cause Necrotic enteritis (NE), an economically important disease of chickens. In the current era of 'no-antibiotic-ever' farming, NE incidences are on the rise and *Lactobacillus*-based probiotics seem to offer a promising non-antibiotic alternative. Here, we used an in-vitro chicken macrophage (MQ-NCSU) cell-based model to evaluate the immunomodulatory and anti-*C. perfringens* properties of four *Lactobacillus* species, *L. crispatus* (Str. C25), *L. animalis* (Str. P38), *L. acidophilus* (Str. P42), and *L. reuteri* (Str. P43). The results showed that P42 stimulation led to an increased expression of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, IFN $\gamma$ ), while P38 and P43 stimulation showed increased IL-10 (anti-inflammatory cytokine) transcription, compared to medium-only control. Additionally, the in-vitro bacteriological assays (Agar-spot, Well-diffusion and Co-culture tests) showed that while P38 showed a reduction in *C. perfringens* growth in all three tests, a growth inhibition in co-culture assay was observed with all the strains. Of note, P38 and P43 showed the highest growth inhibition. Furthermore, broiler chickens orally administered with these *Lactobacillus* strains followed by a *C. perfringens* challenge showed that Str. P38 and P43 were able to significantly prevent NE. Taken together, the present work shows that *Lactobacillus* strains possessing both anti-inflammatory and anti-*C. perfringens* activity in-vitro can be beneficial in-vivo in preventing NE in chickens.

### **Influence of coccidiosis and enteritis challenges on diet productive energy and economics in broilers**

Diego Martinez<sup>1</sup>, Diego Martinez<sup>1</sup>, Craig Coon<sup>1</sup>

University of Arkansas<sup>1</sup>

Intestinal health is a major factor producing performance variability among and within broiler complexes. This study developed a mechanistic model explaining the associations between gut health challenges, performance, the associated diet energy value, and the economic value of the carcass. The study comprised three phases. The first phase developed a mechanistic model based on literature data to quantify the impact of the challenge on maintenance nutrient expenditure and identified processes underlying such connection. It included data from experiments challenged with coccidia or necrotic enteritis that included varying dietary concentrations of amino acids (AAs) or crude protein (CP). The effects of the challenges on AAs or CP requirements were quantified, and literature data on influential ( $P < 0.05$ ) mechanisms were included in a mechanistic model. The second phase consisted of a 56-d broiler study with 1760 day-old chicks fed one of 11 test diets to induce varying body weight gains (BWG) and feed conversion ratios (FCR). Body composition, energy retention (net energy for gain; NEg; kcal/kg diet), and processing weights were determined with Dual-Energy X-Ray Absorptiometry, the market value of the carcass (MKV; cents/bird) with the weight of each processing part and its market price, and fasting heat production (net energy for maintenance; NEm; kcal/kg diet) with calorimetry chambers. Productive energy (PE; kcal/kg diet) was calculated as NEg + NEm. Linear regression was used to quantify the relationships between BWG, or FCR, and PE and MKV. The third phase validated the assumptions of the model with an intraperitoneally administered lipopolysaccharide (LPS) challenge. The changes in body composition and heat production were determined as above. All data was analyzed using linear mixed models in JMP. The results indicated that coccidia vaccination increases the AA requirement by 10% ( $P < 0.05$ ) and actual challenges by 45% ( $P < 0.05$ ) and that acute-phase response, enterocyte turnover, and impaired nutrient absorption ( $P < 0.05$ ) were the main factors explaining the increased AA requirement. Models explaining the relationship between BWG or FCR and NEg, NEm, and PE showed high precision ( $R^2 \geq 0.93$ ) and were introduced into the mechanistic model above. The LPS experiment showed that protein breakdown (17% body protein catabolized;  $P < 0.02$ ) and the associated reduction in body protein content ( $P < 0.02$ ) supported the acute response, increased the NEm (760 to 3148 kcal/kg;  $P < 0.05$ ), and reduced the NEg (1691 to -2622 kcal/kg;  $P < 0.05$ ) and PE (2451 to 527 kcal/kg;  $P < 0.05$ ). In conclusion, coccidia and enteritis challenges decrease the diet PE by increasing protein breakdown to support the acute phase response. PE is sensitive to variations in performance due to coccidia or enteritis challenges, and estimating their impact, or that of interventions to control them, on PE and the MKV is a sensitive approach for economic assessments.

### **Effect of a blend of organic acids (short-chain fatty acids) and oregano oil on gut morphology growth performance and lymphoid organs of broiler chicken**

Frank Ayala-Limaylla<sup>1</sup>, Freddy Farfan-Farfan<sup>1</sup>, Eliana Icochea D'Arrigo<sup>2</sup>, Guillermo Tellez<sup>3</sup>

Laboratorios Drogavet SAC<sup>1</sup>, Universidad Nacional Mayor de San Marcos<sup>2</sup>, Department of Poultry Science, University of Arkansas<sup>3</sup>

**Abstract:** Background: Organic Acids (OA) and essential oils (EO), can substitute antibiotics growth promoters (AGPs) from broiler diets. Also, OA+EO improve body weight gain, feed conversion ratio, enhance villi height, decrease crypt depth and improve the development of lymphoid organs in chickens. Methods: A total of 720 male chicks (one-day-old, weighted  $47.87 \pm 0.34$  g) were selected to determinate the supplementation of OA+EO on histomorphologic measures, lymphoid organs development and growth performance. Chicks were randomly distributed in four treatments with six replicates and thirty birds per replicate. The treatments were as follows: control group (CON, basal diet), antibiotic group (ANT, control + bacitracin 0.25 g/kg), lower dose of organic acids and essential oils (low OA+AE, control + 0.5 g/kg OA+EO) and medium dose of organic acids and essential oils (medium OA+AE, control + 0.75 g/kg OA+EO). In order to obtain a subclinical clostridial lesions, all broilers were challenged with *Clostridium perfringens* ( $5 \times 10^8$  CFU/ ml) + *Eimeria* spp. ( $5 \times 10^4$  oocysts) at 14d, 15d and 16d of the study. The experiment was divided into before (d 1–d 14) and after the challenged (d 14–d 42) phases. Results: Chickens supplemented with bacitracin or OA+AE showed better final body weight and average daily gain in overall phase compared with control group ( $p < 0.05$ ). However, there was not significant statistical difference among ANT group and two OA+AE groups. The supplementation with OA+AE or ANT improved the VH/CD ratio after challenge compare with control group but there was not significant statistical difference between ANT group and OA+AE groups. Finally,

there was not difference in lymphoid organs development among all groups. Conclusion: We concluded that dietary supplementation of OA+AE or ANT could improve the growth performance and VH/CD ratio. Moreover, we demonstrated that OA+AE could replace to bacitracin supplementation as growth promoter. Keywords: Organic Acids (OA), essential oils (EO), growth promoters, *Clostridium perfringens*.

### **Effect of *Campylobacter jejuni* and *Campylobacter coli* co-colonization on the cecal environment in turkeys**

Silke Rautenschlein<sup>1</sup>, Janina Rzeznitzek<sup>1</sup>, Alexandra Rath<sup>1</sup>, Karen Methling<sup>2</sup>, Michael Lalk<sup>2</sup>, Elisa Peh<sup>3</sup>, Sophie Kittler<sup>3</sup>, Frederic J. Hoerr<sup>4</sup>, Alexandra von Altrock<sup>5</sup>

Clinic for Poultry, University of Veterinary Medicine Hannover<sup>1</sup>, Institute of Biochemistry, University of Greifswald, Germany<sup>2</sup>, Institute for Food Quality and Food Safety, University of Veterinary Medicine Hannover, Germany<sup>3</sup>, Veterinary Diagnostic Pathology, Fort Valley, USA<sup>4</sup>, Clinic for Swine and Small Ruminants, University of Veterinary Medicine Hannover, Germany<sup>5</sup>

While *Campylobacter* (*C.*) *jejuni* and *C. coli* are important causes of food-borne gastroenteritis in humans, turkeys as an animal reservoir do not develop clinical disease in the field despite frequent co-colonization with these *C.* species. The impact of *Campylobacter*-colonization on the gut environment is poorly understood, but needs to be elucidated further to improve current control strategies in the field. In three repeat-experiments, six weeks old turkeys were experimentally co-inoculated with a *C. jejuni* and a *C. coli* strain, and the colonization pattern as well as the impact on the gut microflora composition and cecal metabolome was investigated at seven, 14 and 28 days post inoculation (dpi). While the colonization pattern was determined by quantitative microbiology, the microflora and metabolites were assessed by Illumina-sequencing and proton nuclear magnetic resonance spectroscopy, respectively. In addition selected metabolites were also tested in vitro to determine if they are essential for *C. jejuni* and *C. coli* growth by optical density measurements. Both *C.* strains successfully colonized the caecum of turkeys, while *C. coli* showed significantly higher numbers of colony forming units/g cecal content compared to *C. jejuni* ( $p < 0.05$ ). The microbiota composition varied between experiments. In comparison to *C.*-negative control birds, the cecal microbiota of co-inoculated groups showed a shift toward more Oscillospiraceae in all three trials, while the relative abundance of Lachnospiraceae and Clostridia UCG-014 was reduced in two of three experiments. This change coincided with a significant increase in butyrate and glucose in the cecal chyme and a reduction of propionate in the co-inoculated versus the control birds ( $p < 0.05$ ). It is speculate that this change may lead to an increase in the anti-inflammatory response and mucus production as a protective host response. Interestingly the in vitro studies demonstrated that neither glucose nor butyrate were required for or even reduced growth of either *C.*-strain, while propionate led to a clear increase in *C. coli* growth suggesting that metabolites may affect the *C.*-species differently. Overall, animal health was not clinically affected in this study, but gut integrity as determined by histology as well as weight development were compromised in co-inoculated turkeys suggesting subclinical effect of co-colonization with *C. jejuni* and *C. coli*.

### **Blackhead – Disease review: Past and Current Prevention and Control Strategies**

Jaime Ruiz<sup>1</sup>

Elanco Animal Health<sup>1</sup>

Blackhead / histomoniasis, is an enterohepatic disease condition with a unique form of transmission that involves the cecal worm *Heterakis gallinarum* and the protozoan *Histomonas meliagridis*. It is a common disease, mainly in turkeys, peafowl and broiler breeders. Pheasants, ducks and geese are more resistant to severe histomoniasis. Predisposing factors involve the presence of vectors such as earthworms, darkling beetles and other mechanical carriers. A review of the complex biology and pathogenesis of the disease, including recent research and the most current and practical intervention strategies will be presented.

## **Epidemiology**

## **Understanding risk factors associated with broiler breeder MS cases in Georgia**

Kathleen Sary<sup>1</sup>, Dr. Louise Dufour-Zavala<sup>1</sup>, Dr. Dave Fernandez<sup>2</sup>

Georgia Poultry Laboratory Network (GPLN)<sup>1</sup>, Independent consultant<sup>2</sup>

Avian mycoplasmosis is a disease of gallinaceous birds undesirable to the poultry industry, especially in breeding stock for meat type production. Detection of *Mycoplasma synoviae* (MS) in broiler breeder flocks may trigger declassification in National Poultry Improvement Plan (NPIP), major logistics issues, export disturbances and financial losses. In high density areas, one of the major concerns is spread from a positive flock to surrounding broiler, pullet or breeder flocks, exacerbating the problem for the industry. The objective of this study was to determine important risk factors of recent MS introduction on meat type production farms in Northeast Georgia. Methodology, results, and findings will be discussed.

## **Comparison and evaluation of poultry biosecurity plans through the Rapid Access Biosecurity app**

Jason Galvis<sup>1</sup>, Rocio Crespo<sup>1</sup>, Lilian Hutchens<sup>1</sup>, Kelsey Mills<sup>1</sup>, Gustavo Machado<sup>1</sup>

College of Veterinary Medicine, North Carolina State University<sup>1</sup>

A considerable proportion of commercial poultry farms in the United States adhere to the guidelines set forth by the well-established National Poultry Improvement Plan (NPIP), which includes detailed protocols for developing and executing biosecurity plans. However, due to the absence of data standardization and lack of evaluation tools, there is an information gap regarding on-farm biosecurity measures, making it difficult to compare and evaluate the strengths and weaknesses of biosecurity procedures and infrastructure on individual farms. To provide support to the poultry industry, academic scholars, government officials, and members of the poultry industry assembled the Rapid Access Biosecurity (RAB) app (RABapp™) consortium to reduce ambiguity regarding the construction of on-farm biosecurity plans, provide rapid access to standardized biosecurity data at a national level, and allow biosecurity measures benchmarking. Each biosecurity plan enrolled in RABapp™ includes two elements: 1) a written description and 2) a visual map of the farm, which are subjected to multiple protocols designed to enhance data input standardization. To illustrate the app's usability, we used 130 biosecurity plans enrolled in RABapp™ to perform a quality analysis of the maps, identifying missing biosecurity structures. Additionally, we developed a descriptive analysis of a subset of seven variables related to biosecurity measures that could be associated with introducing infectious pathogens to the farms. In total, 98% of the maps were missing biosecurity structures or had inconsistencies. On average, the minimum distance between the perimeter buffer area (PBA) and the barns' boundaries was 4.5 meters ( $\pm 2.4$  meters SD), the distance between barns and the animal disposal location was 35 meters ( $\pm 57.3$  meters SD), the distance between barns and parking areas was 19 meters ( $\pm 9$  meters SD) and the average ratio between barns and barns' access points was 2 ( $\pm 1$  SD). In addition, 93% of biosecurity plans include cleaning protocols for supplies that cross the barn, 13% of them have specific protocols against migratory birds limiting the presence of streams or ponds, and 92% do not allow dogs, cats, or other domesticated animals inside the barns. In summary, the RABapp™ can help producers, veterinarians, and government officials more easily benchmark biosecurity measures implemented at poultry farms to identify critical points for improvement.

## **Mastering Sample Size Determination: Facts, Fallacies, and Best Practices for Use in Veterinary Research and Diagnostics**

Enrique Mondaca<sup>1</sup>, Robert Beckstead<sup>1</sup>

CEVA Animal Health<sup>1</sup>

Commonly asked questions in veterinary practice are: 'How many animals do we have to test to determine if a disease is present in an animal population?'; 'What percentage of animals have received the vaccine?'; 'Is my flock negative for Salmonella?' These enquires, along with others, pertain to the estimation of disease and vaccine presence or the probability of failing to detect infected animals. Assessing the appropriate sample size depends on

nonstatistical and statistical considerations. Among the nonstatistical aspects, we must take into account practical and ethical factors. Regarding statistical matters, we need to consider the expected prevalence of the disease, the size of the flock, and the desired precision of the estimate. Having this information is essential for a comprehensive understanding and assessment of sample diagnostic data, enabling informed conclusions about the flock. In this work, we seek to take a practical approach, using real-world examples, to illustrate the appropriate use and interpretation of sample size determination for: 1. Detecting the presence of disease. 2. Estimating disease prevalence within an intended confidence interval. 3. Assessing the probability of failure to detect disease. For the calculations above, we will also discuss specific circumstances, like adjustments for sample pooling or the bounds on the error of estimation when not reaching the aimed sample size. The goal of the presentation is to serve as a guideline for veterinarians and researchers to properly interpret field and research data.

### **Securely moving duck hatching eggs from a farm out of or within a Highly Pathogenic Avian Influenza (HPAI) Control Area**

Sylvia Wanzala-Martin<sup>1</sup>, Kaitlyn St. Charles<sup>1</sup>, Peter Bonney<sup>1</sup>, Marie Culhane<sup>1</sup>, Rosemary Marusak<sup>1</sup>, Amos Ssematimba<sup>1</sup>, Carol Cardona<sup>1</sup>

University of Minnesota<sup>1</sup>

The ongoing onslaught of Highly Pathogenic Avian Influenza (HPAI) on commercial and backyard poultry has led to devastating consequences for producers and other industries linked to the poultry industry. In order for these critical industries to continue functioning, continuity of business (COB) strategies have been utilized by various poultry commodities (e.g., turkeys, broilers, layers, upland game birds) to ensure that safe, uncontaminated products can be moved to different destinations from HPAI negative farms caught up in a control area. These COB strategies ensure food security by supporting these vital industries and are backed by proactive science-based risk assessments. These risk assessments ensure that not-known-to-be-infected animals and their products can be moved for continued production or marketed. Thus, fewer animals are depopulated, the need for indemnity payments is decreased, and market disruptions related to food shortages are minimized. The University of Minnesota Secure Poultry Supply (SPS) risk assessments and permit guidances provide clear, actionable steps that ensure that COB movements can take place with minimal risk of furthering a disease outbreak. Conventional commodities, i.e., turkeys, layers, broilers and upland game birds, have clear COB movements as outlined on the SPS website - <https://securefoodsystems.umn.edu/>. The commercial duck industry, however, encountered challenges when attempting to move product during the 2022 HPAI outbreak. This work is in response to that need - we have been awarded a NADPRP grant to address the movement of duck hatching eggs from a farm out of or within a control area during an HPAI outbreak. Evaluating the risk of this movement will benefit state and federal animal emergency decision makers by providing clear information on the best risk mitigation strategies for the movement and its potential consequences. Since the inception of the project, we have convened a workgroup of federal and state regulators, duck industry representatives, and other relevant subject matter experts in order to understand the duck commodity and assess the risk of moving duck hatching eggs by identifying pathways, mitigations and risk of HPAI spread from a uninfected farm in a control area (monitored premises) to other facilities with susceptible species.

### **An approach for prioritizing Salmonella serotypes for foodborne outbreak prevention: Using burden and trajectory of outbreak-related illnesses associated with meat and poultry**

Katherine Marshall<sup>1</sup>, Marisa Hast<sup>1</sup>, Zhaohui Cui<sup>1</sup>, Brigette L. Gleason<sup>1</sup>, Cassie Hartley<sup>1</sup>, Matthew E. Wise<sup>1</sup>, Beau B. Bruce<sup>1</sup>, Patricia M. Griffin<sup>1</sup>

CDC<sup>1</sup>

Salmonella causes an estimated 1.03 million foodborne infections in the U.S. each year, and over 40% are attributed to consumption of contaminated chicken, turkey, beef, and pork. Although more than 2,500 Salmonella serotypes have been described, the top 20 cause nearly 70% of U.S. Salmonella infections. Most Salmonella infections are not

linked to a recognized outbreak, but outbreak data provide definitive links to specific food sources. To determine which serotypes cause the most outbreak illnesses associated with specific food products and inform prevention measures, we developed an approach to categorize serotypes using outbreak illness burden (high, moderate, low) and trajectory (increased, stable, decreased). Data were collected from Salmonella outbreaks reported to CDC's Foodborne Disease Outbreak Surveillance System during 2012-2021 with a single confirmed or suspected food vehicle of chicken, turkey, beef, or pork. For each meat and poultry type, the number of outbreak-associated illnesses were calculated for each serotype. Serotypes were considered highest priority for outbreak prevention if they were classified as high burden ( $\geq 75$ th percentile for number of outbreak illnesses among that product type during 2017-2021) and having an increased trajectory (illnesses increased  $\geq 50\%$  from 2012-2016 to 2017-2021). During 2012-2021, we identified 192 foodborne outbreaks linked to chicken, turkey, pork, and beef resulting in 7,077 illnesses, 1,330 hospitalizations, and 9 deaths. Of these, 88 (46%) outbreaks and 2,935 (41%) illnesses were attributed to chicken and 24 (13%) outbreaks and 1,188 (17%) illnesses were attributed to turkey. For chicken, illnesses were caused by 19 serotypes. The serotypes Enteritidis, Infantis, and Blockley had high outbreak burden and increased trajectory so were considered highest priority for outbreak prevention; Typhimurium and Braenderup were classified as moderate outbreak burden and increased trajectory and were also considered high priority. For turkey, illnesses were caused by 12 Salmonella serotypes, with two (Enteritidis, Reading), considered highest priority and one (Hadar) with moderate burden and increased trajectory, considered high priority for outbreak prevention. Two serotypes were identified to be recently emerged (caused outbreak illnesses during 2017-2021 but not during 1998-2011) for chicken (Blockley, Anatum) and two were identified for turkey (Anatum, Schwarzengrund). These results are available on a publicly available tool (CDC's BEAM dashboard: <https://www.cdc.gov/ncezid/dfwed/BEAM-dashboard.html>). By identifying and publishing outbreak illness burden and trajectory data annually, our goal is to facilitate prioritization of serotypes for prevention of outbreaks. Intensified prevention measures may be considered for serotypes with both high or moderate burden and increased trajectory.

## Immunology

### **Immune Responses in the Harderian Gland after Newcastle Disease Vaccination in Chickens with Maternal Antibodies**

Raimundo Espejo<sup>1</sup>, Cassandra Breedlove<sup>1</sup>, Camila Cuadrado<sup>1</sup>, Haroldo Toro<sup>1</sup>

Department of Pathobiology, Auburn University College of Veterinary Medicine<sup>1</sup>

The immune responses in the Harderian gland (HG) were characterized after Newcastle disease virus (NDV) LaSota ocular vaccination in NDV antibody naïve specific pathogen free (SPF) chickens and in chickens of commercial origin with maternally derived antibodies (MDA). In trial 1 using only layer-type SPF chickens, ocular LaSota vaccination elicited serum antibody levels that consistently increased after day 15 post-vaccination, while the specific IgA response in lacrimal fluids was already detectable on day 10 after vaccination. Eleven days post-vaccination, the relative abundance of B cells (Bu-1+) as well as T-helper (CD4+), and cytotoxic T cells (CD8+) in HGs was significantly increased achieving maximum frequencies 16 days post-vaccination. In trial 2, progeny chickens of NDV vaccinated commercial layer breeders were used. LaSota virus RNA was detected in lacrimal fluids and tracheal swabs both in commercial and control SPF chickens after vaccination at 2 or 15 days of age (DOA). Vaccination at 2 DOA did not induce a serum NDV antibody response in chickens of commercial origin. In contrast, seroconversion was elicited in commercial chickens upon vaccination at 15 DOA likely due to waning of MDA. Unlike systemic IgG responses, vaccination at 2 or 15 DOA elicited strong specific IgA responses in commercial chickens. The IgA response was highest 9 days after vaccination and showed a tendency to decline on day 15 post-vaccination. Commercial chickens vaccinated on day 2 of age showed increased B cells both on days 10 and 16 post-vaccination. The expansion of B cells in the HG in these chickens is consistent with increased IgA levels detected in lacrimal fluids. In contrast, control SPF chickens showed a more limited B cell expansion and lower IgA levels. Vaccination on day 15 of age triggered a stronger increase of B cells in commercial chickens than in control SPF chickens. The B cell response was accompanied by T helper (CD4+) cell expansion occurring both in commercial and control SPF

chickens. These cells expanded to a lesser extent when vaccination was performed at 2 DOA compared to vaccination at 15 DOA. Cytotoxic T cells (CD8+) showed significant expansion irrespective of vaccination day and without differences detected between control SPF chickens and chickens with MDA. We conclude that NDV LaSota elicits vigorous humoral and cell immune responses in the Harderian gland. Furthermore, unlike the interference shown by MDA on vaccine-induced serum antibody responses, MDA do not interfere with the mucosal immune response of the HG.

### **Field vaccination of turkey breeder candidates part II: Challenge trial of day old poults from various vaccine strategies**

Ben Wileman<sup>1</sup>, Marissa Studniski<sup>1</sup>, Molly Parker<sup>1</sup>, Jake Carlson<sup>1</sup>, Nathan Freeman<sup>1</sup>, Milos Markis<sup>2</sup>

Select Genetics<sup>1</sup>, AviServe<sup>2</sup>

This is the follow-up presentation to last year's turkey Reovirus field vaccination presentation. Last year we showed the serologic results and shedding data from the use of a live primer followed by two killed booster approach in turkey breeders in the field. This presentation will cover the results of two challenge trials performed examining heterologous protection of day old and 14 day old poults from field vaccinated turkey breeder hens. Various vaccination responses (titer levels) of the turkey breeders and various vaccination programs (live primer, no vaccination, killed vaccine only) were examined in an established foot pad challenge model. The live primer used in this trial was from a chicken 1133 origin strain. The killed vaccine Reo strains were three field origin viruses administered under field conditions. Egg from the various breeder flocks were shipped to a single hatchery and incubated and hatched and transported to the challenge facility. Poults were challenged with two different Reovirus strains in each trial at day of age or 14 days of age. Maternal antibody titers and virus neutralization titers and results from the challenge trials will be shared.

### **Evaluation of Diagnostic Tools for Detection of Egg Drop Syndrome**

Cole Taylor<sup>1</sup>, Ashley Hallowell<sup>1</sup>, Erich Linnemann<sup>1</sup>, Holly Sellers<sup>1</sup>, Jenny Nicholds<sup>1</sup>

University of Georgia<sup>1</sup>

Egg drop syndrome 1976 (EDS-76) is a viral disease caused by duck Atadenovirus A and was considered exotic to the United States. The disease has been detected in domestic commercial layer and broiler breeder chickens resulting in significant economic losses. The clinical presentation is commonly associated with a decrease in egg production and production of soft and shell-less eggs in birds that have no other signs of disease. At this time, diagnostic assays are limited to detection of DNA by PCR and real time PCR (qPCR) or by detection of antibodies, in unvaccinated flock, by ELISA and/or hemagglutination inhibition (HI). Primary isolation of the virus in primary cells prepared from SPF chickens has not been successful. Numerous reports in the literature suggest the use of duck embryo fibroblasts (DEF) which, are not readily available due to the lack of specific pathogen free (SPF) duck populations. The ability to isolate the EDS76 virus is necessary for characterization and the ability to evaluate cleaning and disinfection processes to determine if the premise is virus free. In addition, inactivated EDS76 vaccines have been authorized for limited use in some states. Serological evaluation of flocks, before and after vaccination is an important tool for assessing vaccination. Given the recent emergence of this viral disease in the US and the limited use of inactivated vaccines in some states, additional diagnostic tools are needed for confirmation and detection of viable virus, as well as an understanding of serological tools for use in measuring the immune response following vaccination. The primary objectives of this project are to 1) evaluate avian cells and cell lines (primary chicken embryo liver, LMH ATCC-2117, DEF ATCC CCL-141) for primary isolation of a contemporary EDS76 and 2) evaluate antibody detection in serum from unvaccinated and EDS76 vaccinated flocks using ELISA and HI. At present, isolation of NVSL EDS76 Chicken/PA/20-005075/2020 in successive passages of primary chicken liver cells prepared from SPF embryos has not been successful. However, successive passages of the virus in the LMH cell line suggests viral replication, in the absence of cytopathic effect (CPE), as demonstrated by decreasing qPCR Ct values. In addition, passages of the virus in the DEF cell line also suggests viral replication as demonstrated by decreasing Ct values and with evidence of

CPE characterized by rounding of cells. Clinical samples with low qPCR Ct values will be passaged in the cell lines for attempts to isolate the virus. Evaluation of immune response to EDS76 via ELISA and HI, in vaccinated flocks at predetermined time points of pre-vaccination, post-vaccination and post-placement is currently underway. Assessing serological response on what can be expected on ELISA vs. HI in response to vaccination and/or EDS76 infection is important for the interpretation of serological results and understanding expected response to vaccination.

### **Glycerides of lauric acid supplementation in the chicken diet enhances the humoral and cellular immune response to infectious bronchitis virus**

Wanderley Quinteiro-Filho<sup>1</sup>, Amine Mellouk<sup>2</sup>, Virginie Michel<sup>2</sup>, Nuria Vieco-Saiz<sup>2</sup>, Olga Lemâle<sup>2</sup>, Tim Goossens<sup>2</sup>, Jessika Consuegra<sup>2</sup>

Adisseo LATAM<sup>1</sup>, Adisseo France S.A.S. Center of Expertise and Research in Nutrition (CERN)<sup>2</sup>

Pathogen's infections and medication reduction are important challenges in poultry industry. In addition of vaccination strategies, many solutions to enhance the immune response against pathogens are developed. One of those are glycerides of lauric acid that, besides its anti-microbial and -viral effects, can also improve the immune response through different pathways such as enhancing T lymphocytes activation and differentiation to Th1 and Th17 instead of a regulatory T cells profiles, B cell activation and antibodies secretion. In the current study, we aim to determine the effects of glycerides of lauric acid (GLA) supplementation in chickens' diets on the kinetics and levels of humoral and cellular immune response to a pathogenic aggression using an infectious bronchitis virus (IBV) in vivo model. One-day-old Ross 308 broilers were vaccinated via eye-nose drops with live attenuated IBV and fed diets supplemented or not with GLA at 3 kg/ton. The levels of early (day 7) specific anti-IBV broiler sera (n=12/group) significantly increased in broilers fed GLA supplemented diet compared to the control and non-vaccinated broilers (p< 0.05) showing a better primary immune response. Long term supplementation of GLA in diets (28 days) decreased splenocytes numbers compared to the control group ( $2.5 \times 10^8$  vs.  $4.1 \times 10^8$  p< 0.01) but did not affect the peripheral blood mononuclear cells (PBMC) numbers in chicken's blood. Splenocyte numbers decrease was correlated with the decrease of macrophages markers MCSF (p< 0.05) and their secretion of IL-6 proinflammatory cytokine (p< 0.001) in the spleen. Basal cytokines secretions of pan (IL-2 and IL-16), Th1 (IFN- $\gamma$ ) and Th17 (IL-21) T cell response remained similar in the spleens of two groups. Unlike the basal levels, the splenocytes of broilers fed with GLA, showed higher activation and effector abilities measured by IFN- $\gamma$  ELISpot quantification after 24h exposure to IBV antigens (N-261-280 peptide) or antigen independent mitogen (Con A). In response to N-261-280 peptide, GLA group splenocytes showed a 2-fold increase of spot numbers (p< 0.05) and 3-fold increase of spot surfaces (p< 0.01) compared the control and non-vaccinated groups. Similarly, Con A stimulation showed a 2-fold increase of spot surfaces and numbers in the GLA supplemented group (p< 0.01). In summary, first we show here that GLA supplementation in the feed improves the intensity and the kinetics of primary humoral immune response of broilers. Second, GLA enhanced the levels of global and specific cellular immune response mediated by Th1 and Cytotoxic T lymphocytes. Altogether, we show in vivo how glycerides of lauric acid supplementation in the diet can enhance chicken resilience against pathogenic challenges by strengthening their immune response.

## **Parasitology**

### **Economic Impact of Bed Bug Infestation in Breeder Operations**

Aaliyah Gore<sup>1</sup>, Rocio Crespo<sup>1</sup>, Simon Shane<sup>2</sup>, Jenny Nicholds<sup>3</sup>

North Carolina State University<sup>1</sup>, Private Consultant<sup>2</sup>, University of Georgia<sup>3</sup>

*Cimex lectularius*, also known as the common bed bug, is a pervasive external parasite that plagues people all around the world. This hematophagous parasite not only affects humans but has also been reported in poultry production dating back to the 1940s. *C. lectularius* infestations are one of the most challenging parasitic infestations to treat due to product resistance and rapid reproduction. A survey was released in 2023 to poultry veterinarians and



producers, to determine the extent and impact of the bed bug infestation in the poultry industry. The survey focused on the location and number of affected farms, treatments and controls used, and the negative impact on production. A total of 52 veterinarians and 46 producers responded to the survey. Most of the companies that experienced infestation were in the southeastern portion of the United States and belong to the broiler breeder and layer breeder sectors of the industry. According to the producers, the major production impact was the increase in floor eggs, due to the *C. lectularius* residing in the nest boxes. The goal of this project was to use the data collected to create a predictive model to determine the economic impact of bed bug infestations in breeder operations by focusing on production loss and the cost of successful treatment or control of an infestation. The objective was accomplished by monitoring and collecting production data of infested breeder farms and comparing it to control farms (no bed bug infestation). From this data, we concluded that bed bug infestation may not have high priority when ranked against other external parasites; however, bed bug infestation can have a grave economic impact on a company.

### **Effect of Fenbendazole on Broiler Breeder Sperm Mobility.**

Ivan Alvarado<sup>1</sup>, Jeanna Wilson<sup>2</sup>, Hunter Mason<sup>2</sup>

Merck Animal Health<sup>1</sup>, University of Georgia<sup>2</sup>

Fenbendazole is a benzimidazole antihelmintic currently approved for use in turkeys and chickens for the effective control of *Ascaridia galli* and *Heterakis gallinarum*. Fenbendazole has shown no detrimental effects on semen quality or fertility in cattle, sheep or swine. However, a transitory adverse effect on sperm mobility in mature breeder toms was reported when administered in the feed at a dose of 8 g of Fenbendazole 20% mix/kg of feed for 6 days. The observed decrease in sperm mobility and fertility within 6 weeks after administration was associated with fenbendazole binding of the tubulin protein, interfering with sperm microtubules assembly at the axoneme. In this study, we assessed the impact of a fenbendazole molecule especially formulated for drinking water administration on sperm mobility of mature broiler breeder males when administered as recommended. Mature roosters in production were selected based on normal semen production. The roosters were handled every other day to acclimate to contact with collectors and trained to the abdominal massage technique for semen collection. Prior to the administration of fenbendazole, sperm mobility was assessed by forward motion through a thin gel (6% accudenz, as described by Froman and McLean, 1996). Baseline mobility values were obtained, and rooster numbers were further reduced to utilize roosters in the middle range of mobility (index of 0.380 or greater). The selected roosters were randomly divided into a control group (n=20) and a treated group (n=20). Prior to fenbendazole administration, average baseline mobility indexes of 0.450 and 0.433 were observed, respectively. Males in the treated group received the recommended dose of 1 mg of fenbendazole per kg of body weight by drinking water during 5 consecutive days. Sperm viability and mobility was assessed at 7 and 14 days after treatment. No significant differences in mobility indexes between fenbendazole treated and control groups were observed at 7 (averages of 0.412 and 0.418, respectively) or 14 (averages of 0.404 and 0.409, respectively) days post-treatment. When used at the recommended dose, the fenbendazole molecule used in this study was shown to be safe for sperm mobility in mature broiler breeders.

### **Anthelmintic Support with Natural Products**

Philip Stayer<sup>1</sup>

Dr Phil Stayer Poultry Consulting, LLC<sup>1</sup>

With limited products licensed for poultry anthelmintics, especially poultry destined for specialty markets, United States commercial poultry producers are interested in non-conventional products that may impact worm burdens. A research trial with turkeys as well as field experiences with leghorns and goats have demonstrated reduced worm challenges while animals are fed a mixture of chestnut and quebracho tree extracts. This unique blend of naturally occurring polyphenols has multiple modes of action that aid host intestinal defense and healing that may impact ascarid infestations even if the final product is not labeled as an anthelmintic. The components of this feed additive are generally regarded as safe and the commercially available product is labeled as US organic.

## **Effects of stress factors on histomoniasis in broiler breeder pullets**

Catherine Fudge<sup>1</sup>, Mark Burluson<sup>2</sup>, Chance Williams<sup>2</sup>, Chongxiao (Sean) Chen<sup>1</sup>

University of Georgia<sup>1</sup>, Wayne-Sanderson Farms<sup>2</sup>

With the lack of efficacious drugs to prevent or treat histomoniasis, reports of mortalities in broiler breeder facilities have increased in recent years. Previous research in the current lab found that environmental management factors and nutritional factors impacted histomoniasis severity in turkeys. However, there is a lack of information on broiler breeders. The objective of this study was to explore the role of various stressors on histomoniasis in broiler breeder pullets. 800 YP x Ross 708 pullets were randomly assigned to 1 of 8 treatments (10 birds x 8 replicates). Birds were raised in battery cages from 0 to 4 weeks and provided a diet formulated for broiler breeder pullets following nutrient guidelines containing wheat midds. Feed intake was measured daily, and a skip-a-day feeding program was applied from D14 to D28. Treatments included Non-challenged control (NC); Challenged control (PC); rice-hull based diet (RH); 200 ppm copper chloride (CC); 75% amount of PC fed from day 14-28, (R75); 50% amount of PC fed from day 14-28, (R50); 108 CFU Avian Pathogenic *E. coli* orally gavaged on day one (EC); Neomycin at 0.2mg/mL and tetracycline at 0.6 mg/mL in water from day 14-20 (N+T); early *H. meleagridis* infection at day 7 (D7); Western Red Cedar hydrosol in water at 0.2% from day 18-28 (WRC). On day 7, D7 treatment was intracloacally inoculated with 1 mL of 100,000 histomonads/bird. This was repeated on D18 for all other treatments except NC. Individual body weights were collected on day 0, day 18, and day 28. Mortalities were collected during the trial and scored for histomoniasis. The trial was terminated on D28, and all remaining birds were scored for histomoniasis. Results were analyzed using one-way ANOVA, SAS, and Duncan's MRT for mean separation with a significance of  $P \leq 0.05$ . No significant differences in infection rates were seen between treatments. Early infection (D7) had the highest mortality rate (18%) followed by *E. coli* inoculation treatment (10%;  $P = 0.0021$ ). There were no differences in ceca scores between treatments, but R50, D7, and N+T treatments had higher liver scores than PC ( $P < 0.0001$ ). This data suggests that the timing of broiler breeder pullets exposed to *H. meleagridis*, pathogenic bacteria co-infection, and long-term use of antibiotics play a role in disease severity and mortality. Furthermore, pullets with improper nutrition are more susceptible to histomoniasis.

## **Performance of Broiler Chickens Vaccinated with an In Ovo Coccidiosis Vaccine Compared to Broiler Chickens Treated with Anticoccidial Drugs in-Feed**

Martina Dardi<sup>1</sup>, Joan Molist<sup>1</sup>, Marina Solé<sup>1</sup>, Luis Pantoja<sup>1</sup>

Laboratorios HIPRA S.A.<sup>1</sup>

Avian coccidiosis is one of the main diseases affecting the production of broiler chickens, either clinically or subclinically. The inclusion of anticoccidial drugs in the broiler feed has been the traditional method of prevention of the disease. However, the increased resistance of the *Eimeria* parasites to these drugs, together with recent trends in the market to reduce the use of antibiotics and chemicals, have led to the use of more and more alternative methods of prevention, live coccidiosis vaccines being the main one. These coccidiosis vaccines are also recognized for their efficiency in restoring sensitivity to anticoccidial drugs and controlling field *Eimeria* strains. Regarding coccidiosis vaccination, a new live vaccine against avian coccidiosis, EVANOVO® (HIPRA), containing *Eimeria acervulina*, *E. maxima*, *E. tenella*, *E. praecox* strains attenuated by precociousness and designed for in ovo (IO) administration, has been introduced on to several markets. The objective of the study presented was to evaluate, under field conditions, the performance of broiler flocks vaccinated with the in ovo coccidiosis vaccine in comparison with previous flocks where the preventive treatment against coccidiosis was the inclusion of anticoccidial drugs in the chicken feed. The study was conducted on two farms of a European broiler company and included a total of 31 broiler flocks (724,850 chickens). Of these chickens, 539,410 were treated with anticoccidial drugs and 185,440 chicks were vaccinated in ovo against coccidiosis. In the vaccinated flocks, the excretion of the parasite oocysts was measured, and coccidiosis lesion scoring was conducted to ensure good replication of the vaccinal strains in the chicken gut. The oocyst excretion patterns showed early peaks (between 14 and 21 days of

age), indicating a good development of immunity. In addition, reduced lesion scores were detected on each farm that was evaluated. The parameters used to evaluate the performance of the chickens included Mortality, Body Weight (BW), Average Daily Gain (ADG), Feed Conversion Ratio (FCR) and the European Production Efficiency Factor (EPEF). Regarding these zootechnical parameters, the broilers vaccinated in ovo against coccidiosis showed a statistically significantly better performance compared to those treated with anticoccidial drugs, with a 6.6% higher EPEF in the vaccinated birds. This EPEF result was due to a statistically better performance in terms of growth, with a 4.2% increased AVDG and a 4.5% reduction in FCR corrected to 2.5 kg in vaccinated birds compared to chickens treated with anticoccidial drugs. In conclusion, the broilers vaccinated in ovo against coccidiosis exhibited a superior overall performance compared to chickens protected against coccidiosis using the traditional method of including anticoccidial drugs in the feed, probably due to the reduction of field resistant strains of the parasite.

## Pathology

### **Analysis of the localized immune response in the bursa of Fabricius post infectious bursal disease virus challenge**

Julia Blakey<sup>1</sup>, Maricarmen Garcia<sup>2</sup>, Sujit Mohanty<sup>3</sup>, John Dunn<sup>3</sup>

USDA ARS SEPRL Endemic Unit<sup>1</sup>, UGA<sup>2</sup>, USDA<sup>3</sup>

Previously categorized infectious bursal disease (IBD) susceptible or resistant MHC-B congenic SPF chickens, as well as SPF White Plymouth Rock chickens, were utilized to investigate the effect of MHC-B haplotype on infectious bursal disease immune response in the bursa of Fabricius. Chickens were either challenged at 25 days of age with the rA strain of infectious bursal disease virus (IBDV) and sampled at 1-, 2-, and 3-days post inoculation (dpi), or challenged at 28 days of age with the STC strain of IBDV and sampled at 4-, 8-, and 12-dpi. IBD severity was evaluated based on mortality rates and bursal lesion scoring from H&E stained bursal sections. RT-qPCR was performed on bursal samples from the rA challenge experiments for viral load comparisons during early infection. Two unique multicolor flow cytometry panels were created to analyze the phenotypes of bursal immune cell infiltrates post IBDV inoculation. Results demonstrate that MHC-B haplotype effects on IBD severity and immune response depends on the challenge strain of IBDV as well as the challenge dose utilized.

### **Lesions Associated with Avian Metapneumovirus Subtype B Infection in Commercial Turkeys and Broiler Breeder Chickens**

Dallas Clontz<sup>1</sup>, Frederic Hoerr<sup>1</sup>

Veterinary Diagnostic Pathology, LLC<sup>1</sup>

In 2023, the incidence of upper respiratory disease in commercial turkeys began to increase in multiple areas across the United States. Affected turkeys presented with ocular and nasal discharge, conjunctivitis, rhinitis, swollen infraorbital sinuses with abundant mucus production, coughing, sneezing, and respiratory rales. Often, bacterial coinfection was apparent within 3-7 days after the onset of clinical signs resulting in airsacculitis with or without pneumonia and polyserositis. Histopathology of the conjunctiva, nasal turbinate, infraorbital sinus, and upper trachea included moderate to marked lymphoplasmacytic and heterophilic inflammation with focal to multifocal cilia loss, increased mucus production, epithelial attenuation, loss, and hyperplasia, and rare, intraepithelial, eosinophilic, intracytoplasmic inclusions. These inclusions are consistent with those known to occur with avian metapneumovirus infection. Broiler breeder flocks within these same areas began presenting with swollen heads, torticollis, opisthotonos, and occasionally, respiratory rales. Gross lesions of affected broiler breeders included subcutaneous edema of the head, cranial osteomyelitis, otitis, and meningitis. Aerobic culture of the brain and inner ear yielded pure cultures of *Escherichia coli*. Histopathology of affected broiler breeders included cranial osteomyelitis, otitis media and interna, tracheitis, and rhinitis. RT-PCR of nasal turbinate and choanal cleft swabs from affected turkeys and choanal cleft and oviduct swabs from affected broiler breeders was positive for avian metapneumovirus subtype B.

## **Postmortem diagnostic profile of laying hens with hypocalcemia**

Mayra Tsoi<sup>1</sup>, Allison Gerras<sup>1</sup>, Cara Robison<sup>3</sup>, Courtlandt Lyons<sup>4</sup>, Cory Howard<sup>4</sup>, Dina Joardar<sup>6</sup>, Richard M. Fulton<sup>1</sup>, John P. Buchweitz<sup>1</sup>

Department of Pathobiology and Diagnostic Investigation, Michigan State University<sup>1</sup>, Department of Animal Science, Michigan State University<sup>3</sup>, College of Veterinary Medicine, Michigan State University<sup>4</sup>, Cargill, North Carolina<sup>6</sup>

Hypocalcemia is one of the leading causes of death in commercial egg-laying chickens and a major welfare and economic concern for the egg industry. A large amount of calcium is lost daily in the eggshell and is replenished by intestinal absorption and medullary bone resorption. Despite an adequate diet, calcium reserves can become depleted over time, resulting in hypocalcemia. Postmortem diagnosis is one of exclusion based on the presence of an active ovary, an egg in the shell gland, and no significant lesions to account for the death of the hen. The goal of the present study was to develop a quantitative postmortem test for hypocalcemia in laying hens. Forty, 35-week-old, white leghorn hens were randomly assigned to one of three groups that were fed 6.3%, 4.0%, or 1.5% calcium diets for ten weeks. Hens on the 1.5% calcium diet had significantly lower ionized blood calcium, a decrease in average daily and weekly egg production, thinner eggshells, and thinner tibial cortical bone at 10 weeks, relative to 4.0% or 6.3% diets. Morphometric analysis revealed a significant increase in the amount of medullary tibial bone in the tibia, consisting of unmineralized osteoid, and enlarged parathyroid glands. Mineral analysis revealed a significant decrease in Ca, P, and molar ratio Ca:P in tibia. Using ROC curve, values below 5.71 M for Ca (100% sensitivity and specificity), 3.49 M for P (100% sensitivity and specificity), 1.63 for Ca:P (60% sensitivity, 100% specificity), and 0.84 M for Mg (100% sensitivity and specificity) were consistent with hypocalcemia. Results from this study identified criteria including thin tibial cortical bone, large parathyroid glands, a predominance of unmineralized osteoid in the tibia, and tibia mineral values to be consistent with a postmortem diagnosis of hypocalcemia.

## **Reovirus**

### **Evaluation of Pathogenicity and Antigenicity of Turkey Reovirus Isolates**

Milos Markis<sup>1</sup>, Anna Desmond<sup>1</sup>, Maria Yea<sup>1</sup>

AviServe LLC<sup>1</sup>

Reovirus infections and associated diseases continue to be an economically-significant factor for turkey producers in the U.S. and Canada. Only infections at an early age result in the development of disease, and poults gradually develop resistance to disease as they age. Reovirus infections in turkeys have been associated with development of viral arthritis and tenosynovitis, viral enteritis, aortic ruptures, and hepatitis. Turkey reoviruses range in pathogenicity from mildly pathogenic strains to very pathogenic strains that cause disease of multiple tissues including tendons, intestines, heart, spleen, and liver, and result in elevated mortality. Turkey reoviruses are more antigenically-homogeneous than chicken reoviruses, however, turkey reoviruses can be differentiated into serogroups based on virus neutralization assay. Since 2011, turkey reoviruses have continued to evolve antigenically with shifts occurring in 2014, 2017, and 2019. Recent turkey reovirus isolates from different geographic areas in the U.S. and Canada were inoculated into susceptible poults to evaluate pathogenicity. Majority of isolates were very pathogenic, inducing swollen foot pads, tendon lesions, liver and spleen necrosis, and subsequently mortality. Based on virus neutralization assay, recent turkey reoviruses can be differentiated into several serogroups. Control of reovirus infections in turkeys is difficult due to lack of commercial live and inactivated vaccines. Industry relies on the use of autogenous inactivated vaccines, which typically contain multiple turkey reovirus isolates, to immunize breeding hens and toms, prevent vertical transmission of reovirus and provide progeny poults with maternal immunity. Understanding the pathogenicity and antigenic diversity of turkey reoviruses is crucial for control of reovirus-associated diseases in turkeys.

## **Forgotten turkey avian reovirus isolates decipher naturally occurring co-infections of CRV and TRV in commercial turkeys.**

Sonsiray Alvarez Narvaez<sup>1</sup>

Southeast Poultry Research Laboratories<sup>1</sup>

Avian orthoreoviruses (ARV) are an emerging threat for the poultry industry. ARV infections in turkey have been associated with arthritis, lameness and neurological disorders, and cost the US economy around ~USD 33 million per year. The shortage of turkey ARV (TRV) genomic sequence data hinders the efforts to explore the molecular epidemiology of this virus, although several studies suggest a close relationship between European TRVs and TRVs circulating in the US. In 2009, 18 chicken embryo kidney (CEK) cell culture supernatants containing TRV isolated between 2004 and 2008 from nine districts in Germany were shipped to the Southeast Poultry Research Laboratory (SEPRL, USDA/ARS) in Athens, GA (USA) for genomic characterization. These TRVs were forgotten in a freezer for nearly 15 years until our group discovered them and decided to NGS sequence and analyze their genomes. To our surprise, 82% of our isolates appeared to be coinfecting with a TRV and a chicken ARV (CRV). In fact, we observed that these isolates contained one complete TRV-genome, and fragments of genomic segments that shared their highest similarity with CRVs. These results suggest a naturally occurring co-infection of a CRV and a TRV in commercial turkeys. We next studied the genetic relatedness of the German TRVs with each other and with other ARVs. Our phylogenetic analysis depicted a consistent host-associated ARV clustering, with three main clades: (i) the TRV clade, (ii) the CRV clade, and (iii) the Duck ARV (DRV)/Goose ARV (GRV) clade. This provides evidence that ARV shares a high level of host-species specificity among the main production bird species. Studying the phylogeny of each ARV gene individually, we observed that all the German TRVs but one grouped among other TRVs. TRV S5 genes consistently clustered together with the genes of other CRVs. This suggests that CRVs are genetically equipped to infect turkey but in the majority of cases they do not produce disease in this species. There are no signs of segment reassortment between ARVs isolated from different hosts, and most of the genomic recombination observed in the genomes of the German TRVs happened between each other and with other TRVs firstly reported in the USA. This study shows a snapshot of the genomic diversity of TRV circulating in Germany in the middle 2000's and reports for the first time CRV and TRV co-infections in Turkey.

### **Development of turkey reovirus specific ELISA**

Khaled Abosria<sup>1</sup>, Steve Lawson<sup>1</sup>, Laura Munger<sup>1</sup>, Noreen Bashir<sup>1</sup>, Ben Hause<sup>1</sup>, Ben Wileman<sup>1</sup>, Tamer Sharafeldin<sup>1</sup>

Department of Veterinary and Biomedical sciences, South Dakota State University<sup>1</sup>

Turkey reoviruses (TRVs) impose a great threat to turkey industry in the US. After the emergence of arthrotropic variant of turkey reovirus in 2011, research focused on better ways to diagnose the infection among turkey flocks. Till now, there is no turkey-reovirus specific diagnostic immunoassay to monitor the seroconversion in turkey sera. We developed Turkey reovirus-specific ELISA assay based on the use of recombinant Sigma C protein of turkey arthritis reovirus. SigmaC protein was produced by E. coli expression system and purified twice. The immunoassay was optimized and standardized to meet the requirement of validation and to be used for diagnostic purposes. We tested different serum samples of different turkeys' flocks including specific pathogen free, negative, vaccinated, and infected flocks. The positive (n=592) and negative (n=346) sera were tested with our TRV-specific immunoassay to determine the cut off and relative sensitivity and specificity. Out of the total serum samples (positive = 292 and negative =266) were tested with Avian reovirus based commercial ELISA to detect the diagnostic sensitivity and specificity to be compared with our developed assay. Statistical analysis for our turkey-reovirus based immunoassay showed a diagnostic sensitivity (97.8%) and specificity (98.8%) with cut off value 0.32 while the Avian reovirus based ELISA showed a diagnostic sensitivity (94.5%) and specificity (99.2%) with cut off 0.2. Our TRV-based ELISA assay provides a higher diagnostic sensitivity and specificity in comparison with the commercial assay. Our future plan is to improve our assay so it can distinguish between vaccinated and infected flocks. Keywords: Turkey, Reovirus, ELISA, Sensitivity, Specificity

# Salmonella

## Longitudinal evaluation of Salmonella in broiler breeder flocks

Nikki Shariat<sup>1</sup>, Amy T. Sicheloff<sup>1</sup>, Sean P. Nolan<sup>2</sup>

Poultry Diagnostic and Research Center, University of Georgia<sup>1</sup>, Nolan Integrated Pest Control and Management (NIPCAM) Group<sup>2</sup>

Successful Salmonella control during broiler production relies on robust biosecurity and an appropriate surveillance platform, which includes monitoring breeder flocks. CRISPR-SeroSeq is a PCR-based, next-generation sequencing tool that exploits the native Salmonella CRISPR sequences to detect and determine the relative frequency of multiple serovars in a sample. This deep serotyping approach overcomes significant limitations of traditional Salmonella isolation. This study was designed to determine the incidence of multiserovar Salmonella populations in broiler breeder flocks, and to highlight the importance of maintaining rodent control to limit Salmonella introduction. Across two broiler complexes (A, B), 16 pullet (five farms) and 14 breeder houses (seven farms) were sampled over a 65-week production period. Pullets were sampled at weeks 14 and 21, then breeders sampled every four weeks and weekly during peak production (weeks 29-31). Two bootsock pairs were collected from each house and cultured for Salmonella (n=400). Rodents (mice, plus roof and Norway rats; n=355 carcasses across 49 composite samples) were captured from farms and tested for Salmonella, along with bait station swabs (n=33). Overall Salmonella prevalence in pullets was 17% (11/64), although only houses in Complex B were positive (7/7 houses). All 14 breeder houses were Salmonella-positive at least once; the overall prevalence was 35% (63/182) and 52% (80/154) in Complexes A and B, respectively. A generalized additive mixed effects model showed the expected marginal mean Salmonella prevalence peaked ~38 weeks, indicating that this time would be optimal for surveillance. Deep serotyping showed that 39% (53/137) samples contained multiple serovars (average of 1.5 serovars/sample). The number of serovars differed between Complex A (six serovars) and B (16 serovars) (Shannon diversity index plus Hutcheson t-test (p< 0.05)). In rodents, 35% (17/49) of composite samples and 9% (3/33) of bait station swabs were positive, and eight serovars were identified. Four serovars found in rodent samples were also found in bootsock samples from the same farms/houses, with two serovars matching Salmonella subtypes between bootsocks and rodents. The data show that multiserovar populations often occur, and the increased resolution of CRISPR-SeroSeq can support development of improved Salmonella control strategies based on transmission patterns. The difference in serovars found across complexes may be attributed to management practices as Complex A employs a 3rd party integrated pest control company while Complex B relies on growers for pest control. This study highlights the importance of maintaining on-farm biosecurity to limit Salmonella introduction.

## Re-evaluating the gold standard: evaluating environmental Salmonella surveillance sampling methodologies in commercial broiler live-production

Emily Cason<sup>1</sup>, Marco Reina<sup>1</sup>, David Ayala-Velastegui<sup>1</sup>, Nikki W. Shariat<sup>1</sup>

University of Georgia, Poultry Diagnostic and Research Center<sup>1</sup>

Effective pre-harvest Salmonella control in broilers relies on being able to accurately, reliably, and reproducibly evaluate pre-harvest Salmonella. In this study, six surveillance sampling methodologies were evaluated and compared to assess Salmonella prevalence and quantification during broiler live production across three iterative experiments. In all experiments, two sets of samples were collected, one from each side of the house. In the first experiment, bootsocks, electrostatic pads secured around a paint roller, feather swabs, cloacal swabs, fecal grabs, and litter grabs were collected from 24 houses across 10 farms (n=288 total samples) and evaluated. In the second experiment, bootsocks, bootsocks secured around a paint roller, and feather swabs were collected in 16 houses on seven farms (n=128) and evaluated. Bootsocks and bootsock-rollers were selected as the most reproducible sample type and in the final experiment these were collected in triplicate from 20 houses on 10 farms (n=240). Prevalence was determined by qPCR assay and traditional culture, then compared using a Fisher's Exact test between

methodologies and a McNemar's test between methodologies for each experiment. Salmonella was quantified using a commercial qPCR assay and the Ct-values were compared by an ANOVA (experiments 1 and 2) or a linear mixed effect model (experiment 3). In experiment 1, Salmonella prevalence differed between methodologies by qPCR ( $p=0.015$ ) but not by culture. The three best performing sampling methodologies were bootsocks (42/48 were culture positive and 41/48 were qPCR positive), feather swabs (42/48 and 36/48), and electrostatic pad-rollers (35/48 and 34/48). In experiment 2, Salmonella qPCR prevalence differed ( $p=0.0004$ ) while culture did not. Bootsocks (30/32 by culture and 28/32 by qPCR) and bootsock-rollers (31/32 and 32/32) were the best performing methodologies. In experiment 3, qPCR prevalence (210/240) was greater than culture (167/240) ( $p=0.0021$ ) but there were no differences observed between methodologies or replicates. The average bootsock Ct-value (35.7) was lower than that of bootsock-rollers (36.4), indicating Salmonella quantification was higher in bootsocks ( $p=0.0002$ ). A mixed-effect model found that house contributed 44% of the variance observed while house side and replicate accounted for 15% and 4% of variance, respectively. This study shows that sampling methodology directly influences both Salmonella detection and load. For Salmonella surveillance sampling, bootsocks and bootsock-rollers were found to best indicate the Salmonella present in the live production environment. These two environmental sampling methodologies are highly reproducible, user friendly, and provide the most reliable Salmonella results indicating the Salmonella prevalence and load of broiler flocks in live production. A future goal will be to determine whether these methods are able to correlate Salmonella in live production with Salmonella at processing.

### **SE challenge studies in live ST vaccinated broilers—comparing different vaccination strategies to protection against SE challenge at different ages**

Kalen Cookson<sup>1</sup>, Jon Schaeffer<sup>1</sup>, John Dickson<sup>1</sup>

Zoetis<sup>1</sup>

Two controlled broiler studies will be presented showing the influence of live ST vaccination on colonization rates of broilers when challenged at different time frames with virulent Salmonella Enteritidis (SE)—one of the top 3 serotypes and key performance indicators (KPI) according to FSIS. The first study was designed to measure the effect of a conventional live ST vaccination schedule (day of hatch coarse spray followed by a water boost in the second week) on ceca and liver colonization after either a 4-day or 14-day challenge with SE by oral gavage. In short, live ST vaccinated broilers had lower loads of SE in cecas and lower incidence and loads in livers after both the 4-day and 14-day challenge. The second study was designed to compare a conventional live ST vaccination schedule to ones that could potentially be tailored specifically to higher risk flocks or farms based on either historical or more “real-time” testing. The SE challenge was given at 35 days of age. The SE incidence and loads in both cecas and liver/spleens between the different live ST vaccination schedules were pending at the time of abstract submission but will be presented at the meeting.

### **The use of a nutritional approach to support the control of Salmonella in poultry.**

Raquel Burin<sup>1</sup>, Marco Rebollo<sup>1</sup>

Zinpro Corporation<sup>1</sup>

The poultry industry has been extensively working in ways to reduce the loads of Salmonella being carried by poultry products. It also has faced many challenges when implementing pre-harvest control programs given that Salmonella clinical manifestations in poultry are uncommon, making this pathogen many times ubiquitous to the poultry environment and thereby difficult to control. A potential avenue to overcome this challenge is the use of an intervention capable of stimulating the host's immune response in a way that recognizes Salmonella as a potential threat rather than a normal commensal bacterium. Although Salmonella vaccination program is considered one of the main and most acceptable strategies used with that purpose, its limited efficacy still creates opportunities for exploring new interventions. Given that zinc (Zn) is an essential cofactor for modulating all the branches of the animal's immune response, we thought its supplementation to birds could stimulate a response capable to control

the intestinal colonization and internal organ infiltration of Salmonella. A study conducted at Iowa State University has shown that a more bioavailable source of Zn, such as the Zn-amino acid complex, supported the development of a healthier intestine in broilers challenged with Salmonella Typhimurium. This was evidenced by the significant increased synthesis of the tight junction proteins (occludin and claudin), induction of genes encoding cytokines (IL-6, IL-10 and IL-18), chemokines (CCL4 and CXCL14) and antimicrobials peptides (AvBD5, AvBD9 and AvBD10). Other outcomes such as reduced fecal shedding and reduced internal organs invasion (liver, spleen and reproductive tract) by Salmonella, were observed in studies where chickens were challenged with the serotypes, S. Typhimurium, S. Enteritidis and/or S. Infantis. The direct antimicrobial effect of trace-mineral-amino acid complexes against Salmonella serotypes of human importance was also determined using an in vitro assay at Texas Tech University. In summary, during this presentation the audience will learn how dietary supplementation of trace-mineral-amino acid complexes can support the development of a strong first line of defense in poultry and thereby control the invasion of Salmonella through such barrier. More specifically, we will explore the mode of action of these highly available trace minerals and understand their impact on intestinal microbiota, epithelial integrity, intestinal immunity, and ultimately their influence on cecal recovery and fecal shedding of Salmonella in poultry. Finally, we will propose the in-feed use of trace mineral sources, such as those from amino-acid-complexes, as a potential alternative to reinforce the efficacy of current interventions available to control enteric pathogens in poultry, supported by recent research findings.

## Vaccinology

### **Control of Fowl Cholera in Commercial Layer type birds with Siderophore Receptor and Porin Protein Vaccine**

Charles Hofacre<sup>1</sup>, Dan Domingo<sup>2</sup>, Matthew K. Jones<sup>1</sup>, Jennie Baxter<sup>1</sup>, Tim Hopson<sup>2</sup>

Southern Poultry Research Group, Inc.<sup>1</sup>, Vaxxinoa U.S.<sup>2</sup>

Approximately 38% of the U.S. commercial layers are cage-free or pasture raised. As these pullets and hens are now on the floor, they are exposed to many disease agents such as, *Pasteurella multocida*. The inactivated vaccines with oil emulsion adjuvants have a strong immune response affording protection but the vaccine reaction can cause many pullets to stop eating, thus affecting growth and body weight uniformity, ultimately affecting egg production. The siderophore receptor and porin (SRP<sup>®</sup>) vaccine uses these proteins without LPS. Therefore, the vaccine reaction is reduced and effects on bird growth and performance are minimal. Two hundred (200) Specific Pathogen Free (SPF) layer pullets (AVS Bio) were reared on the floor to 12 weeks of age when 65 were vaccinated by inguinal fold with SRP vaccine (2 time vaccine group). Then at 15 weeks, these were given a second SRP and 65 were given an injection of SRP by inguinal fold (1 time vaccination), all were housed in the same room and were individually wing tagged to differentiate the two vaccine groups from the 65 birds not vaccinated. At 15 weeks, all birds were challenged by intramuscular injection of the USDA challenge strain serotype 1 at  $1.3 \times 10^3$  CFU/bird. All mortality was necropsied and on day 14 post challenge, all remaining birds were examined and any lame were euthanized, necropsied and cultured. There was 61.5% Fowl cholera mortality in the non-vaccinated, challenge control. Both the two SRP vaccination (12 and 15 weeks) and the one SRP vaccination (15 weeks) had 3.1% mortality with a 95% preventive fraction. This study demonstrated that the SRP vaccine is an effective vaccine to prevent Fowl cholera in commercial layer type chickens when given either once or twice prior to egg production.

### **Evaluating the efficacy of novel inactivated and live vaccine approaches for the control of Spotty Liver Disease (SLD) caused by *Campylobacter hepaticus* in layer hens**

Roel Becerra<sup>1</sup>, Roel Becerra<sup>1</sup>, Catherine M. Logue<sup>1</sup>

University of Georgia<sup>1</sup>

Spotty liver disease (SLD) caused by *Campylobacter hepaticus* (*C. hepaticus*) has emerged as an important cause of disease in table egg layers in the United States (US). The disease associated with *C. hepaticus* results in focal lesions on the livers of infected birds, reduced egg production, and increased mortality of layer hens. Currently, there



are no approved treatments, and no commercial vaccine is available for *C. hepaticus*, and there is very limited research available supporting the best approaches for the control of *C. hepaticus* in laying hens. The objective of this project is to evaluate the efficacy of novel inactivated and live vaccine for the control of spotty liver disease. One-Hundred and forty-two commercially available brown hens, free of *C. hepaticus*, 16 weeks of age, will be divided into six groups; two groups (n=18 each) and vaccinated with a live *C. hepaticus* vaccine orally  $10^5$  cfu/ml, and 6 sentinel birds will be added to both groups who receive the live vaccine to determine potential transmission of the live vaccine. Two groups (n=18 each) will receive the inactivated vaccine intramuscularly  $10^{11}$  cfu/ml in the breast muscle and allowed to develop immunity. At 18 weeks of age, one group that received a killed and one group that received a live *C. hepaticus* vaccine will receive a second killed vaccine in the breast muscle. A non-vaccinated but challenged group (n=18) will receive a placebo orally. Sentinels added to the live vaccine group will be euthanized before the second vaccine round to determine if they obtained the live vaccine. All Groups will be placed in battery cages. All groups, live vaccine, killed vaccine, and non-vaccinated groups will be orally challenged 2 times, one day apart with a dose of  $10^{11}$  cfu/ml of *C. hepaticus* at 21 weeks of age. After challenge 6 sentinel birds will be added to the challenge and vaccinated groups. On days 8, and 15 post-challenge, a subpopulation of vaccinated and challenged hens per group will be euthanized to collect tissues for bacterial culture and PCR and to record gross lesions. At 16 days post-challenge, the remaining sentinels will be euthanized to collect tissues for bacterial culture and PCR and record gross lesions. During the entire duration of the study, vaccine reactions, clinical signs, mortality, feed and water intake, and egg production will be recorded. As spotty liver disease has emerged in the last few years, the goal of this study is to develop a vaccine that will protect layer hens against SLD resulting in protection of the flock and reduced economic losses as a result of SLD.

### **The Impacts of a Spotty Liver Disease (*Campylobacter hepaticus*) Autogenous Vaccine on Pasture Raised Production Systems**

Mark Mouw<sup>1</sup>, Dan Wilson<sup>1</sup>, Michaela Olson<sup>1</sup>

Wilson Veterinary Co.<sup>1</sup>

In the last decade, the US laying hen industry has seen a transition to alternative production styles including a number of new free range/pasture access operations. As flocks have gained outdoor access in these alternative systems there has been a notable increase in the incidence of flocks affected by Spotty Liver Disease (SLD) due to *Campylobacter hepaticus* or *Campylobacter bilis* infection. The epidemiologic shift in birds with outdoor access, including soil, that then become affected by SLD has been well-documented in similar production styles in Australia and the European Union. Cases of SLD typically occur around peak egg production resulting in failure to reach peak egg production and the potential for 5-20% hen day egg production declines over the time course of challenge. Depending on a number of factors, such as ambient weather, the same flocks also see an increase in cumulative mortality ranging from 0.5-6% usually over two plus weeks. No specific treatment is approved for SLD and there are currently no commercial vaccines available. At this time supportive care and careful management of flocks are the only suggestions available to affected producers. In following trends in Australia, our clinic has worked closely with autogenous vaccine producers to develop an SLD bacterin in the hopes of preventing or limiting the clinical effects of SLD. Here we will discuss the impacts of the use of a SLD autogenous vaccine in various dosing schemes given to replacement pullets destined for facilities with a previous history of SLD challenge.

### **Protection of Broiler Chickens Against Necrotic Enteritis by Intrapulmonary Delivery of a Live *Clostridium Perfringens* Vaccine Employing Gut-Lung-Axis Concept**

Hemlata Gautam<sup>1</sup>

University of Saskatchewan<sup>1</sup>

[Need abstract](#)

### **Investigation of Low AE titers in Broiler Breeders**

Len Chappell<sup>1</sup>, Dr. Louise Dufour-Zavala<sup>1</sup>

GPLN<sup>1</sup>

Most broiler breeder flocks are expected to have antibodies to AE before they go into production. Vaccines are used in pullets to ensure the seroconversion but often produce low and variable antibody levels by ELISA and high coefficients of variation (CV's). An investigation was done to determine the possible causes for the low titers by comparing different state results, ELISA kits and vaccination programs.

### **Optimising in ovo Herpesvirus of turkey (HVT) - vectored vaccines: Defining the role of the HVT vNr-13 protein in vitro in chicken embryo fibroblasts and in ovo in late stage embryonic tissues**

Vishwanatha Reddy Avalakuppa Papi Reddy<sup>1</sup>, Yaoyao Zhang<sup>1</sup>, Salik Nazki<sup>2</sup>, Andrew J Broadbent<sup>3</sup>, Yongxiu Yao<sup>1</sup>, Venugopal Nair<sup>1</sup>

The Pirbright Institute<sup>1</sup>, Pandemic Sciences Institute, Nuffield Department of Medicine, University of Oxford, United Kingdom<sup>2</sup>, Department of Animal and Avian Sciences, University of Maryland, United States of America<sup>3</sup>

The herpesvirus of turkeys (HVT), an apathogenic alphaherpesvirus of chickens and turkeys, is widely used as a live vaccine against Marek's disease (MD) because of its antigenic relationship with MDV. HVT is also used as a very successful in ovo recombinant vaccine vector (rHVT) to protect against multiple avian diseases, including infectious bursal disease (IBD), Newcastle disease (ND), infectious laryngotracheitis (ILT), and fowl pox. However, although HVT in ovo vaccination has been general practice in the poultry industry for several decades, and billions of doses of recombinant HVT (rHVT) are administered each year globally, our understanding of HVT-induced protection mechanisms is still poorly understood. Moreover, the HVT genome encodes 397 open reading frames, of which approximately 99 are functional genes, and the role of many of these genes remains unknown. It is important to understand the function of these proteins in order to optimise HVT vectors for greater efficacy in the future. The Bcl-2 (B-cell lymphoma-2) family of proteins is known to be important regulators of cell apoptosis. The Bcl-2-related gene, Nr-13, plays a major role in the inhibition of cell death during embryonic development of the avian immune system. HVT has been found to encode a gene product with over 65% sequence similarity to the chicken Nr-13 gene. The functional role of this viral (v)Nr-13 in the context of HVT replication and interaction with the host cell was lacking. Therefore, first, we evaluated the HVT vNr-13 role in vitro in chicken embryo fibroblasts, and the vNr-13 knocked-out HVT (HVT-ΔvNr-13) induced more apoptosis and had a lower viral yield at early time points than the wild-type HVT, suggesting that vNr-13 has an important role in sustaining virus replication by prolonging cell viability for a longer duration. Then, second, to understand whether HVT vNr-13 plays a role in ovo in embryonic tissue replication kinetics, and in innate and adaptive immune response protection mechanisms, viral replication kinetics and transcriptional changes of innate and adaptive immune patterning genes were examined upon in ovo inoculation of wild-type HVT, HVT-ΔvNr-13, rHVT carrying IBDV VP2-protein and NDV F-protein (rHVT-VP2-F), or rHVT-VP2-F-ΔvNr-13. HVT vNr-13 was required for viral replication in ovo in late-stage embryonic tissues, and active virus replication was observed only in the lungs. IFN-inducible Mx1 response was higher with wild-type HVT compared to HVT-ΔvNr-13, and rHVT-VP2-F-ΔvNr-13 in the lungs. In the future, it would be interesting to determine whether vNr-13-mediated MX1-pathways play a role in augmenting the efficacy of HVT, and could be used as molecular-adjuvants to improve the efficacy of HVT-based vaccines, and other avian disease vaccines.

### **A herpesvirus of turkey-based vector vaccine induces the development of antibodies against the HA protein of H5N1 AIV of clade 2.3.4.4b in broiler chickens.**

Aldo Rojas-Neyra<sup>1</sup>, Katherine Calderó<sup>1</sup>, Brighth Carbajal-Lévano<sup>1</sup>, Gloria Guerrero-Fonseca<sup>1</sup>, Julio Ticona<sup>1</sup>, José Goggin-Ortiz<sup>1</sup>, Ángela Montalvan-Avalos<sup>1</sup>, Gisela Isasi-Rivas<sup>1</sup>, Manolo Fernández-Sánchez<sup>1</sup>, Manolo Fernández-Díaz<sup>1</sup>

Farmacológicos Veterinarios S.A.C.<sup>1</sup>

Several outbreaks of highly pathogenic avian influenza (HPAI) H5N1 virus (clade 2.3.4.4b) have been reported in several wild aquatic birds and poultry species in Peru at the end of 2022, which has caused a high mortality rate and economic losses. However, currently, vaccination plays a key role in controlling the avian influenza virus (AIV). For this reason, in this study, we have developed a vector vaccine of the herpesvirus of turkey (HVT) that expresses the hemagglutinin (HA) protein of H5N8 AIV, named rHVT-HA, utilizing the (CRISPR)/Cas9 gene-editing technology via NHEJ repair pathway. The expression of the HA protein was evaluated by Western blotting. Posteriorly, to evaluate the immunogenicity of the vaccine, day-old broiler chickens were divided into two groups. Group 1 (n=10) was vaccinated at 1 day old with 2500 plaque-forming units of the vaccine; group 2 (n=10) was considered as unvaccinated control. Then, the sera were collected at 34 and 41 days post-vaccination (DPV) to be evaluated by H5-specific in-house indirect ELISA and haemagglutination inhibition (HI) test using an HPAI H5N1 virus of clade 2.3.4.4b. The results of Western blotting detected a ~70 kDa band corresponding to the uncleaved HA0, and lower bands around 50 and 25 kDa that represented the HA1 and HA2 subunits of the HA protein respectively in cells infected with the rHVT-HA virus. On the other hand, we showed a high seroconversion rate in the serological samples of the vaccinated chickens; where the ELISA and HI test showed positive results at 34 and 41 DPV. In conclusion, our rHVT-HA vaccine has shown to be an immunogenic vaccine candidate against HPAI H5N1 AIV of clade 2.3.4.4b in commercial broiler chickens.

### **Vaccination of Pullets with an Inactivated Chicken Astrovirus Isolated from a Clinical Case of White Chick Syndrome**

Jason Sousa<sup>1</sup>, Jenny Nicholds<sup>1</sup>, Erich Linneman<sup>1</sup>, Robin Gilbert<sup>2</sup>, Holly Sellers<sup>1</sup>

University of Georgia - PDRC<sup>1</sup>, Wayne-Sanderson Farms<sup>2</sup>

White Chick Syndrome (WCS) is caused by Chicken Astrovirus (CAstV), belonging to genetic Group Biv. Clinical cases of WCS have been observed in broiler breeders exposed to CAstV after the onset of lay. Breeders are not clinically affected, but the virus is vertically shed to progeny and results in significant hatch losses associated with mid and late-dead embryos. Affected embryos that survive will often hatch as small, weak chicks with white down. Green livers have also been observed in the late-dead embryos and white chicks. The decreased hatchability and appearance of white chicks are generally observed until seroconversion occurs, typically 2-4 weeks later. In the absence of a commercial vaccine, control measures have included serological surveillance of pullets then movement of litter from farms with confirmed CAstV seroconversion to farms with seronegative birds. However, litter movement carries the risk of introducing other pathogens to the farms, such as Salmonella. Autogenous vaccines (AV) have included CAstVs, but no data exists to validate their ability to stimulate adequate immunity in breeders to prevent WCS. The objective of this experiment was to 1) develop a CAstV Biv inactivated oil emulsion vaccine using a CAstV isolate from a clinical case of WCS and 2) evaluate antibody production following injection into 3-week-old SPF leghorns. The CAstV isolate was propagated and titrated in LMH cells, then inactivated using beta-propiolactone, formulated into an oil emulsion with Montanide ISA 70 VG adjuvant then injected intramuscularly into 3-week-old SPF leghorns. Birds received a second injection at 8 weeks of age. Serum was collected at 0, 2, 4, and 8 weeks post-vaccination (wpv) for CAstV ELISA (BioChek) and CAstV virus neutralizations (VN). At 0, 2, and 4 wpv no vaccinated birds had developed positive titers via ELISA and VN tests. At 8 wpv, no positive antibodies were detected by ELISA in either groups; however, seroconversion was observed by VN in CAstV vaccinated birds with a geometric mean titer of 168.9. It's not clear why the CAstV ELISA did not detect antibodies in vaccinated birds with neutralizing antibodies. To further evaluate the utility of ELISA and VN, commercial serum samples from breeder flocks with clinical cases of WCS will be tested by both assays. This experiment demonstrated that one injection of inactivated vaccine did not generate detectable antibody levels. However, two injections of a killed CAstV induced neutralizing antibodies in SPF leghorns. Control of WCS by using a CAstV AV may be an option for use in broiler breeders. Progeny studies to detect maternal antibody transfer after vaccination would be useful in confirming this assertion. The ability to determine efficacy would rely solely on field observations as there is no challenge model for WCS.

# Virology

## **Preliminary characterization of avian metapneumovirus subtype B in the U.S.**

Darrell Kapczynski<sup>1</sup>, Kelsey Briggs<sup>1</sup>, Molly Parker<sup>2</sup>, Iryna Goraichuk<sup>1</sup>, Jongsuk Mo<sup>1</sup>, Erica Spackman<sup>1</sup>, David Suarez<sup>1</sup>  
USDA-ARS<sup>1</sup>, Select-Genetics<sup>2</sup>

Avian metapneumovirus (aMPV) is the causative agent of turkey rhinotracheitis, an acute upper respiratory tract infection of turkeys, and is associated with swollen head syndrome in chickens. The disease is usually accompanied by secondary bacterial infections that can increase morbidity and mortality. aMPV infection of turkeys was first reported in the late 1970s in South Africa, and viruses were subsequently isolated in Europe, the U.S., Asia, Central and South America. aMPV belongs to the Metapneumovirus genus within the Pneumovirinae subfamily of the Paramyxoviridae family. It is a single-strand, nonsegmented, negative-sense RNA virus that contains eight genes. Currently only one serotype of aMPV has been described; however, nucleotide sequence analysis has identified four subtypes A, B, C and D. Until recently only subtype C had been identified in the U.S. Here we report on the isolation and characterization of aMPV B in turkeys from North Carolina that were demonstrating respiratory distress and drops in egg production. Swab samples obtained from turkey hens produced a positive reaction by quantitative real-time RT-PCR to aMPV B. Following passage on Vero cells, cytopathic effect was observed that was consistent with metapneumovirus infection. Subsequently, a virus was isolated and its characterization will be discussed. In addition, testing of field samples from North Carolina demonstrated that the virus could first be detected in September of 2023. Taken together these studies report on the first isolation of aMPV B in the U.S.

## **Detection and molecular characterization of avian metapneumovirus subtype B in the US turkey and chicken farms**

Sumit Jangra<sup>1</sup>, Naveen Duhan<sup>1</sup>, Gun Temeeyasen<sup>1</sup>, Muhammad Luqman<sup>1</sup>, Mohamed Helal Selim<sup>1</sup>, Sumit Jangra<sup>1</sup>, Martha Ohnstad<sup>1</sup>, Craig Long<sup>1</sup>

Animal Disease Research and Diagnostic Laboratory (ADRDL), South Dakota State University<sup>1</sup>

Avian Metapneumovirus (AMPV) is a highly contagious virus that inflicts significant economic losses on the poultry industry through respiratory and reproductive disorders. AMPVs are classified into four subgroups (A, B, C, and D) based on the level of genetic variations and antigenic differences. Recently, outbreaks of severe respiratory symptoms and significant decline in egg production were reported in turkey and chicken farms from different states in the USA. The SDSU-ADRDL received tissues and swab samples for detection of pathogens associated with the disease. The next generation sequencing (NGS) using both Illumina MiSeq and Oxford Nanopore MinION of choanal swab samples confirmed the presence of AMPV subtype B. Five whole genomes were assembled from turkey samples and one whole genome from chicken samples. Based on whole genome sequence analysis of these six genomes, subtype B strain from recent outbreak is showing 98.5% nucleotide identity with pathogenic VCO3/60616 and 657/4 subtype B sequences detected in turkeys from France and Hungary, respectively. The whole genomes of subtype B assembled from different farms are 100% identical indicating one type of strain is circulating in chicken and turkeys. Further, realtime RT-PCR kit detecting subtype A/B was used for screening of samples from different farms. 119 out of 157 farms were tested positive by realtime RT-PCR with Ct values ranging from 16.5 to 36.7. Both breeders and commercial flocks were tested positive by NGS and PCR. Positive samples were inoculated on primary and continuous cell lines for virus isolation. This study presents the first documented detection of AMPV subtype B in the US, exhibiting close phylogenetic proximity to European AMPV subtype B strains. These findings warrant further investigation into the emergence, potential consequences, and adaptation of this subtype within US poultry.

## **An Epidemiological look at Infectious Bursal Disease (IBD) in Commercial Broiler Industry in Canada; Geographical Difference and Applied Vaccination programs**

Babak Sanei<sup>1</sup>, Davor Ojkic<sup>2</sup>, Kalen Cookson<sup>3</sup>

Zoetis Canada<sup>1</sup>, Animal Health Laboratory, University of Guelph<sup>2</sup>, Zoetis US<sup>3</sup>

Infection with variant strains of infectious bursal disease virus (IBDV) in commercial broilers in Canada results in moderate to severe bursal atrophy and is consequently involved in an immunosuppressive disease. Typically, in Canada infection occurs after 18-20 days of flock age, although sporadically earlier infections have been observed. Depending on age of IBD infection, affected broiler flocks may go through subclinical infection and/or elevated condemnations at the processing plant, poor growth, increased feed conversion, increased mortality due to concurrent other diseases such as inclusion body hepatitis (IBH), colibacillosis and infectious bronchitis (IB). The objective of this review is to investigate prevalence of variant strains of IBDV across Canadian provinces based on their molecular differences in VP2 region of the virus. The evolving dynamics of variant strains over time across various regions will also be discussed. This data is based on diagnostic submissions of bursal tissue samples to the University of Guelph Animal Health Laboratory for PCR testing and subsequent genotyping of positive samples over the last 13 years. We will briefly address different vaccination programs in several geographical regions that may include hyperimmunization of broiler breeder parents followed by potential in-ovo vaccination of broilers with recombinant or immune complex vaccines, or hatchery spray vaccination and/or on farm vaccination with live intermediate vaccines. One successful approach has been rotation of hatchery and on-farm vaccination regimes, based on seasonal IBD pressure and/or concurrent IBH concerns.

### **Molecular survey of infectious bursal disease virus in Western Europe in 2020-2023 reveals lasting predominance of A3B1 reassortant viruses**

Matteo Legnardi<sup>1</sup>, Francesca Poletto<sup>1</sup>, Claudia Maria Tucciarone<sup>1</sup>, Riccardo Baston<sup>1</sup>, Daniela Pasotto<sup>1</sup>, Giovanni Franzo<sup>1</sup>, Mattia Cecchinato<sup>1</sup>

Department of Animal Medicine, Production and Health (MAPS), University of Padova<sup>1</sup>

Infectious bursal disease (IBD) is an immunosuppressive viral disease causing major losses to the worldwide poultry industry. Its agent, infectious bursal disease virus (IBDV), features a remarkable genetic variability, which in turn may profoundly affect disease manifestation and antigenicity. Recently proposed phylogenetic approaches, through which classification is achieved by sequencing both VP2 (genogroups A0-A9) and VP1 (B1-B5) genes, have proven helpful to perform epidemiological surveys, improving our understanding of current field threats and thus our chance to promptly diagnose and ultimately control IBD. The present work reports the result of molecular diagnostic activities conducted on samples from 9 Western European countries in 2020-2023. In total, 1,443 pooled bursal samples were collected from flocks representing different productive categories and vaccinated following different protocols. Vaccine and field strains were discriminated based on partial VP2 sequencing, then the latter were subjected to partial VP1 sequencing to achieve a full characterization. Demonstrating a considerable infectious pressure, 348 samples tested positive for field strains (24.1%), whereas 599 were positive for vaccine strains (41.5%), 17 proved unsequenceable (1.2%), and 479 were negative (33.2%). Although the sampling intensity varied significantly among the different countries, the obtained results offered valuable insights on which field IBDVs are currently present in the region. Some IBDV types were found to circulate only nationally, such as a segregated clade of genotype A3B1 (17 strains, 4.9%) found solely in Italy and genotype A9B1 (18 strains, 5.2%) which was only detected in Portugal. However, the vast majority of the detected field strains (311, 89.3%) were identified as North-Western European reassortants (genotype A3B1). Lastly, one strain belonging to serotype 2 (genotype A0B1) and another with atypical VP2 features (provisionally classified as genotype AxB1) were detected in France. Despite being first described only in 2017, North-Western European reassortants, originated by a reassortment event involving a very virulent and a classical attenuated virus, have rapidly become predominant in most of Western Europe. The case of Portugal is worthy of particular attention: while only local A9B1 strains were detected at the beginning of the study period, they appear to have been largely displaced by reassortants following their entry in the country in 2021. These findings appear crucial to properly plan monitoring and control activities in the surveyed countries, especially in light of the well-established immunosuppressive potential of North Western European reassortants. Moreover, this information should also serve as a warning for other epidemiological contexts, as an

increasing number of reassortment events is being reported in different parts of the world and the spread of North-Western European reassortants appears not to be over.

### **Decoding the Sequence, Structure, and Antigenicity of Infectious Bursal Disease Virus (IBDV) Isolates in the Delmarva Region Using Whole Genome Sequencing (WGS) and Reverse Genetic Approaches**

Sofia Egana-Labrin<sup>1</sup>, Milos Markis<sup>2</sup>, Shankar P. Mondal<sup>3</sup>, Andrew J Broadbent<sup>1</sup>

Department of Animal and Avian Sciences, University of Maryland<sup>1</sup>, AviServe LLC, Newark, DE, 19702<sup>2</sup>, Salisbury Animal Health Laboratory Maryland Department of Agriculture, Salisbury, MD 21801<sup>3</sup>

Infectious Bursal Disease Virus (IBDV) causes a major immunosuppressive disease in chickens, leading to economic problems in the poultry industry. The Delmarva (DMV) region is a major US poultry-producing area, and while the control of IBDV relies on broiler and breeder vaccination, immunized flocks may harbor subclinical infections with strains that continue to evolve by antigenic drift and/or reassortment, which can lead to immune escape and vaccine failure. Several approaches were taken to assess IBDV field isolates. First, we characterized field isolates currently circulating on the DMV based on genome sequence data and structural modeling of the hypervariable region (HVR) of the VP2 capsid gene, encoded by segment A, and the VP1 gene, encoded by segment B. Second, we evaluated the antigenic cross-reactivity of a panel of recombinant chimeric IBDVs engineered by reverse genetics to define the contribution HVR mutations make to immune escape. Third, we provide up-to-date whole genome sequencing (WGS) information regarding currently circulating strains. Finally, we evaluated the presence of co-infection viruses in the field samples. Bursal samples were obtained from commercial farms between 2018 and 2024, and, after RNA isolation, the samples were subject to reverse-transcription polymerase chain reaction (RT-PCR), amplifying the HVR and VP1 genes. Sanger sequencing revealed that all the strains belonged to genogroup A2B1, typical of US variant strains, however, there was a unique "genetic signature" in the HVR of some sequences, compared to the prototype strain DE Variant E: S215N, I272V, S317R, G322E, and E323D, suggestive of a novel IBDV variant. This variant was present in only 16% of the samples in 2007, but increased in prevalence to over 57% during recent years, suggesting it might have a fitness advantage over other strains. We are currently evaluating the contribution this genetic signature makes to immune escape using a reverse genetics system. Additionally, we detected mutations in and around a key region of VP1 that correlates with virulence (amino acids 145-147), with 7/28 (25%) of the sequences having mutations at position 145, and 20/28 (71%) having mutations at position 147. Interestingly, one isolate had two mutations: D146N and D147S, that have been partially attributed to very virulent strains, suggesting there could be differences in virulence between the strains, which we plan to explore in the future. Furthermore, 2/28 (7%) of the bursal samples were found to be positive for avian reovirus, demonstrating that coinfection with multiple immunosuppressive viruses occurs in the field. We are now conducting WGS of both IBDV segments A and B from the field samples, and we are increasing our sampling to present the results from a larger dataset.

### **Infectious laryngotracheitis viruses (ILT) Genotypes Associated with Cases of the Disease between March 2023 to April 2024.**

Maricarmen Garcia<sup>1</sup>, April Skipper<sup>2</sup>

University of Georgia<sup>1</sup>

Infectious Laryngotracheitis (ILT) is an acute respiratory disease of poultry of endemic nature worldwide. The disease is caused by the infectious laryngotracheitis virus (ILT), also known as Gallid alphaherpesvirus 1 (GAHV-1). Antigenically, ILTV strains exist as a single serotype. Therefore, viral strain differentiation is based on specific genome differences. Knowledge of ILTV genotypes allows veterinarians to determine whether the circulating virus is of vaccine (vaccinal LT) or field origin. This knowledge facilitates investigating events that potentiated the virus introduction and help prevent or anticipate future exposures. Also, periodic viral genotyping during disease outbreaks within a dense poultry production region (State) allows for tracking changes in circulating viruses and correlating these trends with disease severity, transmission, and efficacy of vaccination campaigns. In 2023, our

laboratory received 25 ILT cases for viral genotyping. These submissions came from six states and nine companies. Twenty of the 25 cases that spanned four states (A, B, C, and D) and five companies were determined to be genotype VI; 18 originated from broiler flocks and two from broiler breeder flocks. Viruses belonging to genotype VI are not related to live vaccine usage or circulating vaccine-related viruses and are known for their increased pathogenicity and transmissibility. The remaining five cases belong to genotype IV and originated from broiler flocks in three states (B, E, and F) involving three companies. CEO vaccines and circulating CEO-related viruses belong to genotype IV. So far, these data indicate that Genotype VI viruses are still causing considerable disease outbreaks. Genotype analysis of 2024 cases is ongoing.

### **Binding Ability of the Hemagglutinin-Neuraminidase Protein as a Predictor of Pathogenicity of Newcastle Disease Virus Isolates**

Farjana Saiada<sup>1</sup>, Kellye S Joiner<sup>1</sup>, Vicky van Santen<sup>1</sup>

Department of Pathobiology, College of Veterinary Medicine, Auburn University<sup>1</sup>

The Newcastle disease virus (NDV) hemagglutinin-neuraminidase (HN) glycoprotein is responsible for binding to target cells and triggering the viral fusion (F) protein to initiate viral entry. Although the virulence of NDV is primarily determined by the F protein's cleavage site, the ability of the HN protein to bind to chicken tissues could also be an essential determinant of virulence. We hypothesized that differing pathogenicity of highly virulent NDVs with similar F proteins is associated with the ability of the HN protein to bind to relevant chicken tissues. To compare tissue binding activity of recombinant HNs we developed a protein histochemistry assay. Secreted tetrameric strep-II-tagged recombinant protein representing the HN protein of highly virulent chicken/California/D1806566/2018 (CA18) NDV strain was produced in HEK293T cells and affinity purified using Strep-Tactin Sepharose. Then, the HN protein complexed with streptactin-HPRO was incubated with relevant formalin-fixed chicken tissues, and bound protein detected with chromogenic substrate DAB. Strep-tagged infectious bronchitis virus S1 protein was used as a positive control. We observed binding of the HN from the highly virulent CA18 to respiratory epithelium (in choana, conjunctiva, trachea, and lungs), epithelial cells in the digestive tract (duodenum, jejunum, ileum, cecal tonsil, and cloaca), epithelial cells in kidney, and cells in the spleen and Harderian gland. This technology will allow comparing tissue binding activity of recombinant HNs representing NDVs of differing virulence for chickens and other bird species.

### **Efficacy of Newcastle Disease Virus–Based Vectored Vaccine Against H5N1 Highly Pathogenic Avian Influenza Virus Challenge in SPF Chicken**

Katherine Ivette Calderón Mayo<sup>1</sup>, Aldo Rojas-Neyra<sup>1</sup>, Gloria Guerrero-Fonseca<sup>1</sup>, Brighth Carbajal Lovano<sup>1</sup>, Julio Ticona<sup>1</sup>, Jose Goggin-Ortiz<sup>1</sup>, Suly Montoya-Ortiz<sup>1</sup>, Angela Montalvan-Avalos<sup>1</sup>, Manolo Fernandez-Sanchez<sup>1</sup>, Manolo Fernandez-Diaz.<sup>1</sup>

FARVET, Laboratories of research and development, Chincha, Peru<sup>1</sup>

The H5N1 subtype of highly pathogenic avian influenza virus (HPAIV) was detected in aquatic wild birds and poultry species in Peru at the end of 2022. Vaccination is one of the most effective ways to control avian influenza (AI) outbreaks, and the Newcastle disease virus (NDV)-based vaccines against AI have proven to be efficacious and safe in poultry. In this study, we have developed two NDV-based vaccines (rNDV-H5) that express a codon-optimized (rNDV-H5Opti) and non-codon-optimized (rNDV-H5non-Opti) hemagglutinin (HA) gene of H5N8 virus. The expression cassette of the HA protein was introduced into the genome NDV in the intergenic region between the phosphoprotein (P) and the Matrix (M) genes. PCR, IFI, and Western blotting were carried out to evaluate the expression of the HA protein. Immunogenicity and efficacy of the vaccines were assessed in chicken's specific pathogen-free (SPF); the humoral response was measured at 14 and 27 days post-immunization (dpi); the effectiveness against AI was evaluated at 28 dpi by a challenge using a H5N1 HPAIV of clade 2.3.4.4b isolate in Peru. Viral shedding was assessed at 5 and 10 days post-challenge (dpc), and clinical signs and mortality were monitored until 14 dpc. Results showed that both recombinant vaccines expressed the HA protein in infected cells in vitro. In

animal experiments, both vaccines induced high titer of H5-specific antibodies and offered protection against a lethal challenge with the highly pathogenic H5N1 virus. Both vaccinated groups had no clinical signs and low viral shedding after H5N1 HPAIV. In contrast, non-vaccinated chickens died at 4 dpc. In conclusion, the vaccines of this study offered protection against HPAIV H5N1 of clade 2.3.4.4b.

### **Comparing gene expression in chicken Harderian glands and tracheas with and without maternal antibodies after vaccination with LaSota Newcastle Disease Virus**

Taina Lopes<sup>1</sup>, Taina Lopes<sup>2</sup>, Jannis Nankemann<sup>2</sup>, Andrea Pietruska<sup>2</sup>, Ruediger Hauck<sup>2</sup>

Universidade Federal de Minas Gerais<sup>1</sup>, Department of Pathobiology, Auburn University<sup>2</sup>

Newcastle disease (ND) affects poultry, causing significant economic losses due to trade restrictions. Current vaccines do not prevent infection and virus shedding. However, preventing virus shedding is an important step to minimize the risk of spreading. Understanding the expression of immune-related genes to identify target genes involved with virus shedding pathways will assist vaccines efficiency. Furthermore, another aspect that needs to be better understood is the effects of maternal derived antibodies (MDAs) on vaccinal response. The mechanisms by which MDAs affect the response to the Newcastle vaccine have not yet been fully elucidated. Therefore, the objectives of this study were to evaluate immune gene expression in chicken Harderian glands (hg) and tracheas (tc) after ND vaccination in the presence or absence of maternal antibodies. Fifty-six specific pathogens free (SPF) embryonated eggs and white leghorn eggs (CM) with maternal antibodies obtained from a commercial hatchery were incubated and hatched in a BSL2 incubators. After hatch, the SPF and CM birds were split in two groups each to be vaccinated at 1d or 14d old. Finally, each group of each age was divided into vaccinated or unvaccinated control. Birds received 107 EID50 of NDV strain LaSota in 100 µl via oculo-nasal route, while the other group was mock-vaccinated with PBS. Twenty-four- and 48-hours post-vaccination (hpv), seven birds per group were euthanized for sampling collection. Hg and tc were collected for total RNA extraction, followed by mRNA-sequencing by Illumina sequencing. Differential expression of genes, gene ontology, KEGG pathways enrichment and protein-protein interaction analysis were performed. For transcriptome analysis reads were trimmed and aligned to the NCBI chicken reference genome using hisat2. Reads were counted using HTSeq. Differently expressed genes and regulated pathways related to the innate immune system were identified using edgeR, limma, and Cytoscape. A two-way anova-like test with edgeR package was used to check interactions between bird type and age for each organ, and timepoint. Results are going to be presented.

### **Detecting Infectious Bronchitis Virus Recombinants From Field Samples: A Next Generation Sequencing Approach**

Robert Beckstead<sup>1</sup>, Robert Beckstead<sup>1</sup>, Sean Brimer<sup>1</sup>, James White<sup>1</sup>, Scott Callison<sup>1</sup>, Mark Jackwood<sup>1</sup>

Ceva Animal Health, LLC<sup>1</sup>

The Infectious bronchitis virus (IBV) undergoes rapid evolution, primarily driven by frequent mutations and recombination events, leading to the emergence of new IBV antigenic variants. The majority of these variants result from genetic drift, which involves the accumulation of mutations in the S1 portion of the spike gene. Recombination within the S1 region can also occur, contributing to the rapid development of novel variants. These genetic variations may not be detected when using traditional qPCR identification testing. In a study utilizing a Next-Generation Sequencing (NGS) protocol targeting the entire S1 region of the IBV genome, two recombinant sequences (DMV1639/Ark or "DARK" and GA98/Mass or "GASS") were identified in field samples obtained from commercial broilers. The recombination site for DARK was pinpointed at S1 nucleotide 471, while for GASS, it occurred at nucleotide 627. Following isolation and purification in chicken embryonated eggs, the viruses underwent whole genome sequencing (Illumina), confirming the S1 sequences for both recombinants. Using this sequence information, screening of previous field samples revealed DARK's presence 10 times through NGS in both broilers and broiler breeders from the same state, with all sequenced viruses demonstrating similarity. GASS was identified once. Examining sequences at the recombination site in S1 for both viruses revealed intriguing relationships,



suggesting potential implications for cross-over events within that gene. Because of its more widespread nature and likelihood that it is a recombinant between a field (DMV/1639) and vaccine (Ark) virus (whereas GASS likely arose from recombination between two vaccine viruses), we conducted pathogenicity studies on DARK and found it to be pathogenic in SPF chickens. In addition, a vaccine combination effective against DMV/1639 protected against the recombinant virus indicating that although it was a recombinant within the S1 gene, it was not a new antigenic variant. This study demonstrates the use of NGS technology in detecting new IBV variants in the field and the need for classical virology and animal studies prior to making changes to current vaccine strategies.

### **Avian hepatitis E virus in the U.S. diagnostics, isolation, and characterization**

Rodrigo Gallardo<sup>1</sup>, Evelin Saenz<sup>1</sup>, Rachel Jude<sup>1</sup>, Sofia Egana<sup>2</sup>, Laura Flores<sup>1</sup>, Isa Ehr<sup>3</sup>, Jim Stockam<sup>4</sup>, Andrew Broadvent<sup>2</sup>

University of California, Davis<sup>1</sup>, University of Maryland<sup>2</sup>, Hendrix Genetics<sup>3</sup>, Merck Animal Health<sup>4</sup>

Cases of lingering mortality, in egg layer flocks between 30 to 50 weeks of age, caused by enlarged spleens, fatty livers and bloody exudate in the coelomic cavity reminded us about the existence of avian hepatitis E virus (aHEV). While properly described as a pathology in chickens the virus is poorly studied. Most of the information we have, is due to its use as a model to study Hepatitis E in humans. Since this effort was abandoned, reagents and expertise were lost and as a good re-emerging disease, we need to update our expertise and understanding of the virus and the disease. To understand the seroprevalence, several flocks were screened for the presence of antibodies against aHEV using a commercially available ELISA kit. Out of 210 serum samples screened from hens between 20 and 110 weeks, 30% were seropositive. We developed a RT-qPCR to screen tissues from infected birds looking for the best diagnostic sample. We found that gallbladder had the highest viral load among the tissues tested. We also genetically characterized the virus from samples across the U.S. After full genome sequencing using Oxford Nanopore technology, we found that two genotypes namely 2 and 3. This is the first description of genotype 3 in our country. When we attempted virus isolation, while we were not able to isolate the virus in embryonated eggs inoculated intravenously, we observed replication in LMH cells after 4 passages even though, at low titers. Finally, we were able to prove Koch postulates. Using bile as our virus source and after challenging 7-week-old SPF pullets via oral, cloacal and IV we were able to reproduce a mild disease affecting the liver among other measured parameters in these birds.

## **Wealth of Knowledge**

### **ATP Meter for Evaluating Water Line Sanitation**

Kabel Robbins<sup>1</sup>

Butterball, LLC<sup>1</sup>

Optimal water quality is important for producing healthy turkeys. On-farm water sanitation is necessary to prevent microbial contamination of turkey drinking water from both pathogenic and non-pathogenic microbes. Biofilm buildup is unfortunately common in turkey water lines. Removal of any accumulated biofilm from the lines must be a component of an effective water sanitation program. But just because a line cleaner has been used in the lines between flocks doesn't mean all biofilm has been removed. A verification step is necessary, especially on farms with historical health challenges attributed to water sanitation, to ensure the lines are clean and free of biofilm before the next flock is placed. Monitoring turkey farms for biofilm and evaluating effectiveness of water sanitation programs has traditionally been done by swabbing and enumerating bacteria and fungi. This gold standard evaluation is lengthy, often taking over a week to get results. Data will be shown evaluating an ATP meter for rapid on-farm results for water line sanitation monitoring including demonstration of repeatability, accuracy, and correlation with the gold standard methodology. Based on this research, ATP meters are now being used by our veterinary team to provide on-farm feedback in real-time to more effectively improve water quality and bird health.

## **Hatchery investigations: proposing a protocol to maximize insight and efficiency.**

Isabella Hannay<sup>1</sup>, Donna Hill<sup>2</sup>, Marcela Arango<sup>1</sup>

Mississippi State University<sup>1</sup>, Donna Hill Consulting<sup>2</sup>

The hatchery represents a vital component of vertically integrated poultry operations. Good hatchery practices can maximize the success of a flock. In contrast, poorly performing hatcheries can contribute to substandard flock performance, and in some cases, catastrophic losses involving bird health and livability. In order to ensure problem areas are efficiently identified and resolved, a hatchery investigation protocol has been developed. The protocol and associated data collection sheets were designed and revised following field application and peer feedback. Feedback during the design process highlighted barriers encountered when performing hatchery evaluations. Most veterinarians understood the need to conduct a hatchery evaluation when bird health, performance or livability issues arise. However, a number of participants found it overwhelming to assess individual hatchery components when faced with the hatchery as a whole. Improvement opportunities may be missed due to time constraints and perceived lack of knowledge. Based on these comments, the protocol was designed to be comprehensive, easy-to-use and time efficient. The proposed protocol guides the user to approach the investigation systematically and ensures each component of the hatchery is considered. The results of guided data collection will allow conclusions to be drawn about overall hatchery efficiency. This investigation protocol has been piloted in hatcheries belonging to 5 different broiler production companies in the Southeastern United States. The assessed hatcheries reported a variety of issues relating to topics such as incubation, egg storage and sanitation. A number of the assessed hatcheries reported no issues. Two assessments were completed at each hatchery, one by the primary author and another by a participant blinded to the reported issue. The results of each assessor were compared to determine if this protocol will identify key problem areas irrespective of previous hatchery training. The ability to perform a comprehensive hatchery assessment efficiently using the proposed methodology will encourage routine hatchery assessment as part of wider bird health and performance investigations.

## **A 12-Year Retrospective on Hatchery Applied Vaccines in the Layer Industry**

Ian Rubinoff<sup>1</sup>

Hy-Line North America<sup>1</sup>

Commercial laying hens receive a number of vaccines in the hatchery before being delivered to the pullet farm. Hatchery vaccines are particularly important because they allow the bird to start developing immunity to the chosen pathogen prior to any field exposure. The hatchery is a logical point for vaccination because of automated processing equipment and the need for counting chicks into boxes. Current hatchery vaccines for laying hens can be applied in two categories: spray and injection. Hatchery spray vaccination allows for uniform coverage of each chick box. Injectable vaccines ensure a proper titration of each dose and allow for individual chick inoculation before leaving the hatchery. By looking at historical hatchery vaccination trends, we can understand both the changes in disease pressure and the evolution of vaccine technologies. The last dozen years has demonstrated considerable change in the North American egg industry as we have seen the growth of cage-free production (from under 10% to almost 40% of hens), the adaptation of vectored vaccines, and disease issues such as false layer syndrome, velogenic Newcastle disease, highly pathogenic avian influenza, and infectious coryza. For this study, we will discuss vaccines applied in Hy-Line North America hatcheries. Using serotype 1 Marek's vaccine as a benchmark (as a rule, no laying hen chick in North America should leave the hatchery without this vaccine) we will compare inoculation percentages of various vaccine types. Spray vaccines tracked include coccidiosis, infectious bronchitis, and Salmonella Typhoid. Injectable vaccines tracked include fowl pox, infectious bursal disease (live and vectored), infectious laryngotracheitis vectored, Marek's serotypes 1, 2, and 3, and Newcastle disease vectored. The vaccine with the highest increase in usage percentage was infectious bronchitis which went from none vaccinated in 2012 to over 72% of chicks vaccinated in 2024. The vaccine that decreased the most was serotype 2 Marek's vaccine which went from approximately 20% of chicks vaccinated in 2012 to under 10% of chicks vaccinated in 2024.

## **Development and Implementation of a Framework and Assessment Tool to Evaluate an Organization's Antimicrobial Stewardship Policies and Practices**

Ken Opengart<sup>1</sup>

3 Birds Consulting<sup>1</sup>

As poultry industry veterinarians, we recognize and embrace our responsibility in the production of high-quality, safe, and affordable protein that contributes to the sustenance of society. Our responsibilities outlined within the veterinary oath include the protection of animal health and welfare, the prevention and relief of animal suffering, the conservation of animal resources, the promotion of public health, and the advancement of medical knowledge. Within a poultry production system, these responsibilities are supported by evidence-based preventive health programs, robust biosecurity programs, effective vaccine strategies, appropriate environmental and production system management programs, appropriate therapeutics, and other practices that minimize stress and reduce disease incidence and severity. Even with the best-designed programs, however, animals still become sick, and antimicrobials may be necessary. In these cases, robust antimicrobial stewardship (AS) programs must be maintained to safeguard the efficacy of the limited tools we have to prevent, control, and treat disease. According to the AVMA, antimicrobial stewardship refers to the actions veterinarians take individually and as a profession to preserve the effectiveness and availability of antimicrobial drugs through conscientious oversight and responsible medical decision-making while safeguarding animal, public, and environmental health. For poultry veterinarians, decisions to use antimicrobials should be based on the best available veterinary medical evidence, ethically and legally, and following guidelines for responsible use of antimicrobials established by organizations like the World Organization for Animal Health and the American Veterinary Medical Association. There is no comprehensive tool available for poultry practitioners to evaluate an organization's antibiotic stewardship program. Such a tool could assess relevant programs, policies, and practices associated with antimicrobial use across a production system and identify opportunities for continuous improvement. In support of these desired outcomes, an AS framework and assessment were developed which includes five key elements (commitment and culture, veterinarian guidance and partnership, disease prevention strategies, optimal treatment approaches, and antibiotic use records). Within each framework key element, three core components (foundation, implementation, and evaluation) are used to assess relevant organizational AS programs, policies, and practices. The framework and assessment were used to evaluate the current organizational AS program and identify program gaps, that when closed, would lead to continuous improvement and a more robust AS program. An example of the framework, assessment, and assessment findings will be shared to demonstrate how veterinarians can create, implement, and advance an antibiotic stewardship program within a poultry company.

### **Overview of the AVMA Guidelines for the Depopulation of Animals and the revision process**

Cia Johnson<sup>1</sup>, Michelle Kromm<sup>2</sup>

AVMA<sup>1</sup>, Food Forward LLC<sup>2</sup>

Stemming from discussions of the Panel on Euthanasia, the AVMA convened its Panel on Depopulation (POD) for the first time in 2015. The subsequent AVMA Guidelines for the Depopulation of Animals (2019) reflects the AVMA's on-going commitment to ensure that the treatment of animals during every stage of life, including during emergency situations, is respectful and as humane as possible. These Guidelines provide guidance for veterinarians about 1) options for killing animals in emergency situations, and 2) how to prevent or minimize pain and distress in animals that have been designated for depopulation in accordance with clinical standards of care, local, state and federal regulatory bodies and to ensure a quick and effective depopulation process that respects animals, human beings and the environment. The Guidelines define depopulation as: the rapid destruction of a population of animals in response to an emergency situation, which may include disease control, or natural or human-made disaster. The POD developed these Guidelines for use by members of the veterinary profession who are involved in the rapid destruction of a population of animals in response to urgent circumstances with as much consideration given to the

welfare of the animals as practicable. This session will cover the AVMA Guidelines for the Depopulation of Animals since its release and the process of review and revision, member comment period, and anticipated publication.

### **CRISPR: An easy button for disease mitigation?**

Michelle Kromm<sup>1</sup>, Carol Cardona<sup>2</sup>

Food Forward LLC<sup>1</sup>, University of Minnesota<sup>2</sup>

The use of genetic engineering (GE) techniques to improve the production and sustainability of animal agriculture holds great potential. For example, using the CRISPR/Cas9 system to modify host cell receptors or biological function to make them more resilient to pathogens, making the raising of animals for food more productive, efficient, and sustainable. But when should we deploy these powerful tools to tackle big challenges such as improving the health and welfare of the birds in our care? What considerations beyond the host-pathogen interaction should be considered before utilizing GE technologies? HPAI made headlines in a different way last Fall when a group of scientists from the UK announced they had utilized CRISPR to generate chickens that had increased resistance to infection to avian influenza (1). While there is no doubt that the global epizootics of HPAI have caused tremendous strain on humans and natural resources as well as a profound loss of life in bird populations, there needs to be caution when deploying such powerful tools that affect ultimately the food we eat. In addition, while there are diseases that are amenable to remediation through GE, the highly complicated epidemiology of HPAI combined with a highly mutable virus creates a situation for many potentially unknown consequences of the impact of GE. While advancements in scientific discovery in biomedical research need to be celebrated when we look at applying those advancements outside the laboratory walls, many additional considerations need to be made. How successful will the mitigation be when it's deployed in the unpredictable real world? When the impact is on the food we eat, what considerations need to be made to stakeholders outside the scientific community? How can we take our learnings from No Antibiotics Ever (NAE) marketing and apply them to foods derived from GE animals?

### **Feed Withdrawal: Review and Lessons Learned Over a Lifetime**

Timothy Cummings<sup>1</sup>, Doug Fulnechek<sup>1</sup>

Zoetis<sup>1</sup>

Feed withdrawal programs are utilized by the poultry industry as a management tool to minimize contamination and reduce feed cost/loss. It is designed to allow emptying of the intestinal tract of the birds to allow for the processing of the majority of the birds during a "window of time" before yield loss becomes excessive and intestinal strength starts to decline. Dr Stan Savage pioneered a series of projects in the 1980s to assess the normal, gross physiological changes that occur in the intestinal tract as feed passes through and out of the intestines of broilers during a feed withdrawal period. Over the years, the author has worked with numerous companies in assessing their feed WD program, and will offer insights learned in working with the modern broiler.

## Poster Presentations

### Antimicrobial/Antibiotic Resistance

#### **Agroforestry tree leaves as feed supplements for improving growth and as an alternative to antimicrobial drugs in poultry breed of Jammu and Kashmir, India.**

Mandeep Singh Azad<sup>1</sup>

*Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu<sup>1</sup>*

Antibiotics are being used in poultry feed from last 40-50 years at sub therapeutic doses as a strategy to improve feed conversion rates and to reduce production costs. Although inclusion of antimicrobials in the poultry feed has increased the production potential of poultry birds but at the cost of development of antimicrobial resistant population. Present study was carried out to assess the effect of locally available agroforestry tree leaves in hilly areas of Jammu and Kashmir, India on production potential and to control parasitic load in Kadaknath poultry birds. Kadaknath is only melanin rich black coloured meat breed of India. Kadaknath poultry birds of 28 days old were divided into four groups of 60 birds each in a completely randomized design. The locally available agroforestry trees like, guava (*Psidium guajava*), neem (*Azadirachta indica*), jamun (*Syzygium cuminii*) and mango (*Magniferra indica*) found in hilly areas of Jammu and Kashmir were used to prepared leaf meal mixtures in 1:1:1:1 proportion and used at different inclusion levels (T1=0 %, T2=1.5%, T3=2.5% and T4=3.5%). Four iso-caloric and iso-nitrogenous diets were formulated with inclusion of Agro forest leaf meal mixtures at 0%, 1.5%, 2.5% and 3.5% for T1, T2, T3 and T4 respectively. Experiment lasts for 105 days (15 weeks). Data regarding daily feed intake, adult body weight, Average body weight gain, FCR, body score and fecal egg count were recorded and results were subjected to one way ANOVA accordingly. Results indicate there is no significant change in dry matter intake of Kadaknath birds of T1, T2 and T3 groups but significant ( $p<0.05$ ) decrease in feed intake of T4 group of kadaknath birds. Daily body weight gain, average final body weight, FCR and overall body score were significantly increased in T3 group as compared to T1, T2 and T4 groups of birds. Fecal egg count was significantly ( $P<0.001$ ) decreased in T2 and T3 groups as compared to T1 and T4 group of birds. Overall, the use of leaf meal mixture @ 2.5% improved the overall body score as compared to controlled group. Leaf meal mixture prepared from guava (*Psidium guajava*), neem (*Azadirachta indica*), jamun (*Syzygium cuminii*) and mango (*Magniferra indica*) in 1:1:1:1 proportion when used at 2.5% in the poultry ration significantly increased the overall production performances in Kadaknath poultry birds. Conclusion: The use of agro forest leaf meal mixtures appear as alternative to the use of antimicrobial growth promoter factors. These natural products do not leave residues in poultry derived products. Also, agro forest leaf meal mixtures contain photochemical substances with many bioactive principles that would have fewer chances to induce resistance in microorganisms.

#### **Relationship between antimicrobial use and resistance in Salmonella, Campylobacter, E. coli and Enterococcus from an on-farm U.S. broiler production monitoring system: 2020-2023**

Randall Singer<sup>1</sup>, Iteeshree Mohapatra<sup>1</sup>, Jennifer Staffenhagen<sup>1</sup>, Kari Mattison<sup>1</sup>, Brittany Peters<sup>1</sup>

*University of Minnesota*<sup>1</sup>

The objective of this project was to design a sustainable on-farm antimicrobial use (AMU) and antimicrobial resistance (AMR) monitoring program representative of the U.S. broiler chicken industry. The program was implemented as a cross-sectional sampling of farms. Each company that voluntarily participated selected the complexes to enroll, and then each complex selected 4-8 farms for sampling during each 3-month interval. Litter samples were cultured for Salmonella, Campylobacter, E. coli and Enterococcus. A total of 346, 356, 364 and 357 farms were sampled in 2020, 2021, 2022 and 2023, respectively. Approximately 50% of the sampled farms raised animals without antimicrobials. No medically important antimicrobials were used in the feed of the sampled flocks. S. Kentucky and S. Infantis were the most common Salmonella serotypes identified. Most S. Kentucky isolates were pan-susceptible or resistant to tetracycline (TET) whereas most S. Infantis isolates were multidrug resistant (MDR), typically with resistance to sulfonamide, tetracycline and fluoroquinolone antimicrobials. There was no statistical association between AMU and the presence of MDR S. Infantis. Most Campylobacter isolates were C. jejuni, and most were pan-susceptible or resistant to TET only; approximately one-third had resistance to ciprofloxacin. There was no statistical association between AMU and resistance in C. jejuni isolates. The most common AMR in E. coli was against sulfonamides and tetracyclines, but there was no statistical association between AMU and AMR in E. coli. The prevalence of resistance against gentamicin in E. coli was between 10 and 20%, which represents a substantial decline from the prevalence 5 to 10 years ago. The most common AMR in Enterococcus was against streptogramins and tetracyclines, with some resistance to erythromycin and ciprofloxacin, especially in E. faecium. To capture long-term associations between AMU and AMR, these datasets need to be collected in parallel at the farm level.

### **Phenotypic and genotypic antimicrobial resistance profiling of Staphylococcus xylosus isolated from layer chicken barn bioaerosols in Alberta, Canada**

Awais Ghaffar<sup>1</sup>, Karen Liljebjelke<sup>1</sup>, Sylvia Checkley<sup>1</sup>, Faizal Abdul Careem<sup>1</sup>

*Faculty of Veterinary Medicine, University of Calgary*<sup>1</sup>

Poultry environment has a vast variety of bacterial species and staphylococci are found in highest frequency (90%). S. xylosus is among normal inhabitant coagulase negative Staphylococcal species found predominantly in bioaerosol of poultry. The study objective is to isolate and identify S. xylosus from bioaerosols of layer chicken barns in Alberta along with the phenotypic and genotypic AMR profiling of the isolated bacterial colonies. A total of 18 barns in Alberta will be sampled. The XM-CV air sampler was used to collect air samples. Samples were plated on mannitol salt agar for isolation of S. xylosus. Three presumptive colonies were randomly chosen from the mannitol salt agar and further identified by Staph API strips. Phenotypic AMR profiles of S. xylosus were determined by using the Sensititre<sup>®</sup> automated microbroth dilution method on the standard CMV2AGNF plate (Sensititre<sup>®</sup>). Samples were submitted to University of Montreal for whole genome sequencing. The sequencing results were processed and analyzed by Galaxy software. 14 barns have been sampled so far, and S. xylosus were isolated and confirmed from 10 barns. Ten of the S. xylosus isolates were assessed for phenotypic AMR profile against different classes of antibiotics. Susceptibility results showed 40% (4/10) resistance to Lincomycin, 30.0% (3/10) resistance to tetracycline and 10 % (1/10) resistance to Tylosin. Ciprofloxacin, chloramphenicol, erythromycin, daptomycin, kanamycin, gentamicin, nitrofurantoin, linezolid, penicillin, synergid, streptomycin, vancomycin and tigecycline

were found sensitive against all isolates. There were no virulent genes found in the drafted genome of 1 WGS results and Mph (C) gene encoding for erythromycin, tet (k) gene encoding for tetracycline and rep7a gene and were found on same contigs while Lnu (A) gene encoding for lincomycin resistance and rep21 gene were found on same contigs. Six of the isolates are sent for whole genome sequencing and are waiting for the sequencing results. This is the first report of the isolation of *S. xylosus* and phenotypic and genotypic AMR profiling from layer chicken barn bioaerosols in Alberta, Canada.

### **Trained immunity or innate immune memory induced by CpG-ODN to protect broiler chickens against bacterial diseases**

Iresha Subhasinghe<sup>1</sup>, Khawaja Ashfaque Ahmed<sup>1</sup>, Hemlata Gautam<sup>1</sup>, Shelly Popowich<sup>1</sup>, Ayumi Matsuyama<sup>1</sup>, Suresh Tikoo<sup>1</sup>, Susantha Gomis<sup>1</sup>

*University of Saskatchewan<sup>1</sup>*

Iresha SubhasingheA, Khawaja Ashfaque AhmedA, Hemlata GautamA, Shelly PopowichA, Ayumi MatsuyamaA, Suresh TikooB & Susantha Gomis A\* ADepartment of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, S7N 5B4, CanadaBVIDO-InterVac., 120 Veterinary Road, University of Saskatchewan, Saskatoon, SK S7N 5E3, Canada\*Corresponding author: Email: susantha.gomis@usask.ca Bacterial diseases severely impact the poultry industry leading to economic losses. Alternative strategies to control bacterial infections are a priority. We have demonstrated the immunoprotective ability of oligodeoxynucleotides containing cytosine phosphodiester guanine motif (CpG-ODN) in broilers and its ability as an alternative to antibiotics. We have previous shown that a single dose of CpG-ODN at hatch or by the in ovo route can protect neonatal broilers for the first week of life. This current study explores the possibility of protecting broilers during the entire production cycle by inducing trained immunity or innate immune memory. It has been demonstrated mammals that immune cells undergo profound metabolic changes and metabolic reprogramming while attaining memory characteristics to augment protection against infectious disease by inducing trained immunity. To investigate this possibility, neonatal broiler chickens were injected with CpG-ODN (one, two, three, and four doses), and mitochondrial OXPHOS and cellular glycolysis were measured using Seahorse XFp analyzer to characterize kinetics of immune cells. In another experiment, birds were injected with two doses of CpG-ODN at 1 and 4 days of age and challenged with a lethal dose of *E. coli* at day 27 of age. We observed that immune cells switched to mitochondrial OXPHOS for energy demand from cellular glycolysis which indicated changing phenotype of immune cells to memory cells following two doses of CpG-ODN at 1 and 4 days of age. Remarkably, there was significant protection of broilers against *E.coli* at day 27 of age following two doses of CpG-ODN at days 1 and 4. This indicates that immune cells shift metabolism from glycolysis to mitochondrial OXPHOS resulting in induction of trained immunity. This study provided the possibility of inducing trained immunity in broilers following administration of CpG-ODN and to control infectious bacterial disease beyond the first week of life.

## **Avian Influenza**

**Evaluation of 5 and 10-day-old commercial Pekin ducklings (*Anas platyrhynchos domesticus*) naturally infected with H5N1 highly pathogenic avian influenza**

Veronica Nguyen<sup>1</sup>, Simone Stoute<sup>1</sup>, Carmen Jerry<sup>1</sup>, Carlos Daniel Gornatti-Churria<sup>1</sup>

*University of California, Davis<sup>1</sup>*

Highly pathogenic avian influenza (HPAI) has resulted in significant economic losses in commercial poultry worldwide. Given its major economic impact, many studies have been published in order to better understand the pathogenesis of HPAI. HPAI in waterfowl has been an area of interest as wild waterfowl are a significant component in the spread and maintenance of this virus. However, limited studies have been published that focus on avian influenza infection in ducklings less than 9 days of age. This is an important area of study as this can affect when avian influenza is an appropriate differential and when further testing for avian influenza should be considered. This paper focuses on a case submission from the California Animal Health and Food Safety (CAHFS) Laboratory, Tulare that included ten 5-day-old commercial Pekin ducklings (*Anas platyrhynchos domesticus*) that were positive for HPAI H5N1. Microscopic and macroscopic lesions in this case were compared to those in 10-day-old Pekin ducklings from the same flock. The aim of this study is to emphasize that waterfowl less than 9 days of age can be infected with avian influenza and to provide further insight on how this disease presents grossly and microscopically at different ages in ducklings.

### **Safety and Antibody Response of a RNA Vaccine Against H5Nx HPAI in Commercial Ducks Reared in Field Conditions in France**

Christophe Cazaban<sup>1</sup>, Lavigne Florent<sup>1</sup>, Castagnos Stephanie<sup>1</sup>, Langlade Thierry<sup>1</sup>, Comte Sylvain<sup>1</sup>, Kamrud Kurt<sup>1</sup>, Elattrache John<sup>1</sup>

*Ceva Animal Health<sup>1</sup>*

Highly pathogenic avian influenza (HPAI) of the Goose/Guangdong/1996 lineage is a widespread devastating disease to the poultry industry, backyard flocks, as well as wild birds. For the last five years, outbreaks have occurred in Asia, the Middle East, Africa, as well as in Europe. This lineage further expanded from this geographical area to North and South America in 2022. Although presently limited, these outbreaks are also accompanied with a zoonotic potential. In 2022-2023, outbreaks reached an unprecedented amount, including in high biosecurity facilities like parent stock flocks which is threatening the sustainability of poultry production, preservation of the genetic heritage, and food security. Several non-endemic countries (eg, in Europe) have decided to assess the additional benefits vaccines could provide in addition to biosecurity, monitoring and stamping out. France has experienced severe and regular HPAI outbreaks over the past years, especially in the duck producing industry. Mule ducks (a hybrid of a Muscovy male and a Pekin female duck) is highly popular since it is reared to produce foie gras. Following several experiments done in mule ducks, in field conditions, French authorities decided to vaccinate all production ducks from October 2023 onwards. We present here the results of the field 'experiments' (in approximately 6,000 ducks) followed by commercial use (in approximately 70,000 ducks) of a self-amplifying RNA vaccine (Respons AI H5, Ceva Animal Health) in the southwest of France. Vaccinated ducks were given two 0.2-ml vaccine applications, by intramuscular route in the thigh, at day one in the hatchery, and at 21 to 28 days of age in the farm. Production performance parameters were recorded throughout the rearing period, including body weight, feed consumption, morbidity and mortality. The safety of the tested vaccine was confirmed, according to the recorded parameters. Growth curve, feed consumption, morbidity and mortality were similar between the vaccinated and control groups and



were equal to or better than the production standards. Due to the experimental status of the trials all ducks were sent to rendering and were not further processed resulting in no foie gras yield data. Commercial farms confirmed the previous outcome of safety features, with additional slaughterhouse performance data. Serology monitoring using a specific H5 antibody ELISA kit displayed strong antibody detection after the second vaccine application. Official PCR tests confirmed the lack of field infection with avian influenza virus.

#### **Efficacy of a reverse-genetics derived vaccine against highly pathogenic avian influenza viruses from clade 2.3.4.4**

Leticia Frizzo da Silva<sup>1</sup>, Jeff Rodenberg<sup>1</sup>, Tyler Brown<sup>1</sup>, Jose Portillo<sup>1</sup>, Gregory Nitzel<sup>1</sup>, Jody Kremer<sup>1</sup>, Candyce Pacione<sup>1</sup>

*Zoetis Veterinary Medicine Research and Development<sup>1</sup>*

Highly pathogenic avian influenza (HPAI) viruses from the Goose Guangdong lineage (Gs/Gd), clade 2.3.4.4, are causing unprecedented losses to the poultry industry, besides threatening public health. Zoetis has generated two reverse-genetics derived vaccines with the hemagglutinin (HA) gene homologous to HPAI viruses from the clade 2.3.4.4: A/Gyrfalcon/Washington/41088-6/2014, H5N8 (H5N1rg\_2015) and A/turkey/Indiana/22-003707-003/2022, H5N1 (H5N2rg\_2022). The H5N1rg\_2015 vaccine was demonstrated to induce hemagglutinin inhibition (HI) antibody titers correlated with protection (HI  $\geq$ 40) and was subsequently conditionally licensed and included as part of an US National Veterinary Vaccine Stockpile. In addition, H5N1rg\_2015 vaccine conferred 100% protection from clinical disease and prevented or drastically reduced viral shedding following challenge with the HPAI A/Ck/SA/275401/17 H5N8, Clade 2.3.4.4.b, South Africa\_2017 virus, which HA is 96.1% homologous to the H5N1rg\_2015 vaccine virus. Recently, the H5N1rg vaccine was employed on an emergency vaccination program to help protect the critically endangered California condors after several deaths due to HPAI. The genetic shifts and/or drifts in the HA protein over time requires timely updating of avian influenza vaccines. An updated reverse-genetics vaccine H5N2rg\_2022 is under development and was demonstrated to induce HI antibody titers correlated with protection (HI  $\geq$ 40) when tested against antigen of the HPAI H5N1 currently circulating in US. Reverse-genetics is a versatile technology that allows for streamlined development of safe and efficacious vaccines for HPAI.

#### **Environmental Sampling and Results on Multiple Turkey Farms with Highly Pathogenic Avian Influenza, from 2022 through 2023**

Carrie Cremers<sup>1</sup>

*Jennie-O<sup>1</sup>*

Highly pathogenic avian influenza (HPAI) is a viral, foreign animal disease that is contagious to domestic poultry and wild bird species. The virus is contagious and causes increased mortality to susceptible species of poultry and wild birds. The current outbreak of HPAI in the United States started in 2022 and is persisting. There have now been over 81.8 million commercial and backyard birds that have been depopulated, according to USDA's website. When a poultry premises goes positive with HPAI, all the birds on the whole premises needs to be depopulated due to the fear that the virus may have spread to the other birds on the same premises. In this presentation,

environmental sample results that were taken on positive HPAI turkey farms will be discussed. The environmental samples were taken both inside and outside of the barns for both positive and negative barns that are on the same premises. With the goal of rapid depopulation to have the premises depopulated within 24-48 hours, there is not a lot of data to know how quickly the negative barns would contract the virus. This environmental testing will help to understand how the virus is moving around within the same premises once a site goes positive with HPAI. Using this data will be helpful to continue to learn about HPAI virus and if there are ways to continue to with the stamping out policy while avoiding depopulating healthy birds and decreasing the amount of indemnity that is paid.

### **Antigenic characterization of immune escape mutants of H7 low pathogenic avian influenza virus.**

Jiho Lee<sup>1</sup>, Jiho Lee<sup>1</sup>, Erica Spackman<sup>1</sup>

*Southeast poultry research laboratory, USDA<sup>1</sup>*

The emergence of immune escape mutants, known for reducing the effectiveness of pre-existing immunity provided by vaccination, prompts the exploration of the impacts of amino acid mutations in the hemagglutinin (HA) of avian influenza virus (AIV). In this study, we made an immune escape model by subjecting the A/Turkey/New York/4450/94 H7N2 low pathogenic AIV to antibody pressure via homologous serum during passage of the virus in SPF eggs. Chickens were immunized with escape mutants from several passages to collect anti-sera. One antiserum with antigenically discriminating reactivity was selected and used as a heterologous serum for generating further escape mutants. Antisera were used to perform cross-hemagglutination inhibition assays to calculate antigenic distances. Antigenic distances between viruses were visualized using antigenic cartography. Amino acid differences and antigenic distances between mutants and the parental virus were compared to observe the antigenic determinants and characteristics. Mutations observed in this experiment were selected and applied on the HA of reverse genetically generated virus to observe the accumulative effect of mutations. This study will not only contribute to the understanding of antigenic determinants in AIV but also underscores the broader implications of these findings in the context of AIV evolution and vaccine development strategies.

### **Pathology and viral tissue distribution of high pathogenicity avian influenza H5N1 in wild black-headed gulls (*Chroicocephalus ridibundus*) in France**

Manuela Crispo<sup>1</sup>, Nicolas Gaide<sup>1</sup>, Malorie Dirat<sup>1</sup>, Gregory Jouvion<sup>1</sup>, Pascal Arna<sup>1</sup>, Irene Valverde Dominguez<sup>1</sup>, Julien Hirschinger<sup>1</sup>, Guillaume Le Locah<sup>1</sup>

*IHAP, ENVT, INRAE, University de Toulouse<sup>1</sup>*

Over the last few years, marine birds' populations have been significantly challenged by the emergence of high pathogenicity avian influenza (HPAI) H5N1 clade 2.3.4.4b. viruses. We provide an overview of the pathological and immunohistochemical findings associated with HPAI H5N1 natural infection in wild black-headed gulls (*Chroicocephalus ridibundus*). A total of 13 cases, registered in the North-West of France between January and February 2023, were selected. Affected birds were found dead or humanely euthanized after exhibiting neurological signs. The circulation of HPAI H5N1 clade 2.3.4.4b. among gull populations in the area was confirmed by official molecular testing

conducted by the National Reference Laboratory. A complete necropsy was performed and sections of feathered skin, nasal cavity, eyelid, eye, brain, trachea, lung, heart, liver, spleen, kidney, digestive tract, gonad, nasal, adrenal, Harderian, uropygial and thyroid gland were sampled, formalin-fixed and routinely processed for histopathology. Uropygial and adrenal gland were obtained from 7/13 and 9/13 birds, respectively. Slides were stained with hematoxylin and eosin and an anti-influenza A nucleoprotein immunohistochemistry. Histopathological lesions were recorded and viral antigen distribution was semi-quantitatively scored. Necropsy reports were available for 8/13 birds. Significant lesions included mottled pancreas with pale areas and/or hemorrhagic foci (4/8), and multiple hemorrhages in mediastinum (2/8), meninges (1/8), periocular tissues (1/8) and breast muscles (1/8). Mild to severe necrotizing and/or lymphoplasmacytic encephalitis (13/13), endophthalmitis (10/13) and pancreatitis (9/13) were the most consistent findings identified microscopically. Mild necrotizing uropygial gland adenitis (5/7) and folliculitis (1/13) affecting immature feathers were also present. Viral antigens were most commonly detected in brain (13/13), eye (10/13) and pancreas (8/13), followed by uropygial gland (4/7), lung (3/13), feathered skin (2/13), visceral ganglia (2/13) and adrenal gland (1/9). The highest immunohistochemical scores were recorded in brain (2.2) and pancreas (2.1), followed by eye (1.8), and uropygial gland (1.5). Lung, feathered skin, visceral ganglia and adrenal gland had a score of 1. This is the first report of ocular lesions associated with HPAI H5N1 natural infection in black-headed gulls in France. Cloudy eyes and corneal opacity, resulting from corneal edema and keratitis, and concurrent endophthalmitis were described in several waterfowl species and seagulls experimentally-infected with HPAI H5N1. Our findings also suggest the role of the integumentary system and specifically the uropygial gland as a potential additional shedding route of HPAI in marine birds. Overall, these results will allow to adapt and ameliorate screening protocols in the field, supporting epidemiological surveillance and diagnostic investigation.

### **Breeder farm biosecurity enhancements during the 2022-2023 HPAI outbreak**

Marissa Studniski<sup>1</sup>, Jake Carlson<sup>1</sup>, Molly Parker<sup>1</sup>, Ben Wileman<sup>1</sup>

#### *Select Genetics<sup>1</sup>*

Breeder farm biosecurity enhancements during the 2022-2023 HPAI outbreak Marissa Studniski, Jake Carlson, Molly Parker, Ben Wileman Select Genetics, Willmar, MN, United States Since the HPAI outbreak began in early 2022, over 13.4 million commercial turkeys have been affected in the US, according to APHIS. Around 9.7 million turkeys were lost in 2022, and over 3.7 million turkeys were lost in 2023. The ongoing outbreak indicates the virus may be here for good and it's up to the poultry industry to learn how to adapt and prevent infections. The primary tool and focus for disease prevention has been biosecurity measures. Biosecurity has evolved throughout the HPAI outbreak and strict operational and structural measures have shown to be vital in preventing disease. Breeder farm biosecurity enhancements modified throughout the 2022-2023 outbreak have shown success at preventing HPAI cases. Implementation of enhanced biosecurity reduced the incidence of HPAI cases within a breeder operation from 2022 to 2023. These biosecurity measures will be described to help minimize the impacts of HPAI on commercial poultry operations.

### **Avian influenza virus replication in bone marrow-derived dendritic cells induce cytokine dysregulation with eventual cell death**

Jongsuk Mo<sup>1</sup>, Kelsey Briggs<sup>1</sup>, Klaudia Chrzastek<sup>2</sup>, Karen Segovia<sup>3</sup>, Darrell Kapczynski<sup>1</sup>

*USDA-ARS<sup>1</sup>, Pirbright Institute<sup>2</sup>, CSL Seqiru<sup>3</sup>*

Dendritic cells (DC) function as professional antigen presenting cells, and act as sentinels of the immune system. They are a part of the primary immune response to pathogens and help bridge the innate and adaptive immune responses. They are believed to migrate from bone marrow into the blood stream and eventually reside in most all tissue. Immature DC are especially equipped for antigen uptake and processing, while mature dendritic cells undergo physical and functional maturation to present antigen to naïve T cells. Vaccination against viral pathogens critically relies on DCs for primary and secondary immune responses. DCs are rare cells in all tissues and hard to isolate, which makes the use of primary DCs in functional assays challenging. Avian influenza virus (AIV) is a well-known disease in poultry that can cause severe morbidity and mortality. Here, chicken bone marrow-derived DCs were tested against AIV infection with characterization of innate immune responses and cell morphology. Both high and low pathogenic AIVs (HPAIV, LPAIV) of H5 and H7 subtypes were used in these studies. At 8 hours post infection, strong proinflammatory cytokine expression of chicken interleukin (IL)-1 beta and IL-6 were observed in both HPAIV and LPAIV infected DCs. However, HPAIV infected DCs demonstrated stronger immune responses compared to LPAIV infected DCs. Both types of AIV were able to infect and replicate in DCs and induce cytopathic effect which was microscopically observed as morphological changes in shape and structure. In addition, differences were observed in severity between H5 and H7 subtypes. Overall, we demonstrated both HPAIV and LPAIV can infect and replicate in chicken DCs which can negatively impact their ability to protect birds from disease. The elevated expression of cytokines from infected DCs likely contributes to the immune dysregulation typically observed in HPAIV infections.

### **A Focus on the Basics of the National Poultry Improvement Plan (NPIP) Avian Influenza Compartmentalization Program**

Savannah Busby<sup>1</sup>

*USDA APHIS NPIP<sup>1</sup>*

The NPIP is widely recognized as the gold standard for poultry monitoring and surveillance. APHIS added the avian influenza (AI) programs for breeding chickens and breeding turkeys to the NPIP in the 1990s. Prior to this time, only vertically transmitted diseases were included in the NPIP. However, when the poultry industry began to export large quantities of poultry genetic stock and poultry meat and eggs, major U.S. trading partners wanted assurances that the poultry and poultry products originated from breeding flocks that were free of AI. H5/H7 AI monitoring programs for commercial table-egg layers, broilers, and meat turkeys were added to the NPIP in 2006. In addition to the AI Clean and H5/H7 AI monitoring programs, the concept of Compartmentalization was also adopted by NPIP at the 38th Biennial Conference in 2006. Compartmentalization is a procedure a country may implement to define and manage animal subpopulations of distinct health status and common biosecurity programs within its territory, in accordance with the guidelines found within the World Organization for Animal Health (WOAH) Code, for the purpose of disease control and international trade. The concept of Compartmentalization is a program that was specifically designed for the Primary Breeder sector of the poultry industry, to be utilized in the face of an Avian Influenza outbreak, as a tool when zoning and regionalization fails, in order to maintain continuity of business and international trade. NPIP continues to provide assurance that poultry and poultry products originating in the United States are free of AI. Due to the current Highly Pathogenic Avian Influenza

outbreak and its impact on both domestic and international trade, there are a lot of misconceptions for what the Compartmentalization program is, and its intended purpose. This presentation will provide a broad and basic overview of the NPIP US AI Clean Compartment program, describing what they are, how they operate, maintenance, requirements, and their overall purpose and benefit.

### **Genetic identification of the HPAIV (H5N1) in domestic birds received at the Faculty of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Peru (2022- 2024).**

Rosa Gonzalez<sup>1</sup>, Gina R. Castro-Sanguinetti<sup>1</sup>, Alonso Callupe-Leyva<sup>1</sup>, Katherine Vargas-Coca<sup>1</sup>, Eliana Icochea-DiArrigo<sup>1</sup>, Juan More-Bayona<sup>1</sup>

*Universidad Nacional Mayor de San Marcos<sup>1</sup>*

Twelve viruses were analyzed from the seventeen cases of domestic birds positive for HPAIV (H5N1), which were received at the Avian Pathology Laboratory of the National University of San Marcos from November 2022 to January 2024. The twelve isolates that were selected for analysis by complete genome sequencing were from: 05 domestic ducks, 04 laying birds, 02 quails and 01 backyard birds, all coming from outbreaks that occurred in areas close to the biological corridor of migratory wild birds. Lung, trachea and brain samples were analyzed by real-time RT-PCR and viral isolation in SPF embryonated eggs. The preliminary analysis identified that the isolates correspond to the highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b. The results of the NGS sequencing that are part of the genomic surveillance of the viruses that circulate to date in domestic birds, are being analyzed to determine their phylogeny, genetic characterization and evolutionary changes of the virus in the country.

### **Active surveillance of Avian Influenza Virus from wild birds in Peru after highly pathogenic H5N1 subtype emergence (2023-2024)**

Gina R. Castro-Sanguinetti<sup>1</sup>, Rosa Gonzalez-Veliz<sup>1</sup>, Alonso Callupe-Leyva<sup>1</sup>, Katherine F. Vargas-Coca<sup>1</sup>, Eliana Icochea-DiArrigo<sup>1</sup>, Juan A. More-Bayona<sup>1</sup>

*Universidad Nacional Mayor de San Marcos<sup>1</sup>*

At the end of 2022, South America experienced the emergence of the highly pathogenic Avian Influenza virus subtype H5N1 clade 2.3. 4.4 b. During this period, Peru showed multiple cases of high mortality in resident wild birds, followed by multiple outbreaks in domestic birds. Thus, Peru was one of the most affected countries in the marine ecosystem and national poultry industry. The massive use of vaccination has controlled the high mortality in most commercial farms in Peru. However, sporadic outbreaks are observed in unvaccinated flocks following apparent close contact with wild birds. Therefore, our goal was to pursue a continued-active surveillance of Avian Influenza Virus in order to track the evolutionary changes at the viral genome level that occurred within this period and the distribution of the avian species involved. To this, we deployed an integrative approach using molecular tests, viral isolation through SPF embryonated chicken eggs, and NGS sequencing for genomic characterization from environmental samples. We evaluated more than 20 different species of wildbirds that included Peruvian pelican, Guanay cormorant, Peruvian booby, Peruvian gulls, among others. To date, we have evaluated more than 400 fresh fecal samples from coastal wild birds, detecting 15 PCR-positive samples with varied Ct value (ranging from 22 to 41). Following assays, we propagated and characterized the genome of 8 AIV isolates. Our results showed the presence of H5N1

Influenza virus as the predominant subtype in asymptomatic wild birds associated with varied viral load. This agent was present mainly in avian species such as Guanay cormorant and Franklin's Gull. To date, amino acid changes have been observed in their viral proteins. This work represents the first study of H5N1 Avian Influenza virus evolution in coastal wild birds of Peru in the post-emergence period 2023-2024. Keywords: Avian Influenza Virus; H5N1; wild birds; virus isolation; Peru

### **Monitoring of commercial Layers vaccinated with a rHVT H5 vaccine in Peru**

Claudia Carranza<sup>1</sup>, Gonzalo Castillo<sup>2</sup>, Bruno Gracia<sup>3</sup>, Luiz Sesti<sup>4</sup>

*Ceva Salud Animal Peru<sup>1</sup>, Ceva Salud Animal Peru<sup>2</sup>, Ceva Salud Animal Peru<sup>3</sup>, Ceva Salud Animal LATAM<sup>4</sup>*

The highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b was detected for the first time in Peru in November 2022 in pelicans on the north coast of the country. Twelve days later, the first outbreak in backyard birds was confirmed, as well as a later detection in a commercial laying farm in Huacho. Given the epidemiological situation, the animal health authorities (SENASA) declared a state of emergency in the country which lasted until the end of 2023. Due to the new epidemiological situation in the country, the Peruvian poultry industry and SENASA agreed to start a national vaccination program directed to light and heavy breeders, commercial layers, breeders, broiler turkeys, and fighting cocks. The first authorized vaccine (February 27, 2023) was a vector rHVT H5 vaccine against Marek's and Avian Influenza (Vectormune HVT AIV; recombinant HVT expressing the gene of the hemagglutinin H5 day one application). Along with the outset of vaccination, a monitoring program was implemented to characterize the seroconversion of vaccinated birds. Although in many countries around the world, the hemagglutination inhibition (HI) test is the serological tool available, in the case of Peru the decision was to utilize new commercial ELISA kits which not only measure specific antibodies against subtype H5 but also allow the implementation of a DIVA program (Differentiating Vaccinated from Infected Animals detection of specific antibodies against influenza virus nucleoprotein NP). It was decided that the HI test could generate confusion due to variation intra and inter laboratories and the fact that there was no homologous antigen to be used. The commercial ELISA kits used proved to be very sensitive and specific and capable of generating very uniform results among different laboratories. This allowed an excellent and very reliable follow up throughout time in many companies vaccinating against H5N1, ensuring that the vaccines were applied properly and significant seroconversion was reached. Therefore, not only seroconversion from the vector rHVT H5 vaccine but also the response from the inactivated vaccines applied in the field. The results to be presented and discussed illustrate the seroconversion from 3 flocks which were blood sampled at day 1 and weeks 3, 7, 13, 17, 20, and 30 and serology run by the Innovative Diagnostics (ID) ELISA kits (ID Screen® Influenza H5 Indirect and ID Screen® Influenza A Nucleoprotein Indirect). Likewise, the specific take of the vector rHVT H5 vaccine was determined by a PCR protocol that detects specifically the vaccine in spleen samples collected from birds at 4 and 5 weeks of age. The results were quite welcomed by the poultry industry since companies were able to know exactly what was happening with their flocks as far as vaccine seroconversion and estimated protection against the high path H5N1.

### **Efficacy of an inactivated avian influenza H5N1 vaccine in layer hens**

Melanie Caballero<sup>1</sup>, Juan Carlos Flores<sup>1</sup>, Juana Quispe<sup>1</sup>, Julio Levano<sup>1</sup>, Elizabeth Sianquez<sup>1</sup>, Alfredo Mendoza-Espinoza<sup>1</sup>

*R&D Quimtia*<sup>1</sup>

The highly pathogenic avian influenza virus (AIV) subtype H5N1 is responsible for large economic losses in the poultry industry. This study was performed to determine the protection of an oil-emulsion vaccine against an experimental challenge with the highly pathogenic H5N1 (H5N1AP) strain. 4-week-old laying hens were vaccinated at a rate of 0.5 mL subcutaneously. Serum samples were collected from all birds for antibody detection by ELISA and HI at 2, 3 and 4-weeks post vaccination. The viral challenge was carried out 4 weeks post vaccination with a H5N1AP strain recently inoculated and recovered from specific pathogen-free (SPF) chicks. Cloacal swabs were taken to evaluate viral shedding by RT-PCR and TCID50. The birds were observed for 14 days post-challenge. There was no record of mortality or clinical signs in the immunized birds, while 100% of the unvaccinated group died between 48 and 72 h. In vaccinated group the results of serology showed a positive seroconversion by ELISA, and the HI test titers were greater than Log<sub>2</sub>24, in comparison of control group. Regarding viral excretion, results of the control group were not register because mortality started between 48 and 72 h, while at 7 dpi results of vaccinated group showed a negative result by RT-PCR and a low viral load by TCID50. This study confirmed that inactivated vaccines induced robust protection from mortality and morbidity against H5N1HP circulating in Peru.

#### **Development of an Inactivated Avian Influenza Virus Vaccine against local highly pathogenic H5N1 isolated in Peru**

Melanie Caballero<sup>1</sup>, Juan Carlos Flores<sup>1</sup>, Juana Quispe<sup>1</sup>, Julio Levano<sup>1</sup>, Elizabeth Sianquez<sup>1</sup>, Alfredo Mendoza-Espinoza<sup>1</sup>

*R&D Quimtia*<sup>1</sup>

Avian influenza virus (AIV) causes a wide range of clinical signs and high mortality in poultry. Our objective was to develop an inactivated vaccine against local highly pathogenic H5N1 Avian Influenza circulating in Peru. The vacunal virus candidate was identified by genomic sequence of hemagglutinin protein and the alignment of amino acids of the cleavage site region of the same protein confirmed the IAV was a highly pathogenic H5N1 subtype. The viral isolate was replicated in MDCK cell line at 33°C and 5% of CO<sub>2</sub>, was exposed to 10<sup>-2</sup> of MOI and was harvested at 72 h. The quantification of virus and the hemagglutinin antigen was performed by TCID50 and Hemagglutination assay respectively. The protein profile was evaluated after the replication in MDCK. Inactivation control assays were performed at MDCK, SPF chickens and SPF eggs. By other hand, light mineral oil, PBS, pH 7.2 buffer, surfactants with HLB 4.3 and 15, and WFI water were used. The incorporation of the antigen was carried out with a homogenizer equipment (8000 rpm) to generate the simple emulsion (w/o) and finally with a propeller stirrer to form the double emulsion w/o/w (1200 rpm). The final product complies with quality standards, in addition to obtain a high viral load, complete inactivation without affections in hemagglutinin protein, IAV inoculum was immersed in a double emulsion pharmaceutical form that facilitates the prolonged release of the viral antigen.

## **Bacteriology**

## **Correlation between Clostridium perfringens spore load and prevalence of netB-positive isolates in the poultry environment and necrotic enteritis on broiler chicken farms**

John Maurer<sup>1</sup>, Sydney Kinstler<sup>1</sup>, Margie D. Lee<sup>1</sup>

*Virginia Tech*<sup>1</sup>

Necrotic enteritis (NE) is a significant intestinal disorder of poultry caused by the spore-forming, obligate anaerobe, *Clostridium perfringens*. Antimicrobials have historically prevented NE until concerns over its use and resistance pressured the industry its use in poultry. Antibiotic free (ABF) production has been challenging for some poultry farms to raising successive flocks without breaking with NE as some points. However, some ABF poultry farms can raise multiple flocks without any NE break. Why do some farms break with NE and others don't? Our central hypothesis is high *C. perfringens* spore loads is responsible for recurrent NE on some poultry farms. Capitalizing on *C. perfringens* physiology and metabolism, a media formulation was developed that "poisons" other, aerobic poultry environmental bacteria using potassium chlorate coupled with a physical heat treatment to determine *C. perfringens* spore abundance. The medium was formulated to be selective and differential for *C. perfringens* spores over the other lecithinase-positive, aerobic endospore formers in poultry litter. Combined with a heat treatment, enumeration of clostridial spores and isolation *C. perfringens* from the poultry house environment was achieved. Using our chlorate medium formulation and heat treatment, lecithinase-positive *C. perfringens* was identified from the poultry house environment and isolates were subsequently confirmed as *C. perfringens* by MALDI-TOF. We specifically found a significant correlation between *C. perfringens* spore load in poultry litter and farms with a history of NE ( $p < 0.05$ ). There was also a significant association between prevalence of netB-positive *C. perfringens* and history of NE ( $\chi^2$ -test;  $p < 0.01$ ) The ability to monitor *C. perfringens* spore abundance and prevalence of netB isolates in the poultry environment is important in determining which poultry farms are likely to break with NE

## **Impact of a Postbiotic Containing Saponin, with or without Vaccination, on the Mitigation of Colibacillosis in Broilers Challenged with Avian Pathogenic Escherichia coli Serotype O78**

Evan Chaney<sup>1</sup>, Timothy J. Johnson<sup>2</sup>, Alicia Meyer<sup>1</sup>, Teresia W. Maina<sup>1</sup>, Vivek Kuttappan<sup>1</sup>, S. Ali Naqvi<sup>1</sup>, Matthew Jones<sup>3</sup>, Charles Hofacre<sup>3</sup>

*Cargill, Inc.*<sup>1</sup>, *University of Minnesota, Department of Veterinary and Biomedical Sciences, Saint Paul, MN, USA*<sup>2</sup>, *Southern Poultry Research Group, Inc., Watkinsville, GA, USA*<sup>3</sup>

Commercial poultry operations face significant flock health and economic losses associated with colibacillosis caused by Avian Pathogenic *Escherichia coli* (APEC). Clinical APEC isolates demonstrate broad serotype diversity and virulence characteristics, ultimately challenging vaccine efficacy and risk management strategies. Additional, non-antibiotic solutions are needed to promote resiliency against infections or recovery from existing infections in commercial poultry. In this study, the protective effects of a postbiotic prototype containing saponin, alone or in combination with vaccination, were evaluated in broilers intratracheally challenged with APEC O78 on Day 28. Six hundred day-of-hatch male broilers were randomly allocated across five treatment groups consisting of 4 pen replicates with 30 birds per pen. Control birds were fed a basal diet while challenged birds were fed the basal diet either supplemented with postbiotic (Dia-V™, PTPLUS prototype, Diamond V,



Cedar rapids, IA) or not and administered a live, attenuated vaccine or not. Necropsy and lesion scoring were conducted on Day 35 and Day 42 at study termination. Swab samples of the air sacs and pericardium, blood collection, and tissue collection from lung and liver were collected on Day 35 (n=5 birds/pen) and on remaining birds at Day 42. Blood samples were couriered to the Elk River Innovation Center (Elk River, MN) and processed with a commercial cytokine/chemokine panel (Millipore Sigma, Burlington, MA, USA) and reader (Luminex, Austin, TX, USA). Swab and tissue samples were couriered to the University of Minnesota (Willmar, MN) for microbiological analyses and PCR-based isolate characterization. Day 35 mean lesions scores for perihepatitis, pericarditis and air sacculitis were numerically improved by all treatments. Postbiotic alone significantly reduced perihepatitis lesions over the challenged control (1.15 vs. 1.80; P=0.043) and approached significance for pericarditis (0.95 vs. 1.55; P<0.10) with numerical improvement in air sacculitis (1.25 vs. 1.75). Vaccination alone numerically improved perihepatitis (1.35) and pericarditis (1.30) and approached significance for air sacculitis (1.20; P<0.10). Postbiotic with vaccine numerically improved perihepatitis (1.25), pericarditis (0.90) and air sacculitis (1.20) scores all approaching significance (P<0.10). Cumulative mean lesion scores were lowest for postbiotic with and without vaccine both approaching significance. Postbiotic treatment resulted in lower mean APEC colonization load in liver and lung tissue and % positive heart swabs. By Day 42, viable birds demonstrated challenge recovery and treatments numerically improved overall livability compared to the challenged control. These data suggest the postbiotic with saponin blend prototype alone or in combination with vaccination, may be a viable and additional non-antibiotic solution to support reduction in the severity of APEC induced clinical colibacillosis in broilers.

### **In-vitro Bacillus-based probiotic screening against field *Campylobacter hepaticus* isolates**

Kay Russo<sup>1</sup>, Dr. Eric Sobotik<sup>2</sup>, Lena Capern<sup>3</sup>

*Chr Hansen<sup>1</sup>, Dr. Dorthe Sandvang<sup>2</sup>*

Spotty Liver Disease (SLD) is an emerging disease of laying hens, marked by multifocal acute hepatocellular necrosis, and associated with significant mortality and production losses. *Campylobacter hepaticus* is the species of bacteria identified as the causative agent of SLD in poultry. Our research indicates that while *C. hepaticus* can be cultured from the liver and bile of infected birds, isolation of this bacterium is challenging. The pathogenesis of SLD is currently not fully understood, although transmission of the disease is likely through a fecal-oral route. Control measures for SLD include vaccination and antibiotics, with chlortetracycline being the primary treatment. However, the emergence of plasmid-borne antibiotic resistance presents a significant concern. Probiotics are a promising option for poultry producers, offering an effective strategy to mitigate *C. hepaticus* infections and support flock health. In this study, in-vitro screening was conducted on *C. hepaticus* isolates from commercial flocks using various strains of *Bacillus*-based probiotics. This screening revealed multiple probiotic strains exhibiting effective in-vitro inhibition against these *C. hepaticus* isolates, highlighting the potential of probiotics in controlling this pathogen. The next phase in our evaluation involves assessing the effectiveness of an in-feed probiotic candidate in reducing morbidity and mortality associated with SLD. This step is crucial to determine the practical applicability of probiotics in managing this condition on-farm.

### **Using an Experimental Blend of Mono- and Di-glycerides in the Starter Phase for Efficacy Against a Pathogenic *Enterococcus cecorum* Isolate in Broilers**

Matthew Jones<sup>1</sup>, Charles L. Hofacre<sup>1</sup>, M. Mitsu Suyemoto<sup>3</sup>, Roy D. Berghaus<sup>4</sup>, Luis Gomez<sup>5</sup>, Hector Badillo<sup>5</sup>, William Serna<sup>5</sup>, Kari Saddoris-Clemons<sup>5</sup>, Brooke Humphrey<sup>5</sup>, Kevin J. Bolek<sup>5</sup>

*Southern Poultry Research Group, Inc.*<sup>1</sup>, *Department of Population Health and Pathobiology, CVM, NCSU*<sup>3</sup>, *Department of Population Health, CVM, UGA*<sup>4</sup>, *Phibro Animal Health Corporation*<sup>5</sup>

Enterococcus species are gram-positive commensals of poultry and other monogastrics. There are pathogenic Enterococcus isolates, including strains of *E. cecorum*, which can traverse the intestinal epithelium and leak into the blood stream, later manifesting as infections throughout the body. Pericarditis, infection of the free thoracic vertebra (FTV), and femoral head infections are common tissues in which Enterococcus induces gross pathology. In applied situations, this disease may manifest as an increase in leg issues and greater cull rates among flocks. Some producers have observed an increase in Enterococcus-related issues in recent years. More focus has shifted to products, like monoglyceride compounds, that may help alleviate the diseases resulting from Enterococcus and other similar pathogens. In the current trial, an experimental blend of mono- and diglycerides was added to a starter diet at 3 lbs/US ton (0.15%) and compared directly to a challenge control group for efficacy against Enterococcus cecorum. Each treatment was represented by eight replicate floor pens of twenty-five male Ross broiler chicks. All chicks were coarse sprayed with a 1x dose of a commercial coccidiosis vaccine and placed in floor pens with fresh pine shavings. On day 4, each chick was orally gavaged with 0.1 mL of Enterococcus cecorum (~1 x 10<sup>7</sup> CFU/bird) isolate SA3. At 22 days of age, spleen samples were collected from four birds in each replicate pen. At 42 days, spleen and FTV swab samples were collected from 100 birds in each treatment. Birds were weighed on day 0, 22, 32, and 42 to capture performance over the course of the challenge. The monoglyceride group trended higher in body weight gain compared to the challenge control from 0 to 22 days (P = 0.068). By day 32, the trend became significantly greater for body weight gain in the monoglyceride group compared to the challenge control (P = 0.016), and this was maintained out to 42 days (P = 0.042). At termination, the treated group body weight gain was ~120 grams greater than the challenge control. There was no significant difference between the 22-day Enterococcus spleen prevalence; however, by 42-days there was significantly lower Enterococcus spleen prevalence in the monoglyceride treatment, at 7% positive, compared to the challenge control, at 19% positive (P = 0.022). The prevalence trend in the 42-day FTV swab samples mirrored the spleen data (P = 0.10). Overall, the experimental blend of mono-and diglycerides effectively lowered the bacterial prevalence in the target tissue at termination as well as ameliorating some of the negative consequences (lower body weight gain) of the infection. Despite the product being present only in the starter diet, the influence lasted to the peak observed bacterial challenge at 42 days. This mono- and diglyceride blend has potential to be part of an effective Enterococcus control program.

#### **Assessment of virulence in novel Avian Pathogenic Escherichia coli (APEC) serogroups using in vitro and in vivo assays.**

Klao Runcharoon<sup>1</sup>, Julia Ienes Lima<sup>1</sup>, Yu Yang Tsai<sup>1</sup>, Catherine Logue<sup>1</sup>

*Department of Population Health, College of Veterinary Medicine, University of Georgia*<sup>1</sup>

Avian Pathogenic Escherichia coli (APEC) causes colibacillosis resulting in systemic or localized infections in poultry including airsacculitis, septicemia, pericarditis, perihepatitis, salpingitis, and cellulitis. Serogrouping based on the detection of somatic O-antigens is a useful tool to classify APEC

and relate to disease pathogenicity. There are approximately 188 *E. coli* serogroups and the most common types associated with colibacillosis include O1, O2, and O78. However, our APEC collection from avians diagnosed with colibacillosis in Georgia poultry populations has identified emerging serogroups with limited information on their pathogenicity. Therefore, the pathogenicity features of these emerging serogroups were evaluated using multiple approaches including the serum resistance assay, embryo lethality assay (ELA), and chick challenge assay. We selected 10 novel APEC strains from our collection which included serogroups O25, O15, O91, O152, O161, O86, O88, O115, O62/O68, O45. We tested their ability to grow in chicken serum in 96 well-plates. 30  $\mu$ l of a bacterial suspension was added and adjusted to a concentration of 10<sup>6</sup> colony-forming units (CFU)/mL in chicken serum. 30  $\mu$ l of serum was removed from each well at 0 and after 4 hours incubation at 40°C. The suspensions were diluted and plated and the CFU was determined after 24 hours of incubation. All selected APEC serogroups except O25 were resistant to serum. For the assay, 12 day of age (d.o.a.) embryonic eggs were injected with 300-500 CFU/0.1 mL of each strain via the allantoic fluid. Embryos were candled daily for 5 consecutive days, and deaths were recorded. The results showed that the highest mortality (100%) was found for APEC serogroups O152 and O145 while O88 caused only 50% mortality. Further assessment of the pathogenicity in chicks was also performed using a chick challenge assay. 12 one-day-old chicks per group were inoculated subcutaneously with 100  $\mu$ L (0.1 ml) (10<sup>8</sup> CFU) of the bacterial strain. Times of death and clinical scores were combined to give pathogenicity scores (PS). *E. coli* isolates that killed >50%, 10%-50%, and 0-10% of chicks were considered as virulent, moderately virulent, and avirulent, respectively. Using a one-way ANOVA analysis to compare the PS among APEC serogroups found that O15, O91, and O88 had significantly lower ( $p < 0.05$ ) PS than the positive control group (APEC O18) while O25, O152, O115, and O45 had numerically higher PS than the positive control group. Overall, the novel APEC strains exhibited different degrees of pathogenicity in both in vivo and in vitro assays. Some strains showed high virulence in all assays while some were less virulent warranting further investigation of their pathogenicity in older birds to establish new protection plans against these emerging serogroups.

### **Characterization of emergent *Avibacterium paragallinarum* strains in Brazil**

Priscila Diniz Lopes<sup>1</sup>, Gustavo Schaefer<sup>1</sup>, Edmilson Freitas<sup>1</sup>, Fabio Cristiano Vieira<sup>1</sup>

*Vaxxinova Brazil*<sup>1</sup>

*Avibacterium paragallinarum* (Avib. *Paragallinarum*) is the etiological agent of Infectious Coryza (IC), an acute respiratory disease of chickens. The current epidemiological status of IC was investigated in Brazil over 3 yr (2021 to 2024). Samples were collected from infra orbital sinus swabs of broiler breeders and layer hens suspected of IC, presenting clinical signs of facial edema, nasal discharged, and increased mortality. Swab samples were conducted to bacterial isolation and biochemical confirmation, and after were conducted to polymerase chain evidence (PCR) analysis and genetic sequencing of the hemagglutinin gene. A phylogenetic tree of the *hagA* gene sequences was constructed using 18 sequences. Phylogenetic analysis showed that most field strains belong to group IV (IDs 8670, 19, 20, 21, 89, 8231, 8698, 126, 128, 129, 903 and 1648). The remaining samples belonged to group III (IDs 4016, 4949, 6423, 6424, 127, 2127). In relation to the distribution of isolates across Brazilian regions, those belonging to Group III were isolated in the states of Minas Gerais and São Paulo, and the samples from Group IV were isolated in the states of Rio Grande do Sul, Paraná,

Espirito Santo and Pernambuco. Regarding the estimates of evolutionary divergence between sequences of isolates with the serotypes present in inactivated conventional vaccines, we noticed divergences of the *hagA* gene of up to 7.9% with serotype A1 (221), between 3.7 and 8.7% with serotype B1 (222), and finally, between 3.6 and 9.7% with serotype C2 (Modesto). These results suggest that the main isolated group in Brazil is IV, despite this, it is limited to certain Brazilian regions.

### **In-Vitro Evaluation of a Blend of Three Direct-Fed Microbial Bacillus Strains to Inhibit the Growth of Multiple Enterococcus cecorum and Enterococcus faecalis Field Isolates from Several Commercial Chicken Companies**

Blair Telg<sup>1</sup>, Kevin Bolek<sup>1</sup>, Luis Gomez<sup>1</sup>, Chad Malinak<sup>1</sup>, Ana Maria Villegas<sup>1</sup>, Ashley Hallowell Scarbriel<sup>1</sup>

*Phibro Animal Health*<sup>1</sup>

Enterococcal diseases of commercial chickens have been on the rise for the past decade. The clinical presentation of enterococcus infections has changed over this period from vertebral abscesses to femoral osteomyelitis to the more current systemic presentation with fibrinopurulent pericarditis and airsacculitis. The clinical infection can hit birds of any age, but there are indications that the bird may arrive to the farm with the infection and begin to express clinical signs and symptoms at a very young age (< 2 weeks). This presentation is an indication that intervention strategies may need to begin on the hen farm to minimize vertical transmission from hen to offspring. In addition, once in the broiler house, early intervention strategies are crucial if prevention of systemic infection is to be accomplished. One potential early intervention strategy is the dietary inclusion of a direct fed microbial (DFM) product to provide protection from enterococcus through competitive exclusion and/or the production of compounds that inhibit the growth of enterococcus. Oftentimes, a "generic" Bacillus-based DFM product is used in an attempt to address numerous pathogen and performance deficiencies in the field. The use of a custom blended Bacillus DFM product specific for the control of enterococcus is a novel idea. For the product used in this study, multiple bacillus strains were tested in-vitro for the ability to inhibit the growth of Enterococcus spp. The three best strains were selected and blended into a single DFM product. In this in-vitro study, the novel 3-strain bacillus DFM product was tested against 15 Enterococcus cecorum and 11 Enterococcus faecalis field isolates from 4 different commercial chicken companies. After allowing for growth of both the DFM strains and the Enterococcus isolates, the presence or absence of a zone of growth inhibition was evaluated for each Enterococcus isolate. In-vitro suppression of growth was observed for all Enterococcus isolates by at least one Bacillus strain from the novel DFM product. The result of this study gives evidence and support for taking this DFM dietary blend into complexes that may be experiencing increased Enterococcus infections in the field. Future work can focus on refining the DFM blend for a more targeted anti-Enterococcus approach as well as ascertaining additional isolates from the field to ensure continued growth inhibition.

### **Ecology of Avian Pathogenic Escherichia coli in Commercial Turkey Production Across Time, Space, Tissue, and Vaccination Status**

Lauren White<sup>1</sup>, Kari A. Mattison<sup>1</sup>, Randall Singer<sup>1</sup>, Timothy J. Johnson<sup>1</sup>

*University of Minnesota*<sup>1</sup>

Avian pathogenic *Escherichia coli* (APEC) causes significant disease in poultry production. When virulent strains are present, they have the potential to cause respiratory disease and other systemic issues such as colibacillosis, pericarditis, and septicemia. APEC can also cause decreases in feed efficiency and growth rate, and increases in morbidity and mortality. While many studies have focused on isolates from clinical disease, few studies have examined the overall ecology of *E. coli* on farms. A field trial was performed using eight different turkey barns from two successive flock cycles. All turkeys came from a single brood facility in a vertically integrated system. Barns were split into two equal treatment groups (n=4 for each group): an unvaccinated negative control group and a group sprayed with a commercial *E. coli* vaccine according to manufacturer's recommendations. Cloacal swabs (n=320), tracheal swabs (n=320), boot sock samples (n=161), and litter samples (n=203) were collected at multiple timepoints from each barn, with sampling occurring from 0-19 weeks of age. In total, 808 *E. coli* isolates were examined from the 8 barns sampled. One isolate from each sample was collected. DNA was extracted from each isolate, and multiple PCR assays were used to determine the *E. coli* Clermont phylogroup, APEC 5-gene prevalence, and the presence of high-risk APEC clones for each isolate. Results were analyzed comparing flock-to-flock differences, ages, treatments, and sample type. Significant differences in ecology were observed in most of these comparisons, including shifts in *E. coli* ecology in response to vaccination. Our work highlights the importance of considering the overall ecology of *E. coli* as it relates to preventing disease in poultry production.

#### **Identification, typing and antimicrobial susceptibility of *Gallibacterium anatis* isolates from Colombia.**

Vladimir Morales Erasto<sup>1</sup>, Liman-Gonzalez MM<sup>1</sup>, Luna Castrejen LP<sup>1</sup>, Juarez-Fuentes N<sup>1</sup>, Torres Bernal CC<sup>2</sup>, Delgado-Forero NI<sup>2</sup>, Rey Morales AF<sup>2</sup>

*Facultad de Medicina Veterinaria y Zootecnia - Universidad Nacional Autónoma de México*<sup>1</sup>,  
*Finmark Laboratories S.A., Tocancipa, 251010, Colombia.*<sup>2</sup>

*Gallibacterium anatis* is a gram-negative bacteria within the family Pasteurellaceae and is known to colonize the upper respiratory tract and lower reproductive tract of healthy chickens. However, it was associated with respiratory disease, salpingitis, peritonitis, and septicemia in chickens. Experimental infections in chickens with *Gallibacterium anatis* lead to increased mortality, and lowered egg production in layers. The hemagglutinating activity of *Gallibacterium anatis* for erythrocytes from different animal species and differences between agglutination for isolates has been reported. In addition, the presence of multidrug-resistant *Gallibacterium* isolates has been reported. Additionally, reports suggest that *Gallibacterium* coinfections with other bacteria could be a greater impact on poultry production. In this study, we report the identification, biotyping, typing by enterobacterial repetitive intergenic consensus ERIC-polymerase chain reaction (PCR), determination of hemagglutinating activity and antimicrobial susceptibility of 6 *Gallibacterium anatis* isolates obtained from layers from Colombia, the isolates were obtained from lung associated to respiratory diseases during the years 2016-2023. The identification was carried out by *Gallibacterium*-specific PCR and MALDI-TOF. Furthermore, all isolates were biovar haemolytica and biotyped as biovar 1 (2 isolates) and biovar 4 (4 isolates). The isolates typing by ERIC-PCR showed 3 distinct patterns. There was no association between ERIC-PCR type and biovar. These results

indicate that ERIC-PCR may be a suitable technique for the typing *Gallibacterium anatis* isolates, however, is necessary the inclusion of a greater number of isolates. The hemagglutinating activity of the isolates was evaluated against four types of erythrocytes (chicken, rabbit, quail and pig), three isolates were not hemagglutinating; one isolate agglutinated only erythrocytes chicken, one isolate agglutinated erythrocytes of chicken and quail, and one isolate hemagglutinated all three types of erythrocytes. The antimicrobial susceptibility test was evaluated by the disk diffusion method against 17 antimicrobials corresponding to 5 antimicrobial groups. The isolates showed resistance to antimicrobials from the group of lincosamides, macrolides, sulfonamides and tetracyclines. In conclusion, *Gallibacterium anatis* isolates from Colombia showed phenotypic and genotypic diversity, in addition, the isolates showed resistance to different antibiotics, studies are important that allow us to identify the pathogenic potential of the bacteria in Colombia. The results and tools used can be useful for the typing of isolates in Colombia, the inclusion of a greater number of isolates is necessary.

### **Identification and typing of *Avibacterium paragallinarum* isolates obtained from commercial laying hens in Colombia.**

Vladimir Morales Erasto<sup>1</sup>, Morales-Erasto V<sup>1</sup>, Liman- Gonzalez MM<sup>1</sup>, Luna Castrejan LP<sup>1</sup>, Torres Bernal CC<sup>2</sup>, Delgado-Forero NI<sup>2</sup>, Rey Morales AF<sup>2</sup>

*Facultad de Medicina Veterinaria y Zootecnia - Universidad Nacional Autónoma de México<sup>1</sup>, Finmark Laboratories S.A., Tocancipa, 251010, Colombia.<sup>2</sup>*

*Avibacterium paragallinarum* is the etiological agent of infectious Coryza disease, a disease that affects the upper respiratory tract of chickens and laying hens. Moreover, it causes losses in egg production between 10-40% in laying hens. Currently, serological classification based on the Kume hemagglutinin scheme divides the bacterium *A. paragallinarum* into three serogroups (A, B and C) and nine serovars (A-1 to A-4, B-1, C-1 to C-4). All three serogroups are present in Latin America. Furthermore, genetic diversity by ERIC-PCR has been studied in isolates obtained in Latin America. In addition, biotyping based on the differences observed in the carbohydrates: maltose, mannitol and sucrose, allowed the identification of 5 biovars (I, II, III, IV and V) in *A. paragallinarum* isolates. In this study, three isolates of *A. paragallinarum* obtained from different outbreaks of infectious Coryza of commercial laying hens in Colombia during the years 2015-2021 were recovered. The isolates were identified by culture on blood agar with *Staphylococcus aureus*, biochemical tests and by species-specific HPG-2 PCR. Furthermore, the isolates were serotyped using the Kume scheme. In addition, carbohydrate-based biotyping and molecular typing using ERIC-PCR were performed. All isolates were NAD-dependent and positive for the specific HPG-2 PCR. One isolate belonged to serogroup A, another to serogroup C and one could not be typed. Likewise, in the carbohydrate-based biotyping tests, two isolates were identified that belonged to biovar III and one to biovar II. Furthermore, ERIC-PCR typing identified 3 different genotypes among *A. paragallinarum* isolates. These results confirm the presence of NAD-dependent *A. paragallinarum* isolates that belonged to two different serogroups. In addition, three different ERIC genotypes are identified among the isolates and two different biovars. There was no correlation between serogroups, ERIC-PCR genotypes and biovars. In conclusion, the tools used in this study are relevant for isolate typing and the above provides information for the design of immunogens that protect against the most frequent

serogroups in commercial poultry farms in Colombia. The inclusion of a greater number of isolates is suggested for the evaluation of the genetic diversity of *A. paragallinarum* in Colombia.

### **Whole Genome Sequencing and Characterization of *Enterococcus faecalis* isolated from Pullet Layers with Growth Depression and Amyloid Arthropathy.**

Aaliyah Gore<sup>1</sup>, Rocio Crespo<sup>1</sup>, Grayson Walker<sup>1</sup>, Mitsu Suyemoto<sup>1</sup>, Mike Petrik<sup>2</sup>

*North Carolina State University<sup>1</sup>, McKinley Hatchery<sup>2</sup>*

*Enterococcus faecalis* (EF) is a commensal bacterium that colonizes the intestines of mammals and birds. It is characterized as an opportunistic pathogen and has been commonly associated with late-stage embryo mortality, omphalitis, growth depression, sepsis, and amyloid arthropathy in poultry. In the present study, a flock of 6,000-layer pullets located in Canada exhibited impaired growth, poor uniformity, and sporadic lameness that started at week 1 of age and extended throughout the pullet rearing phase. EF was isolated from swollen tarsometatarsal joints of lame pullets that contained yellow-orange material. Three EF joint isolates from clinically affected birds and 2 EF environmental isolates were submitted for further characterization by whole genome sequencing. After DNA isolation, draft genome assemblies were subjected to *in silico* multilocus sequence typing, virulence gene detection, and phylogenetic analysis. Two sequence types known to be pathogenic to chickens, ST82 and ST49, were identified among the joint isolates. Seventeen known EF virulence genes were detected in these strains, including *gelE* for gelatinase production and the *fsrB* quorum sensing signaling peptide gene required for virulence regulation. Phylogenetic analysis revealed that an ST82 strain isolated from an environmental sample was clonally related to the two joint isolates. To date, cases of amyloid arthropathy caused by EF are reported in European countries but rarely from North American poultry operations. Further characterization of these EF strains is needed to elucidate transmission routes, uncover environmental reservoirs, and identify specific virulence genes in the development of amyloid arthropathy in poultry.

### ***Mycoplasma synoviae* Genotyping and Phylogenetic Analysis: A Comprehensive Study of VlhA Gene Variation in the Middle East**

Husam Bakri<sup>1</sup>, Raghad Amro<sup>1</sup>, Rana Ayoub<sup>1</sup>, Saja Khader<sup>1</sup>

*Vaxxinoa International<sup>1</sup>*

*Mycoplasma synoviae* (MS) became a major threat to poultry producers in the Middle East, causing substantial economic losses each year. MS infection is characterized by causing sub-clinical upper respiratory tract infection, infectious synovitis, eggshell abnormalities, and egg production losses. The MS-specific *vlhA* gene encodes hemagglutinin and other immunodominant membrane proteins involved in colonization, antigenic differences, and virulence. This study aims to understand MS epidemiology, investigating clinical signs and *VlhA* gene variation in isolates from different Middle Eastern regions. Samples were collected systematically from unvaccinated flocks on *Mycoplasma*-related clinical signs for two years (2022 and 2023) from different regions in the Middle East, including Jordan, Lebanon, Iraq, United Arab Emirates, Kuwait, and Oman. These samples were tested against MS. Initially, most samples tested positive for MS using real-time PCR test. Subsequent sequencing analysis for the positive samples classified all detected PRR as type A, C, D, or E among reference strains. Phylogenetic analysis grouped samples into 2 closely related clusters (group 3 and 4).

*Mycoplasma synoviae* (MS), was detected in different regions in the Middle East, implicating MS as the underlying causative agent for the observed clinical signs. This study sheds light on the prevalent threat of MS in the poultry production in the middle east. The identification of the MS-specific *vlhA* gene variation provides a useful diagnostic tool. The systematic analysis, using clinical signs, real-time PCR, sequencing and phylogenetic analysis, confirms MS presence in the studied areas. Detection and classification of MS types highlight similarities among circulating strains in the Middle East, emphasizing the ongoing economic threat to the poultry industry. MS is a major economic threat to the poultry industry in the Middle East and remains an ongoing challenge that requires effective strategies to control this disease. The results of this study provide valuable contributions to understanding and addressing the impact of *Mycoplasma* on poultry production. The data presented here are an important step toward developing targeted strategies. It highlights the importance of epidemiological analysis of the disease and identifying the strains circulating in the region, paving the way for the development of better diagnostic tools, and improved therapeutic response.

### **Importance of *Mycoplasma Synoviae* Vaccination in performance of breeders challenged with Infectious bronchitis virus: A field report from Colombia.**

Francisco Perozo<sup>1</sup>, Hernan Niao<sup>2</sup>, Laura Robayo<sup>3</sup>

*Boehringer Ingelheim*<sup>1</sup>, *Agroavícola Sanmarino*<sup>2</sup>, *Carval*<sup>3</sup>

*Mycoplasma Sinoviae* (MS) and Infectious Bronchitis (IB) challenges are increasingly predominant in broiler breeder farms. They are responsible for aerosaculitis, synovitis and shell abnormalities, affecting the number and quality of the hatching egg. The effect of MS on the number of hatching eggs and chick quality is often underestimated or covered by the metaphylactic use of antibiotics. Eradication is the best way to keep the birds free from the MS; nevertheless, it is not economically plausible in South America. As an alternative, vaccination (single dose of Ms-H) is implemented. In this abstract the effect the Ms-H vaccine (VAXSAFE MS) on total hen mortality, culling percentage, total and hatchable hen housed eggs and total chicks produced was assessed. Consecutive flocks in a MS and IB positive farm (non-vaccinated/antibiotic treated vs MS vaccinated/not medicated) were compared. The vaccinated flock showed 2.6% less mortality, 2.1% less culling, 8.3 and 7,6 additional eggs (total and hatching, respectively). Ultimately, the vaccinated flock produced 123,6 chicks per hen during the production cycle when compared with the unvaccinated that produced only 115,3. These results arise despite the fact that a four weeks-long IB clinical challenge was fully diagnosed, the suitability of MS vaccination demonstrated, indicate that when mycoplasmas are effectively controlled the deleterious effects of IB can be mitigated allowing for economically sound results in a breeder operation.

### **The Effect of Sample Pooling on the Detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by Real-time PCR**

Michael Davis<sup>1</sup>, Naola Ferguson-Noel<sup>1</sup>

*UGA Poultry Diagnostic Research Center*<sup>1</sup>

Surveillance testing for important poultry respiratory pathogens like *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), avian influenza (AI) and Newcastle disease (ND) is carried out regularly in the US as part of national control plans. It has been established that up to eleven swabs



from a flock may be pooled for real-time PCR (qPCR) screening for AI and ND, while only five swabs are pooled for MG and MS real-time PCR testing. Multiple screenings for these pathogens contribute to high cost of poultry production and increased stress on birds. The objective of this research was to compare the sensitivity of detection of avian mycoplasma (MG and MS) by real-time PCR of pools of 5 and 11 swabs with varying concentrations of avian mycoplasma. Specific real-time PCR for MG and MS was conducted on 5 (containing 4 negative and 1 positive) and 11 (containing 10 negative and 1 positive) pooled tracheal swabs. Preliminary results indicate that there may be no significant difference in the sensitivity of detection of avian mycoplasma between pools of 5 swabs and pools of 11 swabs using real-time PCR. However, further research investigating different transport media, extraction methods and PCR protocols is required to establish broadly applicable protocols towards the goal of collecting one set of samples when screening for poultry respiratory pathogens.

## Case Reports

### **Weather or Management: A Series of Erysipelas Cases in Commercial Turkeys During an Unseasonable Midwest Winter**

Ashley Poissant<sup>1</sup>

*Jennie-O Turkey Store<sup>1</sup>*

In March of 2023, unseasonably warm weather in southeast Minnesota presented several disease and management challenges for raising commercial toms. Over the course of the month, with the ground was still frozen, rainstorms brought several inches of precipitation. This caused rainwater to seep into barns, making litter management extremely difficult. This contributed to several cases of acute and chronic erysipelas diagnosed shortly after these weather events in both curtain sided and tunnel barns. Due to the No Antibiotics Ever (NAE) intended placements of these flocks, management changes, non-antibiotic interventions and antibiotic treatment strategies were utilized to control morbidity and mortality.

### **Case Report: Glaucoma, Corneal Edema, Cataracts, and Retinal Degeneration in Broiler Breeder Chickens**

James Davis<sup>1</sup>, James F. Davis<sup>1</sup>, Roscio Crespo<sup>2</sup>, Oscar Fletcher<sup>2</sup>, Louise Dufour-Zavala<sup>1</sup>

*Georgia Poultry Laboratory Network<sup>1</sup>, North Carolina State University<sup>2</sup>*

Broiler breeder chickens were presented to the Georgia Poultry Lab in Gainesville, Ga with gross lesions consistent with severe glaucoma. Upon histologic examination, a number of unusual lesions were detected in the affected eyes. These included, corneal edema, anterior uveitis, cataracts, infiltration of lymphocytes and plasma cells with extramedullary hematopoiesis, glaucoma, and retinal degeneration. Immunohistochemistry and quantitative PCR testing was used to try and determine the etiology of these lesions.

### **Avian Coryza and Laryngotracheitis co-infection in layer chicken: A case report**

Ahmed Achhal<sup>1</sup>

*Clinique vétérinaire Tit Mellil<sup>1</sup>*

Avian respiratory diseases pose significant threats to the poultry industry, affecting both productivity and welfare. This case report details a simultaneous infections of avian laryngotracheitis and infectious coryza in a commercial layer flock. The affected layers exhibited clinical signs including respiratory distress, nasal discharge, decreased egg production, and a rise in mortality rates. Diagnostic investigations, encompassing molecular assays, confirmed the presence of both avian laryngotracheitis virus and Avibacterium paragallinarum, the etiological agents responsible for ALT and coryza, respectively. This dual infection presented challenges in disease management, necessitating a comprehensive approach to control and prevent further dissemination. This case report contributes valuable insights into the complexities of managing concurrent respiratory infections in layer flocks, urging a proactive stance towards disease prevention and control in the poultry industry.

### **Outbreaks of duck viral enteritis in breeding mallards for the British game sector in 2023**

Christopher Poulos<sup>1</sup>, Scott Reid<sup>2</sup>, Dan King<sup>3</sup>, Zoe Treharne<sup>4</sup>, Marco Falchieri<sup>5, 6, 7, 8, 9, 10</sup>

*Pathology Department, Animal and Plant Health Agency<sup>1</sup>, Virology Department, Animal and Plant Health Agency - Weybridge, New Haw, Addlestone, UK<sup>2</sup>, Sandhill Veterinary Services, Ripon, UK<sup>3</sup>, Avian Expert Group, Surveillance Intelligence Unit, Animal and Plant Health Agency<sup>4</sup>, Virology Department, Animal and Plant Health Agency - Weybridge, New Haw, Addlestone, UK<sup>5</sup>*

Duck viral enteritis (DVE), caused by an infection with Anatid alphaherpesvirus-1, is a highly contagious disease of ducks, geese, and swans. It has worldwide distribution and is reportable to the World Organisation for Animal Health. Outbreaks of DVE in Great Britain have been sporadic, with varying clinical presentations that depend on species and age affected. The majority of these outbreaks investigated by the endemic diseases surveillance network of Animal and Plant Health Agency has involved Muscovy ducks, a species that is particularly sensitive. These outbreaks present with gross and microscopic lesions typical for DVE, with inclusion bodies frequently identified in hepatocytes. Vaccination can also protect from infections, which are harboured in the environment or may occur after exposure to wild waterfowl shedding the virus. In April 2023, increasing mortality, weeping eyes, and egg drop were reported on a site with 10-month-old homebred breeding mallards. There was a significant drop in egg production over three days (25 to 40%), but mortality in this flock was relatively low (1.5%). Post-mortem examinations by the private practitioner identified necrotising oesophagitis in one bird and mild multifocal pancreatic lesions, which prompted consideration of avian influenza as a differential diagnosis. Histopathological findings in the oesophagus were consistent with DVE, with acute mucosal necrosis and very rare intranuclear inclusion bodies in epithelial cells. Inclusions bodies were not identified in other submitted tissues. Additional findings included amyloidosis as the cause of pancreatic necrosis, myocardial necrosis consistent with acute heart failure, and systemic bacterial infections which were considered secondary to DVE. Another outbreak occurred on a nearby mallard breeding site in May 2023. Whilst most findings were similar to those described in the previous outbreak, a predominant finding in five examined birds was necrotising cloacitis. Neither site was recently vaccinated for DVE. Real-time PCR for DVE virus was positive and confirmed the diagnosis. Testing also yielded positive PCR results from oesophageal, tracheal, or cloacal samples or swabs, but not from other common samples used for PCR, such as liver or spleen. In summary, the main presenting clinical sign for DVE in adult mallards was egg drop. It was suspected that these outbreaks were

triggered by stress associated with the breeding season in commercial set-ups. Cloacal lesions may be more prevalent than those in the oesophagus during post-mortem examination, but the identification of inclusion bodies within mucosal lesions can be very limited. PCR is recommended to confirm a diagnosis, and consideration should be given to sample selection to obtain a confirmatory result.

### **Ocular Marek's Disease in a Black Shouldered Peafowl**

Hailey Quercia<sup>1</sup>, Jessica Walters<sup>2</sup>, Sheryl Coutermarsh-ott<sup>3</sup>

*Virginia Department of Agriculture and Consumer services<sup>1</sup>, Virginia Department of Agriculture and Consumer services<sup>2</sup>, Virginia Department of Agriculture and Consumer services<sup>3</sup>*

Marek's disease has been diagnosed in chickens, Japanese quail, and more rarely in turkeys and peafowl. In chickens four different forms of the disease are recognized, including an ocular form. With the ocular form neoplastic T cells invade nervous tissues of the eye, disrupting normal appearance and function. A similar presentation was identified in two related peahens. In 2022, a peafowl breeder submitted a four-year-old female Black-shouldered peahen with complaints of wasting and blindness for euthanasia and necropsy. Histopathology identified a disseminated lymphoma in multiple tissues, including the iris and uvea of one eye. One year later, in June of 2023, the same breeder submitted a five-year-old female sibling of the first peahen, with the same clinical presentation of blindness and weight loss. Gross lesions were suggestive of lymphoma, and histopathology identified neoplastic lymphocytes in numerous organs including the peripheral nerve, ocular nerve, iris, choroid, and sclera of one eye. Sections of liver infiltrated with neoplastic cells from this second case were PCR positive for Marek's Disease Virus DNA. These cases illustrate that peafowl are susceptible to ocular forms of Marek's disease, in addition to the visceral form. Interestingly, both birds were much older than the age range where Marek's disease is most often seen in chickens. Both the close relationship and similar presentation of these birds may suggest a shared genetic susceptibility in this particular line.

## **Diagnosics**

### **Modified Extraction Method Reduced Variation in Feather Corticosterone Replicates In Combination With High Extraction Efficiency**

Nikolas Faust<sup>1</sup>, Mazette Croom<sup>1</sup>, Nathaniel Ward<sup>1</sup>, Sara Barnes<sup>1</sup>, Rosemary Walzem<sup>1</sup>

*Poultry Science Department, Texas A & M University<sup>1</sup>*

Robust, non-invasive methods are needed to objectively assess stress to aid in documenting welfare status of commercial poultry. Feather corticosterone (F-CORT) has emerged as a longer-term sample source that is potentially less affected by acute handling stress. However, extraction methods display undesirable degrees of variation. We sought to optimize feather extraction protocols for subsequent F-CORT analysis by enzyme immunoassay (EIA) and to apply the optimized protocol to feathers collected from three strains of 16-week-old chicken pullets (n=15 per breed assayed in duplicate). Parallel extractions using published methanol (M) and keratinase (K) treatments led to development of an optimized K extraction protocol (K+) using a common feather pool stemming from a modified evaporation/resuspension step within the original K protocol. The K+ protocol was tested

with a spike/recovery test. The common feather pool was created from mature primary contour feathers of 60-week-old layer chickens. The calamus and rachis were removed from the vanes prior to their maceration into pieces <4-5mm<sup>2</sup> via razor blade. All extractions used a feather to solvent ratio of 2mg/1mL. F-CORT content of prepared extracts was determined using an EIA (K014-H1/K014-H5, Arbor Assays) kit and Synergy H1 Microplate reader set to read at 450nm. Spike/recovery test targeted five central points of the EIA standard curve with samples in triplicate containing known CORT standard (Corticosterone EIA Standard, Cayman Chemicals, EIA standard curves R<sup>2</sup> = 0.999). Protocol intra-assay variation (ICV) tests used duplicates of 10 separate feather extracts. Data = means ± SEM. All concentration data log transformed for normality and linearity assessed using Pearson correlation coefficient. Variation of M, K and K+ was compared using Bartlett's test. Chicken strains were compared using one-way ANOVA with post-hoc means separation by Tukey's HSD. In all comparisons significance was assessed at p ≤ 0.05. M, K, and K+ extracted 6.42 ± 1.39 (CV = 61.2%), 4.10 ± 0.42 (CV = 33.0%), and 1.42 ± 0.0737 (CV = 5.2%) pg CORT/mg feather, respectively. Reduction in ICV in K+ was significant compared to K (28%, p=0.024), and M (88%, p<0.0001). K+ spike/recovery test found ≥ 90% recovery with a significant positive correlation, y = 0.97x + 36.8 (R<sup>2</sup> = 0.91), p=0.002, to theoretical yield. Average feather CORT of Breed 1(layer) = 2.52 ± 0.96 A, Breed 2(broiler breeder) = 1.77 ± 0.51B, Breed 3(broiler breeder) = 1.79 ± 0.68 B pg/mg. The experiments demonstrate K+ reliability through positive correlation of theoretical returns and reduced ICV of F-CORT values compared to published protocols. Further studies will be needed to investigate variables of feather hormone deposition and breed specific responses.

### **New Infectious Bronchitis indirect ELISA, based on well conserved recombinant protein, for improved detection of live vaccines and challenge including variant strains**

Rafael Forero<sup>1</sup>, Stephanie Lesceu<sup>1</sup>, Marina Gaimard<sup>1</sup>, Chloa Redal<sup>1</sup>, Jean-Emmanuel Drus<sup>1</sup>, Catherine Lefebvre<sup>1</sup>, Philippe Pourquier<sup>1</sup>

*Innovative Diagnostics<sup>1</sup>*

Avian infectious bronchitis virus (IBV) is a coronavirus, which infects poultry and causes infectious bronchitis. It is a highly infectious avian pathogen, which affects the respiratory tract, gut, kidney and reproductive systems of chickens. Important mutations could be observed for avian Coronavirus which lead to the appearance of new variants worldwide. To control the disease, vaccination is largely used and based on classic or circulating variant strains. Thus, diagnosis and monitoring of vaccination require laboratory testing, and ELISA may be used for monitoring serum antibody responses. The new ID Screen<sup>®</sup> Infectious Bronchitis Indirect ELISA, based on well conserved recombinant protein, allows for the detection of IBV antibodies in samples. This kit is specifically used for the detection of antibody response after IBV vaccination (including variants: 4/91, 793B, QX, Italy 02, BR1...), and improve detection of challenge in vaccinated flocks (by classic or variants strains).

### **Rapid Disease Diagnostics in Broiler Chickens Using Metabolomics**

Asha Ranaraja<sup>1</sup>, Mihiprabha Rathnayake<sup>1</sup>, Iresha Subhasinghe<sup>1</sup>, Hemlata Gautam<sup>1</sup>, Noor Shaik<sup>1</sup>, Shelly Popowich<sup>1</sup>, Betty Chow- Lockerbie<sup>8</sup>, Khawaja Ahmad<sup>1</sup>, Susantha Gomis<sup>1</sup>

*University of Saskatchewan<sup>1</sup>, Abbotsford Veterinary Diagnostic Lab, British Columbia<sup>9</sup>*

The Canadian broiler industry suffers considerable economic losses due to outbreaks of infectious diseases that may also threaten human food safety and animal welfare. In addition, the discontinuation of prophylactic antibiotic usage has led to an increase in poultry bacterial infections. At the same time, detecting subclinical and simultaneous bacterial and viral infections remains a major challenge. Therefore, there is a need for early diagnostic techniques that can detect diseases, both bacterial and viral, within 1-2 days post-infection. To address this, a novel approach of metabolic biomarker-based diagnosis can detect infections by considering pathogen-induced early metabolic changes even before clinical signs appear. While metabolic biomarker-based studies are common in human medicine, such studies are still in their early stages in veterinary medicine. Therefore, the objective of this study was to diagnose bacterial and viral infections in broiler chickens using metabolic biomarkers as a rapid diagnostic tool. Serum samples were collected from broiler chicken at 24 h post-challenge with avian pathogenic *Escherichia coli* and avian reovirus. The control birds were kept uninfected and sampled for serum at the same time points. Liquid Chromatography mass spectrometry was used for metabolic profiling of serum samples. Data analysis was performed using multiple statistical approaches including univariate analysis, multivariate analysis, feature selection, and machine learning techniques. We were able to observe a clear separation between the metabolite profiles of the infected and control birds. The pathway analysis revealed a significant downregulation of the purine metabolism in the infected birds in comparison to the control. Purine metabolism is an essential process in the body that contributes to energy generation, DNA synthesis, and signaling. During the initial stages of infection, the body undergoes a reprogramming of its energy generation and biosynthesis processes. This includes an increase in glycolysis, which helps in faster ATP production and biosynthesis, necessary for damage repair and defensive response. Additionally, there are epigenetic modifications that occur, connected to immune cell proliferation, signaling, activation, and differentiation. As a result, purine metabolism will likely change during the early phases of infection. Therefore, it can be used as a potential biomarker for the early detection of colibacillosis due to Avian pathogenic *Escherichia coli* and avian reovirus infection in chickens to reduce the severity of outbreaks.

### **Advantages of Genotyping Infectious laryngotracheitis virus (ILTV) from Clinical Samples Using MinION**

April Skipper<sup>1</sup>, Yi Chen-Lou<sup>1</sup>, Maricarmen Garcia<sup>1</sup>

*University of Georgia*<sup>1</sup>

Infectious laryngotracheitis (ILT) is a highly contagious poultry respiratory disease that causes substantial economic losses to the poultry industry during outbreaks. The disease is caused by the infectious laryngotracheitis virus (ILTV), also known as Gallid alphaherpesvirus 1 (GAHV-1). Antigenically, ILTV strains exist as a single serotype. Therefore, viral strain differentiation is based on specific genome differences (Genotyping). ILTV strains currently circulating in the United States belong to four main categories: vaccines and vaccine-like viruses (Genotypes II and IV), virulent vaccine revertant (Genotypes III and V), and virulent no-vaccine related viruses (Genotype VI). Advancements in next-generation sequencing and the demand for more accurate genotyping led to the development of a Multiplex PCR and MinION sequencing assay that amplifies the viral genome's unique short (Us) region. As compared to the previously used PCR-Sanger sequencing assay, the Multiplex PCR MinION assay increased the genome coverage from 2.6% (4Kb/154Kb) to 8.4%

13Kb/154Kb), increased the depth of sequencing to more than 50X per nucleotide, and increased the number of informative single nucleotide polymorphisms (SNPs) that allow to differentiate among genotypes II/III, IV, V, and VI from 15 to 30. We have routinely used the Multiplex PCR and MinION sequencing assay to genotype ILTV from clinical samples in the past year. Compared to the PCR Sanger sequencing assay, which needs a viral genome load with a Ct value of 25 or lower to accurately genotype ILTV from a clinical sample, the multiplex-MinION assay sensitivity is superior as accurate genotyping is achievable in clinical samples of genome load with Ct values of 30 to 32. Moreover, the broader coverage and deeper sequencing provided by the Multiplex PCR and MinION sequencing assay have allowed the tracking of unique SNPs within the same genotype outbreak-related viruses circulating in specific poultry production sites.

### **Enhancing Diagnostic Efficiency: Implementation of a Multi-Laboratory Platform for Poultry Diagnostics**

Dimitri Popov<sup>1</sup>, Dr. Frederic Hoerr<sup>2</sup>

*Aggio<sup>1</sup>, Veterinary Diagnostic Pathology, LLC,<sup>2</sup>*

Technological advancements have revolutionized diagnostic processes in human and companion animal health. The food production animal health and diagnostics industries in the USA lag in technology adoption, relying on paper forms for diagnostic submissions contrasting with the seamless digital submissions facilitated by Veterinary Practice Management software in companion animal diagnostics. The use of PDFs for result delivery contributes to a manual and less automated process, leading to increased costs, potential human errors, and a lack of uniformity that impedes aggregate data analytics. Innovative laboratories have resorted to creating customized proprietary client portals or request Laboratory Information Management System (LIMS) vendors to integrate this functionality. While beneficial for those poultry diagnostic participants associated with a particular lab, such approaches fall short when catering to multi-lab submitters. A survey conducted in 2022/23 within the poultry industry, including managers, veterinarians, and private and public laboratories, revealed a compelling need for a unified solution for poultry diagnostics. In response, a diagnostic (Dx) multi-laboratory platform was developed and successfully launched in October 2023. Presently, two prominent poultry laboratories are actively participating, with over 1,200 users, many of whom regularly log in to access results or submit diagnostics digitally. Initial feedback has been positive. The implementation of a Dx multi-laboratory platform marks a pivotal step in improving diagnostic efficiency within the poultry industry.

### **Optimizing Metagenomic Approaches for Enhanced Food Safety in Poultry Production Environment.**

Mostafa Ghanem<sup>1</sup>, Dhiraj Chundru<sup>1</sup>, David Erickson<sup>1</sup>, Xinyang Huang<sup>1</sup>, Jianghong Meng<sup>1</sup>

*University of Maryland, College Park<sup>1</sup>*

Monitoring foodborne pathogens in agricultural environments is critical for regulatory compliance, source tracing, and outbreak management. Current methods, such as pathogen-specific culture-based or molecular assays, are time-consuming and may overlook vital foodborne pathogens not targeted by these tests. While Whole Genome Sequencing (WGS) has enhanced outbreak investigation accuracy, it still carries limitations from culture-based approaches. Shotgun

Metagenomics (SGM) is proposed as a comprehensive solution for faster, holistic microbial community analysis to bolster food safety. However, implementing Metagenomics for this purpose faces challenges due to the absence of standardized workflows in laboratory procedures and data analysis pipelines. In this experiment, we aimed to validate standardized protocols for DNA extraction, and library preparation using mock microbial communities with targeted samples like fecal droppings and litter samples from a broiler farm. This validation assesses relative and absolute taxonomic species diversity, along with alpha and beta diversity, for three replicates of test sample. The samples undergo processing using two distinct DNA extraction methods, each coupled with two different library preparation methods, and subsequent analysis involving three different analysis pipelines. Subsequently, all libraries underwent sequencing on Illumina HiSeq. Additionally, Truseq libraries were also sequenced on Miseq for preliminary results. Preliminary results analyzed by Metaphlan4, revealed better alpha diversity using the Qiagen kit for DNA extraction. Principal Coordinate Analysis (PCoA) plots (bray-Curtis) displayed dissimilarities among samples extracted using Zymo kits across various sample types. Moreover, relative taxa abundance from DNA extracted by the Qiagen kit from Zymo bacterial community standards closely matched the expected abundance. These findings highlight the impact that DNA extraction and library preparation kits could have on diversity metrics and taxa abundance in similar experiments. These experiments are initial steps to create a standardized workflow for laboratory procedures and data analysis to monitor Foodborne pathogens in poultry production environments. This could enhance food safety in agriculture by improving pathogen detection, contamination source identification, intervention assessment, and risk assessment models. It could pave the way for widespread adoption across agricultural systems, benefiting regulatory monitoring and food safety. Furthermore, this research could uncover inherent biases associated with each sample preparation step, offering valuable considerations for future studies.

### **Use of High-Resolution Melting Point RT-PCR for Confirming the Effectiveness of Two Live IB Vaccine Strains Application When Administered Simultaneously at the Hatchery and at Farm Level in Layers**

Francisco Rios-Cambre<sup>1</sup>, Alberto Estrada-Loza<sup>1</sup>, Alfredo Mercado<sup>3</sup>, Aurora Romero-Tejeda<sup>4</sup>, Richard Currie<sup>4</sup>

*MSD Salud Animal Mexico<sup>1</sup>, Independent consultant<sup>3</sup>, X Ovo Limited<sup>4</sup>*

Infectious Bronchitis is caused by a gammacoronavirus which is highly contagious and is distributed worldwide causing serious economic burden in broilers and layers. The high capability of this RNA virus to mutate and recombine has caused the emergence of many genotypes, as well as the generation of antigenic variants that make their control very difficult by vaccination. The use of homologous vaccines for controlling these variants may also induce the emergence of novel variants. The concept of Protectotype™, briefly, the use of two antigenically different vaccine viruses for controlling variants, has proven successful in many areas of the world, including the Mexican layer industry. Since the two vaccine strains used require effective reconstitution and application techniques, it is important to confirm that both replicate in the bird post-application. By using a High-Resolution Melting Point Reverse-Transcriptase Polymerase Chain Reaction (HRMP-RT-PCR) test, we were able to confirm the presence of both IB Ma5 and 4/91 vaccine strains in layers vaccinated both

at the hatchery and at farm level. The results of this study will allow the use of this technique to monitor the effectiveness of vaccine application crews in long-living birds.

### **Looking beyond implementing Oxford Nanopore Technologies (ONT) in poultry diagnostics: Lessons Learned from Years of Working with ONT**

Amro Hashish<sup>1</sup>

*Iowa State University<sup>1</sup>*

The poultry industry confronts a myriad of health challenges, with the continual emergence of novel variant strains of infectious agents. This presents a continuous need to improve available laboratory diagnostics using newly emerging technologies for accurate and timely diagnosis of these diseases. After the debut of the MinION device of Oxford Nanopore Technologies (ONT), Nanopore sequencing emerges as a game-changer in the field of molecular diagnostics. Nanopore sequencing distinguished by its rapid data generation, real-time data acquisition, generation of long reads, portability and affordability. While the versatility of nanopore sequencing allow customization to specific applications is one of the major advantages, it can pose a significant challenge, particularly for new users to the technology. This presentation focuses on conveying experience gained for our team from working with ONT to diagnose poultry pathogens "viral and bacterial" during the last four years. This experience not only provides insights but also addresses the challenges encountered from working with this platform. The diversity in the employed methods spans various aspects, including different extraction strategies, various enrichment steps, library preparation, sequencing methodologies, and data analysis tools. This diversity serves as a valuable resource for researchers, particularly those new to the technology, aiding in their better understanding and optimization of this sequencing platform towards their intended application. Ultimately, this knowledge contributes to enhanced preparedness in combating infectious agents across different poultry species.

### **The Advantages of Hemagglutination Inhibition (HI) MGMS Test Automation Using a Lynx LM900 by Dynamic Devices Versus Manual Testing**

Sara Cooper<sup>1</sup>

*Georgia Poultry Laboratory Network<sup>1</sup>*

1.HI test on the Lynx runs faster and saves time. All tips, HI stock plate, reagents and HI test plates are placed on the Lynx deck and remain on the deck during the duration of the test. Manual testing requires configuration of tips, changing pipettes and size of tips. HI test plates are moved from one place to another during dispense of reagents and serial dilution. 2.The 96-channel head on the Lynx can dispense the same volume into multiple wells. Manual testing can dispense only a maximum of 12 volumes at one time. 3.Volumes are more accurate for antigen and red blood cell dispenses using the Lynx. Antigen and red blood cells are dispensed directly into the buffer and test samples. Tips are washed in between each HI test plate. Manual testing uses reverse pipetting above the buffer and test samples for antigen and red blood cell dispenses. HI test plates are tapped after dispenses, but no guaranteed full volume reaches the liquid in the plate. 4.No timers need to be set. Thirty minute and one hour incubation periods are built into the Method Manager 4 software of the Lynx. Music will play at the end of each incubation period in the automated MGMS HI Test (NPIP). After thirty minutes music plays to indicate the red blood cells need to be added to the red blood cell reservoir for red



blood cell dispense and after one hour music plays to indicate the HI test plates are ready to be read. The Method Manager 4 software has also built into the MGMS HI Test (NPIP) to begin the thirty-minute timer or one hour timer when antigen or red blood cells is dispensed into the first HI test plate.

### **Use of a new veterinary diagnostic tool for ELISA titer data analysis and interpretation**

Reshma Ramachandran<sup>1</sup>, Martina Drown<sup>2</sup>

*Professional Services Veterinarian-II<sup>1</sup>, Sr. Marketing Manager Global Software<sup>2</sup>*

Enzyme-linked immunosorbent assay (ELISA) is a rapid serological test used for detecting and quantifying antibodies or antigens. It is a valuable technique for poultry disease diagnostics and vaccination program monitoring. However, to improve the overall poultry flock health it is important to streamline serologic data collection and analysis as well as to develop poultry company-specific serologic baselines. Hence, the objective of this study was to analyze and interpret the average ELISA geometric mean titers (GMTs) to various poultry viral diseases using a new veterinary diagnostic tool, xChek Vet. For this, Newcastle disease virus (NDV), infectious bronchitis virus (IBV), infectious bursal disease virus (IBD) and reovirus (REO) ELISA GMT data were collected from a commercial poultry company with multiple broiler breeder and broiler chicken flocks across the nation. Data was filtered such that only flocks (multiple complexes) from a specific region were used. Further, the data was analyzed using xChek Vet software and then, age-specific serologic baselines for each virus ELISA titers were developed. Finally, the average ELISA GMT data was compared with serologic baselines allowing to determine the company titer trends over time and to identify strategies to improve overall flock health.

### **HVT-NDV hatchery vaccination evaluation using a fusion protein-specific ELISA**

Duane Murphy<sup>1</sup>, Sara Stephens<sup>1</sup>

*Farbest Farms, Inc.<sup>1</sup>*

Background: Our operations experienced an increased incidence of respiratory disease associated with rising titers to Newcastle disease virus and/or positive PCR NDV-matrix results on oropharyngeal swabs, despite hatchery-applied HVT-NDV vaccine. We questioned efficacy of the vaccine, or its application. Because standard NDV ELISA fails to detect the NDV-fusion protein of HVT vaccines, we used a fusion protein-specific ELISA to measure serologic response to vaccination. Because we found no data on use of this NDV-F ELISA in turkeys, a second purpose of this study was to see if it works on turkey serum. Protocol: Study was initially conducted on a single flock of tom turkeys vaccinated with Product A, and then repeated on a second flock vaccinated with Product B. For each flock, we collected blood from 25 birds at 1, 19, 28, 42, and 56 days of age, and from 6 birds at 140 days (slaughter age). Samples were held at room temperature overnight until a clot formed and began to contract, then centrifuged, and the serum collected and stored in a freezer. For each flock, serum samples were held until all samples had been collected up to day 56, at which time they were shipped to a private commercial lab which ran all the serum samples for that flock at the same time. (The 140-day samples were run separately.) Each serum sample was tested on the fusion-protein specific NDV-F ELISA, as well as the standard NDV ELISA. Results: 1. The NDV-F ELISA was able to detect a serologic response in these turkey flocks. 2. Both NDV-F and std NDV ELISA's detected maternal antibody, which waned by 28 days of age. 3. Both vaccines induced a serologic

response to fusion protein, though slow ( $\geq 56$  days) to protect a significant percentage of birds.  
4. Vaccine A seemingly produced a stronger serologic response to fusion protein than vaccine B.  
5. Serologic immunity to fusion-protein persisted for the life of the flocks (up to 20 weeks).

### **Developing Third Generation Sequencing Assays to Rapidly Analyze Whole Genome and Hexon Genotypes from Field Samples and Isolates of Fowl Adenovirus**

Derek Moormeier<sup>1</sup>, Julia McElreath<sup>1</sup>, Curtis Tobaben<sup>1</sup>, John Goza<sup>1</sup>, Scott Reed<sup>1</sup>, Fraser Combe<sup>1</sup>, John El-Attrache<sup>1</sup>, Scott Callison<sup>1</sup>

*Ceva Animal Health*<sup>1</sup>

Fowl Adenoviruses (FAdV) have been implicated in causing a variety of poultry diseases, including hepatitis hydropericardium syndrome, inclusion body hepatitis, and gizzard erosion. The FAdV ~43kb genome is double-stranded, non-segmented linear DNA that encodes approximately 40 proteins. FAdVs are divided into five species (A-E) that include 12 serotypes largely classified by nucleotide changes in the coding region of the variable L1 loop of the hexon protein. Given that specific serotypes are often associated with particular diseases, rapid identification and genotyping of FAdV serotypes within field and isolates are becoming increasingly more important. Here, we developed two separate assays to sequence either the whole genome or target the hexon gene for rapid genotyping. To test the utility of each assay, field samples and known adenovirus serotyped isolates were collected. For the whole genome approach, a modified host depletion method combined with non-specific amplification and Oxford Nanopore rapid barcoding were used to rapidly assemble the entire FAdV genome. For the hexon genotyping approach, a targeted PCR combined with Oxford Nanopore native barcoding enabled sample genotyping. These two methods combined provide additional sequencing tools to enable faster identification of emerging genotypes to environmental treatment and vaccine development.

### **Incidence of airsacculitis in newly hatched broiler chicks in Brazil: possible causes and potential consequences**

Jorge Chacon<sup>1</sup>, ISABEL MINOLI<sup>2</sup>, GRAZIELA SANTOS<sup>3</sup>, LUIZ SESTI<sup>4</sup>

*Ceva Animal Health - Veterinary Services*<sup>1</sup>

In Brazil, in the last two years, there has been a significant increase in the incidence of broilers condemnation at slaughter due to airsacculitis lesions, especially during wintertime. The clinical picture starts with respiratory distress in chicks as young as one week old. An investigative diagnostic work was designed to try establishing possible etiologies of such clinical condition at so early age. The work involved extensive post-mortem investigation, scoring of macroscopic findings and sampling for laboratory analyses. A total of 61 broiler flocks from nine production systems (companies A through I) located in four geographical regions were included in the study. Seven from those nine farms were having high condemnation rate at slaughter due to airsacculitis lesions and two production systems (C & G) had no problems. The 61 broilers flocks were progeny of breeders with age ranging from 25 to 67 weeks. Forty newly hatched chicks were randomly selected from each of the broiler flocks involved in the study, being 20 good quality and 20 runt chicks destined to be discarded. Presence of foam and caseous materials on air sacs were registered and scored (0 to 2). Bacterial isolation for aerobic and anaerobic species was carried out in four production systems (E

through H). The range of incidence of airsacculitis ranged from 0 to 100%. Airsacculitis was observed in 75% of good quality and 85% of runt chick flocks. High airsacculitis score (2) was found in 8.5 and 24.3% of good quality and runt chick flocks, respectively. Companies C & G that were not having condemnation at slaughter presented the lowest percentage of airsacculitis. Higher incidence of airsacculitis in newly hatched chicks did not correlate with the age of producing breeder flocks. A total of 18 bacterial species were isolated from sampled chicks with higher detection rate of *E. coli*. The isolates were done from affected air sacs having foam and/or caseous materials. According to previous surveillances, many of the bacterial species isolated from the newly hatched chicks came from the breeder farms and/or were residents on the hatcheries. Very early airsacculitis in broilers chicks may not cause overt clinical signs and loss of performance throughout the broiler cycle but a large % of the birds will never be completely cleared out of air sacs lesions and will be condemned at slaughter. The losses may be significantly higher if broiler flocks presenting very early airsacculitis are not properly vaccinated against Infectious Bronchitis (IB). In conclusion, if rate of condemnation at the slaughter increases it is imperative to go back and investigate early incidence of airsacculitis and possible sources in addition of revising and adjusting vaccination programs against IB. The methodology showed be a useful tool that can evaluate quality of newly hatched chicks, detect health challenges in breeder flocks that can affect the eggshell, and predict respiratory disease in old bird flocks.

#### **Nanopore sequencing protocol for enhanced genome recovery of Newcastle disease virus from clinical samples**

Maria Chaves<sup>1</sup>, Amro Hashish<sup>1</sup>, Mostafa Shelkamy<sup>1</sup>, Eman A. Gadu<sup>1</sup>, Yuko Sato<sup>1</sup>, David Suarez<sup>2</sup>, Mohamed El-Gazzar<sup>1</sup>

*Iowa State University<sup>1</sup>, US National Poultry Research Center, Agricultural Research Service, USDA, Athens, GA<sup>2</sup>*

Newcastle disease viruses (NDV) result in significant losses to the poultry industry worldwide, and their circulation in domestic poultry and wild birds leads to a constant rise in genetic diversity. NDV is classified into low virulence or virulent strains, and the classification is based on the pattern of amino acid residues in the fusion protein cleavage site. In addressing viral diagnosis and classification, molecular diagnostic tools, such as genome sequencing, become essential. They play a crucial role in revealing the genetic and virulence patterns of NDV strains, which guides the veterinary decisions of prevention, control, and eradication of the disease in the field. In this context, Oxford Nanopore Technologies (ONT), a third-generation sequencing platform, stands out for its unique features, such as long read generation and real-time analysis of generated sequence data. However, performing Nanopore sequencing from clinical samples in a metagenomic Next Generation Sequencing (mNGS) approach leads to lower diagnostic sensitivity due to the overabundance of host DNA/RNA. Hence, the implementation of enrichment strategies to increase the viral reads becomes necessary. Therefore, in this study, we aimed to compare two different enrichment strategies for NDV, which included (i) target-independent enrichment using sequence-independent single primer amplification (SISPA) and (ii) a target-dependent approach using a tiling PCR for the viral fusion gene. We selected five known NDV-positive samples and pre-processed them using both approaches. Following this, we prepared the sequencing library from the treated samples using the Ligation Sequencing kit (LSK110) and the PCR barcoding expansion (PBC001). Samples

were sequenced using the MinION Mk1B device. Data analysis will include the evaluation of the percentage of NDV reads generated, as well as genome coverage (depth and breadth) to assess the different performance from the different enrichment approaches. The final goal of this study is to formulate an optimum workflow for NDV diagnosis using Nanopore sequencing, reducing the time for diagnosis, without compromising diagnostic sensitivity and accuracy.

### **Developing a novel method for sequencing the whole genome of infectious bursal disease virus**

Julia McElreath<sup>1</sup>, Derek Moormeier<sup>1</sup>, John Goza<sup>1</sup>, Scott Reed<sup>1</sup>, Fraser Combe<sup>1</sup>, John El-Attrache<sup>1</sup>, Scott Callison<sup>1</sup>

*Ceva Animal Health*<sup>1</sup>

Infectious bursal disease virus (IBDV) is a ubiquitous virus that causes immunosuppression and mortality in poultry. It is common practice in the poultry industry to submit samples (primarily bursal tissues) that originated in the field for viral isolation, detection, and genomic classification of IBDV using classical and PCR laboratory methods. However, these methods can consume valuable time and resources. Thus, we sought to develop an accurate and cost-effective method for sequencing the entire IBDV genome of IBDV-positive field samples. By combining novel lab protocols, nanopore-based sequencing, and custom bioinformatic tools, we can sequence, assemble, and analyze genome Segments A and B directly from field samples, autogenous vaccine, and isolates. This information can be used to genotype IBDV that is present in field samples and culture. Analysis of this information can help stakeholders to make informed decisions as it pertains to protecting poultry flocks from IBD.

### **Evaluating the role of Vitamin E in Turkey Degenerative Myelopathy Syndrome**

Jonathan Elissa<sup>1</sup>, Kayla Niel<sup>1</sup>, Megan Lighty<sup>1</sup>

*Penn State University*<sup>1</sup>

Turkey degenerative myelopathy syndrome is an emerging non-inflammatory disease affecting turkeys of both major commercial genetic lines between 6 - 12 weeks of age. Both male and female flocks have been affected. Affected flocks are also typically from organic or antibiotic-free production systems. The syndrome is characterized by 3 - 6% increased mortality over the life of the flock with progressive ataxia leading to lameness and gross lesions secondary to incoordination, such as bruising of the metacarpal region and skin lacerations. The syndrome is likely to reoccur in subsequent flocks on farms with previously affected flocks. Histopathologic examination of spinal cord revealed myelin sheath swelling, spheroid formation, and axonal swelling, fragmentation, and degeneration. Lesions are most concentrated at the level of the cervical spinal cord. No other significant neurological or musculoskeletal lesions have been noted. To date, no causative agent has been identified. However, vitamin E deficiency is known to present with ataxia and may cause demyelination within the central nervous system. Liver vitamin E levels in clinically affected turkeys were lower than non-clinical turkeys from the same flock by an average value of 2.3ppm ( $p = 0.007$ ), and lower than unaffected control flocks of similar age by an average value of 3.6ppm ( $p < 0.001$ ). This preliminary study discusses the significance of liver vitamin E levels as a characteristic factor of

turkey degenerative myelopathy syndrome. Further data collected during ongoing investigation will be presented.

## Enteric Health

### **Colonization of Probiotic Bacteria in the Intestine of Chicken Embryos Following Coarse Spray on Incubating Eggs**

Mihiprabha Rathnayake<sup>1</sup>, Asha Ranaraja<sup>1</sup>, Shelly Popowich<sup>1</sup>, Hemlata Gautam<sup>1</sup>, Iresha Subhasinghe<sup>1</sup>, Khawaja Ashfaque Ahmed<sup>1</sup>, Betty Chow-Lockerbie<sup>1</sup>, Lisanework Ayalew<sup>1</sup>, Susantha Gomis<sup>1</sup>

*University of Saskatchewan<sup>1</sup>*

The preventive use of antibiotics has been phasing out in Canada and other countries, hence alternatives to antimicrobials and innovative technologies are needed, especially to control yolk sac infections in neonatal chickens. This study examined a novel technique of introducing probiotics to developing embryos as a coarse spray application on hatching eggs, to minimize pathogenic bacterial infections of newly hatched chicks. The objective of this study was to explore the migration and colonization of probiotics in chicken embryos following application of probiotics as a coarse spray onto eggshells. *Enterococcus faecalis*, *Bifidobacterium gallinarum*, *Pediococcus acidilactici* and *Lactobacillus salivarius* were applied on the eggshell of embryonating specific pathogen free (SPF) as a coarse spray. Spray solutions containing probiotics were maintained at 100C and were sprayed on the eggshell continuously for 30 seconds on days 15 and 17 of incubation. The control group was sprayed with saline. Groups of eggs received coarse spray with different probiotics were incubated in separate incubators to minimize cross contamination of probiotics during incubation. The migration of probiotics through the eggshell into intestine was identified by culturing intestines of embryos at day 20 of incubation using selective media, matrix assisted laser desorption-time of flight (MALDI-TOF) and whole genome sequencing. No embryo mortality or hatchability was noted with this technique of coarse spray application. *E. faecalis* was isolated in the intestine of chicken embryos at day 20 of incubation following coarse spray on eggshell at days 15 and 17. Direct cultures from 75% of embryos at day 20 of incubation revealed colonization of *E faecalis*. None of the other probiotics were identified by direct culture at this time point. No bacteria were cultured from the group exposed to saline. Whole genome sequencing was conducted to compare probiotics applied by coarse spray on eggshells and probiotics recovered by direct culture on day 20 of incubation. One hundred percent nucleotide identity was received between probiotics recovered from the intestines of embryos at day 20 of incubation and coarse spray application. This study demonstrated the possibility of delivering probiotics on incubating eggs without interfering with hatchability and health of embryos. This is a noninvasive industry feasible technique of delivering probiotics to chicken embryos. We hypothesize colonization of probiotics will reduce yolk sac infections in neonatal chickens.

### **Effect of RfC in the relative genetic expression of mRNA for: IgG, IgM, and INF $\gamma$ , and histomorphometry in the intestine of broiler chickens.**

Sebastian Mena<sup>1</sup>, Vladimir Egas<sup>1</sup>, Oscar Porras<sup>2</sup>, Jose Oaate<sup>2</sup>, Pablo Jaramillo<sup>2</sup>, Mateo Lucio<sup>2</sup>, Mayco Arce<sup>2</sup>, Diego Chaves<sup>2</sup>, Marcelo Mosquera<sup>2</sup>, Alejandro Torres<sup>2</sup>

*Dimune, Veterinary Services<sup>1</sup>, Pronaca, Poultry Nutrition<sup>2</sup>*

The Ecuadorian and global poultry industry are continually exploring viable alternatives for producing broiler without the use of antibiotic growth promoters (AGPs), tailored to the specificities of intensive production systems, while maintaining efficiency and food security for consumers. The use of Refined Functional Carbohydrates (RFC) in industrial poultry farming has grown in the past years due to their multiple effects on bird intestinal integrity and immunity, emerging as an alternative in broiler regimen without AGPs. This study evaluated the impact of feeding broilers with RFC in the genetic expression of mRNA through RT-QPCR (conducted by Labigen-Ecuador) for IgG (or IgY in chickens), IgM, and INF $\gamma$  in the Jejunum of thirty-five days old broilers. This was done by evaluating the expression ratio of the chicken  $\beta$ -actin gene, using the relative gene expression methodology (Livak and Schmittgen, 2001). Intestinal integrity was assessed through jejunal histomorphometry (conducted by HDX-Ecuador), to examine its effects on: villus length, crypt depth, relative absorption surface, and the degree of exfoliation and inflammation. The study considered three treatments, with isoprotein and isoenergetic diets with of a modification in the positive control that included an antibiotic growth promoter. The RFC treatment excluded AGPs and was included in a dosage of 500g/Tm. The negative control excluded AGPs and RFC. Sixty-four experimental cages were set up in a controlled environment poultry farm, where 704 Ross 308AP broilers were placed. The treatments were randomly assigned to experimental units, with 16 replicates for each treatment and 44 birds per cage. Zootechnical data showed no significant differences between treatments. The broilers of the group with RFC exhibited a relative genetic expression for IgM at 256.47%, for IgG at 154.6%, and for INF $\gamma$  at 163.21% compared to the negative control, respectively. In histomorphometry, the RFC group had longer villi than the positive control ( $P < 0.05$ ), and the RFC group showed the shallowest crypt depth among all groups ( $P < 0.05$ ). The L/P ratio (villus length/crypt depth) in the RFC group was higher than the positive control ( $P < 0.05$ ). However, there were no significant differences in relative absorption surface, exfoliation, and inflammation between groups ( $P > 0.05$ ). In conclusion, RFC has an effect in gut on the gene expression and histomorphometry in broilers feeding without AGPs. The mayor gene expression could protect the birds for diseases or others challenges in diet and environment, and changes in large and depth of the villi could improve the nutrients absorption from the diet.

### **Crude Protein and Starch Analyses of Fecal Feed Particles obtained from 60 Day-old Broilers**

Samuel Christenberry<sup>1</sup>, Mike Glass<sup>2</sup>, University of Missouri Extension Analytics Laboratory<sup>3</sup>

*Alabama Veterinary Diagnostic Laboratory<sup>1</sup>, Live Production Manager, PECO Foods, Gordo, AL<sup>2</sup>,  
University of Missouri Extension Analytics Laboratory<sup>3</sup>*

Feed passage, or the presence of undigested feed in the feces of broilers, has been, until recently, a worldwide problem for decades. Feed passage is so common that it does not seem to garner the attention that it used to, when it was first recognized. Based on its gross appearance in broiler fecal droppings, it is commonly held that these feed particles are made up of undigested corn, but to this author's knowledge, a definitive determination has never been made. It is with this in mind, that fecal feed particles were collected and assayed at the University of Missouri Extension Service Laboratory, and a comparison made of the crude protein and starch content of the fecal particles to corn meal and soy bean meal (SBM) obtained from the same feed mill that manufactured feed for the broilers that consumed the corn meal and SBM.

## **Ex vivo assessment of the direct and indirect antimicrobial capacities of glycerides of lauric acid using gastrointestinal fluids**

Wanderley Quinteiro-Filho<sup>1</sup>, Nuria Vieco-Saiz<sup>2</sup>, Virginie Michel<sup>3</sup>, Amine Mellouk<sup>4</sup>, Olga Lemale<sup>5</sup>, Tim Goossens<sup>6</sup>, Jessika Consuegra<sup>7</sup>

*Adisseo LATAM<sup>1</sup>, Adisseo France S.A.S. Center of Expertise and Research in Nutrition (CERN)<sup>2</sup>, Adisseo France S.A.S. Center of Expertise and Research in Nutrition (CERN)<sup>3</sup>, Adisseo France S.A.S. Center of Expertise and Research in Nutrition (CERN)<sup>4</sup>, Adisseo NL<sup>5</sup>, Adisseo France S.A.S<sup>6</sup>, Adisseo France S.A.S. Center of Expertise and Research in Nutrition (CERN)<sup>7</sup>*

Alpha-monolaurin is an antimicrobial agent with a potent in vitro activity against gram-positive (G+) but less against gram-negative (G-) bacteria. To this reason, glycerides of lauric acid (C12G) are used as a feed additive to prevent intestinal infections, thereby enhancing animal resilience against pathogen challenges, and improving animal performance. Here, we aimed to evaluate the antimicrobial activity of C12G ex vivo, using fluids of the gastrointestinal tract (GIT) obtained from animals receiving a C12G-supplemented diet. A total of 100 chicks were divided in two experimental groups, both receiving the same standard diet supplemented or not with C12G at 3 kg/ton. Contents from crop, ileum and caeca were sampled on day 28 and GIT fluids were extracted and filter-sterilized. Their antimicrobial capacity was assessed using Minimal Inhibitory Concentration (MIC) assays against *Enterococcus faecalis* (G+) and *Escherichia coli* APEC (G-). MIC values of samples from C12G-treated and control animals were compared with Student's t-test. Differences were considered statistically significant  $P \leq 0.05$ . MIC analyses showed that for all the GIT segments tested, fluids from animals receiving C12G have a significantly higher antimicrobial activity against *E. faecalis*. For example, the average concentration of ileal fluids required to inhibit bacterial growth was 15% in the samples from C12G-fed birds and 60% in the control samples. Significant antimicrobial activity against the G- *E. coli* APEC in the other hand, was restricted to the caeca. We therefore further analysed the caecal contents, by performing microbiota analyses through 16s sequencing. Interestingly, we found a significant increase of Lactobacillaceae relative abundance in the C12G-fed birds. Species of this family have been reported to limit pathogen intestinal colonization through to the production of lactic acid and other antimicrobial molecules such as bacteriocins and peptidoglycan hydrolases. Altogether, using ex vivo assessment of GIT fluids, we further characterized the potential in vivo antimicrobial activity of C12G. A higher antibacterial effect against the G+ *E. faecalis* was found throughout all tested GIT segments of birds supplemented with C12G. On the other hand, a stronger bacteriostatic effect against the G- *E. coli* was only found in the caeca of these animals. Further studies will be needed to evaluate whether this effect can be attributed to a shift in hindgut microbiota and metabolite composition.

## **Effect of *Bacillus subtilis* DSM29784 secreted metabolites on poultry resilience**

Wanderley Quinteiro-Filho<sup>1</sup>, Nuria Vieco-Saiz<sup>2</sup>, Tim Goossens<sup>3</sup>, Olga Lemale<sup>4</sup>, Jessika Consuegra<sup>5</sup>

*Adisseo LATAM<sup>1</sup>, Adisseo France S.A.S. Center of Expertise and Research in Nutrition (CERN)<sup>2</sup>, Adisseo France S.A.S.<sup>3</sup>, Adisseo NL<sup>4</sup>, Adisseo France S.A.S. Center of Expertise and Research in Nutrition (CERN)<sup>5</sup>*

*Bacillus subtilis* DSM 29784 (Bs29784) is a probiotic used in poultry nutrition, able to maintain intestinal health and enhance animal resilience and performance. It can produce bioactive metabolites such as hypoxanthine (HPX), Niacin (NIA), and pantothenate (PTH). It has been assessed previously in *in vitro* and *in vivo* conditions. This study aimed to establish a connection between those bioactive metabolites produced by Bs29784 and their impact on animal resilience and intestinal health. To this end, immune response, intestinal barrier, and microbiota were analyzed. We evaluated *in vitro* Bs29784 vegetative cells (metabolic active form), spores and metabolites capacity to modulate global immune regulators and intestinal integrity using HT-29 reporter cell lines both in the presence and absence of a pro-inflammatory challenge. Finally, we simulated chickens' ileal and cecal fermentations to determine the effect of Bs29784 metabolites on the microbiota and their fermentation profile. Bs29784 vegetative cells reduced inflammatory response more significantly than the spores ( $p < 0.0001$ ), showing that its beneficial effects are linked to its metabolic activity. To test this hypothesis, we analyzed Bs29784 metabolites individually. The results suggest that each metabolite had specific beneficial impacts. Specifically, PTH and NIA reduced inflammation (23.2% and 9.7% respectively;  $p < 0.0001$ ) and HPX enhanced mucin production by increasing 19.3% MUC2 expression ( $p = 0.011$ ). Furthermore, PTH and HPX increased epithelial resilience to an inflammatory challenge by limiting permeability increase ( $p = 0.024$ ). Concerning intestinal fermentations, PTH increased butyrate levels to 0.31mM ( $p = 0.076$ ) in ileal fermentation, while cecal PTH increased 5.1%, 6.8% and 16.1% the levels of acetate, butyrate, and propionate respectively ( $p = 0.087$ ); NIA increased propionate production by 6.7% ( $p = 0.016$ ) and HPX increased butyrate with 5.7% ( $p = 0.019$ ). All molecules lead to changes in microbiota explaining the different fermentation patterns such as the increase of *Ruminococcus* and *Clostridium\_XIVb* in presence of NIA or PTH and the increase of *Ruminococcus*, *Anaerotruncus* with HPX ( $p \text{ adj.} = 0.040$ ) in the cecal fermentation. The different tests performed *in vitro* have shown that Bs29784 modulates intestinal health by acting on the three lines of resilience via its secreted metabolites.

### **Microbiome Changes and Horizontal Transmission of *Salmonella infantis* in Broilers Consuming Feed Treated With Feed Sanitizers, Organic Acid Blends, Probiotics and Combinations**

Enrique Montiel<sup>1</sup>, Callie Selby<sup>1</sup>, Jose Ramirez<sup>1</sup>

*Anitox Corporation*<sup>1</sup>

Groups of six-day old broilers were infected by gavage with *Salmonella infantis* and placed as seeders with twenty-four uninfected hatch mates using 8 replicates per treatment. Feed was formulated identically for all experimental group and the following treatments were applied: a) untreated non-challenged control; b) untreated-challenged control; c) formaldehyde-based feed sanitizer; d) non-formaldehyde feed sanitizer; e) organic acid blend; f) probiotic; g) probiotic+feed sanitizer. Feed samples from each group was tested for coliform and *Salmonella* contamination and *Salmonella* load and prevalence and microbiome analysis were assessed at 7, 14, 26, 35 and 49 days of age. Results indicated a significant decrease in *Salmonella* loads and prevalence in the groups consuming feed treated with feed sanitizers alone or in combination with probiotics. The beta microbiome analysis indicates that bacterial families found in birds consuming treated vs untreated feed are different. These results suggest that feed treatment with feed sanitizers help reduce *Salmonella* prevalence and loads in broilers and influence the development of different microbiome



makeup in crop and ceca from 14 to 49 days of age. Results suggest that feed treatment with feed sanitizers help controlling Salmonella horizontal transmission in broilers infected at day of age and stimulate the development of different microbiome profiles.

### **Microbiome Shifts in Birds Fed Quillaja saponaria and Yucca schidigera Biomass that are Related to Performance Outcomes**

Luis Gomez<sup>1</sup>, Ana Maria Villegas<sup>2</sup>, Blair Telg<sup>3</sup>, Chad Malinak<sup>4</sup>, Ashley Hallowell<sup>5</sup>, Tim Buisker<sup>6</sup>, Brett Lumpkins<sup>7</sup>

*Phibro Animal Health Corporation<sup>1</sup>, Phibro Animal Health Corporation<sup>2</sup>, Phibro Animal Health Corporation<sup>3</sup>, Phibro Animal Health Corporation<sup>4</sup>, Phibro Animal Health Corporation<sup>5</sup>, Smart Data Science Solutions<sup>6</sup>, Southern Poultry Feed and Research<sup>7</sup>*

Analysis was conducted to determine contrasting changes in the microbiome of the ileum based on a previous trial that compared the performance effect of feeding broilers Magni-Phi<sup>®</sup> nutritional special product, a combination of Quillaja saponaria and Yucca schidigera (QY) biomass. The birds fed QY were significantly heavier compared to the control birds (2.13 vs 1.97,  $P < 0.05$ ), had significantly lower feed conversion ratio (FCR) (1.65 vs 1.77,  $P < 0.05$ ), and significantly lower mortality (5.0% vs 9.0%,  $P < 0.05$ ). The group fed QY had significantly lower total lesion scores compared to the control group (0.07 compared to 0.26,  $P < 0.05$ ). This difference was driven by the decreased Eimeria acervulina (EA) lesion score (0.46 compared to 0.08,  $P < 0.05$ ). The main difference observed in the group fed QY on morphometrics appeared to be prevention of the worst deterioration of villi width. When comparing the thinnest villi between treatment groups, the most damaged villi in the group fed QY were 26% larger compared to the most damaged villi in the control group. On the microbiome front, QY use was associated with both a lower Shannon score and higher d42 body weight. A lower Shannon score was also associated with a higher d42 weight independent of feeding QY, suggesting a partial pathway for these beneficial effects. There were no differentially abundant organisms or groups of organisms between the treatment and control animals in any taxa (phylum, class, order, family, genus or species). However, there were statistical associations between several organisms in both villi width and EA lesion score, both of which were statistically significantly related to treatment.

## **Infectious Bronchitis Virus**

### **Detection of Avian Infectious Bronchitis GI-23 (Variant 2) Virus in Mexico During 2023**

Juan Carlos Valladares de la Cruz<sup>1</sup>, LUIS ETCHARREN MARQUEZ<sup>1</sup>

*Phibro Animal Health<sup>1</sup>*

Infectious Bronchitis virus of the GI-23 lineage has circulated in the Middle East since 1998 and has spread to Eastern Europe and Africa in recent years. In Brazil, the first report of the GI-23 virus occurred in 2022. In Mexico there is no record of the GI-23 lineage; previous studies indicate that strains isolated between 2007 and 2021 belonged to 4 lineages of Genotype I (GI-3, GI-9, GI-13 and GI-30) and to two groups tentatively classified as new lineages GVIII-1 and GIX-1 (Mendoza et al. 2022); recently Valladares et al. (2023), from 86 samples collected in 2021 and 2022, found 5 lineages of Genotype I (GI-1, GI-9, GI-13, GI-25 and GI-27), the GI-9 virus being the most prevalent.

Due to the fact that in Mexico, during the period from May to December 2023, an increase in cases suggestive of Infectious Bronchitis (IB) was observed with increased mortality and severe kidney lesion in broilers and cystic oviduct in layers, the present study was carried out for the detection and typing of the IB virus from 69 cases suggestive of the disease (32 of broilers, 31 of layers and 6 of breeders), using the conventional 3' UTR based PCR method and the S1 gene-based conventional PCR method, at the Clinic for Poultry and Fish Medicine, Clinical Unit for Poultry Medicine, University of Veterinary Medicine, Vienna, Austria. 76.81% of the samples were positive for the detection of IB, 96.87% in broiler and 64.5% in layer. GI-23 (Variant 2) lineage was detected in 20.28%, GI-13 in 14.49%, GI-1 in 2.89%, and GI-19 in 1.44%. A GIV lineage 1 virus and a novel virus that could not be assigned to any of the lineages described in the literature were also detected. Interestingly, no virus of the GI-9 lineage was detected, as had been previously described. 40.62% of positive broiler cases were positive for GI-23 and were associated with renal degeneration and inflammation with severe uratoses and moderate respiratory disease, while in the case of layer only one case of GI-23 was detected, however, these birds presented a very severe cystic lesion indicating a possible early age infection with GI-23. In 38.7% of the positive IBV layer hen, the genetic material was insufficient to perform viral typing. This is the first report of the detection of the GI-23 lineage in Mexico and the results indicate that it is becoming the predominant strain in Mexico. **Keywords.** Infectious Bronchitis Virus, IB GI-23, Mexico-

### **Epidemiological assessment of Infectious Bronchitis Virus after the detection of strains of the GI-23 lineage in Brazil**

Jose Emilio Dias<sup>1</sup>, Eva Laurice Pereira Cunha Hunka<sup>2</sup>, Jorge Augusto Petrolini Marchesi<sup>3</sup>, Alberto Back<sup>4</sup>

*Phibro Animal Health, Instituto Federal Catarinense, Concórdia – SC, Brazil<sup>1</sup>, Phibro Animal Health Corporation<sup>2</sup>, MercoLab Laboratorios Ltda, Brazil<sup>3</sup>, MercoLab Laboratorios Ltda, Brazil<sup>4</sup>*

Avian Infectious Bronchitis (IB) is a respiratory disease, but it can also affect organs such as the kidneys and reproductive system. Infectious Bronchitis Virus (IBV) is its etiological agent and several variants (Genotype I - lineages 1 to 27 or GI-1 to 27) can be widely found in poultry production systems around the world, including in Brazil where the most prevalent strains in the last 70 years were Brazilian (BR) (GI-11) and Massachusetts (GI-1). Variants belonging to the GI-23 lineage of IBV have circulated since 1998 in the Middle East, spreading to several countries over time. In Brazil, the first report of IBV GI-23 occurred in 2022 after research conducted to understand the cause of the increase in respiratory diseases and condemnations in poultry slaughterhouses due to severe cases of airsacculitis. In February 2023, a new homologous vaccine using the live IBV GI-23 lineage was introduced in Brazil. The present study aimed to report the epidemiological dynamics of IBV in Brazil between 2022 and 2023. For this study, 2059 tracheal, kidney, cecal tonsils and cloaca swab samples from commercial flocks (broilers, breeders, and layers) presenting or not presenting clinical signs of IBV were analyzed between January 2022 and December 2023. The samples came from 12 Brazilian States (BA, ES, GO, MG, MT, PB, PE, PR, RO, RS, SC, and SP) and were evaluated for detection and genotyping of IBV by RT-qPCR. Of the 2059 samples evaluated, 34.6% were positive only for BR strains (GI-11); 33.9% were positive only for GI-23; 15.3% were positive for both GI-11 and GI-23; 6.8% were positive for both GI-23 and Massachusetts (GI-1) strains; 4.2% were positive for GI-1; 4% were positive for both GI-11 and GI-1 and 1.3% were positive for GI-11, GI-23, and GI-1. Field strains of GI-23 lineage were detected in four Brazilian States (PR, RS, SC and SP) before the

introduction of the live homologous vaccine in 2022. In seven Brazilian States (BA, GO, MG, MT, PR, SC, SP) IBV from GI-23 lineage were detected in 2023. Evaluating the frequency of identification of each strain, the most prevalent was GI-23, which was present in 1179 samples (57.3%), followed by the GI-11, present in 1135 samples (55.1%) and lastly the GI-1, present in 334 samples (16.2%). Considering the frequency of identification of each strain before and after the vaccination (February 2023), GI-23 lineage strains went from the second most prevalent (43.1%) before the start of vaccination (GI-11 69.3%; and GI-1 22.1%), to the most prevalent (62.8%), followed by GI-11 (49.6%) and finally GI-1 (13.9%). These results give us an idea of the current IBV distribution panorama in the main poultry producing regions in Brazil and relevant insights into the evolution of vaccination programs in the country.

### **Reduction on airsacculitis condemnations in commercial broiler and griller flocks after introduction of homologous vaccine against IBV GI-23 lineage in Brazil**

Josiane Griebeler<sup>1</sup>, Jose Emalio de Menezes Dias<sup>2</sup>, Eva Laurice Pereira Cunha Hunka<sup>3</sup>, Leandro Biancheti<sup>4</sup>

*MSc. In veterinary science in Poultry Health - UFRGS<sup>1</sup>, Instituto Federal Catarinense, Concordia SC, Brasil<sup>2</sup>, Phibro animal health corporation<sup>3</sup>, Phibro animal health corporation<sup>4</sup>*

Infectious Bronchitis Virus (IBV) is one of the most important and economically devastating pathogens to chickens. As a continuously evolving virus, Infectious Bronchitis (IB) is one of the most complicated problems for the poultry industry all over the world. The GI-23 lineage from IBV has proven to be one of the most important in recent years with circulation in the Middle East, Turkey, Russia, Gulf States, Iran, North Africa, Baltic States, Poland and recently in Brazil. Based on the failure of commercial heterologous vaccines to control IB, the use of a homologous vaccine with GI-23 started as a tool to reduce losses and improve zootechnical results. The aim of this study was to evaluate the effect of introducing a homologous vaccine against IBV of the GI-23 lineage in commercial flocks of chickens and grillers on airsacculitis condemnations in the slaughterhouse. For this study the database refers to 6,641 flocks, with 2,683 broilers flocks (slaughter age close to 40 days) and 3,958 grillers flocks (slaughter age close to 28 days). Together they totaled 52,655,029 and 84,417,600 birds respectively. Data on airsacculitis condemnations were compared from February to November of 2022 in which flocks were vaccinated in the hatchery with vaccines containing the Massachusetts (GI-1) and BR-1 (GI-11) strains, to data from February to December 2023 in which the flocks were vaccinated with the Massachusetts and Variant 2 (GI-23) strains at the hatchery. The airsacculitis data from 2022 and 2023 were subjected to analysis of variance (ANOVA) with values of  $P > 0.05$  being considered significant. Data variability was also calculated using the coefficient of variation. A significant reduction ( $P < 0.0001$ ) of 44.3% in airsacculitis condemnations in broilers flocks in 2023 was observed after the introduction of the homologous vaccine against IBV GI-23 lineage. A significant reduction ( $P < 0.0001$ ) of 34.7% in airsacculitis condemnations was also identified in the griller flocks in the year 2023. The coefficient of variation of airsacculitis condemnations was lower in 2023 than in 2022, demonstrating greater uniformity. These results show that the use of a homologous vaccine against IBV GI-23 lineage reduced the percentage of condemnations due to airsacculitis in both broilers and grillers flocks, when compared to the vaccination program based on heterologous vaccines. In addition to the reduction, a better

uniformity in airsacculitis parameters was observed with the use of the homologous vaccine. Key Words: IBV; BR-1 strain; Massachusetts strain; IBV vaccine, slaughterhouse

### **Improvement in the airsacculitis condemnation, antibiotic therapy, and mortality after introduction of a homologous vaccine against IBV - Var 2 (IS/1494/06 strain)**

Andre Marca<sup>1</sup>, Jose Emilio de Menezes Dias<sup>2</sup>, Eduardo Rother<sup>3</sup>, Eva Laurice Pereira Cunha Hunka<sup>4</sup>, Udi Ashash<sup>5</sup>

*DVM, University of Santa Catarina State (UDESC) - Brazil<sup>1</sup>, Phibro Animal Health; Instituto Federal Catarinense, Concordia, SC - Brazil<sup>2</sup>, Phibro Animal Health<sup>3</sup>, Phibro Animal Health<sup>4</sup>, Phibro Animal Health<sup>5</sup>*

In 2022, the molecular characterization of Infectious Bronchitis Virus (IBV) strains belonging to the GI-23 (Var 2) lineage in commercial broiler flocks in Brazil was published. This virus belongs to the genus Gammacoronavirus and Coronaviridae family. It remains a serious source of illness and economic losses for the poultry industry worldwide. The presence of GI-23 lineage strains was highly associated with an increase in condemnations at the slaughterhouse due to airsacculitis and an increase in field mortality from primary and secondary causes. In 2023, vaccination with a homologous vaccine had started with the aim of helping to control this new variant present in the country. This study aimed to evaluate the reduction in condemnation rates due to airsacculitis, antibiotic use and mortality after the use of the homologous vaccine in commercial broilers flocks in Paraná State - Brazil. For this study, the months of March to December 2022 were used as a comparison, when the vaccination program included an association of two vaccines containing strains from the GI-1 (Massachusetts) and GI-11 (BR-1) lineages and the months of March to December 2023, when the vaccination program included a combination of two vaccines containing strains from the GI-1 and GI-23 (Var 2) lineages. This study included data from 16,619 broiler flocks, located in the State of Paraná – Brazil, totaling 415,261,580 birds (184,408,710 in 2022 and 230,852,870 in 2023). Data relating to mortality (%), condemnations due to airsacculitis in the slaughterhouse (%) and the number of flocks treated with antibiotics for respiratory causes in the year were compiled. The data were subjected to descriptive statistics analysis and subsequently to the analysis of variance test (One-Way ANOVA) and mean comparison using the Tukey test. P-value  $\leq 0.05$  were considered significant. A significant reduction ( $P < 0.001$ ) in airsacculitis condemnations was noticed after the introduction of the GI-23 homologous vaccine in 2023, with a result of 1.03% compared to 3.12% identified in 2022. A significant reduction ( $P < 0.001$ ) was detected in mortality after using the new vaccination program, with a result of 6.0% against 7.81% identified in 2022. In agreement with the other results, a significant reduction ( $P < 0.001$ ) was evidenced in the number of flocks treated against respiratory symptoms, with results from 160 flocks in 2023, compared to 419 flocks in 2022. These results suggest that the use of a homologous vaccine against IBV of the GI-23 lineage, in a high-challenge region, presented better results, compared to the program with heterologous vaccines.

### **Evaluation of Two Bronchitis Vaccines under field conditions**

Lucas Colvero<sup>1</sup>, Ha Jung Roh<sup>2</sup>, Fernando de Castro Vargas<sup>3</sup>, Alexandre Jacques<sup>4</sup>

*MSD Saúde Animal<sup>1</sup>, Merck Animal Health, Rahway, NJ, USA<sup>2</sup>, Merck Animal Health, Rahway, NJ, USA<sup>3</sup>, MSD Saude Animal<sup>4</sup>*

Infectious bronchitis (IB) is a respiratory disease affecting poultry, caused by the avian infectious bronchitis virus (IBV), a member of the gammacoronavirus family. IBV is widespread wherever the poultry industry operates, significantly impacting poultry health and performance. Globally, over 9 genotypes and 38 lineages of IBV have been identified and are in circulation. The spike glycoprotein of IBV, encoded by the S gene, is a structural protein and a major determinant of virus tissue tropism. The S1 subunit of the spike protein is a primary target for neutralizing antibodies and exhibits the highest genetic diversity among IBVs. Amino acid changes within the S1 region can disrupt the neutralizing antibody binding. A study by Leyson et al. (2016) revealed variations in tertiary S1 structures among the same Massachusetts-type IBV, affecting the virus's antigenicity. Ma5 demonstrated higher antigenicity scores near the receptor binding area compared to other Massachusetts-type viruses such as M41, H120, and H52, emphasizing differences in antigenicity among vaccines of the same genotype. Building on this knowledge, we aimed to assess the performance of two Massachusetts-type vaccines, Nobilis® IB Ma5 and H120, under field conditions using two commercial broiler flocks in southeast Brazil. Both flocks, located on the same location, followed identical vaccine programs except for the IB vaccine. Farm A received the Nobilis® IB Ma5 vaccine, while Farm B received the H120 vaccine at the hatchery. Monitoring continued throughout the grow-out period until slaughter at 46 days of age, including assessments of mortality and bleeding every 7 days. Swab samples (choanal, kidney, and cloacal swabs) were taken for IBV PCR before processing. Although both flocks had no other health concerns, birds at Farm B exhibited increased mortality from 35 days of age until processing, compared to Farm A, with a 1.29% difference. IBV presence was confirmed in cecal tonsil and trachea by traditional PCR in both farms, but serology results showed a significant increase in IBV titer in birds at Farm B at slaughter age. The economic impact of the mortality difference exceeded 1 metric ton of chicken meat. In conclusion, both farms faced IBV challenges at a later age. Under IBV challenge conditions, birds vaccinated with Nobilis® IB Ma5 demonstrated better performance in terms of mortality compared to those vaccinated with H120. These results align with historical field observations and suggest a potential influence of Ma5 antigenicity in the vaccine efficacy.

### **Efficacy of TABic® IBVAR206 vaccine against challenge with Infectious Bronchitis Virus BR-1 field strain in broiler chickens**

Dana Goldenberg<sup>1</sup>, Udi Ashash<sup>1</sup>, Elina Aizenshtein<sup>1</sup>, Avner Finger<sup>1</sup>

*Phibro Animal Health<sup>1</sup>*

Infectious bronchitis, caused by the Infectious bronchitis virus (IBV), is a difficult disease to control due to the existence and emergence of many different variants. Most commercially available IBV vaccine strains protect against related viruses, cross-protection against heterologous viruses must be evaluated for each virus. Knowledge of the circulating IBV types causing outbreaks in a specific geographic region is beneficial to selecting the appropriate vaccines and vaccination programs. GI-23 lineage has circulated in the Middle East since 1998 and has spread to Europe and Africa in recent years. In 2022, Brazil reported for the first time that the IBV GI-23 was isolated from chickens with typical respiratory clinical signs. Our studies have shown that the TABic IBVAR206 vaccine belonging to the GI-23 lineage can provide cross-protection against different IBV field variants. We know, for

example, TABic IBVAR206 vaccine shows very strong cross-protection against the QX and 793B strains. Therefore, we conducted a study to evaluate the efficacy of TABic IBVAR206 alone or combined with IB H120 vaccine against the circulating Brazilian strain BR-1 in commercial broiler chickens. The evaluation of the protection was based on a cilliosstasis test and clinical signs post-challenge. All challenged control chickens presented extreme loss of vigor of ciliary activity. The vaccinated groups had normal ciliary activity, and no abnormal clinical symptoms were observed. Vaccine protection was efficacious in all treatments, 90% of the birds presented normal ciliary movement. There was no significant difference between the groups treated with the Tabic IBVar206 + Mass (H-120) vaccine combination and the groups vaccinated only with the Tabic IBVar206 vaccine alone on Day 0 or Day 14. There was also no significant difference between the groups vaccinated on the first day compared to the groups that received late vaccination (Day 14). In conclusion, the TABic IBVAR206 vaccine can contribute to the control of the heterologous BR-1 variant. Continuous monitoring of the IBV types circulating worldwide and evaluation of the protection induced by vaccines continue to be necessary to control IBV infections.

### **Effect of spraying supplemental passive IBV antibodies in the protection against early IBV exposure in chicks**

Rachel Jude<sup>1</sup>, Laura Flores<sup>1</sup>, Rodrigo Gallardo<sup>1</sup>

*Department of Population Health & Reproduction, University of California, Davis<sup>1</sup>*

Robust passive immunity against infectious bronchitis virus (IBV) at hatch has been shown to be instrumental in preventing chronic urogenital issues in layers that can lead to costly production losses. At hatch, commercial flocks do not have uniform maternal antibody titers and maternal protection also disappears between two and three weeks of age. To fill the gap in protection between hatching and vaccination, we investigated the efficacy of spray application of IBV hyperimmune serum to day-of-hatch SPF chicks and found that antibody spraying reduces the respiratory distress and trauma to tracheas and airsacs but does not affect viral replication rate or shedding. Here, we assessed whether 1X or 2X doses of IBV specific antibodies delivered via spray affect the degree and duration of the protection provided. We also evaluated whether the mechanism of challenge, ocular-nasal vs. intramuscular, affects the ability of supplemental antibodies to protect against disease caused by the virus. These investigations are integral to showing the effectiveness and mechanisms of protection provided by supplemental application of antibodies and are the initial steps needed before assessment of their effects in protecting against long-term issues, such as false layer syndrome, in commercial layers.

### **Identification and Geographic Location of Variant Strains of Infectious Bronchitis Virus in Broiler and Layer Farms in Peru 2023**

Cesar Reyes<sup>1</sup>, Lucas Pedroso Colvero<sup>2</sup>, Ha Jung Roh<sup>3</sup>, Taylor Barbosa<sup>4</sup>

*MSD Salud Animal - Peru<sup>1</sup>, MSD Salude Animal - Brazil<sup>2</sup>, Merck Animal Health, Rahway, NJ, USA<sup>3</sup>, MSD Salud Animal - Peru<sup>4</sup>*

The increasing prevalence of variant strains of Infectious Bronchitis Virus (IBV) in commercial poultry farms and broiler facilities throughout Peru over the past decade has raised concerns about their potential impact on flock health. Compounding this issue is the restriction on the use of live variant

vaccines in the country, with scientific evidence highlighting the limited efficacy of the permitted Massachusetts vaccine strains against these variants. This study emphasizes the critical importance of IBV surveillance in the commercial poultry sector, given the challenges posed by circulating variants. IBV monitoring was conducted on seemingly healthy flocks in various regions of Peru from September through November 2023. Choanal and cloacal swabs were collected at various ages (between 28-42 days) and applied to FTA cards for analysis at the X-OvO laboratory in the UK. RNA extracted from FTA cards underwent RT-PCR and sequencing. Out of the 33 FTA cards analyzed, 25 samples tested positive for IBV, while 8 samples were negative, with 3 having insufficient genetic material for sequencing. Among the positive samples, Latin American Q1 was identified in birds from the northern part of Peru, representing 27% of positive samples. The 793B type field virus was isolated from all regions sampled, comprising 33% of IBV-positive samples. Previous surveillance from 2020-2022 also detected both Q1 and 793B type field viruses in commercial flocks in Peru (data not shown), confirming their widespread distribution among commercial broilers and layers. Given that the Massachusetts-type vaccine alone does not offer sufficient protection against these two variants, the prevalence of these IBV types raises concerns about their impact on production performance for poultry producers in Peru. Additionally, a new IBV similar to the CA1737/04 strain previously reported in California, US, was isolated from layer farms in this study, highlighting the dynamic nature of IBV distribution and the threat of new IBV challenges. While the impact of this virus on poultry health is currently unknown, this finding underscores the urgency of comprehensive IBV surveillance strategies to address the evolving challenges faced by commercial poultry farms in Peru and the need for improvements in protection regimens.

**Cross-protection studies using GA08 and Mass type IBV vaccines against four different antigenic variant viruses currently circulating in poultry in the USA and Canada.**

Mark Jackwood<sup>1</sup>, Robert Beckstead<sup>1</sup>, Jimmy White<sup>1</sup>, Adrian Bustamante<sup>1</sup>, Sean Brimer<sup>1</sup>, Marshall Putnam<sup>1</sup>

*Ceva Animal Health*<sup>1</sup>

Avian infectious bronchitis virus (IBV) is a gamma coronavirus that causes a highly contagious upper-respiratory disease, resulting in airsacculitis, production losses and condemnations at processing. Some strains can replicate in the kidneys causing an interstitial nephritis and flushing. In addition, the virus has been implicated in testicular lesions resulting in decreased fertility in breeding males and when female chicks are infected within the first 2 weeks of life, damage to the immature oviduct can occur resulting in false layers which are hens that look normal but fail to lay eggs. The disease is difficult to control because many different antigenic variants of IBV can be found in poultry with only a few vaccine types available. The objective of this study was to determine if two licensed commercially available vaccines (GA08 and Mass types) can protect against 4 different currently circulating IBV variants. The variant strains of IBV used in this study consisted of two viruses that were identified some time ago (PA/1220/98 and CA/1737/04) but continue to circulate in commercial poultry as well as two recently identified viruses, NC/DARK/23 and Canada/DMV/1639/23. Vaccine challenge studies were conducted to examine the level of cross-protection afforded by GA08 type and Mass type vaccines given simultaneously to 1 day of age SPF chicks against challenge at 4 weeks of age. In the vaccinated/challenged birds, clinical signs were reduced and the level of challenge virus detected was significantly lowered for each of the challenge viruses. This study is important

because it provides poultry veterinarians with information on the level protection that can be expected against 4 important currently circulating IBV variants.

### **Polymorphisms of S1 Sequence following Single or Dual Infectious Bronchitis Virus Strains Passage in Embryonated Chicken Eggs**

Ming-Kun Hsieh<sup>1</sup>, Tsung-Ting Chiu<sup>1</sup>, Pei-Fang Hsieh<sup>1</sup>, Jui-Hung Shien<sup>1</sup>, Shan-Chia Ou<sup>1</sup>

*Graduate Institute of Microbiology and Public Health, National Chung Hsing University, Taiwan<sup>1</sup>*

Infectious bronchitis virus (IBV) causes infectious bronchitis (IB) in chickens and leads to severe economic loss. Since the poor cross-protection among different serotypes of IBVs, two different serotypes of attenuated IBV vaccines are regularly co-administered to protect chicks against field IBVs. Recombination between vaccine strains may alter the antigenicity of vaccine strains. Moreover, vaccine strains may serve as heterologous RNA donors and result in the appearance of new IBV serotypes. The objective of this study is to investigate the polymorphisms of S1 sequence of IBVs after single or dual virus strains passage in embryonated chicken eggs. The vaccine strains of Ma5, H120, and 4/91 as well as field strain TW100 were inoculated in specific pathogen free embryonated chicken eggs allowing free replication of IBVs without host adaptive immunity. The S1 genes of IBVs obtained from embryonated eggs were amplified by reverse transcription polymerase chain reaction. Two single nucleotide polymorphisms (SNPs) were found in 4/91 strain during single passage in embryonated eggs. However, by analyzing sequencing chromatograms, the secondary peaks were found at these two SNP sites in original virus before passage. Sixteen out of 26 SNPs were observed during dual passage in embryonated eggs without secondary peak in original viruses, illustrating that the mutations occurred or the minor subpopulations were selected during dual passage in embryonated eggs. The intertypic recombination were not identified in all inspected S1 sequences. In conclusion, the mutations or selections of IBVs may be promoted in the circumstance of co-infection of dual IBVs

## **Infectious Bursal Disease**

### **Disease Awareness Program : Field survey of Infectious Bursal Disease Virus from broiler and layer farms in Peru during 2020 and 2022**

Claudia Carranza<sup>1</sup>, Bruno Garcia<sup>2</sup>, Istvan Kiss<sup>3</sup>, Luiz Sesti<sup>4</sup>

*Ceva Salud Animal - Peru<sup>1</sup>, Ceva Salud Animal - Peru<sup>2</sup>, Ceva Animal Health - SSIU Phylaxia<sup>3</sup>, Ceva Salud Animal - LATAM<sup>4</sup>*

Infectious bursal disease (IBD) continues to be a major threat to poultry worldwide. In Peru, the very virulent form of IBD was detected in broiler farms located in the eastern part of the country in the late '90s. However, recent studies have shown that the most dominant strains are now present in a so called subclinical form related to the presence of variant IBD virus (IBDV). With the absence of clinical signs, routine monitoring becomes more important than ever, to identify the virus present in the field and the age of birds at infection. Therefore, the "Disease Awareness Program" created by Ceva Animal Health was implemented in Peru aiming to identify circulating IBDVs in all poultry producing regions. This survey represents the first wide reaching Gumboro survey in broilers and layer farms in the country. The selected farms lacked a recent history of IBD V sampling with Ceva, had



concerns regarding their vaccination program against Gumboro, and were located in different geographical regions. A total of 343 bursas were collected from broilers 21 to 36 days of age (doa) and layers 14 to 86 doa. Samples were individually analyzed using RT PCR (Reverse Transcription Polymerase Chain Reaction) for IBD V detection. Positive samples were typed by RFLP (Restriction Fragment Length Polymorphism) and confirmed by sequencing. Our results detected 21 1 positive samples (61.52%). Of which 35.54% were identified as variants. Regardless of the age or type of bird, the only detected variant was Variant Alike (VarA/85US). In broilers, variant detection was at 21, 22, 23, 24, 26, 28, 32, and 35 doa. In layers, the variant strain was detected at 28, 35, 42, and 56 doa. This variant seemed to infect birds early and was present in all poultry producing regions. Its detection represented the first step to implement more suitable biosecurity measures and vaccination protocols.

### **Infection by Genogroup 4 strains of Infectious Bursal Disease Virus negatively affects mortality and feed conversion in commercial broiler flocks in Brazil**

Jose Emilio Dias<sup>1</sup>, Eva Laurice Pereira Cunha Hunka<sup>2</sup>, Udi Ashash<sup>3</sup>, Diogenes Dezen<sup>4</sup>, Breno Castello Branco Beiro<sup>5</sup>

*Phibro Animal Health, Instituto Federal Catarinense, Concórdia – SC, Brazil<sup>1</sup>, Phibro Animal Health Corporation<sup>2</sup>, Phibro Animal Health Corporation<sup>3</sup>, Instituto Federal Catarinense, Concordia SC, Brazil<sup>4</sup>, Instituto Federal Catarinense, Concordia SC, Brazil<sup>5</sup>*

Infectious Bursal Disease Virus (IBDV) is a Birnavirus with worldwide prevalence throughout areas with high poultry density. It causes clinical and subclinical immune suppression due to the infection of lymphocyte B progenitors and macrophages. In the last decade, several studies have demonstrated a high prevalence in South America of strains from a distinct lineage of IBDV (dIBDV), belonging to Genogroup 4. These studies have identified Genogroup 4's pathogenicity, antigenicity, and immunosuppression profile. The aim of this study was to compare the zootechnical performance and lesions in the immune system organs in commercial broiler flocks infected by Genogroup 4 strains vs non-infected flocks. Samples of Bursa of Fabricius (BF) were collected from thirty commercial broiler flocks from three different companies in Paraná (the main broiler producing state in Brazil, responsible for 35% of Brazil's production). Ten broiler flocks between 18 and 23 days of age were randomly chosen from each of three companies. Five BF samples were collected per flock, totaling 150. The samples were assessed in pools of five BF (one pool per flock) and evaluated using the nested RT-PCR method followed by RFLP based on a fragment of the hypervariable region of the VP2 protein. Additionally, five samples of Bursa of Fabricius, spleen, thymus, cecal tonsils and bone marrow were collected at the same ages, for histopathology analysis, following the European Pharmacopoeia standard of lymphoid depletion scores (0 to 5). Scores from 0 to 3 were attributed to the parameters of necrosis, inflammatory infiltrate, epithelial hyperplasia, hyperemia, edema, and cystic follicles. Bursal histopathology and performance data were subjected to descriptive statistical analysis, followed by the Kruskal-Wallis method and mean comparison by the DSCF method. Histopathological data from the spleen, thymus, bone marrow and cecal tonsils were subjected to the chi-square association test. Out of thirty samples, ten (33.3%) were positive for dIBDV. Flocks infected by dIBDV had significantly higher Bursa of Fabricius mean lesion scores than flocks negative for dIBDV. There were no significant differences in the frequency of lesions found in the spleen, thymus, cecal tonsils and bone marrow. Flocks positive for dIBDV had their performance negatively

impacted, with a poorer feed conversion rate of 1.77 compared to 1.67 in flocks negative for dIBDV. Flocks positive for dIBDV had a 42% increase in mortality compared to flocks negative for dIBDV. These results suggest that flocks infected by strains of dIBDV (Genogroup 4) in Parana, Brazil have their zootechnical performance negatively affected, in addition to experiencing greater damage to the Bursa of Fabricius. This corroborates the scientific literature on the immunosuppressive potential of these strains. New studies are needed to confirm these hypotheses, while blocking other variables that could interfere with these results.

### **Generation of avian paramyxovirus type 1 TS09 strain-based recombinants expressing the VP2 protein of infectious bursal disease virus and a chicken cytokine as dual vaccines for in ovo vaccination**

Qingzhong Yu<sup>1</sup>, Sujit Mohanty<sup>1</sup>, Lei He<sup>1</sup>

*USDA/ARS, US National Poultry Research Center<sup>1</sup>*

Infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV) are two economically important avian pathogens threatening the poultry industry worldwide. Vaccination combined with restricted biosecurity has been a common practice to control these infectious diseases. However, due to the virus evolution of IBDV, commonly used IBDV vaccines become less effective against infectious bursal disease caused by virulent IBDV variants, and live NDV vaccines cannot be administered in ovo because of reduced hatchability and embryo mortality. Recently, we developed an avian paramyxovirus type 1 vaccine TS09 strain-based vector, which has been proven safe for in ovo vaccination. In the present study, we generated two TS09 recombinants expressing the IBDV VP2 protein of a very virulent IBDV strain with or without a chicken cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF), as in ovo dual vaccine candidates. The VP2 gene of the IBDV UK661 strain was synthesized and inserted into the TS09 vector with or without GM-CSF as an independent transcription unit between the P and M genes. Two recombinant viruses, rTS/IBDV-VP2 and rTS/IBDV-VP2-GM-CSF, were rescued using reverse genetic technology. Biological characterization showed that these two recombinant viruses retained the non-virulence pathotype with similar growth kinetics as their parental virus. IBDV VP2 expression was detected in DF1 cells infected with the recombinants by immunofluorescence assays. These vaccine candidates' safety and protective efficacy against IBDV variant and NDV challenges are under evaluation through in ovo vaccination trials.

### **Genetic and epidemiological characteristics of Infectious Bursal Disease Virus circulating in broiler flocks in Brazil**

Jorge Chacon<sup>1</sup>, CESAR NUNES<sup>1</sup>, CRISTIANO TRONCOSO<sup>1</sup>, ALEX LAURINDO<sup>1</sup>, JAIME SARABIA<sup>1</sup>, MARCO LOPES<sup>1</sup>, LUIS SESTI<sup>1</sup>

*Veterinary Services - Ceva Animal Health<sup>1</sup>*

Infectious Bursal Disease Virus (IBDV) is a very resistant and persistent viral agent of the poultry industry. IBD vaccines applied at the hatchery have been reasonably effective for controlling the clinical form of IBD. However, a more in-depth diagnostic work is needed to characterize the epidemiology of the subclinical form caused by variant strains. An epidemiological investigation was carried out to determine the efficacy of current vaccination programs for preventing the circulation

of subclinical IBDV variant strains. Seven broiler production systems located in different geographical regions of Brazil were included in the study. Bursa of Fabricius of 156 vaccinated flocks were collected between 30 and 32 days of age and submitted to IBDV molecular detection and typing based on VP2 gene analysis. Flocks were previously vaccinated at the hatchery as follows: with two Immune Complex (IC) vaccines (both having the Winterfield 2512 strain; Vaccine 1 - 72 flocks and Vaccine 2 - 22 flocks) and the remaining flocks received an HVT-based vector vaccine (rHVT-IBD; 62 flocks). Conventional live vaccine was applied as booster dose at 14 days of age by drinking water in 34,7%, 45,5% and 16,1% of flocks of each vaccine program, respectively. Vaccine and variant strain viruses were detected in 21,2% and 47,4% of flocks, respectively. IBDV was not detected in 25,6% of flocks, while the vaccine virus applied at the field was detected in 5,8% of flocks. Macro and microscopic lesions were scored as moderate. Genetic analysis showed that the 74 variant viruses sequenced in the study belong to Genogroup 4. The nucleotide and amino acid sequence analyses revealed 92,1-100% and 94-100% identity among them, respectively. The genetic homology of the field IBDV variants sequenced in this study ranged from 86.1 to 97.2% for nucleotide and from 90.4 to 98.8% for amino acids when compared with the values determined for variant IBDVs previously detected in the country. Three subclusters were observed in the phylogenetic tree, that were grouped according to geographical origin. Variant IBDVs were detected mostly in flocks vaccinated with rHVT-IBD vaccine (62,9%). On the other hand, a lower detection rate was in flocks receiving IC vaccines (vaccine 1:34.7% and vaccine 2:45.5%). No significant effect on reduction of variant IBDVs detection was observed in flocks further vaccinated via drinking water in the field. Interestingly, broiler production systems reusing litter for more than a year presented a higher detection of variant strains (45.8%) than those reusing litter for less than a year (13%). In conclusion, even though the IC vaccines appeared to be more effective in controlling field IBDVs, this investigation showed an ample geographical distribution of variant viruses in flocks properly vaccinated, suggesting a need to improve biosecurity and prophylactic measures in the broiler production systems investigated.

### **Datamining an infectious bursal disease field trial database comparing bursal atrophy effect on performance data, serology and biochemistry profile. Putting it all together Part 2**

Daniel Venne<sup>1</sup>, Brenna Tuer<sup>2</sup>, Chanelle Taylor<sup>3</sup>

*Ceva animal health*<sup>1</sup>, *Cargill Canada*<sup>2</sup>, *Cargill Canada*<sup>3</sup>

A database from a trial with performance data, biochemical profiling, serology for IBD, IBD+ and IBV, bursa size, qPCR, sequencing and vaccine information was datamined. When grouped according to bursal size at processing for average bursa weight < 3g and over 3g, a significant effect was observed for albumin, globulin and albumin to globulin ratio indicating a shift from albumin production to globulin production when bursa were smaller. Processing age was significantly lower when bursa were large. Large bursa had an average qPCR ct of 36.49 and small bursas had an average of 23.83 which translates into a reduction of viral load difference of 4.5 logs. 100% of the flocks vaccinated with vaccine B and positive for variant E strains had bursal atrophy while 20% of flocks vaccinated with vaccine A and positive for variant E had bursal atrophy. This data shows bursal atrophy associated with variant IBD virus have a physiological cost shifting protein production from albumin to globulins. Bursal atrophy is also associated with higher viral loads in the bursa. This increase viral load and immunological cost of protein shift influence ADG.

## **Molecular detection and characterization of emerging novel variant IBDV (genotype A2dB1b) in the Middle East prompts concern of spread of subclinical disease forms**

Matteo Legnardi<sup>1</sup>, Francesca Poletto<sup>1</sup>, Giovanni Franzo<sup>1</sup>, Claudia Maria Tucciarone<sup>1</sup>, Mattia Cecchinato<sup>1</sup>

*Department of Animal Medicine, Production and Health (MAPS), University of Padova<sup>1</sup>*

Infectious bursal disease (IBD) is a highly contagious immunosuppressive disease that has a severe impact on the worldwide poultry industry. It is caused by infectious bursal disease virus (IBDV), a pathogen with a double stranded, bi-segmented RNA genome which is highly prone to mutation and reassortment events. Adding to the traditional classification based on pathogenicity and antigenicity assessment, recently proposed phylogenetic criteria, by which strains are classified based on sequencing both VP2 (genogroups A0-A9) and VP1 (B1-B5) genes, contributed to a greater understanding of IBDV epidemiology, leading to the characterization of several novel subtypes in different parts of the world. Among the most notable epidemiological events of the last decade is the emergence of genotype A2dB1b, also known as novel variant IBDV. First reported in 2015 in China, this genotype is thought to have originated from the spread and divergent evolution of antigenic variant IBDVs from North America to East Asia. Subsequently, it has been responsible for large-scale IBD epidemics in China, Malaysia, South Korea, and Japan. Infection by novel variant IBDVs consistently results in severe immunosuppression, but its subclinical course may hamper diagnosis, impact estimation and control efforts. The present study reports the recent detection of novel variant IBDVs in the Middle East, a region historically characterized by very virulent IBDV circulation. Following multiple identifications of A2dB1b strains in Egypt (earliest known detection in March 2023), molecular diagnostic activities also highlighted their presence in Jordan and Lebanon (earliest detection in December 2023) in flocks showing diminished performance and increased susceptibility to respiratory diseases. The sequenced strains from the three countries showed a high genetic identity between each other (above 98.7% and 99.1% identity at VP2 and VP1 level, respectively). Looking at amino acid sequences, all Middle Eastern strains featured a distinctive Thr321Val change compared to most Asian A2dB1b IBDVs. Residue 321, located within the PHI hydrophilic loop, is known to influence the reactivity to neutralizing antibodies, possibly altering antigenicity. Since novel variant IBDV has never been signaled outside of East Asia and now the Middle East, the mode of transmission between these two regions remains unexplained. Nonetheless, these preliminary results represent an epidemiological update of global concern. Considering the epidemic potential and antigenic divergence displayed by novel variant IBDV, further research efforts appear crucial to track its current and future spread and understand its actual impact in productive contexts different from the .

## **Title Comparing recombinant infectious bursal disease virus vaccines: field observations, bursal size, DIVA serology, qPCR and sequencing. Putting it all together. Part 1**

Brenna Tuer<sup>1</sup>, Chanelle Taylor<sup>2</sup>, Daniel Venne<sup>3</sup>

*Cargill Canada<sup>1</sup>, Cargill Canada<sup>2</sup>, Ceva animal health<sup>3</sup>*

A trial was setup to evaluate the effect of vectorized vaccines on production parameters in 3.2kg broilers. 82 flocks from 3 groups IBD vector vaccine A, vector vaccine B and a control group were

compared for age at processing, average weight, ADG, condemnations, total mortality and 10 day corrected mortality. 16 flocks from the three groups were also analyzed for bursal size, bursal weight, IBD Diva serology using IBD and IBD+ serology, and IBV serology. Vectored vaccines had a significant effect on reducing age at processing and increasing ADG. No significant effect were observed between groups for average weight, percent condemnations and mortality. When corrected for feed mill effect vectored vaccines improved average condemnations. Diva serology results indicated that some flocks were misidentified for vaccination groups. One of the vaccinated flocks was poorly vaccinated. qPCR Ct and sequencing showed that variant E viruses were most prevalent. When infected with genetically identical viruses Vaccine B had lower Ct values than vaccine A. Vaccine A 33.73 Ct and 39.05 Ct respectively and Vaccine B 27.44 Ct and 20.82 Ct and translates into a 2 to 3 log reduction in viral replicates. This works shows the importance of taking into account factors such as feed source, vaccine classification and virus type when comparing vaccines in field studies.

### **Reassortant strains of infectious bursal disease virus (IBDV) belonging to genogroup A3B1 predominate in British broiler flocks**

Vishwanatha Reddy Avalakuppa Papi Reddy<sup>1</sup>, Carlo Bianco<sup>2</sup>, Christopher Poulos<sup>3</sup>, Andrew Brodrick<sup>4</sup>, Salik Nazki<sup>5</sup>, Alex Schock<sup>6</sup>, Andrew J Broadbent<sup>7</sup>

*The Pirbright Institute or Keele University<sup>1</sup>, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, UK<sup>2</sup>, Animal and Plant Health Agency (APHA), Lasswade, UK<sup>3</sup>, Department of Animal and Avian Sciences, University of Maryland, College Park, MD, 20742, USA<sup>4</sup>, Pandemic Sciences Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UK<sup>5</sup>, Animal and Plant Health Agency (APHA), Lasswade, UK<sup>6</sup>, Department of Animal and Avian Sciences, University of Maryland, College Park, MD, 20742, USA<sup>7</sup>*

Infectious bursal disease virus (IBDV), a member of the Avibirnavirus genus in the Birnaviridae family, is an immunosuppressive virus that infects poultry. The segmented nature of its genome allows for reassortment in birds that are coinfecting with different strains, and this has been reported with increasing frequency worldwide. To establish the status of IBDV variants in Great Britain, the endemic disease surveillance network of Animal and Plant Health Agency (APHA) collected sets of bursal samples from birds on British broiler farms between 2020 and 2021. After avian pathologists at APHA Lasswade established a morphological diagnosis consistent with IBDV infection, RNA was extracted from the bursal tissue and subject to reverse transcription-polymerase chain reaction (RT-PCR) to amplify the hypervariable region (HVR) of the VP2 capsid gene encoded by segment A, and a region of the VP1 polymerase gene encoded by segment B. Twenty sample sets from 16 farms were consistent with an IBDV challenge. The PCR-positive samples were sent for Sanger sequencing, after which phylogenetic analyses, and sequence alignments were performed. Of the 16 farms, none contained very virulent (vv) strains belonging to genogroup A3B2, which was consistent with their clinical history of low mortality. Genogroup A3B1 reassortant strains were predominant (13/16) on these farms, and the majority (8/13) were found to be coinfecting with genogroup A1B1 strains. A subset of farms with reassortant viruses (5/13) had HVR mutations consistent with recently described Western European reassortant strains (mutations Q219L, G254D, D279N, and N280T), whilst the remaining majority (8/13) had no mutations or other mutations compared to UK661. Overall, this molecular epidemiology study suggests that A3B1 reassortant strains may have a fitness advantage over vv strains, and that multiple clades of reassortant viruses are now co-circulating in

British broiler flocks. It also establishes a baseline on which to improve our understanding of the consequences of IBDV reassortment, antigenic drift, and co-infection in British broiler flocks.

### **Immunoprotection of broiler chickens against variant infectious bursa disease virus (varIBDV SK09) and associated B cell, T cell subsets and macrophage profile in the bursa of Fabricius**

Ayumi Matsuyama<sup>1</sup>, Shelly Popowich<sup>1</sup>, Asha Ranaraja<sup>1</sup>, Mihirabha Rathnayake<sup>1</sup>, Betty Chow-Lockerbie<sup>1</sup>, Susantha Gomis<sup>1</sup>

*University of Saskatchewan<sup>1</sup>*

Variant infectious bursal disease viruses (varIBDVs) do not cause high mortality in broiler chickens but causes severe immunosuppression. Although studies have explored the immune cell profile in the bursa of Fabricius (BF) following very virulent IBDV infection, little is known in the BF following varIBDV infection in broiler chickens. The objective of this study was to identify the immune cell profile in the BF following varIBDV SK09 challenge in broiler chickens with and without maternal antibodies (MatAb) against varIBDV SK09. Broiler progenies were obtained from naïve parent broiler breeders which were thereafter vaccinated with varIBDV SK09. The immune cell profile in the BF of broiler progenies with and without MatAb against varIBDV SK09 was compared following oral exposure of broiler chickens to varIBDV SK09 at day 6 of age. T cells, B cells, and macrophages in the BF were evaluated at 1, 5, 14, 21, 28, and 35 days post-infection (dpi) using flow cytometry. MatAb negative (MatAb-) varIBDV SK09 challenged chickens had a higher percentage of CD4+ T cells and MHC II+ macrophages in the BF compared to the varIBDV SK09 uninfected group at 1 dpi. A remarkable increase of CD8α+ T cells was observed in the BF in MatAb- varIBDV SK09 challenged chickens at 5 dpi. Additionally, a significant increase in CD8αα+ and CD8αβ+ γδ T cells was observed in the BF of MatAb- varIBDV SK09 challenged chickens at 5 dpi. In contrast, the CD8αα+ γδ T cell population was predominantly induced in MatAb+ varIBDV SK09 challenged chickens at 5 dpi. A marked B cell depletion was noted in MatAb- varIBDV SK09 challenged chickens while it was not observed in MatAb+ varIBDV SK09 challenged chickens through the study period. These results indicate that B and T cell subsets and macrophages have a unique role in pathogenesis and protection of broiler chickens against varIBDV SK09.

## **Parasitology/Coccidiosis**

### **Effects of a proprietary essential oil product on body weight, feed conversion, and mortality of Ross 708 broilers that are either vaccinated or unvaccinated against coccidiosis at placement**

Sharon Heins Miller<sup>1</sup>, John Schleifer<sup>1</sup>, Mark LaVorgna<sup>1</sup>, Mahmoud Masadeh<sup>1</sup>, Gary Reznik<sup>1</sup>, Shameer Rasheed<sup>1</sup>, Kristy Dorton<sup>1</sup>

*Devenish Nutrition LLC<sup>1</sup>*

A study was conducted to evaluate the effect of a proprietary essential oil product (EO; DeviSTAT Broiler NA, Devenish Nutrition, Fairmont, MN) on body weight, feed conversion, and mortality of Ross 708 broilers that are either vaccinated or unvaccinated against coccidiosis at placement. Broiler chicks (n = 1,800; 1-day old chicks, a hatched) were randomly assigned to 36 pens (50 birds/pen; 0.88 ft<sup>2</sup>/bird from d 0 to 31; 1.08 ft<sup>2</sup>/bird from d 31 to 56). There were four diet phases: starter (d 0 to 16), grower (d 17 to 31), finisher (d 32 to 45) and withdrawal (d 46 to 56). Birds were fed corn-soy

based pelleted diets that did not contain coccidiostats. The four treatments were: A) Control (no EO or vaccine), B) vaccine applied at placement (CocciVac B52, Merck Animal Health, Madison, NJ), C) EO at 0.75, 0.5, and 0 lb/ton in the starter/grower, finisher, and withdrawal diets, and a combination of treatments B and C. Body weight (BW) was measured at d 0, 16, 31, and 56. Mortality adjusted feed conversion ratio (FCRM) and mortality were determined from d 0 to 16, d 17 to 31, d 31 to 56, d 0 to 31, and d 0 to 56. Adjusted feed conversion (FCRM+BW; mortality and body weight adjusted to 4.5 lb and 10.5 lb, respectively) was determined from d 0 to 31 and d 0 to 56. Data was analyzed using a two-way ANOVA (Mixed procedure of SAS 9.4; 2018) to determine the main effects of EO and vaccination and their interaction effects. The experimental unit was pen and initial body weight was used as a covariate in the analysis. Means were separated by Fisher's protected least significant difference. Differences were considered significant at  $P \leq 0.05$ . There was only one interaction effect between vaccine and EO. When supplemented with EO, non-vaccinated broilers had better FCRM ( $P = 0.0007$ ) than vaccinated broilers. These two EO supplemented groups had better FCRM ( $P = 0.0007$ ) than broilers not supplemented with EO (control and vaccine only). When examining the main effects of vaccine, vaccinated broilers had lower BW ( $P = 0.04$ ), FCRM ( $P = 0.02$ ), and FCRM+BW ( $P = 0.01$ ) compared to non-vaccinated broilers at d 31. Supplementation of EO resulted in better BW (d 0 to 16:  $P = 0.0004$ ; d 0 to 31:  $P = 0.05$ ), FCRM (d 0 to 16:  $P < 0.0001$ ); d 0 to 31 ( $P = 0.04$ ), and FCRM+BW (d 0 to 31:  $P = 0.03$ ). Mortality tended to be lower (d 0 to 31:  $P = 0.10$ ); d 0 to 56;  $P = 0.07$ ) when broilers were supplemented with EO. With the exception of mortality, there were no significant effects of vaccine or EO overall (d 56). Based on the results of the study, EO can have a positive impact on performance and mortality of broilers when no coccidiosis vaccine is used. However, EO may need to be included in the diet throughout the entire feeding period to maintain these effects. This will be examined in future studies.

### **Comparison of live attenuated and live-non attenuated vaccines on the safety of the administration of an overdose using the model of the European Monograph in SPF chickens**

Martina Dardi<sup>1</sup>, Marc Pagas<sup>1</sup>, Andreu Alba<sup>1</sup>, Joan Molist<sup>1</sup>, Massimiliano Baratelli<sup>1</sup>

*Laboratorios HIPRA S.A.<sup>1</sup>*

Avian coccidiosis poses significant challenges in broiler chicken production. Live vaccines are commonly employed to control the disease. Depending on the type of *Eimeria* strains they contain, these vaccines can be classified as wild-type (non-attenuated) vaccines and attenuated ones. Limited information is available on the safety of non-attenuated vaccines, especially in comparison to live attenuated ones. The aim of this study was to compare the safety of an overdose (10X) of EVANT® (Group A) with two non-attenuated coccidiosis vaccines (Group B - Vaccine with 3 strains of *Eimeria* oocysts; Group C - Vaccine with 5 strains of *Eimeria* oocysts) and a negative control group (Group D - Phosphate Buffered Saline Solution) under experimental conditions. The overdosage study was performed according to the European Monograph model for safety trials which resemble similar conditions to the replication of vaccinal oocysts in the litter. On day 0, 160 SPF birds (14 days old) were randomized by weight, proportionally distributed in cages, and orally inoculated with the corresponding treatment. The birds were blindly monitored for 14 days. Parameters evaluated to assess the safety of the treatments used included Clinical Signs (CS), Mortality, Intestinal Lesions (IL), Body Weight (BW) and Feed Conversion Ratio (FCR). The excretion of the vaccinal oocysts in fresh faeces was measured through Oocysts per gram counts (OPG) from 3 to 9 days post-

inoculation (DPI). The OPG elimination profiles showed a greater abundance of oocysts shed in Groups B and C compared to Group A, as well as different peaks for oocyst shedding. Groups B and C showed some birds with blood in the faeces. Regarding mortality, Group C had one dead bird attributable to the treatment with confirmed grade 4 intestinal lesion scoring at necropsy. No abnormal signs of disease or deaths were recorded in Groups A and D. The IL evaluation performed at 6 and 14 DPI (12 birds per time point) indicated higher intestinal lesions in Groups B and C compared to groups A and D. Statistically significantly lower BW were detected at 14 DPI in groups B and C compared to groups A and D. Groups A and D showed similar FCR from 6 to 16 DPI (0.70 and 0.73 respectively), while groups B and C showed higher FCR in the same period (1.07 and 1.13 respectively). In conclusion, the results obtained in this study indicate that the vaccine administered in Group A is clearly safer compared to the vaccines administered in Groups B and C. Moreover, an overdose of the vaccine in Group A (attenuated) does not cause major clinical signs of disease or mortality, only low and mild intestinal lesions are detected and has similar body weights and feed conversion rate to the control group. It has been demonstrated that non-attenuated vaccines have a higher oocyst shedding and produce more severe and frequent clinical signs and intestinal lesions on the animals, resulting in a loss of body weight and a poorer feed conversion rate.

#### **Comparative analysis of broiler chicken productivity following vaccination with different coccidiosis vaccines and using different administration methods**

Martina Dardi<sup>1</sup>, Jesus Fernandez<sup>1</sup>, Juan Grau<sup>1</sup>, Rocio Sanchez<sup>1</sup>, Marina Sola<sup>1</sup>, Lorena Nodar<sup>1</sup>, Luis Pantoja<sup>1</sup>, Joan Molist<sup>1</sup>

*Laboratorios HIPRA S.A.<sup>1</sup>*

Avian coccidiosis remains a significant challenge in the poultry industry, impacting global poultry production and welfare. Various tools have been developed to mitigate the negative effects of the disease. Strategies such as the rotation of anticoccidial drugs with coccidiosis vaccines have been widely implemented in different countries and by producers worldwide to reduce the resistance of *Eimeria* field strains against anticoccidial drugs and restore sensitivity. The administration of avian coccidiosis vaccines has traditionally been performed in hatched birds by methods such as coarse spray or gel spray. Recently, a live vaccine administered in ovo (IO) against avian coccidiosis, which contains attenuated precocious strains, has been registered in several countries. The aim of this study was to evaluate, under field conditions, the performance of broiler flocks vaccinated with the IO coccidiosis vaccine compared to coccidiosis vaccination with a live attenuated vaccine administered by coarse spray within the same company, which implemented a yearly rotation of anticoccidial drugs with attenuated coccidiosis vaccines (2 cycles of production). The study included 659 standard broiler flocks of a Spanish poultry company, totalling 13,790,496 chickens. Of these, 6,546,090 (333 flocks) were vaccinated in 2022 with the coarse spray vaccine (Group A – Attenuated vaccine with 5 strains of *Eimeria* oocysts), while 7,244,406 chicks (326 flocks) were vaccinated IO with EVANOVO® (Group B). Evaluation parameters included Slaughter Age, Mortality, Body Weight (BW), Average Daily Gain (ADG), Feed Conversion Ratio (FCR) and the European Production Efficiency Factor (EPEF). Seasonality and correlations between slaughter age and ADG, as well as BW and FCR, were considered. Productivity parameters revealed that Group B broilers exhibited a statistically significantly higher ADG compared to Group A ( $65.2 \pm 3.45$  g in Group B compared to  $64.83 \pm 3.05$  g in Group A). Additionally, a significantly lower FCR was observed in Group



B ( $1.612 \pm 0.064$  compared to  $1.652 \pm 0.073$  for Group A), and the EPEF was significantly higher in Group B ( $391.85 \pm 34.18$  compared to  $380.83 \pm 33.80$  in Group A). No significant differences were detected in mortality. No clear effect of seasonality during vaccination periods was identified. Interestingly, no clear correlation was seen between slaughter age and ADG. A positive correlation was detected between BW and FCR. In conclusion, broilers coccidiosis vaccinated in ovo exhibited a more efficient performance compared to the previous year's chickens vaccinated with a coarse spray vaccine, possibly due to more accurate administration, reduced handling of hatched chicks, and effective coverage of field *Eimeria* challenges.

### **Use of an attenuated *Eimeria* vaccine in broilers with in-ovo application for on-farm hatching**

Martina Dardi<sup>1</sup>, Kristof van Hoye<sup>2</sup>, Joan Molist<sup>1</sup>, Luis Pantoja<sup>1</sup>, Marta Busquet<sup>1</sup>, Marina Sola<sup>1</sup>, Marc Tomas<sup>1</sup>, Nikolai Ongena<sup>1</sup>

*Laboratorios HIPRA S.A.*<sup>1</sup>, *Pehestat*<sup>2</sup>

The non-integrated broiler market in Belgium is experiencing a significant transition towards in ovo vaccination, propelled in part by the introduction of a coccidiosis vaccine specifically formulated for in ovo use. This evolving trend is coupled with a simultaneous shift towards on-farm hatching systems, making conventional hatchery coarse spray or gel-spray vaccination impossible. Administering coccidiosis vaccines on the farm with chicks hatched on-site may result in insufficient vaccine intake and suboptimal replication of oocysts. When it comes to coccidiosis vaccination, the crucial factor is ensuring optimal oral intake of the vaccine. What really matters for its effectiveness is making sure that the embryonated eggs receive the vaccine through amniotic vaccination, allowing the chicks to take in the vaccine and the oocysts orally. Embrex<sup>®</sup> has established itself as a leader in the Belgian in ovo machine market, despite the lack of a 100% guarantee for amniotic vaccination. The effectiveness of these machines ranges between 64% and 100% in the amnion, depending on factors such as the specific machine, incubation age, and breeder age. With careful consideration of these variables and diligent follow-up through a hatchery monitoring programme, achieving a +90% amniotic vaccination rate is feasible. A comprehensive trial conducted in Belgium revealed that in ovo vaccination using attenuated *Eimeria* strains contributed to a reduction in the use of coccidiostats, leading to improvements in technical performance. This included a notable 3.4-point enhancement in the average Feed Conversion Ratio (FCR) and a 5-point increase in corrected FCR (1.5kg). Additionally, the intervention resulted in slightly lower mortality rates and an extra daily weight gain of 2 grams. Collectively, these improvements contributed to a remarkable 16-point enhancement in the average European Production Efficiency Factor (EPEF). Despite the perceived cost of vaccination, use of a vaccine instead of coccidiostats resulted in a €0.01 lower average cost to produce one kilogram of meat.

### **Single fecal collection for oocyst shedding does not predict infection for all *Eimeria* species**

Robert Beckstead<sup>1</sup>, Samantha Schmitt<sup>1</sup>, Megan Regier<sup>1</sup>, James White<sup>1</sup>, James Mills<sup>1</sup>, Robert Beckstead<sup>1</sup>

*Ceva Animal Health, LLC*<sup>1</sup>

*Eimeria* sp. in chicken causes intestinal disruption hindering nutrient absorption and can lead to mortality. Coccidiosis vaccines minimize disease by controlled low dose infection. It is important

that all birds are infected at the same time minimizing unvaccinated birds receiving a high dose of oocysts during cycling. Typically, around 7 days post vaccination, a fecal sample is collected to observe the presence or absence of oocysts. However, oocyst shedding and cycling fluctuates due to differential pre-patent periods and localization of infection in the intestines which differs by species. The objective of this study was to provide better insight into the detection of oocysts as a measure of successful vaccination of poultry against coccidiosis. Two separate groups of day of age chicks were vaccinated with a known number of oocysts and then housed individually. On the 7th day post vaccination fecal droppings were collected from each bird every two hours. There were six different time points throughout the day of collection. Quantitative polymerase chain reaction (qPCR) was performed on the samples to detect *E. acervulina*, *E. maxima*, and *E. tenella*. The results revealed that detection of oocysts for different species varied throughout collection period and is dose dependent. For group 1, detection for all time points was 100%, 70% and 53.3% while the 7-day positivity rate was 100%, 100% and 100% for *E. acervulina*, *E. maxima*, and *E. tenella* respectively. For group 2, at a lower dose, detection for all time points was 96.7%, 56.7%, and 23.3% while the 7-day positivity rate was 100%, 100% and 80% for *E. acervulina*, *E. maxima*, and *E. tenella* respectively. This data suggests that vaccine take protocols for *Eimeria* detection via qPCR, relying on a singular timepoint of fecal collection, may result in false negatives for certain *Eimeria* species.

### **Prevalance of Gastro-Intestinal Parasites in Backyard Chicken at Bharatpur-11, Nepal**

Naresh Sah<sup>1</sup>

*Nepal polytechnic Institute, College of Veterinary Science<sup>1</sup>*

Gastro-Intestinal parasitic infestation remains one of the major problems for backyard chicken farmers which can lead to huge economic loss for the farmers. To estimate the prevalence of gastrointestinal parasite in backyard chicken, a study was conducted from July 15, 2023 to August 24, 2023. A Total of 164 fecal samples of chicken were collected from backyard of Bharatpur-11, Bharatpur metropolitan city, Chitwan. The samples were examined by using direct fecal smear method, floatation method and sedimentation method by using purposively selectively sampling. 53.0% samples were found to be positive for gastrointestinal parasites and 47.0% samples were negative. Out of positive samples, *Heterakis* sp. 30%, *Railletina* sp. 22%, *Eimeria* sp. 17%, *Ascaridia* sp. 15%, *Capillaria* sp. 10% and Unidentified 6%. The phylum-wise prevalence on gastrointestinal parasites in backyard chicken showed that nematode 55%, cestode 22%, apicomplexa 17%, unidentified 6% and trematode 0%. Statistically there was a significant difference in the prevalence of Phylum wise ( $\chi^2 = 150.8$ ,  $p < 0.05$ ) with higher prevalence of phylum nematode. In age wise prevalence, 6-7 months aged group chickens 22% were positive, 16% were positive for 8-9 months, 15% positive in 9-10 months and 14% positive in both 1-2 months as well as 7-8 months. Statistically there was a significant difference in the prevalence of age wise ( $\chi^2 = 23.08$ ,  $p < 0.05$ ) with higher prevalence of age in chicken age of 6-7 months. Statistically there was a significant difference in the dewormed chicken and parasite present ( $\chi^2 = 10.39$ ,  $p < 0.05$ ). Thus, present study showed that the backyard chicken is mostly infected by one or more parasite and results indicated that proper deworming practices were not adopted with backyard chicken. Hence, it is recommended to carry out proper deworming practices and maintain good bio-security and proper feeding system to reduce the parasitic infection.

### **Leucocytozoonosis: Diagnosis and PCR analysis in avian species in Canada**

Emily Martin<sup>1</sup>, Andrew Brooks<sup>1</sup>, Felipe Regetti<sup>1</sup>, John R. Barta<sup>1</sup>, Natasha Slawnych<sup>1</sup>, Perryn Kruth<sup>1</sup>, Alecia Finateri<sup>2</sup>

*Animal Health Laboratory, University of Guelph<sup>1</sup>, Alecia Finateri, Algoma Veterinary Services, Desbarats, ON<sup>2</sup>*

In the spring and summer of 2023, the AHL received submissions of avian species with a history of sudden death (cygnets, hawk, duckling) that were either confirmed or suspicious for Leucocytozoonosis. The presence of thin watery blood at postmortem examination prompted preparation of peripheral blood smears to examine for the presence of macrogametocytes. On histopathology, the presence of large protozoal organisms in various stages of development within multiple tissues also prompted further investigation. Consultation with researchers in the Department of Pathobiology at OVC led to tissues being forwarded to their research lab for PCR analysis. The diagnosis, parasitic life cycle and PCR results will be discussed.

## Reovirus

### **Understanding the variability of avian reovirus through adaptation attempts of the virus by serial passages in cell culture and embryonated chicken eggs**

Abdul Rehman Bilal<sup>1</sup>, Rachel Jude<sup>2</sup>, Dr. Rodrigo A Gallardo<sup>3</sup>

*Department of Population Health and Reproduction, School of Veterinary Medicine, UC Davis<sup>1</sup>,  
Department of Population Health and Reproduction, School of Veterinary Medicine, UC Davis<sup>2</sup>,  
Department of Population Health and Reproduction, School of Veterinary Medicine, UC Davis<sup>3</sup>*

Avian reovirus causes viral arthritis and tenosynovitis in chickens causing significant economic losses to the poultry industry and welfare issues. It is a non-enveloped virus and has a double-stranded RNA segmented genome that makes it vulnerable to point mutations, insertions and deletions, recombinations and genetic reassortments. These events play an important role in the variability of the virus causing the emergence of its variants. It is extremely important to better understand ARV variability. Key information regarding the variation of specific regions of the genome is important to better characterize and potentially associate antigenicity. This study aims to investigate variability of plaque purified avian reovirus isolates by analyzing mutations at the whole genome level, during viral adaptation to cell culture and chicken embryonated eggs through serial passaging. Viral genome sequences will be obtained using nanopore technology and compared using available software. The results of this experiment may confirm variable genes of this virus as S1, M2, and L3, and determine hypervariable regions in those genes that might be important in the determination of the antigenicity of the virus.

### **Determination of Purity of an Avian Reovirus Isolate following Plaque Purification**

Zubair Khalid<sup>1</sup>, Sonsiray Alvarez Narvaez<sup>3</sup>, Telvin Harrell<sup>3</sup>, Erfan U. Chowdhury<sup>5</sup>, Steven Conrad<sup>6</sup>, Ruediger Hauck<sup>7</sup>

*Auburn University<sup>1</sup>, United States Department of Agriculture, Athens, GA<sup>3</sup>, Thompson Bishop Sparks State Diagnostic Laboratory, Auburn<sup>5</sup>, United States Department of Agriculture, Athens, GA<sup>6</sup>, Auburn University, Auburn, AL<sup>7</sup>*

Plaque purification technique has been widely employed to obtain clonal viral population from complex samples. Three rounds of plaque purification are generally recommended to ensure purity of the virus being isolated. We attempted at purifying a field strain of avian reovirus (ARV) isolated from the heart of a 4-week-old broiler chicken. The virus was propagated in chicken embryo liver (CELi) culture and subjected to three rounds of plaque purification following traditional protocols. The plaque-picked virus was propagated in CELi culture and passaged once in specific-pathogen-free chicken embryos. Upon next generation sequencing (NGS) and de novo assembly, a mix of divergent ARV sequences were obtained. Upon mapping to the reference vaccine strain S1133, most of the sequences belonged to the field isolate but a lower proportion of sequences were found to be nearly identical to S1133. No viral RNA was amplified with RT-PCR using primers widely used to type ARV strains based on the S1 gene, but not expected to amplify the isolate. Specific primers were designed against the divergent sequences of the isolate to amplify its partial S1 gene. The sequence was confirmed with Sanger sequencing. In conclusion, PCR using conventionally utilized primers had lower sensitivity for divergent sequences than single primer amplification used as part of the NGS protocol. The results indicated the unreliability of plaque purification technique to obtain clonal population of avian reovirus. In conclusion, we suggest an optimized NGS protocol to determine purity of the ARV isolates.

### **Amino Acid Sequence Confirmation and Peptide Analysis of Avian Reovirus Proteins by Mass Spectrometry**

Steven Conrad<sup>1</sup>, Sonsiray Alvarez-Narvaez<sup>2</sup>, Telvin L Harrell<sup>3</sup>

*USDA<sup>1</sup>, USDA<sup>2</sup>, ORISE<sup>3</sup>*

Avian reovirus (ARV) is a double-stranded RNA virus that poses a significant challenge to the poultry industry. It infects broiler and breeder chickens and causes substantial economic losses worldwide. Infection usually occurs before 3 weeks of age as a result of either horizontal or vertical transmission. ARV-infected chickens and turkeys present with a diverse range of symptoms, including and especially lameness (tenosynovitis), atrophy of the lymphoid organs, gastrointestinal damage, respiratory complication, and, recently, neurological complications. The results of ARV infection can range from subclinical (no visible symptoms) to large-scale mortality. Control of ARV is accomplished by both routine vaccination and biocontainment. However, the available vaccine options are limited, and consist of either older-generation serotype 1 vaccine strains (such as s1133) or autogenous vaccines, which are narrowly-protective, costly, and time-consuming to produce. This report contributes to the fight against ARV by providing valuable insights into its protein makeup. The study examines the post-translational modifications of ARV proteins. This data has been made publicly available in a protein database. This resource will support further research endeavors focused on ARV proteins, paving the way for improved diagnostics and ultimately, more protective vaccines.

### **Construction of recombinant Marek's disease virus vaccine expressing sigma C proteins of avian reoviruses**

Taejoong Kim<sup>1</sup>

*Southeast Poultry Research Laboratory, US National Poultry Research Center, USDA-ARS<sup>1</sup>*

Avian reovirus (ARV), a significant economic burden on poultry production, is implicated in various production-limiting diseases including runting-stunting syndrome, malabsorption syndrome, impaired feed conversion, and increased condemnation rates. In young chickens and turkeys, ARV manifests as arthritis/tenosynovitis, with co-infections by other viruses exacerbating clinical severity. Control primarily relies on inactivated and live attenuated vaccines, but their efficacy is hampered by antigenic drift between circulating ARVs and vaccine strains. The ARV sigma ( $\sigma$ ) C protein, encoded by the S1 segment and crucial for virus attachment to target cells, serves as a major neutralizing antibody target. To broaden vaccine protection against diverse ARV groups, a novel recombinant Marek's disease virus (MDV) expressing multiple  $\sigma$ C proteins was constructed using a single bacterial artificial chromosome (BAC) and BAC recombineering. The presentation will explore the expression analysis of  $\sigma$ C proteins from vaccine and field ARV isolates, as well as the stability of the recombinant MDV-ARV  $\sigma$ C construct.

### **Identification of the Role of Avian Reovirus Infection in Various Poultry Diseases in India**

Namdeo Bulbule<sup>1</sup>, Vaibhav V. Deshmukh<sup>3</sup>, Milind Madhukar Chawak<sup>4</sup>

*Assistant General Manager<sup>1</sup>, Manager<sup>3</sup>, General Manager<sup>4</sup>*

Avian reovirus (ARV) is a ubiquitous pathogen with a diverse range of effects on poultry health. This study aimed to identify the role of Avian Reovirus infection in various diseases affecting poultry in India. Employing a multidisciplinary approach, the comprehensive diagnostic protocol was applied to detect and confirm ARV infections in commercial broiler and layer birds experiencing various disease conditions like tenosynovitis, respiratory infections, runting-stunting, pericarditis, nephritis, gout and low egg production. Virus isolation using chicken embryo liver cells and RT-PCR assays targeting sigma ( $\sigma$ ) C of the ARV gene and further its sequencing were employed for accurate and reliable identification. Subsequently, an in-depth analysis was conducted to explore the correlation between ARV presence and the manifestation of various poultry diseases. Using representative ARV isolates, in vivo pathogenicity study was carried out to know the tissue tropism and disease severity. Several Avian reoviruses were successfully isolated in Chicken embryo liver culture from field samples. Sequence and Phylogenetic analysis of ARV isolates clade into different genogroups and predominantly clade into genogroup IV. As most of the ARV isolates were clade in to different groups from vaccine group (genogroup I) and such studies and findings were not reported previously in India. ARV isolated from Malabsorption Syndrome showed reduced live weight and uneven growth varying up to 62% when infected SPF day old chicks by intraperitoneal route. In experimental study, Intraperitoneal route (I/P) was found most significant route rather than oral and foot pad. The virus was detected in pancreas, spleen, liver, kidney and heart. The ARV was isolated from nephritis/gout affected grower broilers and negative for other pathogen associated with nephritis or gout. The pathological examinations and clinical assessments were done to characterize the spectrum of diseases associated with ARV infection. Understanding the impact of ARV on the poultry health landscape in India is crucial for implementing effective disease management strategies. In conclusion, this study provides valuable insights into the identification of the role of Avian Reovirus in various poultry diseases and development of an inactivated ARV vaccine (including variants) for preventing multiple ARV genogroup/serotype infection, transmission, and losses due to disease in India. Keywords – ARV, RT-PCR, Tenosynovitis, Nephritis

### **First Seroprevalence Survey of Avian Reovirus in Broiler Breeders Chicken Flocks in Morocco**

Ahmed Achhal<sup>1</sup>, Amal ZHARI<sup>2</sup>, Noura AIT LAYDI<sup>3</sup>, Mohammed BOUSLIKHANE<sup>4</sup>, Asma Fagrach<sup>5, 6</sup>, Siham FELLAHI<sup>7</sup>

*Clinique vétérinaire Tit Mellil<sup>1</sup>, Institut agronomique et vétérinaire Hassan II<sup>2</sup>, Veterinary Clinic Tit Mellil<sup>3</sup>, Institut agronomique et vétérinaire Hassan II<sup>4</sup>, <sup>5</sup>, Cabinet vétérinaire Dr. MOUAHID<sup>6</sup>, Institut agronomique et vétérinaire Hassan II<sup>7</sup>*

Avian reovirus (ARV) is a prevalent infectious agent that has the potential to cause respiratory and gastrointestinal illnesses in poultry, leading to substantial financial losses in the poultry sector. Until now, there have been no investigations conducted to examine the epidemiological status of ARV infections in Morocco. The aim of this study was to investigate the seroprevalence of ARV infections with respect to area, types of chickens (broiler breeder, and broiler), vaccination status, and age of chickens. A total of 826 serum samples were collected from 36 broiler and broiler breeder flocks, with 14 of them being unvaccinated, from six different regions of Morocco, namely Casablanca-Settat, Rabat-Salé-Kénitra, Tanger-Tétouan-Al Hoceïma, Oriental, Marrakech-Safi, and Fez-Meknès between 2021 and 2022. These serum samples were screened using a commercial indirect ELISA ARV antibody test kit. The study found that all tested flocks were positive for ARV-specific antibodies, indicating that the virus was present in these flocks. Out of the 826 serum samples tested, 782 were seropositive for ARV-specific antibodies. The overall prevalence of ARV seropositivity in breeder and broiler flocks was calculated to be 94.6% ± 0.78. To summarize, the current study provides evidence of the prevalent seropositivity of ARV in Morocco, suggesting a high interaction of the poultry industry in the country with ARV.

### **Correlation Study Exploring the Relationship Between Maternal Titer and Maternal Antibody Levels in Turkeys for Reovirus**

Evan VanBeusekom<sup>1</sup>, Elana Huong<sup>1</sup>, Andrew Sweet<sup>1</sup>

*Hendrix Genetics - Hybrid Turkeys LLC<sup>1</sup>*

Correlation Study Exploring the Relationship Between Maternal Titer and Maternal Antibody Levels in Turkeys for ReoVirus Evan VanBeusekoma, Elana Huongb, Andrew Sweetca University of Minnesota College of Veterinary Medicineb University of Guelph, Ontario Veterinary Collegec University of Guelph Turkey Arthritis Reovirus (TARV) is a serious disease with economic and welfare consequences. It is estimated that the economic impact of the disease can reach up to 33 million USD annually and more than 150 million birds have been affected over the last 5 years. TARV is now thought to have more economic and welfare effects than Mycoplasma infections. Vaccinations, heightened biosecurity, robust farm clean out, and serological monitoring have been used in efforts to help the turkey industry understand the mechanism of transmission. Reovirus serology of breeding hens are commonly used to both evaluate flock Reovirus status and at this time it is assumed that increased maternal antibody levels predict poult protection in the field. As such, vaccination of breeder hens has been utilized with some success by the turkey breeder industry to protect progeny from field challenge. However, most studies have not evaluated the direct relationship between a breeder hen's titer level and her direct progeny's maternal antibodies. This goal of this study was to compare the titers of specific breeder hens and their progeny's maternal antibody level with the use of trap nesting. Serum samples from 60 individually identified vaccinated breeder turkey hens were taken and eggs were collected during a two week period. All eggs from the two week collection period

were set and hatched together. All the progeny were individually identified and serum samples were collected at 3, 9, 16, 23, and 30 days of age. The progeny were bled until the maternal antibodies hit zero or until the end of the study at 30 days. All serum was analyzed utilizing the IDEXX reovirus ELISA kit, and Gmean and CoV values were recorded. Statistical analysis of the data is pending and will be reported upon completion.

### **Genotypic and pathological characterization of emerging avian reoviruses isolated from clinical cases in Georgia, USA**

Sujit Mohanty<sup>1</sup>, Islam Nour<sup>1</sup>, Julia Blakey<sup>1</sup>, Arun Kulkarni<sup>4</sup>

*USDA-ARS<sup>1</sup>, Georgia Poultry Laboratory Network<sup>4</sup>*

Avian orthoreovirus is an immunosuppressive pathogen that imposes critical economic issues to the poultry industry. For this study, pathogenic strains were obtained from Georgia Poultry laboratory Network for molecular characterization including genotyping, evolutionary analysis, and variation analysis of genomic migration patterns. Moreover, histopathological examination of different tissues and embryonic mortality were conducted to assess the probable virulence of these strains. Interestingly, the strains were found to be genotypically diverse since a single strain (22-806) was found belonging to the vaccine group genotype 1, whereas the other strains were found distributed within genotypes V (22-835, 22-087 and 23-272) and VI (22-861, 22-460 and 23-002). Evolutionary analysis showed the close relationship of the study strains to previously detected strains in the USA (K1600600, d = 0.029779) and Canada (D12, d = 0.00519 - 0.037245) in 2016 and 2014, respectively. Moreover, capsid proteins encoding genomic segments (M2 and S3) showed different migration patterns that may indicate their molecular diversity among the tested strains. Remarkably, the strain 22-806 showed a unique migration pattern in extra genomic segments including a spike protein encoding S1 and M1 segment. The birds from which these viruses were isolated showed varying histopathological manifestations such as focal necrotic enteritis in the small intestine, tenosynovitis, multifocal ulceration with fibrinoheterophilic crusting in tendons as well as multifocal necrotizing hepatitis in liver. When injected to the embryo, two reovirus strains (23-087 and 22-460) showed the earliest 100% mortality at two days postinfection. In addition, significant embryonic lesions were observed including mild to moderate hemorrhage, liver discoloration and stunting. Collectively, this study revealed genotypic divergence among the circulating field strains and the associated virulence indicated by various histopathological observations and high embryonic mortality. Besides, the current study emphasized the continuous emergence of novel strains from the vaccine strain that mandates the continuous monitoring programs for updating the vaccination regime to include the indigenous field isolates.

### **Proteomics of Avian Reovirus**

Telvin Harrell<sup>1</sup>, Sonsiray Alvarez-Narvaez<sup>1</sup>, Steven J Conrad<sup>1</sup>

*United States Department of Agriculture<sup>1</sup>*

Avian reovirus (ARV) is a double-stranded RNA virus that imposes a costly burden on poultryproducers. It primarily infects broiler and breeder chickens, via horizontal transmission routes, before 3 weeks of age. Birds infected with ARV can exhibit a wide range of symptoms,including subcutaneous hemorrhaging, atrophy of the spleen, bursa, and thymus,

gastrointestinal damage, respiratory complications, and tenosynovitis. A particularly concerning aspect of ARV is the possibility of subclinical infections; in which, the virus is present in the bird without the outward presence of clinical symptoms. This makes it difficult to assess the true scope of the viral burden and implement early and effective control measures. Controlling ARV primarily relies on two strategies: routine vaccination and biocontainment. However, there is a need for further research into the rising ARV virulence and ARV proteomics to better understand the scope of the problem. This report contributes to the general knowledge of ARV by providing valuable insights into its protein makeup. In this study ARV proteins were separated by polyacrylamide gel electrophoresis to assess their migration patterns and interactions. The proteins were subsequently analyzed by mass spectrometry (MS-MS) to confirm their amino acid sequence and assess the protein peptides detected. This data has been made publicly available in a protein database and will support further research endeavors focused on ARV proteomics, pathogenesis, and vaccine development.

## Salmonella

### **Cross-protection conferred by a live-attenuated *Salmonella* Enteritidis vaccine against *Salmonella* Heidelberg and *Salmonella* Infantis challenge**

Luiz Sesti<sup>1</sup>, Patricia Joaquim<sup>2</sup>, Facundo Balbiani<sup>3</sup>, Maria Laura Socas<sup>4</sup>, David Arenas<sup>5</sup>, Lucas Sara<sup>6</sup>, Jaime Sarabia<sup>7</sup>, Pascal Paulet<sup>8</sup>, Pablo Chacana<sup>9</sup>

*Ceva Animal Health*<sup>1</sup>, *INTA, Argentina*<sup>2</sup>, *INTA, Argentina*<sup>3</sup>, *INTA, Argentina*<sup>4</sup>, *INTA, Argentina*<sup>5</sup>, *Ceva Animal Health, Argentina*<sup>6</sup>, *Ceva Animal Health, France*<sup>7</sup>, *Ceva Animal Health, France*<sup>8</sup>, *INTA, Argentina*<sup>9</sup>

Paratyphoid *Salmonella* infection may cause enteric and systemic illness in humans and animals, and, in poultry production, these bacteria are considered quite relevant food-borne pathogens. Different serotypes are found in poultry worldwide. Besides *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST), other serotypes such as *Salmonella* Heidelberg (SH) and *Salmonella* Infantis (SI) show high prevalence in some countries. Vaccination is one of the main strategies to control *Salmonella* in the farms to improve the food safety and to reduce the use of antimicrobials. Live attenuated vaccines can be administered during the first days of life of chicks in layers and breeders to reduce the impact of non-typhoidal serotypes in the poultry industry. The aim of this study was to evaluate the protection conferred by a live SE vaccine (strain auxotrophic for adenine and histidine) vaccine in laying hens challenged either with SH or SI field strains. Day-old chicks were assigned randomly to vaccinated or unvaccinated groups. Birds from the vaccinated group (n=50) were immunized with Cevac<sup>®</sup> Salmovac at day 1, week 6 and week 13 by the oral route, while birds from the unvaccinated group (n=50) did not receive the vaccine. At week 16, birds from each group were divided into two subgroups and challenged with an infectious dose of SH (10<sup>8</sup> cfu/bird) or SI (10<sup>9</sup> cfu/bird). At 3, 5, 10 and 14 days post challenge (dpc), excretion of the pathogen was determined by individual cloacal swabbing and samples were enriched using tetrathionate broth and thereafter detection of the strain was done on XLD agar plates. In addition, at 5 and 14 dpc, five hens from each group were euthanized and enumeration of *Salmonella* was done in the cecal contents. SH excretion was significantly reduced in birds from vaccinated group at 3 and 5 dpc (both, p<0.0001) in comparison to the unvaccinated birds. SH strain could not be detected in the caeca from the



vaccinated birds analyzed on both sampling days. Conversely, SH recovered from all unvaccinated birds at 5 dpc and from 2/5 of the birds at 14 dpc. Similar significant reduction of the excretion was observed in vaccinated birds challenged with SI. Shedding of SI was significantly reduced in birds from vaccinated group at 3 (p=0.0031), 5 (p=0.0001), and 10 dpc (P=0.0079). SI could not be detected in ceca from vaccinated birds, but it was recovered from 4/5 of unvaccinated birds at 5 dpc and from 1/5 birds at 14 dpc. These results demonstrate that Cevac® Salmovac was able to significantly reduce SH & SI challenge strains replication in vaccinated birds. SH and SI belong to different antigenic serogroups (“group O:4, B” and “group O:7, C1”, respectively) than the SE vaccine strain (“group O:9, D1”).

### **Evaluating Salmonella Serotype Bias in Various Enrichment Protocols**

Katie Murray<sup>1</sup>, Doug Waltman<sup>2</sup>

*Georgia Poultry Laboratory Network<sup>1</sup>, Georgia Poultry Laboratory Network<sup>2</sup>*

Previous studies have shown that specific enrichment methods in combination with plating media can produce a bias towards specific Salmonella serotypes for carcass rinse samples. A potential bias has not been investigated in the sample types submitted for monitoring of poultry house and hatchery environments. For this study, multiple types of poultry house and hatchery samples were chosen from a variety of companies for testing. Each sample was then split for testing with buffered peptone water (BPW) or tetrathionate (TT). The BPW enrichment was then inoculated into Rappaport Vassiliadis (RV), Modified Semi-solid Rappaport Vasiliadis (MSRV), and Tetrathionate Hanja (TTH). The TT enrichment was inoculated into MSRV. All enrichment protocols including direct TT were plated onto 4 media types including xylose lysine tergitol 4 (XLT4), brilliant green with novobiocin (BGN), xylose lysine deoxycholate (XLD), and Brilliance (BRIL) agar. Up to 5 Salmonella suspect colonies were then struck onto MacConkey (MAC) agar for serogrouping via agglutination testing and a representative from each serogroup was then moved forward for antisera-based serotyping. By comparing the results obtained for each enrichment method it can be determined if a potential serotype bias is present.

### **A Novel Approach to the Salmonella ISR Genotyping of Field Isolates Using Nanopore Sequencing Technology**

Scott Callison<sup>1</sup>, Scott Reed<sup>1</sup>, Julia McElreath<sup>1</sup>, Derek Moormeier<sup>1</sup>, John Goza<sup>1</sup>, Fraser Combe<sup>1</sup>, John El-Attrache<sup>1</sup>

*Ceva Animal Health<sup>1</sup>*

Salmonella serotyping is an integral part of surveying potentially pathogenic serovars within the poultry industry. Microbiological culture and serotyping through lipopolysaccharide O-antigen and flagellar H antigen anti-sera serves as the gold-standard. These methods require five to seven days of laboratory work with subjective interpretations of the results when differentiating Salmonella serovars. The advent of Salmonella Intergenic Sequence Ribotyping (ISR) provides a targeted PCR approach by amplifying the Salmonella intergenic sequence enabling genotyping of Salmonella through an ISR reference database. Our team has employed a novel ISR PCR barcoding assay utilizing nanopore sequencing. To test the utility of the assay, nucleic acid from known, cultured, Salmonella field isolates were extracted and sent through our targeted ISR barcode PCR using a 96-well plate

format. In-house bioinformatic pipelines parceled reads according to sequence labels for Salmonella genotyping to occur in less than 24 hours of wet-lab work. This approach can allow for a faster, high throughput, Salmonella typing assay to be developed in the future.

### **Evaluation of different inactivated vaccines to control Salmonella on the performance and productivity of laying birds up to 37 weeks of age**

Eva Hunka<sup>1</sup>, Eric Andrade Culhari<sup>2</sup>, Igor Vellano<sup>3</sup>, Ellen Fukayama<sup>4</sup>, Rafael Neme<sup>5</sup>, Udi Ashash<sup>6</sup>

*Phibro Animal Health Corporation<sup>1</sup>, Phibro Animal Health Corporation<sup>2</sup>, Phibro animal health corporation<sup>3</sup>, BITA - Busca Inteligente em Tecnologia Animal<sup>4</sup>, BITA - Busca Inteligente em Tecnologia Animal<sup>5</sup>, Phibro Animal Health Corporation<sup>6</sup>*

Inactivated Salmonella vaccines are used in layers and breeders, at the pullet stage, with the aim of inducing an immune response, which should reduce the chances of salmonella infection and vertical transmission. Current Inactivated Salmonella vaccines are oil emulsion bacterins or an aluminum hydroxide bacterins. The use of multiple serovars vaccines can offer partial cross-protection for serotypes from different antigenic groups that have some similarities in their antigenic formula, which is why their use is becoming increasingly frequent. One of the negative aspects of using these vaccines is the risk of injury due to the inflammatory reaction at the site of injection that can lead to production losses. This work aims to compare the performance until peak egg production of commercial layers, vaccinated with 4 different commercial salmonella bacterins. 1,200 Lohmman Brown – Lite lineage chicks of the were distributed to 4 treatment groups: T1 - S. enteritidis (2 strains), S. typhimurium and S. infantis (0.30 ml – SALMIN PLUSR), T2 - S. Enteritidis, S. Typhimurium and S Infantis (0.50 ml – aluminum hydroxide), T3 - S. enteritidis and S. typhimurium (0.50 ml- oil emulsion) and T4 - S. enteritidis and S. typhimurium (0.30 ml – oil emulsion). All groups were vaccinated at 10&14 weeks of age by IM injection. The overall management and health programs was according to the nutritional and health requirements for each stage of the bird's life. The parameters observed were: live weight (g), feed consumption (g/bird/day), egg production (%), egg weight (g) and mortality. All data was subjected to homogeneity and normality assessment , and statistically analyzed using SAS® Studio software (2022), and the means compared using the Tukey test at 5% significance. The average weight of the birds, as well as daily consumption, did not differ significantly between the treatments studied and was in line with what is recommended by the lineage guidelines. Regarding productivity and average egg weight, no significant differences were found and were in accordance with the lineage guidelines. The type of vaccine also did not impact mortality, being: 4.00; 2.33; 4.33 and 3.00% for treatments T1, T2, T3 and T4, respectively, until the 32nd week. In view of this, it can be concluded that the use of an inactivated vaccine with an oily emulsion containing 4 bacterins (SALMIN PLUS) does not impact the performance of birds during the reproduction phase, in the productive phase until peak production, nor does it interfere with accumulated mortality of birds. poultry, proving that it is a safe and important product for Salmonella control.

### **Correlation between titers obtained in ELISA and Widal test for serological analysis of Salmonella groups B, C1 and D.**

Eva Hunka<sup>1</sup>, Jose Emilio de Menezes Dias<sup>2</sup>, Eric Andrade Culhari<sup>3</sup>, Udi Ashash<sup>4</sup>

*Phibro Animal Health Corporation<sup>1</sup>, Instituto Federal Catarinense, Concordia SC, Brasil<sup>2</sup>, Phibro animal health corporation<sup>3</sup>, Phibro animal health corporation<sup>4</sup>*

The measurement of immune responses induced by vaccines can be carried out using the Enzyme-linked Immunosorbent Assay (ELISA) technique, in which the levels of antibodies (IgG/IgY and IgM) circulating (in serum) to a given pathogen are determined, including Salmonella (Mirhosseini et al., 2017). However, today there is a limited number of ELISA kits for serological assessment to Salmonella, basically limited to commercial kits for group D, which often makes monitoring of Salmonella vaccination programs unfeasible. The Widal test has been used for many years to diagnose Salmonella spp. in humans by measuring serum antibodies, using specific antigens in this methodology (ex.: somatic antigen, flagellar antigen, Vi, etc.). Although it is an old technique, and with lower sensitivity and specificity than the ELISA test, it can be used to demonstrate a correlation between serological responses for different serogroups. The objective of this study was to compare the results obtained through the ELISA technique for Salmonella group D, considering it as the gold test for monitoring the vaccination program, with the results of the Widal test for Salmonella groups B, C1 and D in search for correlations and agreements between the tests. For this study, 20 blood samples were collected from a batch of layers breeders from the Hy Line® lineage raised under commercial conditions, at ages: 15, 20, 25 and 29 weeks, totaling 80 samples. The birds received one dose (15 weeks) of the trivalent inactivated Salmin Plus® vaccine. The samples were submitted to the ELISA technique (Biochek®) for Salmonella of group D (standard) and to the Widal technique for groups B, C1 and D. The results were subjected to descriptive statistics analysis and subsequently, to correlation analysis of Spearman to determine whether there was a relationship between the variables in addition to linear regression (Jamovi®). To assess agreement, we used the Kappa coefficient, where samples from group 1 were considered positive in ELISA, and in Widal, samples equal to or greater than 1:160. All data had a strong and positive correlation, ranging from 0.829 to 0.975, and a high level of significance ( $p < 0.001$ ), highlighting the relationship between the data. The agreement assessment (Kappa coefficient) ranged from 0.62 to 0.85, indicating substantial agreement of the results. A strong correlation and substantial agreement were observed between the results of the ELISA and Widal techniques evaluated. Based on these data, we can infer that the ELISA results can be used as serological monitoring of batches vaccinated with inactivated vaccine against Salmonella groups B, C and D. Key words: Salmonella, ELISA, Serology, Widal test

### **Live Salmonella Vaccine Compatibility with Competitive Exclusion Solution**

Elizabeth Krushinskie<sup>1</sup>, F. Barbe<sup>2</sup>, A. Sacy<sup>3</sup>, A. Nozeran<sup>4</sup>, S. Castagnos<sup>5</sup>, P. Creach<sup>6</sup>, T. Langlade<sup>7</sup>, V. Pain<sup>8</sup>

*Food Safety Risk Management Consulting LLC<sup>1</sup>, Lallemand SAS<sup>2</sup>, Lallemand SAS<sup>3</sup>, Lallemand SAS<sup>4</sup>, Ceva Sante Animale<sup>5</sup>, Ceva Sante Animale<sup>6</sup>, Ceva Sante Animale<sup>7</sup>, SOCSA Elevage<sup>8</sup>*

Introduction In poultry production systems, vaccination has become a complementary strategy to control Salmonella prevalence with efficacy reported on the reduction of pathogenic Salmonella in the feces, tissues, and eggs of chickens. Due to the specificity of the strains targeted by the vaccines, they are often used in conjunction with alternative microbial solutions to achieve the best results along with strict biosecurity measures. The objective of this study was to demonstrate the compatibility of a competitive exclusion product (AviGuard, Lallemand) with live Salmonella vaccine. The recovery in the liver and ceca of broiler chicks of the Salmonella strain used in the vaccine was

used to demonstrate vaccine uptake. Materials & Methods Forty (40) Ross 308 day-old chicks were dosed by gavage at day 1 (0.5 mL/chick) with live attenuated Salmonella vaccine (Ceva Salmovac, Ceva:  $8 \times 10^8$  cfu/chick) and with either water (control:  $n = 20$ ) or combined with AviGuard ( $n = 20$ ). The birds were humanely euthanized on day 4 and live body weight, liver weight, and ceca weight were individually measured. The number of birds positive for Salmonella vaccine strain (*S. enteritidis*) in liver and ceca samples was determined for each group and Salmonella counts were additionally performed in 5 chicks per group and expressed as log<sub>10</sub>/g of tissue. Live body weight, liver weight, ceca weight, and Salmonella count in liver and ceca were analyzed by T-test, comparing the 2 groups. Moreover, Pearson correlations were analyzed between the different parameters. All statistical analyses were performed with IBM SPSS Statistics 26.0. Significance level was set at 5% ( $P \leq 0.05$ ) and statistical trends were reported at 10% ( $P \leq 0.1$ ). Results All birds from the control and the AviGuard groups were found to be positive for the translocation of the Salmonella vaccine strain to the liver and to the ceca. Salmonella counts were similar, not statistically different, between the two groups in the liver (control: 4.42 log<sub>10</sub>/g liver; Aviguard: 4.68 log<sub>10</sub>/g liver) and in the ceca (control: 6.94 log<sub>10</sub>/g ceca; Aviguard: 6.84 log<sub>10</sub>/g ceca). Discussion The competitive exclusion solution tested in this study had similar Salmonella vaccine strain positivity in the liver and the ceca compared to control group (vaccine alone) showing no interference in the ability of the Salmonella vaccine to translocate to the liver and to colonize the ceca. This product is then compatible with live Salmonella vaccine. Moreover, it is important to notice that this product, Aviguard, is able to prevent the translocation of wild Salmonella strains and other opportunistic pathogens, but allows the translocation of the Salmonella vaccine strain because it is attenuated and does not induce inflammation. This solution, by reducing and modulating the inflammatory response, prevents wild pathogenic strains from multiplying and colonizing the host while retaining vaccine strain efficacy.

### **Evaluation of an Alpha-Monoglyceride Consumed in Broiler Chickens**

Chad Malinak<sup>1</sup>, Luis Gomez<sup>2</sup>, Blair Telg<sup>3</sup>, Ana Maria Villegas<sup>4</sup>, Ashley Hallowell<sup>5</sup>, Charles Hofacre<sup>6</sup>

*Phibro Animal Health Corporation*<sup>1</sup>, *Phibro Animal Health Corporation*<sup>2</sup>, *Phibro Animal Health Corporation*<sup>3</sup>, *Phibro Animal Health Corporation*<sup>4</sup>, *Phibro Animal Health Corporation*<sup>5</sup>, *Southern Poultry Research Group*<sup>6</sup>

Alpha-monoglycerides are short and medium-chain fatty acids linked to the first position of a glycerol molecule by an ester bond. Alpha-monoglycerides are pH independent molecules that are active throughout the entire intestinal tract. Research has shown them to have antimicrobial properties in both acidic and neutral environments. This study was conducted in broiler chickens to determine the effectiveness of a dietary addition of a propriety blend of alpha-monoglycerides when used in conjunction with a coccidiosis vaccine to reduce colonization of salmonella, as well as, impact growth performance parameters and intestinal morphometrics.

## **Vaccinology**

### **HVT-ILT-IBD: A Double Recombinant HVT-Based Vaccine for Protection Against IBDV and ILTV Plus Marek's Disease Virus**

Paula Dyas<sup>1</sup>, Stephanie Cook<sup>1</sup>, Cynthia Pietsch<sup>1</sup>, Kylee McCord<sup>1</sup>, Alicia Brown<sup>1</sup>, Henk Pouwels<sup>1</sup>, Linda Gergen<sup>1</sup>, Julio Cruz-Coy<sup>1</sup>, Mohamad Morsey<sup>1</sup>, Brooke Ledesma<sup>1</sup>

Infectious laryngotracheitis virus (ILTV) and Infectious bursal disease virus (IBDV) are two of the most important pathogens afflicting the poultry industry and infections with these viruses result in substantial economic losses worldwide. First-generation recombinant HVT-based vaccines such as HVT-IBD and HVT-ILT have proven their utility in the control of diseases and production losses caused by these two viruses. Previous attempts to combine these monovalent vaccines for dual protection indicated that these vaccines may interfere with each other. To provide simultaneous protection against IBDV and ILTV from one viral vector, we have constructed a double recombinant HVT vaccine that concurrently express the VP2 protein from IBDV and two proteins from ILTV, gD+gI. Construction of the new Bursal Disease-Infectious Laryngotracheitis-Marek's Disease Vaccine, Serotype 3, Live Marek's Disease Vector, HVT-ILT-IBD and experiments describing the characterization for stability in cell culture and other parameters related to animal safety and environmental impact are described. A set of vaccination/challenge experiments to further assess the protection afforded by this recombinant virus when SPF birds were challenged with virulent viruses were also conducted and the extent of protection was evaluated following in-ovo and subcutaneous routes of administration. These data provide support for the safety and efficacy of HVT-ILT-IBD vaccine for use by the in-ovo and subcutaneous routes.

**Induction of protective mucosal immunity against avian coronavirus via nanoparticle-synchronized antigen and adjuvant delivery to the chicken Harderian gland**

Hui-Wen Chen<sup>1</sup>, Che-Ming Hu<sup>2</sup>, Bing-Yu Yao<sup>3</sup>, Shu-Yi Lin<sup>4</sup>

*National Taiwan University*<sup>1</sup>, *Academia Sinica*<sup>2</sup>, *Academia Sinica*<sup>3</sup>, *National Taiwan University*<sup>4</sup>

Mucosal immunization is a convenient and cost-effective route for vaccinating large numbers of chickens. Potent mucosal immunity protects chickens from infection through mucosal sites of the ocular and respiratory tissues and blocks the spread of pathogens by aerosols. Compared to rapidly degraded soluble antigens, mucosally administered nanoparticles accumulate more effectively in mucosal lymphoid tissues through engulfment and transportation by migratory dendritic cells lining the mucosal sites. In this study, polymeric hollow nanoparticles co-encapsulating avian coronavirus recombinant receptor-binding domain (rRBD) and CpG ODN for synchronized adjuvant and antigen delivery (rRBD-CpG-NP) were developed and administered oculonasally to chickens to evaluate mucosal immunity stimulation. For the nanoparticle vaccine group, antigen-specific IgA and IgG levels in tears were elevated 3-fold and 6-fold, respectively, compared to those in the tears of the rRBD plus free CpG ODN group. Through immune-related gene expression screening and antibody-secreting cell staining, enhanced B-cell differentiation and proliferation in the Harderian gland were noted. After challenge with infectious bronchitis virus, the rRBD-CpG-NP-immunized chickens showed ameliorated clinical signs. In the respiratory system, the rRBD-CpG-NP vaccine protected against tracheal ciliary damage and reduced viral replication in the lungs. Immunization with the nanoparticle vaccine also decreased systemic infection, thereby reducing renal lesions and cloacal shedding. In conclusion, this is the first study to analyze the immune response of the chicken Harderian gland elicited by a non-live subunit vaccine. The synchronized antigen and adjuvant delivery achieved in this study enabled effective mucosal immunity against avian coronaviruses.

## **Differences on Broilers Productive Performance Parameters Between Different Vaccination Protocols Against IBD and ND as Measured by a Statistical Analysis Model in Colombia**

Camilo Andres Medina Santos<sup>1</sup>, FABIAN QUINTERO<sup>2</sup>

The inclusion of recombinant vaccines has become a necessity for the global poultry industry, and the Colombian context is increasingly aligned with this premise, due to handling and logistics conditions. Infectious Bursa Disease and Newcastle continue to be a concern in the Colombian countryside. Controlling them can facilitate the management of other diseases, such as Infectious Bronchitis that affects the respiratory system. A statistical analysis was carried out comparing flocks before and after including an HVT-ND-IBD in three Colombian companies, located in Cundinamarca, Boyacá and Antioquia. Forty-five flocks 3,526,769 broilers with zootechnical and serological data and 98 flocks 7,167,959 broilers only with zootechnical data were used. Results showed a statistically significant difference in mortality, which may indicate an improved health status with the inclusion of the recombinant HVT-ND-IBD vaccine. This is supported by the statistical multivariate model, where it is adjusted for confounding variables. In addition, when serologies are included, mainly the variability of titers, a better performance of feed conversion was observed, which may also indicate that by having sanitary control the performance may be improved with the inclusion of the HVT-ND-IBD recombinant vaccine. This analysis supports the literature, where it is evident that the use of this biological in farms that maintain an adequate biosafety status, zootechnical and health results are obtained that express the potential of high-performance birds.

## **Comparison of two vaccination programs against fowl adenovirus serotype 8b (FAdV-8b) in broiler breeders in Peru**

Bruno García<sup>1</sup>, Claudia Carranza<sup>2</sup>, Luiz Sesti<sup>3</sup>

*Ceva Animal Health Peru<sup>1</sup>, Ceva Animal Health Peru<sup>2</sup>, Ceva Animal Health Latin America<sup>3</sup>*

Fowl Adenovirus (FAdV) may cause different clinical situations and syndromes in poultry production worldwide. There are 12 serotypes belonging to 5 different viral groups which are responsible for the different disease outcomes seen in the field. FAdV is a non-enveloped double-stranded DNA that can persist for long periods in farms and disseminates via both vertical and horizontal transmission. Vertical transmission from broiler breeders to progeny is, most of the times, the way of entrance of the virus in each commercial broiler production system. And control of vertical transmission is accomplished through vaccination of broiler breeder flocks. In Peru, the most prevalent serotype for nearly 30 years was the serotype 4 (FAdV-4) and therefore, most of vaccination programs were directed against this serotype. Around 10 years ago serotype 8b has established itself as the most prevalent virulent serotype in the industry. As a result of this change, many poultry companies have added, to the broiler breeders' vaccination program, 2 or more doses of inactivated vaccines against FAdV-8b. Here, we report a field trial involving 90,000 broiler breeders which was carried out in two different farms in the same company. In one of the farms breeders were vaccinated with the standard program of oil-emulsion inactivated vaccines at 6 weeks (FAdV-4) and at 14 and 20 weeks (multivalent vaccine containing FAdV-4,7,8a,8b). In the second farm, the multivalent vaccine applied at 14 and 20 weeks of age was replaced by another commercial oil-emulsion containing only FAdV-8b (Cevac® IBH 8K). Blood sampling for serology was carried out in the breeders at 14, 18, 20 and 24 weeks of age. All blood samples were analyzed with a commercial Elisa kit (BioCheck – detects

antibodies against group 1 FAdV) and with an in-house virus-neutralization assays using FAdV-8a or FAdV-8b antigens (Ceva Phylaxia, Hungary). Seroconversion results from both broiler breeders' farms will be reported and compared.

### **Efficacy of different inactivated vaccines against infection with the B serovar variant of *Avibacterium paragallinarum* from Argentina in laying hens**

Martina Dardi<sup>1</sup>, Y. Huberman<sup>2</sup>, L. Mendez<sup>3</sup>, A. Mendez<sup>4</sup>, J. Lomonaco<sup>5</sup>, A. Gulle<sup>6</sup>, C. Gulle<sup>7</sup>, M. De-Soler-Pinart<sup>8</sup>, M. Baratelli<sup>9</sup>, J.L. Criado<sup>10</sup>

*Laboratorios HIPRA S.A.<sup>1</sup>, Instituto de Innovación para la Producción Agropecuaria y Desarrollo Sostenible, Balcarce, Argentina<sup>2</sup>, Instituto de Innovación para la Producción Agropecuaria y Desarrollo Sostenible, Balcarce, Argentina<sup>3</sup>, Instituto de Innovación para la Producción Agropecuaria y Desarrollo Sostenible, Balcarce, Argentina<sup>4</sup>, Instituto de Innovación para la Producción Agropecuaria y Desarrollo Sostenible, Balcarce, Argentina<sup>5</sup>, Instituto de Innovación para la Producción Agropecuaria y Desarrollo Sostenible, Balcarce, Argentina<sup>6</sup>, Instituto de Innovación para la Producción Agropecuaria y Desarrollo Sostenible, Balcarce, Argentina<sup>7</sup>, Hipra Scientific S.L.U., Amer, Spain<sup>8</sup>, Hipra Scientific S.L.U., Amer, Spain<sup>9</sup>, Laboratorios Hipra S.A., Amer, Spain<sup>10</sup>*

To assess the efficacy of two inactivated vaccines against *Avibacterium paragallinarum* serovar B variant infection in laying hens. Day-old pullets were allocated into 5 groups (G): G1 received CORIPRAVAC® (HIPRA), containing serovar A, B and C, at 8 and 12 weeks old. G2 received the same vaccine through an early vaccination plan at 5 and 12 weeks old. G3 received a commercial vaccine against infectious coryza, which contains in its formulation also a serovar B variant strain, apart from serovar A, B and C. Chickens in G4 were not vaccinated and were used as a positive control, while G5 was also not vaccinated and served as a negative control. At 25 weeks of life, chickens from G1 to G4 were challenged with a B variant of *Av. paragallinarum* through inoculation into the infraorbital sinus. Clinical signs were monitored daily up to 5 days post-challenge, while body weight was monitored until 26 weeks of age. Bacteriological analyses were conducted on both inoculated sinuses and non-inoculated sinuses on day 5 post-challenge to determine the presence of *Av. paragallinarum*. The interpretation of clinical signs involved grading on a scale from 0 to 4, depending on the severity of conjunctivitis, swelling of the periorbital area and paranasal sinuses. Grades 0 and 1 were considered negative, while grades 2, 3, and 4 were considered positive. For the evaluation of results, a bird was considered sick when showed clinical signs equal to or greater than 2 and/or if it was bacteriologically positive in one or both paranasal sinuses. There were no significant differences in body weight between vaccinated and unvaccinated birds. On day 2 post-inoculation, the highest number of birds exhibiting clinical signs was recorded, gradually decreasing over the following days. Unvaccinated groups had significantly ( $\chi^2$ ,  $p < 0.05$ ) more birds with clinical signs compared to vaccinated birds, regardless of the immunization plan. No statistically significant differences were found between G1 and G3 although both had significantly fewer birds with clinical signs compared to the group with the early vaccination plan G2. Comparing the results of the non-inoculated sinuses, no differences were found among vaccinated birds in G1, G2, and G3, while significantly more infected sinuses were found in the non-vaccinated birds. In conclusion, the three serovars vaccine demonstrated efficacy in significantly reducing clinical signs, both with the conventional vaccination plan at 8 and 12 weeks of age as well as with early vaccination starting at 5 weeks of age, compared

to non-vaccinated birds. It also showed effectiveness in reducing the presence of *Av. paragallinarum* in the infraorbital sinuses following the experimental challenge with a B serovar variant. This indicates that the three serovars vaccine is effective against the B serovar variant of *Av. paragallinarum*, specifically demonstrating its efficacy in protecting against clinical signs associated with this particular serovar.

### **Correlation between the spray dye levels on the body of broilers and the corresponding viral load in the trachea 6 days after vaccination against Infectious Bronchitis Virus**

Jose Emilio Dias<sup>1</sup>, Andre Marca<sup>2</sup>, Mario Luiz Manichi Junior<sup>3</sup>, Eduardo Rother<sup>4</sup>, Jessica Caroline Staffen Wammes<sup>5</sup>, Leandro Bianchet<sup>6</sup>, Eva Laurice Pereira Cunha Hunka<sup>7</sup>

*Phibro Animal Health, Instituto Federal Catarinense, Concórdia – SC, Brazil<sup>1</sup>, DVM<sup>2</sup>, DVM<sup>3</sup>, Phibro Animal Health<sup>4</sup>, Phibro Animal Health<sup>5</sup>, Phibro Animal Health<sup>6</sup>, Phibro Animal Health<sup>7</sup>*

Vaccination with live vaccines against Infectious Bronchitis Virus (IBV) variants has been shown to be an effective and useful tool to protect chickens in the first weeks of life. The success of the vaccination program depends heavily on the vaccination process. Most of the chicken producers in Brazil vaccinate their birds against IBV in the hatchery (day-old chick) and the primary way to monitor the effectiveness of the vaccine application is by the presence of dye in the bird's face, especially in the eyes, nostrils, and tongue. However, many producers correlate the efficiency of the process with the dye level on the bird's body. The aim of this study was to seek correlation between the level of spray dye present on the body of the birds and the vaccine viral load in the trachea after vaccination. Samples were collected from five commercial broilers flocks in the Southern Region of Brazil. Commercial day-old chicks in southern Brazil were vaccinated against IBV GI-23 lineage vaccine (TABic® IBVAR206), by spray with a red dye in the hatchery. Tracheal samples were collected individually from 5 birds per flock, totaling 25 tracheal samples, from 6-day-old chickens. These samples were submitted to the RT-qPCR technique, using specific primers for strains of the GI-23 lineage, based on the glycoprotein S1 gene. At the time of sampling birds were randomly selected for a dye intensity test. A score from 1 to 3 was created according to the level of dye. The levels were: 1 (head dyed), 2 (head and back dyed), and 3, (head, back and wings dyed). The PCR and dye scoring results were submitted to the Spearman's correlation matrix. Of the 25 PCR samples, 24 (96%) were positive for the vaccine strain of the GI-23 lineage. Among the positive samples, the average cycle threshold (CT value) results were 24.6 ranging from 17.9 to 30.3. No correlation was identified between the results of CT value and dye level in the broilers bodies ( $r=0.047$ ;  $p=0.829$ ). These results suggest that it is not possible to associate the level of dye present on the body of the birds (head, back and wings) with good or poor vaccination. Monitoring the vaccination process in the hatchery through the visualization of the spray dye in the eyes, tongue, and nostrils, and the evaluation of the vaccine "take" (by RT-qPCR), continues to be an important tool for the success of an IBV vaccination program.

### **Evaluation of vaccine take in commercial broilers flocks vaccinated with a homologous vaccine against Infectious Bronchitis Virus GI-23 lineage in Brazil**

Jose Emilio Dias<sup>1</sup>, Andre Marca<sup>2</sup>, Daiane Horn<sup>3</sup>, Daniel Dalla Costa<sup>4</sup>, Maurício Braga Puccinelli<sup>5</sup>, Vinicius Duarte<sup>6</sup>, Leandro Bianchet<sup>7</sup>, Eduardo Rother<sup>8</sup>, Eva Laurice Pereira Cunha Hunka<sup>9</sup>



*Phibro Animal Health, Instituto Federal Catarinense, Concórdia – SC, Brazil<sup>1</sup>, DVM<sup>2</sup>, Universidade Federal do Paraná, Palotina, PR - Brazil<sup>3</sup>, DVM<sup>4</sup>, DVM<sup>5</sup>, Universidade Federal de Santa Maria, Santa Maria, RS - Brazil<sup>6</sup>, Phibro Animal Health<sup>7</sup>, Phibro Animal Health<sup>8</sup>, Phibro Animal Health<sup>9</sup>*

Infectious Bronchitis (IB) is a highly contagious viral disease, causing heavy economic losses in the world poultry industry. The Infectious Bronchitis Virus (IBV) belongs to the Gammacoronavirus genus, and it has high capacity for horizontal dissemination. Its main control tool is vaccination with live vaccines. In 2022, IBV GI-23 (Variant 2) lineage was identified by PCR in commercial broiler flocks in Brazil. The presence of this strain was highly correlated with mortality from primary or secondary causes, and an increase in condemnations at the slaughterhouse due to airsacculitis. In 2023, vaccination with a homologous live vaccine belonging to the GI-23 lineage, started with the aim of controlling this new variant present in the country. The purpose of this study was to evaluate the efficiency of vaccination carried out in the hatchery, based on the detection of the vaccine virus in the trachea of vaccinated commercial broiler chickens originating from different companies in the Southern Region of Brazil. Day-old chicks were vaccinated in the hatchery with the IBV GI-23 lineage vaccine, TABIC® IBVAR206 by coarse spray 104.1 EID50/dose. Tracheal samples were collected at 6 days of age, individually from 5 birds per flock, totaling 120 samples. These samples were submitted to a RT-qPCR analysis, using specific primers for strains of the GI-23 lineage based on glycoprotein S1. Of the 120 samples collected, 115 (96%) were positive for the vaccine strain of the GI-23 lineage. Among positive samples, the average cycle threshold (CT value) results were 23.6, ranging from 12.9 to 33.3. The average quantification result was 2.05x10<sup>7</sup> copies/mL, varying between 135 and 5.97x10<sup>8</sup>. These results demonstrate good vaccination efficiency, since at least 95% of birds are expected to be positive at 6 days post-vaccination. In addition, the CT value results demonstrate the presence of a good viral load in the trachea of vaccinated birds.

### **Monitoring of a rHVT vaccine against Marek's disease and Avian Influenza H5 in Broiler Breeders**

Bruno García<sup>1</sup>, Claudia Carranza<sup>2</sup>, Luiz Sesti<sup>3</sup>

*Ceva Animal Health Peru<sup>1</sup>, Ceva Animal Health Peru<sup>2</sup>, Ceva Animal Health Latin America<sup>3</sup>*

In November 2022, Peru reported its first case of Highly Pathogenic Avian Influenza (HPAI) H5. The country immediately declared an emergency and approved the use of vaccines for birds with longer lifespans. Out of the various vaccines used over the year, the vector vaccine containing the H5 gene stood out because it is extensively used from day one by major poultry companies. This preference is due to its ability to protect against different virus types, trigger both cellular and humoral immune responses, and allow for the use of the DIVA (Differentiating Infected from Vaccinated Animals) strategy, among other advantages. Studies have shown that birds vaccinated with the recombinant HVT-H5 (rHVT-H5) vaccines shed significantly less virus than unvaccinated birds when they become infected by field HPAI viruses. While most research has been conducted under laboratory conditions, it is crucial to observe these vaccinated birds in the field to ensure that the Avian Influenza virus is absent. In the field, birds face various diseases, and their immune responses can be affected by additional vaccines they receive. Mass vaccination in the field is also more challenging compared to individual vaccination in a lab setting. Hence, it is important to utilize tools that can monitor large bird populations and support the DIVA strategy. In our trial, we examined 135,000 broiler breeders across three different farms belonging to the same company. Each bird was vaccinated with the

vector rHVT-H5 vaccine (Vectormune® HVT AIV) via a subcutaneous injection on the first day. We collected spleen samples at 28 and 35 days to perform RT-PCR tests to confirm the vaccine's effectiveness. Additionally, we conducted blood tests at 4, 8, 12, 16, and 20 weeks using a commercial Elisa kit (IDVET-H5) that detects antibodies against the specific H5 gene found in the vaccine. Because the birds also received an oil-emulsion inactivated vaccine at 4, 12, and 20 weeks, we tested the blood samples with another Elisa kit (IDvet-NPS) to identify antibodies against the virus's nucleoprotein (NP). This study will enhance our understanding of the tools available for monitoring Avian Influenza in farm conditions and will serve as a reference point for future field investigations.

### **Effects of homologous vaccination against IBV GI-23 lineage on tracheal lesion, serology, antibiotic therapy, mortality, and airsacculitis condemnation in commercial broiler flocks in Brazil**

Daiane Horn<sup>1</sup>, Jose Emilio de Menezes Dias<sup>2</sup>, Vinicius Duarte<sup>3</sup>, Eduardo Rother<sup>4</sup>, Eva Laurice Pereira Cunha Hunka<sup>5</sup>, Udi Ashash<sup>6</sup>

*Universidade Federal do Paraná, Palotina - PR, Brazil<sup>1</sup>, Phibro Animal Health; Instituto Federal Catarinense, Concordia, SC - Brazil<sup>2</sup>, Universidade Federal de Santa Maria, Santa Maria - RS, Brasil<sup>3</sup>, Phibro Animal Health<sup>4</sup>, Phibro Animal Health<sup>5</sup>, Phibro Animal Health<sup>6</sup>*

Infectious Bronchitis remains a source of economic losses for the poultry industry worldwide. Infectious Bronchitis Virus (IBV) strains belonging to the GI-23 lineage (Variant 2) were identified in Brazil since 2022. The use of a homologous vaccine to control this disease started in 2009 when a vaccine was developed from the IS/1494/06 strain, an Israeli pathogenic isolate. IBV belonging to the GI-23 lineage is currently circulating in many parts of the world including the Middle East, Turkey, Russia, Gulf States, Iran, North Africa, Baltic States, Poland and recently also in Brazil. The aim of this study was to evaluate the effects of homologous vaccination against IBV GI-23 lineage on histopathological lesions, serology, antibiotic use, mortality, and condemnations results in commercial broiler flocks in Brazil. Twenty commercial broiler flocks were randomly chosen from a company located in the State of Paraná (largest chicken meat producer in Brazil), where the challenge by IBV field strains of GI-23 lineage was previously proven. These flocks were monitored for two cycles. Cycle 1 was before the introduction of the homologous vaccine from the GI-23 lineage. Their vaccination program consisted of: Massachusetts (GI-1) and BR-1 (GI-11). Cycle 2 was after the introduction of the homologous vaccine. Their vaccination program consisted of: Massachusetts (GI-1), BR-1 (GI-11) and TABic® IBVAR206 (GI-23). These 2 cycles totaled 40 flocks (1,060,342 birds). At 40 days of age, 18 serum samples were collected for an ELISA serological test for IBV. At the same age, 5 tracheal samples were collected per flock for histopathological analysis and measurement of the degree of injury identified in the trachea. Mortality, condemnations for airsacculitis and use of antibiotic therapy (mg/kg) data from these flocks were also compiled. Data were subjected to the Kruskal-Wallis test followed by mean comparison using the DSCF test, with results of  $P \leq 0.05$  considered significant. Flocks vaccinated with the homologous GI-23 vaccine had significantly lower mean antibody titers (1579) than flocks without the homologous vaccine (3197). These flocks also had less necrosis and epithelial desquamation in their trachea at 40 days compared to flocks without the homologous vaccine. No significant difference was identified, however a 30% reduction in mortality was shown in flocks vaccinated with the homologous GI-23

vaccine (P=105). A significant reduction (P>0.001) in airsacculitis condemnations was identified in flocks vaccinated with the homologous GI-23 vaccine (0.98%) when compared to flocks without the homologous vaccine (4.15%). No significant difference was identified although a 71% reduction in antibiotics usage (mg/kg) was shown in flocks vaccinated with the homologous GI-23 vaccine (P=102). After the use of the GI-23 homologous vaccine, there were reductions in infection pressure, antibody titers, tracheal injuries, and condemnations due to airsacculitis.

### **Investigation of procedures and water quality impacting vaccine effectiveness in egg layers in Alberta**

Muhammad Farooq<sup>1</sup>, Faizal Abdul Careem<sup>2</sup>, Sylvia Checkley<sup>3</sup>, Awais Ghaffar<sup>4</sup>, Susantha Gomis<sup>5</sup>, Frank Marshall<sup>6</sup>

*University of Calgary<sup>1</sup>, University of Calgary Faculty of Veterinary Medicine, Calgary, Canada<sup>2</sup>, University of Calgary Faculty of Veterinary Medicine, Calgary, Canada<sup>3</sup>, University of Calgary Faculty of Veterinary Medicine, Calgary, Canada<sup>4</sup>, Department of Veterinary Pathology, University of Saskatchewan, Canada<sup>5</sup>, Marshall Swine and Poultry Health Service, 3831G 44 Avenue, Camrose, Alberta T4V 3T1, Canada<sup>6</sup>*

This study aimed to investigate the potential correlation between water quality and the immune response in commercial layer poultry farms in Alberta. The analysis included physical, chemical, and microbiological properties of the drinking water accessible to the birds. Techniques such as Inductively Coupled Plasma (ICP), Optical Emission Spectrometry (OES), ion chromatography, and titration were used for water analysis. The bacteriological culture technique was used for bacteria detection and ELISA for the antibody titers. The water analysis revealed variations in key parameters, including pH levels, alkalinity, electrical conductivity, chloride levels, and nitrate levels. More than 50% of the farms investigated had hard water for poultry consumption. One the farm had higher water nitrate and nitrite levels. Despite variations, all sulfate levels and dissolved minerals remained within acceptable limits. Bacteriological analysis indicated satisfactory water quality, with minimal coliform counts and undetectable Pseudomonas, except one farm has higher levels of E.coli indicating fecal contamination in the water. Simultaneously, the study investigated the humoral immune response before and after vaccination. Blood NDV titers showed variations among farms before vaccination, and while overall post-vaccination titers increased, the difference was not statistically significant. For IBV, overall titers from all farms combined did not show significant differences. To further elucidate these relationships, additional samples will be collected from Alberta, and in-vivo control experiments will be conducted by spiking water to assess the impact of different water qualities on specific-pathogen-free (SPF) chickens under controlled conditions. These controlled experiments aim to provide a clearer understanding of the direct effects of water quality on the immune response.

### **Preliminary Evaluation of InvG as a Novel Salmonella Vaccine Candidate: Immunogenicity and Efficacy in Layer Chickens**

Roshen Neelawala<sup>1</sup>, Chaitanya Gottapu<sup>2</sup>, Lekshmi Edison<sup>3</sup>, Subhashinie Kariyawasam<sup>4</sup>

*University of Florida<sup>1</sup>, University of Florida<sup>2</sup>, University of Florida<sup>3</sup>, University of Florida<sup>4</sup>*

Salmonella is a leading cause of foodborne disease worldwide, ranking among the four primary contributors to diarrheal disease in humans. Poultry eggs and meat have been identified as the most common sources of foodborne salmonellosis in humans. Consequently, the poultry industry is consistently under pressure to implement rigorous measures to reduce Salmonella colonization of poultry and fecal shedding. Currently, the poultry industry relies on vaccination, biosecurity practices, and screening as primary tools for controlling Salmonella. Although vaccines improved pre-harvest control of Salmonella in poultry, they were not as effective as the industry and regulatory authorities desired. Therefore, there is a need for novel Salmonella vaccines that can help poultry producers to achieve their food safety targets. Our laboratory has previously recognized InvG, a structural protein of the type 3 secretion system of Salmonella, as a potential vaccine candidate. This study entails a preliminary assessment of the immunogenicity and efficacy of InvG in commercial layer chickens. Chickens were vaccinated three times intramuscularly with recombinant InvG at the ages of 2, 4, and 6 weeks and then challenged with Salmonella Enteritidis orally one-week post-vaccination. The vaccinated chickens elicited a robust serum antibody (IgG) response against InvG. Further, there was a reduction of Salmonella counts in the ceca and spleen of vaccinated chickens compared to the unvaccinated control group. However, the mucosal antibody response (IgA) in the intestinal mucosa was poor. Based on our preliminary data, the next step is to develop a live-attenuated vaccine expressing InvG that would further improve the immune response, including the intestinal IgA response, and confer better protection against Salmonella colonization of poultry and their fecal shedding.

#### **Field evaluation of the efficacy of live E. coli and live Salmonella Typhimurium vaccination by parenteral administration in commercial layers**

Fernando Ruiz-Jimenez<sup>1</sup>, Kalen Cookson<sup>2</sup>, John Dickson<sup>3</sup>, Jon Schaeffer<sup>4</sup>

*Zoetis<sup>1</sup>, Zoetis<sup>2</sup>, Zoetis<sup>3</sup>, Zoetis<sup>4</sup>*

Live E. coli and Salmonella Typhimurium (ST) vaccines have been used in the commercial layer industry for almost two decades to help reduce mortality from colibacillosis and aid in the control of Salmonella, which are some of the main concerns in the egg industry. Currently, these live vaccines are only applied by mucosal application, either coarse spray or drinking water. However, due to their unique attenuation properties, recent controlled studies have shown that these vaccines may provide additional benefits when administered parenterally. This presentation will summarize the results of two field trials where live E. coli and ST vaccines were administered parenterally in caged and cage-free commercial egg-type pullets. The parenteral application's safety, protection, and serological response will be evaluated and compared to the conventional application methods.

#### **Before and after comparison from using Nobilis® SG9R on a commercial layer, using the Friedman test for repeated measurements, ANOVA non parametric equivalent of repeated measurements**

Camilo Andres Medina Santos<sup>1</sup>

Salmonella enterica subsp. enterica serovar Gallinarum, specifically identified as S. Gallinarum, serves as the etiological agent responsible for fowl typhoid. Nobilis® SG9R is a commercially

available live vaccine designed to combat fowl typhoid. This study aimed to assess the performance of commercial layer flocks following the implementation of a vaccination regimen incorporating Nobilis® SG9R as a prophylactic measure during the rearing phase (administered at weeks 6 and 12). The study was performed on a farm situated in the northwestern region of Colombia. Among the five flocks subject to assessment, two were observed before the introduction of the vaccine, and an additional three flocks were monitored post-implementation of vaccination protocols. A total of 361,837 birds of the Lohmann Brown Classic strain were included in the study. The houses were conventional open-sided structures with natural sunlight and were situated at an elevation of 2,125 meters above sea level. The temporal scope of the dataset spanned from week 19 to week 78 of production across all observed flocks. A comprehensive evaluation of zootechnical parameters was carried out, including mortality rate, eggs per hen housed, average egg weight and feed conversion, which conclusively showed statistically significant disparities in favor of Nobilis® SG9R. This suggests that the prophylactic integration of the vaccine positively impacted the zootechnical performance of the flocks when combined with good management and biosecurity standards and that on average it went from having 20.15% mortality and 346.05 eggs housed to 4.36% and 370.16 respectively.

### **Evidence of booster response of rFP-gB (ILT) in high ILT prevalence areas in Peru**

Claudia Carranza<sup>1</sup>, Alberto Paredes<sup>2</sup>, Bruno Garc a<sup>3</sup>, Luiz Sesti<sup>4</sup>

*Ceva Salud Animal - Peru<sup>1</sup>, La Calera - Peru<sup>2</sup>, Ceva Salud Animal - Peru<sup>3</sup>, Ceva Salud Animal - LATAM<sup>4</sup>*

Infectious Laryngotracheitis virus is circulating in Peru since 2008, causing severe economic losses to the poultry industry. During the first months of ILT outbreaks no vaccine was available to prevent the disease, only specific biosecurity measures were implemented. However, biosecurity was not enough to control the spread of the disease in the Peruvian territory. That same year, the introduction of a vector fowl pox vaccine carrying the glycoprotein B gene of Infectious Laryngotracheitis virus (ILTV; rFP-gB) represented the first tool to effectively prevent and control the presentation of the disease. With the density increase in poultry areas, increasing the risk of disease, more robust vaccination protocols were required for long living birds and HVT vector vaccines for ILT were introduced. However, in some areas ILT outbreaks were observed, specially in the more densely populated ones. Leading poultry veterinarians to break one industry paradigm: the use of two or more rFP-gB (ILT) vaccines in the field. This work aims to show the serologic response to booster vaccination with an rFP-gB, in seven commercial layer flocks. Humoral response was measured using the IDVet commercial kit that detects specific antibodies against glycoprotein B of the ILTV. For the statistical data analysis and data visualization, the Python programming language coupled with Numpy/Scipy modules were used. The results showed an increase in the serologic response after a booster application of the vector vaccine, versus the control. Evidencing a positive stimulation of the immune system.

### **Mucosal and Serum Antibody Responses Elicited by Single Vaccination with Recombinant LaSota Virus Expressing IBV Spike in Chickens with NDV Maternal Antibodies**

Camila Cuadrado<sup>1</sup>, Haroldo Toro<sup>2</sup>, Cassandra Breedlove<sup>3</sup>, Raimundo Espejo<sup>4</sup>

*Department of Pathobiology, College of Veterinary Medicine, Auburn University<sup>1</sup>, Department of Pathobiology. College of Veterinary Medicine. Auburn University<sup>2</sup>, Department of Pathobiology. College of Veterinary Medicine. Auburn University<sup>3</sup>, Department of Pathobiology. College of Veterinary Medicine. Auburn University<sup>4</sup>*

Vaccination with a recombinant Newcastle disease (ND) LaSota (LS) strain expressing Arkansas (Ark) -type infectious bronchitis virus (IBV) spike protein (rLS/ArkS) was evaluated in chickens of commercial origin with ND virus (NDV) maternally derived antibodies (MDA). Chickens with MDA were vaccinated ocularly either with rLS/ArkS or the empty LS virus at 2, 8, 15 or 30 days of age (DOA). In addition, specific pathogen free (SPF) chickens were vaccinated with each virus at 2 DOA. NDV RNA was determined in lacrimal fluids, indicating successful replication of the recombinant virus at periocular mucosal sites. IBV IgA in lacrimal fluids and serum IgG were determined by ELISA using recombinant IBV Ark S1-protein-coated plates. Vaccination at 2 DOA with rLS/ArkS in chickens with MDA elicited a vigorous IBV IgA response in lacrimal fluids without significant differences between commercial and SPF chickens. Chickens with MDA vaccinated with rLS/ArkS at 8 DOA showed IgA levels in lacrimal fluids not differing significantly from levels achieved upon vaccination at 2 DOA. Vaccination at 30 DOA did not result in increased IBV IgA levels in tear fluids of commercial birds. Chickens with MDA vaccinated at 2 DOA with the empty LS vector developed significantly higher NDV IgA levels in tears compared to chickens vaccinated with rLS/ArkS. Chickens with MDA vaccinated with LS at 8 DOA show slightly higher NDV IgA in tears compared to chickens vaccinated with rLS/ArkS. LaSota vaccination in chickens with MDA at 15 or 30 DOA elicited an ND IgA response in tears similar to chickens vaccinated with rLS/ArkS. Vaccination with rLS/ArkS at 2 or 8 DOA in chickens with MDA resulted in absence of IBV IgG responses in sera. However, vaccination with rLS/ArkS at 15 or 30 DOA elicited IBV serum IgG response. Chickens with MDA vaccinated with rLS/ArkS at 15 DOA displayed increased NDV IgG antibody level in sera. We concluded that vaccination with rLS/ArkS induces both IBV and NDV IgA at periocular mucosae and elicits serum IgG in chickens with NDV MDA.

## Virology

### **Respiratory and gastrointestinal microbiome analysis of chicken infected with infectious bronchitis virus (DMV/1639)**

Heshanthi Herath<sup>1</sup>, Ahmed Ali<sup>2</sup>, Motamed Elsayed Mahmoud<sup>3</sup>, Muhammad Farooq<sup>4</sup>, Ishara M. Isham<sup>5</sup>, Awais Ghaffar<sup>6</sup>, Susantha M. Gomis<sup>7</sup>, Dongyan Niu<sup>8</sup>, Mohamed Faizal Abdul-Careem<sup>9</sup>

*Faculty of Veterinary Medicine, University of Calgary<sup>1</sup>, Faculty of Veterinary Medicine, University of Calgary, Canada<sup>2</sup>, Faculty of Veterinary Medicine, University of Calgary, Canada.<sup>3</sup>, Faculty of Veterinary Medicine, University of Calgary, Canada.<sup>4</sup>, Faculty of Veterinary Medicine, University of Calgary, Canada.<sup>5</sup>, Faculty of Veterinary Medicine, University of Calgary, Canada.<sup>6</sup>, Western College of Veterinary Medicine, University of Saskatchewan, Canada<sup>7</sup>, Faculty of Veterinary Medicine, University of Calgary, Canada.<sup>8</sup>, Faculty of Veterinary Medicine, University of Calgary, Canada.<sup>9</sup>*

Infectious bronchitis virus (IBV) is a coronavirus, which initially replicate in respiratory tract of chicken and ultimately reach reproductive, renal and lymphoid organs to establish systemic infection. Capacity of viral spread and mortality depend on the viral strain. Respiratory secretions and fecal droppings of infected birds are the source of viral transmission. IBV DMV/1639 variant has

shown viral titers in oropharyngeal swabs and cloacal swabs up to 14 weeks and 16 weeks post infection respectively. IBV has the potential to persist in the cecal tonsils and maintain the viral shedding even when the respiratory tract has cleared the infection. It is known that microbiome play a major role in protection against viral infections and viral infections can alter microbiome composition. We are aiming to understand the relationship between IBV infection and respiratory and gut microbiome alterations. In this study, the chickens were infected with IBV maintaining a control group. One, four- and ten-days post-infection, tracheal, lung and cecum samples were collected for respiratory and gastrointestinal microbiome analysis using 16S rRNA gene sequencing. The work is in progress. Funding: Results Driven Agriculture Research (RDAR) and Alberta Chicken Producers

### **Inclusion Body Hepatitis (IBH) vaccines in Peru: an immunogenicity check**

Claudia Carranza<sup>1</sup>, Bruno Garc a<sup>2</sup>, Istvan Kiss<sup>3</sup>, Luiz Sesti<sup>4</sup>

*Ceva Salud Animal - Peru<sup>1</sup>, Ceva Salud Animal Peru<sup>2</sup>, Ceva Animal Health - SSIU Phylaxia<sup>3</sup>, Ceva Salud Animal Latin America<sup>4</sup>*

Fowl Adenoviruses (FAdV), the etiologic agents of IBH and hepatitis hydropericardium syndrome (have long been affecting animal welfare and causing heavy clinical and economic losses in the poultry industry worldwide. The most efficient way to control this disease is applying inactivated vaccines during rearing in heavy breeders to avoid vertical transmission of the virus to the progeny during the production phase as well as to produce progeny with high levels of maternal antibody at day 1. Vaccination protocols must include serotypes that are epidemiologically relevant to each country. In Peru, several reports demonstrated the presence of serotype 4 (FAdV 4; most related to HHS) as the most prevalent strain; therefore, this serotype was added to the vaccination protocols with mixed results. In mid 2014, the emergence of serotype 8b outbreaks, mostly related to IBH, caused the industry to update its vaccination programs during 2015-16, once again with mixed results. Vaccination programs were not sufficient effective and field clinical outbreaks in broilers continued to occur even with vaccines containing both serotypes 4 & 8b). The situation prompted the poultry industry and its veterinarians to question the situation: why were the vaccination programs not working? Many factors can affect the efficiency of inactivated FAdV vaccines, and a main one is the immunogenicity of the isolates used as antigens in the vaccines. To better clarify the issue, a study set up to evaluate the immunogenicity of 5 multivalent inactivated vaccines available to the industry. SPF birds were vaccinated with each product and seroconversion evaluated without the presence of maternal antibodies. Twenty 2 week SPF birds were vaccinated subcutaneously with one dose of each vaccine according to the instructions of manufacturers and 10 SPF birds were kept as negative control. Blood samples were collected at 21 and 28 days post vaccination and seroconversion was measured by a commercial Elisa kit (BioChek Fowl Adenovirus Group 1 Antibody test kit CK132 FADV) or by virus neutralization test using as antigens the same serotypes present in the vaccines. A viral titer of  $10^{2.5}$  /well was used and the effect on the virus was evaluated based on the viable cell ratio. The titer of the serum was considered the highest dilution where at least 50% protection was provided against the neutralization effect on the virus antigen. All chickens in the non vaccinated control group remained sero negative against the tested antigens. The results showed a mixed response rate in vaccinated birds. Most vaccines failed to generate a detectable serologic response with the BioChek kit. When the VN test was used, titers

were found for only one of the two serotypes tested (4 & 8b). These results have helped to understand the lack of protection seen in the field when breeders (and sometimes broilers) were vaccinated with some of the commercial vaccines tested.

### **Vaccine Takes Evaluation of Turkey Herpesvirus vector - Newcastle Disease Recombinant Vaccine (HVT-ND) by Using on-site iiPCR Analyzer**

Keat Fu<sup>1</sup>, Hsiao-Yun Chen<sup>2</sup>, Wei-Fen Tsai<sup>3</sup>, Ping-Han Chung<sup>4</sup>, Simon Chung<sup>5</sup>

*Aviagen Inc<sup>1</sup>, GeneReach Biotechnology Corp<sup>2</sup>, GeneReach Biotechnology Corp<sup>3</sup>, GeneReach Biotechnology Corp<sup>4</sup>, GeneReach Biotechnology Corp<sup>5</sup>*

Post recombinant vaccine of Marek's Disease virus of serotype 3 (Turkey Herpesvirus or HVT) vector with Newcastle disease (ND) vaccination efficacy monitoring can be done by detection of vaccine (HVT) viral replication in the host. To elucidate the appropriate detection timeframe, this trial was designed to collect spleen and feather pulp specimens from birds at 10, 17, 24, and 31 days post vaccination (dpv). Ten chickens for sample collection were randomly chosen in each flock and every sample mandatory undergo individual PCR testing to accurately identify true positives on an individual basis. On-site data was generated using the fully-automated iiPCR analyzer, along with the primers and reagents designed for detecting the HVT as an indication of HVT-ND vaccine takes. Based on the results of feather pulp samples, an average positive rate reached 70% at 24 dpv, further increasing to 88% at 31 dpv. In the context of spleen samples, an average positive rate of 70% emerged at 17 dpv, elevating to 88% at 31 dpv. In summary, the spleen samples showed earlier evidence in vaccine virus replication as compared to feather pulp. The results demonstrated that spleen samples at 17 dpv afford an overview for vaccination uniformity. Moreover, an expectation of over 90-95% of positive rate compared to 88% could be set up for future improvement in this study. These scientific data might empower farmers and veterinarians with approximate understanding of the immunization status of their poultry flock health.

### **US-UK Collab: Influence of vaccines, host genetics, and mutation rates on the evolution of infectious diseases**

John Dunn<sup>1</sup>, Jody Mays<sup>2</sup>, Hans Cheng<sup>3</sup>, Cari Hearn<sup>4</sup>, Margo Chase<sup>5</sup>, Sam Lycett<sup>6</sup>, Andrea Doeschl-Wilson<sup>7</sup>

*USDA-ARS, US National Poultry Research Center<sup>1</sup>, USDA-ARS, US National Poultry Research Center<sup>2</sup>, USDA-ARS, US National Poultry Research Center<sup>3</sup>, USDA-ARS, US National Poultry Research Center<sup>4</sup>, Roslin Institute, University of Edinburgh<sup>5</sup>, Roslin Institute, University of Edinburgh<sup>6</sup>, Roslin Institute, University of Edinburgh<sup>7</sup>*

Imperfect vaccines or host genetic resistance may alter the balance of selection between pathogen transmission and virulence by allowing more divergent but still virulent strains to be transmitted at reduced cost. Our objectives are 1) determine the influence of imperfect vaccines and host genetics on viral transmission and evolution; 2) validate viral genome polymorphisms associated with increased virulence; 3) build models to develop strategies to control the ecology, evolution and economic burden of Marek's disease (MD); and 4) disseminate information on Marek's disease virus (MDV) and infectious bronchitis virus (IBV), and the impact of vaccination to the public using various tools. We used a shedder-sentinel challenge model to naturally passage MDV through 10



successive groups. Each group consists of 10 birds kept in an individual isolator and replicated 3-6x. Viral replication and transmission are assessed by sampling shedder (donor) birds that transmit infectious virions prior to, at, and following co-housing with the contact (recipient) birds. Birds infected in Passage 1 transmit virus to recipients in Passage 2, and so on. Variables include host genetics, vaccination status and dosage. Statistical analyses of the experimental data showed that the infection and transmission dynamics of birds that have been inoculated with MDV differ substantially from those of birds that have become naturally infected through contact with infected shedder birds, highlighting the importance of mimicking modes of transmissions representative of field conditions in vaccination and other MDV challenge experiments. Furthermore, experiment 1 demonstrated that HVT vaccination does not prevent MDV transmission within all 10 subsequent passages. However, vaccination with the full recommended HVT dose was found to not only provide direct protection from MD and death to the vaccinated birds, but also indirect protection for non-vaccinated contact birds. MDV is being transmitted through serial passage in all groups of chickens, however, without clinically observable increase in virulence. Additional experiments are underway designed to increase pressure on virus evolution as well as compare methods with an avian coronavirus, infectious bronchitis virus.

### **Comparative Transcriptome Analysis of Chicken Embryos after Infection with Low Pathogenic Newcastle Disease Virus (LoNDV) from Wild Birds**

Deepa Chaudhary<sup>1</sup>, Andrea Pietruska<sup>2</sup>, Saiada Farjana<sup>3</sup>, Chang-Won Lee<sup>4</sup>, David Suarez<sup>5</sup>, Ruediger Hauck<sup>6</sup>

*Auburn University<sup>1</sup>, Auburn University, Auburn, USA<sup>2</sup>, Auburn University, Auburn, USA<sup>3</sup>, Southeast Poultry Research Laboratory, Athens, GA 30605, USA<sup>4</sup>, Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, Athens, GA 30605, USA<sup>5</sup>, Auburn University, Auburn, USA<sup>6</sup>*

Newcastle Disease Virus (NDV), classified as avian ortholavirus serotype-1, is an economically important causative agent of Newcastle Disease (ND) affecting various bird species, including both domestic chicken and wild birds. NDV typically has effects on either the respiratory and digestive systems (viscerotropic) or the nervous system (neurotropic). Studies of low pathogenic NDV (LoNDV) in wild birds indicate that LoNDV sourced from waterfowl adapts to chickens, but it is incompletely understood how the virus interacts with the host and induces immune response after adapting to chicken embryos. Therefore, this study aims to identify and characterize the changes in the host transcriptome with emphasis on the immune responses to LoNDV wild bird isolates and compare them after the virus adapts in chicken embryos. Four LoNDV isolates from waterfowl were propagated in specific pathogen-free (SPF) chicken embryos for 10 passages. The transcriptome in lungs and spleens of 20-day-old embryos after infection with passages 1 and 10 at 18 days of embryonation were assessed and examined through RNA sequencing (RNA-seq). Analysis of differentially expressed genes will be performed, and changes in functional pathway will be analyzed. The results will be presented and discussed.

### **Genetic and pathogenic characterization of avian Paramyxovirus isolated from domestic birds in Perú (2004-2022)**

María Eliana Icochea D'Arrigo<sup>1</sup>, Gina R. Castro-Sanguinetti<sup>1</sup>, Rosa Gonzalez-Veliz<sup>1</sup>, Alonso Callupe-Leyva<sup>1</sup>, Katherine Vargas-Coca<sup>1</sup>, Juan More-Bayona<sup>1</sup>

Newcastle disease (ND) is considered one of the most important diseases of birds, because velogenic strains of the virus can cause outbreaks with high morbidity and mortality and restriction of international trade, causing large economic losses to the poultry industry. It is caused by strains of avian Paramyxovirus type 1 (APMV-1), of the genus Orthoavulavirus. The wide circulation of viruses in poultry populations has caused genetic diversity and a constant emergence of new genotypes, and given the great importance of ND for the poultry industry and the wide use of live vaccines in the world, sequencing and analysis Phylogenetic have become the methods of choice to characterize the strains circulating in the field. In Peru there is a law that requires vaccination with live vaccines and inactivated vaccines to poultry industry and backyard birds. However, even when several types of effective vaccines are applied, ND continues to be one of the main diseases that affect the domestic birds. The objective of the present study was to characterize genetically and pathogenically the ND viruses isolated from 21 cases received in the Avian Pathology Laboratory of the FMV-UNMSM during the years 2004 to 2022. The study included ten cases of fighting birds, six of broiler chickens, three of layers and two backyard birds. Two viruses of different virulence were isolated in a single case, making a total of 22 isolates analyzed. The analysis of the genotype of the 22 viral isolates obtained in domestic birds determined that eleven were of genotype XII, seven of genotype XII.1, three of genotype II, one of genotype I, and only one isolate obtained from an outbreak of fighting birds in 2022 was identified as VIId. These results demonstrate that among the virulent viruses in the country there is a predominance of genotype XII, followed by XII.1 and finally VIId, also showing that in Perú there is a genetic diversity of virulent viruses capable of infecting domestic birds. Lentogenic viruses were detected in broilers and commercial layers with clinical respiratory signs and hemorrhages in the proventriculus, caused by causes other than virulent NDV infection, highlighting the need to perform definitive diagnosis with PCR in vaccinated bird populations. All viruses detected in fighting birds were of the virulent type, also showing that these birds carry the greatest diversity of genotypes, which is why they constitute the main source of contamination and reservoir of the virus in Perú. The results of this study have allowed us the identification of viral reservoirs, types of circulating viruses and greater knowledge of the epidemiology of NCD in Perú. Keywords: Wild and domestic birds, Newcastle disease, virulent strains, genotype.

### **Detection of Infections Laryngotracheitis Virus (ILTV) circulating in broilers with and without clinical signs**

Jose Linares<sup>1</sup>, Matilde Alfonso<sup>2</sup>, Mark Jackwood<sup>3</sup>, Robert Beckstead<sup>4</sup>, Marshall Putnam<sup>5</sup>

*Ceva Animal Health*<sup>1</sup>, *Ceva Animal Health*<sup>2</sup>, *Ceva Animal Health*<sup>3</sup>, *Ceva Animal Health*<sup>4</sup>, *Ceva Animal Health*<sup>5</sup>

A survey of commercial broilers was conducted to determine if field isolates of ILTV are circulating in flocks without the producer's knowledge. In this study, tracheal or eyelid swabs were collected placed in RNA/DNA Shield and shipped to the diagnostic laboratory for multiplex RT-PCR testing that included detection of ILTV, IBV and NDV. Identification of ILTV positive birds in flocks that are clinically normal or diagnosed with other upper-respiratory disease pathogens could help mitigate the risk of unknowingly spreading ILTV to susceptible birds. In addition, ILTV surveillance in poultry operations will present opportunities to help guide vaccination decisions to control the pathogen.

## **Identification and Functional Characterization of a Virokine Homologous to Chicken Interleukin-4 in Infectious Laryngotracheitis Virus**

Stephen Spatz<sup>1</sup>, Volkening, J.D<sup>2</sup>, Garcia, M.<sup>3</sup>, Zamora, A.C.<sup>4</sup>, Skipper, A.<sup>5</sup>, Ross, T.A.<sup>6</sup>

*US National Poultry Research Center, ARS-USDA<sup>1</sup>, BASE2BIO. 1945 Arlington Dr. Oshkosh, WI, USA<sup>2</sup>, University of Georgia, Poultry Diagnostic Research Center, 953 College Station Road, Athens GA, USA<sup>3</sup>, National University of San Marcos, College of Veterinary Medicine, San Borja, Peru.<sup>4</sup>, University of Georgia, Poultry Diagnostic Research Center, 953 College Station Road, Athens GA, USA<sup>5</sup>, US National Poultry Research Center, ARS-USDA, 934 College Station Road, Athens, GA, USA<sup>6</sup>*

Background: Herpesvirus genomes encode functional homologs of host cytokines (virokines) to modulate the immune response for viral benefit. This study investigates a putative virokine in infectious laryngotracheitis virus (ILTV). Methods: Full-length cDNA sequencing identified a novel gene in both vaccine and virulent ILTV strains, exhibiting 34.7% amino acid identity to chicken interleukin-4 (IL-4). Sequence and structural analyses confirmed similarities across avian and vertebrate IL-4 homologs. Phylogenetic analysis suggests gene capture of six exons from the host 60-70 million years ago. The vIL-4 function was assessed by its ability to stimulate nitric oxide (NO) production in a macrophage cell line (MD11) comparable to chicken IL-4. To investigate virulence, a recombinant ILTV ( $\Delta$ vIL-4) lacking exons 3-6 was generated using CRISPR/Cas9. Results: vIL-4 stimulated macrophage NO production, confirming its functional activity. In vitro,  $\Delta$ vIL-4 replicated slightly better than the parental strain. In vivo,  $\Delta$ vIL-4 exhibited reduced pathogenicity in chickens compared to the wild-type virus, as evidenced by milder clinical signs and lower viral shedding. However, complete attenuation was not achieved. Conclusions: ILTV encodes a functional vIL-4 homolog acquired from its avian host, contributing to viral pathogenesis. This finding expands our understanding of herpesvirus immune modulation strategies and offers insights for potential ILTV vaccine development.

## **Sequence and phylogenetic analyses of chicken astroviruses (CAstVs) detected at Mississippi State University between 2016 to 2023 and associated with digestive and hatchability issues.**

Alejandro Banda<sup>1</sup>, Rebecca Mackey<sup>2</sup>, Candy Zhang<sup>3</sup>, Robin Gilbert<sup>4</sup>, Phil Stayer<sup>5</sup>

*Poultry Research and Diagnostic Lab Mississippi State University<sup>1</sup>, Mississippi Veterinary Research and Diagnostic Lab Mississippi State University. Pearl, MS 39208.<sup>2</sup>, Mississippi Veterinary Research and Diagnostic Lab Mississippi State University. Pearl, MS 39208<sup>3</sup>, Wayne-Sanderson Farms. Inc. Laurel, MS 39441<sup>4</sup>, Poultry Consultant<sup>5</sup>*

Chicken astroviruses (CAstVs) were isolated from tissue samples of chickens suffering from digestive problems, stunting and "white chick disease". Chicken astrovirus detected from intestinal samples were associated with runting, poor condition, poor feathering problems and diarrhea. CAstVs were also detected in progeny and unhatched eggs from breeder flocks experiencing "White chick" condition. These viruses were associated with histopathological lesions including hepatocellular vacuolar degeneration, glycogen accumulation and heterophilic and lymphocytic interstitial nephritis. During viral isolation, chicken astroviruses induced severe congestion, hemorrhages, and edema of abdominal muscles in embryos. A conventional RT-PCR method

targeting CAstV ORF-1b and ORF-2 that corresponds to the viral capsid protein was carried out to detect CAstVs was carried out. Nucleotide sequences were generated and analyzed by phylogenetic analysis using Neighbor-Joining method. The phylogenetic analysis separated the different astrovirus into two phylogenetic groups, according to the system proposed by Dr. V. Smyth (Avian Pathol. 41:2, 151-159, 2012). Astroviruses associated with white chick syndrome clustered in a separate clade of group B. Enteric astroviruses clustered in different clades of groups A and B. According to this study, the capsid protein of astroviruses associated with hatchability issues is genetically different from those astroviruses associated with enteric problems.

### **Molecular characterization of emerging variants of turkey coronavirus associated with outbreaks in turkeys in 2023**

Muhammad Luqman<sup>1</sup>, Selim, M<sup>1</sup>, Duhan, N<sup>1</sup>, Temeeyasen, G<sup>1</sup>, Khaled, A<sup>1</sup>, Sharafeldin T<sup>1</sup>, Jangra, S<sup>1</sup>, Mor, S.<sup>1</sup>

*South Dakota State University<sup>1</sup>*

Turkey coronavirus (TCoV) is a member of the avian coronavirus species with infectious bronchitis virus (IBV), which is considered to be the source of TCoV. This virus is associated with poult enteritis syndrome characterized by enteritis, anorexia, diarrhea, growth depression, retarded development, impaired feed conversion, and sometimes increased mortality. Due to these adverse effects, the virus is responsible for significant economic losses in the turkey industry. In Fall 2023, turkey farms in different states were reported with enteritis and high mortalities mainly associated with TCoV infection confirmed by realtime RT-PCR of intestinal samples collected from affected poults. The 25 PCR positive samples with ct values varied from 18 to 30 were submitted to the Animal Disease Research and Diagnostic Laboratory, South Dakota State University (SDSU-ADRDL). One sample from North Carolina was submitted for detection and whole genome sequencing of TCoV. Samples were processed for whole genome sequencing by doing viral RNA extraction, library preparation and sequencing on Illumina Miseq. The raw fastq files were analyzed using our bioinformatics pipeline for QC, denovo assembly and annotation. TCoV whole genomes were assembled from one NC and four AR samples. The phylogenetic analysis based on whole genome sequence and N gene, revealed high identity (95%) of the new strains with previously reported TCoVs and about 90% with IBVs. However, based on spike protein and S1 subdomain, despite 90% identity with other TCoVs, it is only 30% with IBVs. The NC and AR strains clustered separately hence indicates that two variants are circulating in the turkey population. Interestingly, the new AR strains were clustered in a separate sub-lineage away from the other TCoVs with evidence of some divergence even between the new Arkansas strains. In conclusion, this study highlighted the continuous evolution of TCoVs and emergence of new variants causing outbreaks in turkeys.

## **Wealth of Knowledge**

### **National Poultry Improvement Plan (NPIP): A Review of the Proposed Changes for the 46th Biennial Conference and the Federal Rulemaking Process**

Elena Behnke<sup>1</sup>

*USDA APHIS VS NPIP<sup>1</sup>*

The NPIP is widely recognized as the gold standard for poultry monitoring and surveillance for Salmonella, Mycoplasma and Avian Influenza. The 89-year-old federal-state-industry cooperative program is accepting proposed changes until March 27, 2024, for the 46th Biennial Conference set for Providence, RI, August 27-30, 2024. This presentation will cover some of the reasons behind the proposed changes and will also outline the cumbersome federal rulemaking process.

### **The Importance of Contact Time in Using Ultraviolet Light as a Disinfectant**

Katherine Schaeffbauer<sup>1</sup>

*Jennie-O Turkey Store<sup>1</sup>*

The Importance of Contact Time in Using Ultraviolet Light as a Disinfectant K. R. Schaeffbauer Jennie-O Turkey Store, Willmar MN The cleaning and disinfection step is a crucial part in raising safe and healthy turkeys. Keeping the microbial load as minimal as possible in barns can increase performance, reduce antibiotics, and minimize bacterial and viral expansion and evolution. Ultraviolet light when applied correctly can be beneficial in maintaining low microbial loads. Multiple trials were conducted to test two different ultraviolet light technologies. The first lights (Light A) were a UVC light with a lower wavelength allowing for safe human interaction. These lights require more contact time but can be on and disinfecting 24/7. The second lights (Light B) were a UVC light that has a higher wavelength and are unsafe for human interaction. These lights require less contact time and cannot be operational 24/7. Swabs of barns and service rooms were placed on EMB and Blood agar plates prior to light installation and reswabbed at several time frames during the first 24 hours post light installation. Compared to pre-light exposure swabs, Light A saw an increased proportion of no growth on the EMB plates by 64% and 80 % at 3 and 24 hours post installation, respectively. Light B saw an increase proportion of no growth on the EMB plates by 42.5% at 3 hours post installation, and a decrease by 4.8% compared to the pre-installation swabs. These findings show that contact time and exposure to the UVC light is more beneficial than the intensity of the wavelength when reducing the microbial load present in a barn.

### **Multi-causal respiratory disease and co-infection profiles in Latin America (2nd semester 2023): Brazil, Colombia and Peru as examples**

Adam Jbenyeni<sup>1</sup>, Christophe Cazaban<sup>2</sup>, Cesar Nunes<sup>3</sup>, Jorge Chacon<sup>4</sup>, Leonardo Alvarado<sup>5</sup>, Claudia Carranza<sup>6</sup>, Luiz Sesti<sup>7</sup>

*Ceva Animal Health, France<sup>1</sup>, Ceva Animal Health, France<sup>2</sup>, Ceva Animal Health, Brazil<sup>3</sup>, Ceva Animal Health, Brazil<sup>4</sup>, Ceva Animal Health, Colombia<sup>5</sup>, Ceva Animal Health, Peru<sup>6</sup>, Ceva Animal Health, Latin America<sup>7</sup>*

Respiratory diseases are responsible for major economic losses and health problems in commercial poultry worldwide. Under field conditions, respiratory diseases caused by a single infection are the exceptions. The co-circulation of different pathogens in the field makes co-infections very common, and respiratory diseases are multi-causal. Experimental studies have shown that co-infections increase the clinical outcome compared to single infections with the same pathogens. This is explained by the synergistic effect between pathogens, the immunosuppressive effect of certain pathogens or even the effect due to the destruction of the respiratory epithelium. The pathogens involved in the multi-causal disease are difficult to identify

on the basis of clinical signs and gross lesions, so molecular biology tests are essential. In Latin America the circulation of the main respiratory pathogens has already been reported, yet studies of respiratory diseases most often focused on a single pathogen. The aim of this study was to investigate the frequency of multi-causal respiratory disease in Brazil, Colombia, and Peru and to describe the co-infection profiles in acute respiratory disease episodes. Tracheal swabs were collected from commercial poultry flocks showing spontaneous respiratory disease and within the first five days after the onset of clinical signs. Swabs were smeared into FTA® cards and shipped to the virology department of Toulouse vet school, France. Samples were screened for a panel of seven respiratory pathogens: infectious bronchitis virus (IBV), Newcastle disease virus (NDV), avian metapneumovirus (aMPV), infectious laryngotracheitis virus (ILTV) and *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), *Ornithobacterium rhinotracheale* (ORT) using commercial qPCR ID Gene kits (IDSolution, Montpellier, France). Samples positive for IBV or NDV were genotyped. Specific PCR assays were performed to amplify S1 and F gene of IBV and NDV, respectively and then sequenced by Sanger sequencing. Preliminary results from 25, 24, and 14 flocks tested from Colombia, Brazil and Peru respectively showed co-infections in 30 flocks (48%). Twelve of these cases included co-infections with viral pathogens among which 6 were only viral. In 17 flocks, co-infections were mixed and in 7 flocks only bacterial co-infections were detected. IBV was the most frequently detected virus (75%) followed by NDV (10%), aMPV (6%) and ILTV (5%). ORT was the most detected bacterial pathogen (29%) whereas MG and MS were equally detected (22%). This study showed that respiratory disease is multi-causal in half of the investigated acute respiratory episodes in Brazil, Colombia, and Peru and one out of five only showed viral co-infections. This confirms that respiratory diseases must be thoroughly investigated since more than one pathogen is commonly involved.

### **Management of vaccination program against Infectious Bronchitis Virus as a tool to reduce antibiotic therapy in broilers in Brazil**

Josiane Schuvank Maculan Salvo<sup>1</sup>, Daniel Dalla Costa<sup>2</sup>, Jose Emilio de Menezes Dias<sup>3</sup>, Eva Laurice Pereira Cunha Hunka<sup>4</sup>, Udi Ashash<sup>5</sup>

*Universidade Estadual do Centro Oeste (UNICENTRO) - Brazil<sup>1</sup>, Universidade Federal do Parana, Palotina - PR, Brazil<sup>2</sup>, Phibro Animal Health; Instituto Federal Catarinense, Concordia SC, Brazil<sup>3</sup>, Phibro Animal Health<sup>4</sup>, Phibro Animal Health<sup>5</sup>*

Infectious Bronchitis (IB) is still perceived as one of the most significant diseases in poultry production. Infection of the nasal and tracheal mucosa, and kidney lesions causes a rapid loss of ciliated epithelium, impaired mucociliary clearance and decreased kidney function that causes direct damage and predisposes chickens to secondary bacterial infections. In 2022, the Infectious Bronchitis Virus (IBV) from GI-23 lineage (Variant 2) was detected in Brazil, and since then it has been identified as responsible for increased bacterial infection and medication rates mainly in Paraná State, which represents around 35% of the broiler production in the country. In 2023, vaccination with a homologous GI-23 lineage vaccine started in Brazil, with the aim of controlling IB Variant 2 infections. The purpose of this study was to evaluate the efficacy of vaccination with a homologous GI-23 vaccine in a region with high pressure of infection by IBV GI-23 lineage and evaluate the impact of a change in the IBV vaccination program on antibiotic therapy. This study was conducted in a company located in the State of Paraná - Brazil. This company's slaughters on

average 21 million chickens monthly and the average age at slaughter is 42 days. The parameter measured was the cost of antibiotic medication in US\$/ bird. We compared the medication cost between February to July of 2022, in which the company's vaccination program only included vaccine strains of the IBV GI-1 lineage (Massachusetts) and GI-11 (BR-1) and February to July of 2023, in which the company used the IBV homologous vaccine GI-23 lineage (TABic® IBVAR206) plus an IBV GI-1 (Massachusetts) vaccine. A total of 12,533 broiler flocks, with 261,093,647 birds were evaluated: 124,963,634 in 2022 and 136,130,013 in 2023. This was an average of 20,832 birds per flock. Medication cost data were compiled and subjected to the Shapiro Wilk normality test, followed by analysis of variance using the One-Way ANOVA test and comparison of means using the Tukey test, with a result of  $P \leq 0.05$  being considered significant. The average cost per bird of antibiotic treatments between the months of February to July 2022 was US\$ 0.0084, while during the same period in 2023, it was US\$ 0.0060. This represents a significant reduction ( $P=0.014$ ) of 29% in the use of antibiotics. If we consider the value of US\$ 0.0024, which was the difference between the same period in the two years, and if we consider the sample size of this study (261,093,647 birds), we show a savings of US\$ 625,624.75 in the cost of antibiotic treatments. These results demonstrate the importance of a vaccination program adjusted to the epidemiology of each region. In addition to disease prevention, vaccines can also help to reduce the use of antibiotics to treat secondary infections especially in cases of respiratory diseases.

#### **Developing Sterile Insect Technique (SIT) using Wolbachia for potential biocontrol of Litter beetles (*Alphitobius diaperinus*)**

Teresa Dormitorio<sup>1</sup>, Ruediger Hauck<sup>2</sup>, John Beckmann<sup>3</sup>, Richard Murphy<sup>4</sup>

*Auburn University, Poultry Science Department<sup>1</sup>, Dept. of Poultry Science, Auburn University, Auburn AL USA<sup>2</sup>, Dept. of Entomology & Plant Pathology, Auburn University, Auburn AL USA<sup>3</sup>, Dept. of Entomology & Plant Pathology, Auburn University, Auburn AL USA<sup>4</sup>*

Litter beetles are ubiquitous insect pests in chicken houses and transmit many important poultry diseases. Beetles and their larvae also cause structural damage to poultry houses, and the consumption of too many beetles by the birds can lead to indigestion and impaired feed conversion. Control relies heavily on pesticides, but resistances of beetles against insecticides are increasing. Sterile insect technique (SIT) is the use of sterile males to suppress populations of insects. Wolbachia cytoplasmic incompatibility (CI) has been used as a tool for SIT. When Wolbachia-infected male mates with an uninfected female, eggs produced will not hatch. We collected litter beetles from the field and established colonies in the lab. Beetles are reared in plastic tubs on wood shavings and chicken feed with apple slices. To obtain eggs for Wolbachia injection, 150-200 beetles (3-6 weeks old) and 100g rice are placed on a petri dish (150 x 15 mm) containing 6.35 X 6.35 cm cardboard cutout. An apple slice (~2.5 cm) is placed on top of rice as source of moisture. Beetles lay 20-100 eggs underneath the cardboard within 2 hrs. Eggs are collected and microinjected with CI inducing Wolbachia strains reared in *Drosophila* using cytoplasmic transfer. We plan to inject 5000 beetle eggs with each Wolbachia strain. With a hatch rate of ~10% we would expect to screen 500 F0 injected survivor lines for Wolbachia. More experimental parameters of microinjection need to be optimized to improve hatch rates and viability of infected beetle eggs.

## **Significance of Biosecurity Audits in Addressing High Pathogenic Avian Influenza (HPAI) Challenges**

Mostafa Ghanem<sup>1</sup>, Mostafa Ghanem<sup>2</sup>, Nathaniel Tablante<sup>3</sup>, Madhusudan Timilsina<sup>4</sup>

*University of Maryland, College Park<sup>1</sup>, Department of Veterinary Medicine, University of Maryland, College Park, MD, USA<sup>2</sup>, Department of Veterinary Medicine, University of Maryland, College Park, MD, USA<sup>3</sup>, Department of Veterinary Medicine, University of Maryland, College Park, MD, USA<sup>4</sup>*

Highly Pathogenic Avian Influenza (HPAI) poses a significant threat to poultry health and industry stability. To effectively manage this threat, robust biosecurity measures are necessary to prevent the introduction and spread of the disease. Biosecurity audits serve as essential tools in assessing and enhancing farm biosecurity practices, playing a crucial role in mitigating challenges associated with HPAI. This presentation provides an overview of the benefits, conceptual frameworks, and practical approaches involved in conducting biosecurity audits, adhering to the National Poultry Improvement Plan (NPIP) standard biosecurity guidelines. Additionally, the discussion encompasses online tools designed for biosecurity audits and farm biosecurity risk assessment. The presentation also reports on ongoing efforts to improve biosecurity compliance through audits, specifically focusing on preventing HPAI outbreaks in the Delmarva region. In conclusion, the contribution of poultry farms to industry-wide prevention and preparedness efforts through biosecurity audits is emphasized. The presentation will delve into the various benefits and practical aspects of biosecurity audits, highlighting their crucial role in safeguarding poultry health and ensuring economic well-being.

## **The Great, the Good, the Untestable Serum**

Jaime Hamrick<sup>1</sup>

*Georgia Poultry Laboratory Network<sup>1</sup>*

High quality serum submissions result in accurate and full completion of requested tests in a timely manner. Hemolyzed serum does not affect ELISA results but may interfere with the HI test. A certain minimum amount of serum is necessary to run multiple tests. This poster will present some of the problems encountered in a high throughput laboratory with submissions for serological testing and the consequences of submitting poor quality samples.

## **Safety in Necropsy: Human Protection for Poultry Dissection**

Adriana Guzman<sup>1</sup>

*Georgia Poultry Laboratory Network<sup>1</sup>*

Safety is one of the most important components in a necropsy suite. Due to sharp instruments, pathogens, and biosecurity, the need for a safety program is pertinent. In this project, various aspects of the safety program are explored. It is imperative that those working in the necropsy suite are kept safe so they can continue their work in diagnostics and research.

## **Part 2: NPIP Salmonella Program Overview Specific to Pullorum-Typhoid**

Katy Burden<sup>1</sup>



## *USDA NPIP<sup>1</sup>*

This second installment is a continuation of the presentation that was given at the 2022 AAAP Conference in Jacksonville. In Part 1 presented in 2022, we went over the basics of the NPIP Program and barely scratched the surface. In this second installment we will dive into the details of the Pullorum-Typhoid testing scheme under the National Poultry Improvement Plan. The National Poultry Improvement Plan is one of the oldest standing surveillance programs present in the United States of America. Incorporated into the United States Department of Agriculture by an Act of Congress in 1935, the NPIP program was initially created as a means to diminish the spread of Pullorum-Typhoid in the breeder-hatching industry. Later the program was expanded to include, in the program diseases, Salmonella enteritidis, Mycoplasma gallisepticum, Mycoplasma synoviae, Mycoplasma meleagridis, and Lowly Pathogenic Avian Influenza. The NPIP program and its testing schemes are considered the gold standard model of poultry surveillance for many international trading partners.

# Online Oral Presentations

## **911 Poultry Call: we have a HPAI positive**

Eric Orozco<sup>1</sup>, Becky Tilley<sup>1</sup>, Molly Parker<sup>2</sup>

*Butterball, LLC<sup>1</sup>, Select Genetics<sup>2</sup>*

Highly Pathogenic Avian Influenza (HPAI) is one the most disruptive diseases for the worldwide poultry industry. Since the first U.S.A. case, in this second outbreak, confirmed in February of 2022, more than 300 cases have been reported in turkeys. In the spring of 2022, a total of 6 turkey tom cases were confirmed in North Carolina over a period of 10 days. Birds were between 17 and 21 weeks of age. High mortality, low activity and unusual quietness were the major clinical signs observed in the birds. Over 100,000 Butterball tom turkeys were destroyed with a total of 2 internal strike teams along with the State crews. A series of multiple efforts involving the veterinarians, managers, flock supervisors, and technicians led to a quick and efficient response, not only for the depopulation of the farms, but also for the next steps. These next steps included farms surveillance and the movement of poultry products (birds, eggs, semen). The objective of this study is to share my first experience with HPAI as a turkey veterinarian and the challenges I faced along the way, mostly communication and organization.

## **How the Ducks Stole Thanksgiving: A Natural HPAI Infection in Raised for Release Mallard Ducks**

Emily Pittman<sup>1</sup>

*Georgia Poultry Laboratory Network<sup>1</sup>*

In this presentation, the infection, clinical signs, diagnosis, and response to a natural infection of HPAI H5N1 2.3.4.4b in raised for release mallard ducks in Georgia will be discussed.

## **Highly Pathogenic Avian Influenza in a Commercial Table Egg Layer Flock**

Katie Schlist<sup>1</sup>

*Forsman Farms, Inc.<sup>1</sup>*

Highly Pathogenic Avian Influenza (HPAI) is devastating the US poultry industry since February 2022. In contrast to the 2015 HPAI outbreak, epidemiology suggests that infection has primarily spread directly from wild bird reservoirs to domestic poultry with minimal farm to farm spread, frustrating production systems previously thought to be at low risk due to various biosecurity advantages. This case report presents the HPAI response at a commercial table egg layer farm considered both geographically and conceptually isolated from risk. Hypotheses of disease introduction, on-farm spread, and lessons learned in the response effort will be discussed.

## **Circulation of non-pathogenic Avibacterium paragallinarum in the USA confounds the current diagnostics**

Mohamed El Gazzar<sup>1</sup>, Mostafa Shelkamy<sup>1</sup>, Amro Hashish<sup>1</sup>, Maria Chavez<sup>1</sup>, Mariela Srednik<sup>1</sup>, Nubia Macedo<sup>1</sup>, Eman Gadu<sup>1</sup>, Yuko Sato<sup>1</sup>, Stephan Schmitz-Esser<sup>1</sup>, Qijing Zhang<sup>1</sup>

*Iowa State University*<sup>1</sup>

*Avibacterium paragallinarum* (AP), is a primary bacterial pathogen that causes respiratory disease in chickens known as Infectious Coryza (IC). IC has recently emerged in commercial poultry in the Midwest, an area of the country that did not have this disease. This emerging disease caused significant economic losses to the poultry industry in general, but particularly affected the table egg industry. Around this progressing IC outbreak, surveillance efforts uncovered the positive PCR detection of AP in commercial layers without causing any disease. Multiple AP isolates were acquired from healthy layer flocks, with no history of IC or any history of ever being exposed to or vaccinated with AP. The whole genome sequence (WGS) of these isolates still identified as AP at the WGS level, however, it showed significant differences in comparison with typical AP. Therefore, this newly discovered AP population was dubbed non-pathogenic *Avibacterium paragallinarum* (npAP). Existing diagnostic methods lack the capability to distinguish between these two populations which confounds the diagnosis of IC and severely hinders control efforts. The goal of this study is to estimate the prevalence of the npAP and to define the magnitude and the geographic distribution of this diagnostic challenge within the layer industry. We targeted layer complexes that are completely normal, no clinical signs, normal water and feed intake, normal egg production, no previous IC infection or vaccination. At least one flock represented each layer complex and oropharyngeal swabs were collected from 30 birds per flock in six separate pools. We collected 773 oropharyngeal swab pools from 81 layer complexes distributed in 14 states, including 8 out of the top 10 egg producing states. In terms of the time zones, Eastern, Central, Mountain and Pacific, a total of 38, 39, 2, and 2 layer complexes were sampled, respectively. Samples were tested by the recN screening qPCR assay. The positives were confirmed by our differential qPCRs (npHMTp210 and hctA assays). Unexpectedly, 28 layer complexes out of the 81 were positive for the npAP (34.56%). In proportion to the number of tested complexes per each zone, the vast majority of positives were concentrated in the Eastern and Central zones by 11 and 15 positive complexes, respectively. In conclusion, the percentage of positive sites is unexpectedly large and confirm the existence of a significant problem that should be addressed to restore the diagnostic integrity of IC in the face of an expanding outbreak in the USA.

### **The Use of a Bacterial Litter Amendment to Delay Gangrenous Dermatitis and Reduce Barn Ammonia Levels**

Meagan Abraham<sup>1</sup>, K.R. Schaeferbauer<sup>1</sup>

*Jennie-O Turkey Store*<sup>1</sup>

Gangrenous dermatitis (GD) is considered one of the most significant diseases affecting commercial turkeys. GD, primarily caused by *Clostridial* spp., causes significant economic losses each year due to death loss and plant condemnations. This disease manifests as fever, ataxia and recumbency, and subcutaneous edema with dark reddish, purple, or greenish discoloration to the skin and muscle. Poor litter conditions may predispose birds to this disease, so litter management is critical to preventing GD and limiting the spread of *Clostridial* spp. in the litter. This trial utilized a bacterial product applied on top of the litter with the goal of reducing ammonia levels and delaying the onset

of GD on farm. At the onset of this trial, eight grow barns of conventional, commercial toms were cleaned and disinfected, and fresh shavings placed. Four barns had no litter amendments added and served as controls. Four barns had the bacterial litter amendment applied on top of the shavings. Ammonia was recorded daily, and litter moisture was evaluated every 2 weeks using a hand grab method averaged over 9 areas throughout the barn during the growout period. Ammonia levels were lower in the trial group compared to the control ( $P < 0.001$ ) but there were no differences in litter moisture ( $P > 0.05$ ). The average age at onset of treatment for GD was 120 days of age regardless of treatment group. There were no differences in adjusted livability, gain per day, or medication costs. As the bacterial fauna in the litter grows, we expect that the second round in this trial will have a delayed onset of dermatitis in the treatment barns with similar ammonia levels between the control and treatment barns.

### **Nanopore MinION sequencing of emerging *Castellaniella* spp. associated with mortality in broiler breeders**

Yi Chen Luo<sup>1</sup>, Tiffani Allen<sup>1</sup>, Jenny Nicholds<sup>1</sup>, Grazieli Maboni<sup>1</sup>

*The University of Georgia*<sup>1</sup>

Uncommon bacterial infections in broiler breeders have been repeatedly identified at the Poultry Diagnostic and Research Center (PDRC), University of Georgia, since 2018. Animals from 20 different cases presented with increased mortality, lameness, and swollen wattles, resembling clinical signs of fowl cholera caused by *Pasteurella multocida*. However, further examination of the 16S rRNA gene sequencing disclosed a 99% homology with *Castellaniella* spp. Initially perceived as an environmental bacterium thriving in soil, *Castellaniella* spp., a gram-negative rod, was not deemed pathogenic. However, *Castellaniella* has been associated with two case reports of fatal inflammatory disease in a marine mammal and Daurian pikas. To our knowledge, *Castellaniella* spp. has neither been acknowledged as a pathogen in poultry nor documented in any avian species. Our goal is to provide a genotypic characterization of *Castellaniella* spp. isolated from poultry to assist in understanding its antimicrobial susceptibility, pathogenicity and phylogenetic relationship with other genera within the Alcaligenaceae family. Genomes of approximately 2.9 million base pairs for all 20 isolates were obtained using Nanopore sequencing technology and denovo assembly. We utilized ResFinder and AMRFinderPlus databases for antimicrobial resistance gene detection. Both databases yielded consistent results detecting *sul2* gene, indicating resistance to sulfonamides, and *aadA7* and *aph* genes, indicating resistance to streptomycin in 3/20 isolates. To our knowledge, this is the first study to investigate the AMR gene profile of *Castellaniella* spp. Further work will include Illumina sequencing to perform hybrid genome assembly, and the detection of virulence genes to elucidate mechanisms of pathogenicity and the phylogenetic relationship of *Castellaniella* with other Alcaligenaceae members. Meanwhile, we will set up the foundation to establish an animal model to study *Catellaniella* spp. infection in poultry.

### **An Unusual Case of Diphtheritic Pox in a Small Commercial Layer Flock**

Jarra Jagne<sup>1</sup>, Gavin Hitchener<sup>1</sup>, Alina Demeter<sup>1</sup>, Darian Gordon<sup>1</sup>

*Cornell University*<sup>1</sup>

Cornell University Animal Health Diagnostic Center, College of Veterinary Medicine, Ithaca, NY 14850 Avian pox is a common viral disease of various species of birds with great economic significance. It is a slow spreading disease that is manifested in two forms, a proliferative skin form called dry pox and a diphtheritic form or wet pox with lesions in the upper digestive and respiratory tract. The causative agent is a double-stranded DNA virus of the genus Avipoxvirus belonging to the family Poxviridae. The infection can have adverse effects on flock performance such as reduced egg production and reduced growth. It can also cause a marked increase in mortality. In the Fall of 2023, a case of high mortality in a commercial layer flock was referred to the Animal Health Diagnostic Laboratory at Cornell University for avian influenza testing. The owner had concerns about rising mortality of up to almost 300/day in an old-style layer barn with three-tier A-frame cages holding 50,000 laying hens. The birds had not been vaccinated for avian pox, but the farm also had a history of necrotic enteritis and the owner had started the birds on anticoccidial treatment. The history included lack of respiratory signs and sudden death of birds in the barn. The gross necropsy results for three birds submitted did not reveal any significant findings. After the first submission of birds and a negative avian influenza test, the owner reported that mortality continued to increase, and the birds had difficulty breathing. A visit to the farm and field necropsy of a dozen birds revealed the presence of caseous tracheal plugs and a diffusely roughened tracheal mucosa with raised yellow plaques similar to ones found in the oral cavity. Histopathology revealed the presence of avian pox virus intracytoplasmic inclusions in the trachea. PCR results were positive for avian pox.

### **Know your neighbors - Mycoplasma gallisepticum and Mycoplasma synoviae dual infection in broiler breeders**

Karen Grogan<sup>1</sup>, Kathleen Sary<sup>2</sup>, Naola Ferguson-Noel<sup>1</sup>, Susan Williams<sup>1</sup>, Louise Dufour Zavala<sup>2</sup>, Jenny Nicholds<sup>1</sup>

*UGA PDRC<sup>1</sup>, Georgia Poultry Laboratory Network<sup>2</sup>*

This case report will outline a recent submission of concomitant Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) infections in a flock of commercial broiler breeders. Live birds were submitted for post-mortem evaluation to the diagnostic lab at Poultry Diagnostic and Research Center (PDRC). Two birds in the submission displayed significant respiratory noise and a mild to moderate tracheitis. Suspecting Infectious Laryngotracheitis Virus (ILTV), tracheas were collected for ILTV PCR and tracheas and eyelids were collected for rapid histopathology of affected tissues. Tissues were PCR negative for ILTV and microscopic lesions correlated with Mycoplasma infection in the tracheas. MG and MS PCR were then conducted on the product from the previous PCR, yielding positive results for both infectious agents. The case report will focus on the case background, molecular testing outcomes and epidemiological investigation.

### **Atypical Presentation of Streptococcus Infection in Commercial Turkey Poults**

Elise Gerken<sup>1</sup>

*Butterball, LLC<sup>1</sup>*

Streptococcus is a common bacterial infection in young turkey poults in the commercial industry leading to acute septicemia. Typically, cases present as a sudden onset septicemia in otherwise healthy commercial flocks, with consistency in age of onset as well as clinical appearance and gross

lesions. Case prevalence has increased across the United States since 2018. Recently, one specific farm, has had an atypical presentation of streptococcus infection in poults showing neurologic signs in two subsequent flocks. Lab results identified *Streptococcus gallolyticus* as the cause of encephalitis and meningitis in these birds contributing to the neurologic lesions and signs. Genomic sequencing work comparing these isolates was conducted. Field observations and laboratory findings will be presented for these flocks.

### **Neurologic Signs in Broilers Associated with Myopathy Lesions**

Sabrina Hurst-Proctor<sup>1</sup>, Thomas Gaydos<sup>1</sup>, Oscar Fletcher<sup>3</sup>

*Gaydos Technical Services<sup>1</sup>, North Carolina State University<sup>3</sup>*

An integrated broiler company in the southeast reported sporadic flocks of broilers with neurologic signs and lameness. The age of presentation was variable, but most commonly was approximately 19 days of age. The affected barns had increased mortality up to four birds per thousand. Upon physical evaluation, the birds had tremors, torticollis, and were unable to stand. Affected birds were humanely euthanized and necropsied. The birds were grossly normal excluding soft bones and severe tibial dyschondroplasia. These findings stimulated an investigation into feed ingredient quality. During this investigation, it was discovered that there was variability in meat and bone meal calcium and phosphorus ratios from one of the suppliers. The nutritionist was notified of these findings and changes were made in the supplier of meat and bone meal. Three weeks after the initial cases, another visit was made and new flocks were seen affected with these symptoms. At this visit, gross striations were noted in the pubo-ischio-femoralis muscle of affected birds. Samples were taken and histopathology showed multifocal to multiregional myopathy consistent with pathologist suspected ionophore toxicity. However, ionophore toxicity was considered extremely unlikely because of the long term exclusion of ionophores from the system. Six weeks from the initial visit, ten birds from another flock were submitted for blood chemistry and necropsy and details are provided in this presentation. Ultimately, cases ended approximately 9 weeks after initial findings without further action.

### **Increased Incidence of Rickets in Broilers and Thin Eggshells in Broiler Breeders**

Thomas Gaydos<sup>1</sup>, Sabrina Hurst-Proctor<sup>1</sup>, Panchan Sitthicharoenchai<sup>3</sup>

*Gaydos Technical Services<sup>1</sup>, North Carolina State University College of Veterinary Medicine<sup>3</sup>*

Increased reports of lameness in organic broilers initiated a field investigation. The flocks visited showed signs of lameness and unwillingness to move. Varying between farms, 10-25% of the birds in the broiler barns were lame. Field necropsy revealed bones that were softer than expected for the age with grossly abnormal growth plates on the tibio-tarsus. Sections of tibia were submitted for histopathology which revealed failure of endochondral ossification with expansion of the growth plate in the zone of hypertrophy and the zone of proliferation consistent with rickets. At the same time, an elevated number of thin shells and cracked eggshells were reported by the hatchery. Eggs were evaluated and the shells were significantly thin and mottled for their age. Feed samples were collected from the impacted farms and the mill. While waiting for feed analysis, vitamin D was administered via water and oyster shells were spread in the hen houses. The organic feed and hen feed was produced at the same mill, and all diets from the affected mill were reformulated to include

additional calcium as a precaution. Feed samples were taken from affected flocks and the retained samples at the mill. The results of feed analysis showed variable calcium in the hen diets from 6% above to 51% below target. Similar results were seen with phosphorus values ranging from target to 18% below target. Investigation at the mill revealed a leak into the dicalcium phosphate bin of other bulk ingredients ultimately diluting the dicalcium phosphate and creating a deficiency. The leak was repaired and the dicalcium phosphate bin was emptied and cleaned. The breeder eggshell quality and broiler bone strength returned to normal within two weeks of the correction.

### **Case Report: Reimerella anatipestifer Outbreak in Breeder Ducklings**

Alexander Strauch<sup>1</sup>

*Four Star Veterinary Service<sup>1</sup>*

The following abstract details a case report surrounding a *Reimerella anatipestifer* outbreak at a breeder duckling farm. During the summer season at an Amish contract grower farm in the Midwest US, a flock of 2000 ~3 week old specialty breeder ducklings began exhibiting an increased mortality rate with ante-mortem clinical signs consisting of ataxia, obtundation, leg paddling, and head tremors. Field necropsy revealed some ducklings with fibrinous pericarditis and polyserositis. Based on the presumptive field diagnosis of reimerellosis, empirical antibiotic treatment with penicillin was administered via waterline while laboratory diagnostics were performed - where eventually a final diagnosis of *Reimerella anatipestifer* infection was confirmed. Fortunately, the flock responded well to medication and experienced a quick resolution of clinical signs and returned to normal mortality rates. Using the bacterial isolates, an autogenous vaccination program was developed for future duck breeder flocks at this same farm site so as to avoid similar morbidity and mortality issues.

### **Ask and You Shall Receive. Part 1: A Case Report on Vent Gleet Clinical Manifestations in Commercial Egg Layers as a Result of Chronic Excess Calcium Supplementation**

Daniel Wilson<sup>1</sup>, Montana Oubre<sup>2</sup>

*Wilson Vet Co.<sup>1</sup>, DSM<sup>2</sup>*

Modern laying hen genetic progress in the last century has created dramatic impacts on flock profitability and sustainability. The genetic progress in laying hen primary breeders has demanded parallel improvements in management, nutrition, and health of commercial flocks. As such, the egg industry has always faced a delicate balance of maintaining skeletal health and shell quality with the contending possibility of ingredient over usage and potential negative health effects. Veterinarians and nutritionists have long fought over the base needs of egg layers in regards to balancing the above parameters. Veterinarians have asked, if not begged, nutritionists to feed more calcium to prevent bone pathology and secondary effects. Historically one hallmark indicator of excess calcium usage or CA:P imbalance was that of gout. There has always been a deserved fear that overuse of calcium, while not only costly from a production standpoint, could contribute to higher mortality from gout and kidney damage. With the proper use of enzymes, appropriate feed schedules, and appropriate calcium particle size visceral gout has shifted as a much more rare primary mortality finding by US laying hen veterinarians. Visceral gout cases now more typically manifest from accidental events such as water deprivation, excess sodium levels, or feeding a laying ration to an immature pullet.

## **Use of Next Generation Sequencing for the diagnosis of poultry tumor viral diseases**

Isabel Gimeno<sup>1</sup>, Allison N Dickey<sup>2</sup>, Andrew S. Mason<sup>3</sup>

*North Carolina State University<sup>1</sup>, Bioinformatics Research Center, NCSU, Raleigh, NC, 27695-7566  
USA<sup>2</sup>, Biomedical Research Institute University of York York YO10 5DD, England<sup>3</sup>*

Tumor diseases in poultry are mainly induced by viruses; including herpesviruses (Marek's disease) and retroviruses (avian leukosis/sarcoma group and reticuloendotheliosis). They are all diseases of great economic relevance for the poultry industry and fast and accurate diagnoses are critical. Currently, the diagnosis of poultry viral tumor diseases is a multistep process that involves the clinical history and gross lesions, histopathology, and real time PCR to detect and quantify the most common viruses (Marek's disease, ALV-A, ALV-B/D, ALV-J, and REV). However, none of these assays will detect novel ALV subgroups or acutely transforming retroviruses. In such cases, time consuming and expensive virus isolation and identification, using specific cell lines susceptible for each subgroup, is needed. These limitations could be overcome using whole genome sequence (WGS) metagenomic analysis. In the current study, we have demonstrated that it is possible to obtain good quality DNA samples for further sequencing from samples collected on FTA cards. We could detect Gallid HV 2 in MD tumors but not in tissues latently infected with MDV. Such separation could be used in the diagnosis of MD although the same results could be obtained with qPCR and, as of now, it is more economical. On the other hand, WGS would enable sequence-level resolution of the specific MDV isolate which will be crucial for epidemiological studies. We could not detect Gallid HV 2 vaccines (CVI988) or Meleagrid HV (HVT), regardless if the samples were MD tumors or normal tissues. However, we could readily detect Gallid HV 3 in tumors induced by endogenous retroviruses. It has been previously reported that SB-1 replicates readily in retrovirus-induced tumors and our results confirm those findings. Evaluation of tumors induced by retroviruses requires specific pipelines. Standard bioinformatic analysis offered by commercial sequencing providers was able to detect endogenous avian viruses (EAV) at high frequency but it could not differentiate between EAV and ALV-J, or between tumors induced by endogenous retrovirus and normal tissues. Targeted bioinformatics pipelines (such as obsERVer) are required for high-throughput and specific diagnostics.

## **Cytopathologic diagnosis of inclusion body hepatitis in chickens via Romanowsky staining of liver impression smears**

Kathryn McCullough<sup>1</sup>, Subhan Ullah<sup>1</sup>, Adrea Mueller Slay<sup>1</sup>, Maurice Raccoursier<sup>1</sup>, Reece Bowers<sup>1</sup>, Cole Taylor<sup>1</sup>, Jason Sousa<sup>1</sup>, Tiffani Allen<sup>1</sup>, Daniel Sebastian<sup>1</sup>, Jenny A. Nicholds<sup>1</sup>

*University of Georgia<sup>1</sup>*

Aviadenoviruses are the etiologic agents of inclusion body hepatitis (IBH) and hepatitis-hydropericarium syndrome (HHS) in chickens. Emergence and re-emergence of these diseases internationally and recently within the United States pose an economic and health threat to commercial flocks. Presumptive diagnosis of IBH relies on clinical signs and gross lesions, and confirmatory diagnosis is made by histopathology, PCR, or virus isolation. Romanowsky-stained impression smear cytopathology is a rapid, technically simple, and cost-effective test that can be performed in the laboratory or in a field setting with minimal reagents and equipment as an accessory



to necropsy or when histopathology services are not available. We evaluated 108 pairs of cytopathologic preparations and histopathologic sections of livers from chickens inoculated or mock-inoculated with Fowl Adenovirus (FAV) or submitted as clinical suspects for IBH to the Poultry Diagnostic and Research Center (PDRC). Cytopathologic findings consisted of enlarged hepatocyte nuclei up to 10  $\mu\text{m}$  in diameter, containing clumped, acidophilic material that peripheralized and disrupted the native chromatin. These putative inclusion bodies were morphologically consistent with the intranuclear Adenoviral inclusion bodies observed on corresponding histopathology sections. The presence or absence of putative inclusion bodies per cytologic sample was compared to the corresponding histologic section. An 85.7% positive agreement and 89.1% negative agreement was present between sample types from the FAV inoculated and mock-inoculated birds, which were pooled, randomized, and evaluated blindly. A 100% positive agreement and 100% negative agreement was present between sample types from the clinically submitted cases, which were evaluated on a non-blinded, rolling basis. Per cytologic and histologic sample, inclusion bodies were counted across 114 fields at 40x magnification (27  $\text{mm}^2$  total). Of cytologic samples that contained putative inclusion bodies, the average number of inclusions per field was 0.07 for experimental samples and 1.5 for clinical samples. In positive histologic samples, the average number of inclusions per field was 0.13 for experimental samples and 11.0 for clinical samples. Eight non-pathologist observers with minimal (student) to extensive (10+ years) veterinary clinical experience evaluated a subset of 20 unknown cytologic samples and diagnosed each as "consistent with IBH" or "not consistent with IBH", with 66% positive agreement ("sensitivity") and 98% negative agreement ("specificity") to the previously determined histopathologic diagnosis. As such, we conclude that Romanowsky-stained impression smear cytopathology of the liver is an option to aid in the positive diagnosis of IBH in chickens; however, a negative smear cannot rule-out IBH and should be paired with ancillary testing for definitive diagnosis.

### **Non-invasive sampling for early detection and surveillance of avian reovirus infection in turkey flocks**

Mohamed Selim<sup>1</sup>, Jangra, S<sup>1</sup>, Luqman, M.<sup>1</sup>, Temeeyasen, G.<sup>1</sup>, Ohnstad., M<sup>1</sup>, Long., C<sup>1</sup>, Mor, S.<sup>1</sup>

*South Dakota state university*<sup>1</sup>

Turkey reoviruses (TRVs) continue to cause disease in turkeys with varied pathogenicity and tissue tropism. This study was conducted to evaluate the effectiveness of the non-invasive sampling as a unique way for screening of turkey reovirus (TRV) in hatcheries. One day old poults free from TRV is one of the most pivotal targets of the breeders companies, and the position of hatcheries in the center of the turkey industry between breeders and commercial units makes their screening an exceptional way to confirm that the produced poults from these hatcheries are free from TRV. In Fall of 2023, a total of 350 samples (meconium, fluff, and smashed eggshells) from five different hatcheries were sent to the Animal Disease Research and Diagnostic Laboratory, South Dakota State University (ADRDL-SDSU) for detection of TRV on weekly basis. These samples were properly processed and examined for the detection of TRV by using a universal avian reovirus realtime RT-PCR assay as well as virus isolation by inoculation on QT35 cell line. Additionally, the negative samples were spiked by TARV-O'Neil strain, and the RNA detection and virus survivability were examined immediately and after spiking by 1, 3, 5, 7 and 10 days under different storage temperatures 4 C, 24 C and 37 C. Thus, the potency of both rt-PCR assay as well as QT35 cell line was assessed in

detection of TRV from non-invasive samples. The results showed that the five hatcheries were completely free from TRV. Furthermore, the spiking experiment revealed that there were no any inhibitory substances in all the hatchery's samples which affect the efficacy of the PCR. Moreover, the detection of viral RNA as well as survivability of TRV can be persistent till 10 days regardless of the storage temperatures and the limit of detection (LOD) was 102 TCID<sub>50</sub>. In conclusion, this study reported that the non-invasive sampling can be used as an appropriate tool for routine screening of TRV in turkey hatcheries.

### **Use of feather pulp in the diagnosis of Marek's disease: viral replication and immunopathological characterization**

Federico Bonorino<sup>1</sup>, Juan Francisco Garcia Marin<sup>2</sup>, Abdelhamid Fares<sup>3</sup>, Nagwa Khaled<sup>4</sup>, Deanna Emmanuel<sup>5</sup>, Raveendra Kulkarni<sup>6</sup>, Isabel Gimeno<sup>7</sup>

*Universidad de León<sup>1</sup>, Universidad de Leon, Facultad de veterinaria, Leon, Spain, 24008<sup>2</sup>, NCSU, College of Veterinary Medicine, Raleigh, NC, 27607<sup>3</sup>, NCSU, College of Veterinary Medicine, Raleigh, NC, 27607<sup>4</sup>, NCSU, College of Veterinary Medicine, Raleigh, NC, 27607<sup>5</sup>, NCSU, College of Veterinary Medicine, Raleigh, NC, 27607<sup>6</sup>, NCSU, College of Veterinary Medicine, Raleigh, NC, 27607<sup>7</sup>*

In the last decade, the use of feather pulp (FP) as a gold standard sample for monitoring Marek's disease (MD) vaccination and for the diagnosis of MD has been well established. Our laboratory had previously demonstrated that FP can be used not only for the diagnosis of chickens that have already MD but also as an early diagnostic tool (as early as 21 days of age) to predict the outcome of the disease in apparently healthy chickens. Chickens that are not properly protected against MDV have very high levels of MDV DNA load in the FP at 21 dpi. In the current study, our objective was to elucidate the immunopathological changes that occur in the FP of chickens that were vaccinated with CVI988 vaccine (healthy), those that were vaccinated and challenged with a vv+MDV strain 648A (well protected), and unvaccinated chickens and challenged with vv+MDV strain 648A (no protection) when compared to non-infected naïve chickens. The viral DNA load, viral gene expression, characterization of the lesions by histopathology and immunohistochemistry, and immunophenotypic characterization of the FP infiltrates by flow cytometry was conducted. Our results demonstrate that 648A infected non-vaccinated chickens had a significant increase in the percentage of CD3+ T cells, mainly CD4+MHC-II+ cells and CD8+MHC-II+ cells, when compared to all other groups. They also had a significantly decreased number of CD8β+ T cells compared to all other groups. Interestingly, infection with 648A reduced the percentage of macrophages not only in the unvaccinated but also in the vaccinated and challenged group. In addition, groups that were vaccinated with CVI988, regardless of the challenge status, had higher levels of CD8β+ T cells, suggesting that the vaccine has an enhancing effect on the CTL cells. Our results confirm that the qPCR results obtained in chickens that were inadequately protected against MDV had a correlation with the histological lesions detected in the FP and provide further confirmation that FP is indeed an appropriate sample for the diagnosis of MD.

### **Genetic Evolution of Avian Reoviruses in France (2016-2023), First Applications of Oxford Nanopore Sequencing.**

Guillaume Croville<sup>1</sup>, Aurolie SECULA<sup>1</sup>, Mathilda WALCH<sup>1</sup>, Mattias DELPONT<sup>1</sup>, Sebastien SOUBIES<sup>1</sup>, Jean-Luc GUERIN<sup>1</sup>

*University of Toulouse<sup>1</sup>*

Avian orthoreoviruses (ARVs) are responsible for a variety of clinical conditions in poultry. Among these, orthoreovirus viral arthritis is associated with locomotion problems and consequent reduced performance in broiler chickens. For almost ten years, despite the vaccination of breeding stock, new outbreaks have been regularly identified due to the emergence of strains that escape vaccine protection. Vaccine escape can be explained by the variability of gene encoding the Sigma C surface protein, the main target of neutralizing antibodies. The variability of this gene makes it the basis for the classification of the strains in one of the 5 genogroups or "clusters" described to date. The emergence of wild strains of ARVs underlines the need for efficient detection and typing tools to refine diagnosis and epidemiological investigations. The challenge also lies in developing tools to assess the performance of commercially available vaccine solutions against wild strains. Between June and November 2023, 78 cases were referred to our laboratory thanks to the cooperation of veterinarians, production organizations and breeders in 9 French departments. Among the 78 suspected cases of reovirus tenosynovitis, 69 were confirmed by a positive PCR result, and 48 were subjected to sigma C gene sequencing for phylogenetic analysis. This phylogenetic analysis made it possible to classify the strains analyzed in our laboratory since 2016 into clusters 1.2, 2 and 4, but also to establish that by 2022 a strain very close to those currently responsible for clinical cases had already been identified. In addition, preliminary results from Oxford Nanopore sequencing of complete genomes have validated the interest of this method for studying the genetic diversity and the identification of evolution mechanisms of ARV. Briefly, a random RNA enrichment using the SISPA method was performed prior to preparation of the sequencing library with the Native Barcoding V14 kit. The library was loaded on a R10.4 flowcell and sequenced on the MinION MK1C. The complete sequence of the 10 genomic segments of 12 ARV field strains was generated in just a few hours, and phylogenetic analysis was carried out on the S1, M2 and L3 segments, enabling comprehensive genetic clustering of the ARVs. These results illustrate the value of genetic monitoring of ARVs, combined with detailed genetic characterization of pathogenic strains, for early detection of emerging variants, and for helping to update vaccines. These data will also be used to enrich genomic databases to better understand the spread and evolution of wild strains.

### **The Effect of High Dose Level of Amprolium Following Prolonged Use of Low Dose Level**

Leslie Johnson<sup>1</sup>, Nicholas Brown<sup>1</sup>, David Smith<sup>1</sup>, Bradley Turner<sup>4</sup>

*Huvepharma, Inc.<sup>1</sup>, Poultry Research Partners, LLC<sup>4</sup>*

In modern poultry production, chemotherapeutic agents serve as an indispensable tool for controlling and preventing coccidiosis. However, the extensive use of these compounds has led to the emergence of resistance in coccidia strains. The purpose of this study was to evaluate the impact of increasing the dose of amprolium to 0.025% (227 g/ton) after prolonged use at the 0.0125% (113.5 g/ton) level, with a focus on zootechnical performance and coccidiosis lesion scoring. In these experiments, Cobb 500 broiler chicks were evenly distributed among three treatment groups and reared for 6 weeks: Trt 1- no treatment, Trt 2- amprolium 25% at 113.5 g/ton, and Trt 3- amprolium 25% at 227 g/ton. Pen weights and feed consumption were analyzed to determine body weight gain

(BWG) and feed conversion ratio (FCR). Coccidiosis lesions for *E. acervulina* and *E. maxima* were assessed at 21 and 28 days of age using the Johnson and Reid scoring method. The comprehensive results of this study are pending.

### **Intestinal Integrity Overview in Latin America from 2021 to 2023**

Bauer Alvarenga<sup>1</sup>, Jessica Reple<sup>2</sup>, George Gould<sup>3</sup>, Isis Pasian<sup>4</sup>

*Elanco Animal Health<sup>1</sup>, Elanco Global Poultry<sup>2</sup>, Elanco Global Poultry<sup>3</sup>, Elanco Brazil<sup>4</sup>*

The Intestinal Integrity (I2) Index is a necropsy-based comprehensive index developed by Elanco Animal Health through their global surveillance platform, Health Tracking System (HTSi). This I2 Index is generated by assessing 23 key intestinal conditions known to impact the bird's intestinal health and performance resulting in a weighted algorithm. Kasab-Bachi et al (2017), showed that using a large database in HTSi can be a valuable indicator for flock performance. The purpose of I2 Index, combined with other information contained in the HTSi database, is to identify the yield gaps in production and suggest potential interventions to maintain flock health and optimize broiler performance. The aim of this study is providing an overview of I2 focusing on coccidiosis profile in Latin American countries that used HTSi from 2021 to 2023. In this period, 33,738 broiler flocks ranging from 7d to 49d were evaluated in three different Latin America regions – 1. Brazil; 2. South America (excluding Brazil); 3. Central America and Mexico representing 57%, 32% and 11% of the analyzes, respectively. South America presented the highest I2 Index with 97.2 points, followed by Central America and Mexico with 96.9 points and Brazil with 96.2 points ( $P < 0.0001$ ), not suffering significant variations over the years. In South America, the most prevalent coccidia and the peak of challenge was *Eimeria tenella* (6th wk), followed by *Eimeria acervulina* (5th wk) and *Eimeria maxima* (7th wk), with 0.055, 0.054 and 0.030 points respectively. In Central America and Mexico, the most prevalent coccidia and the peak of challenge was *E. acervulina* (4th wk), followed by *E. maxima* (5th wk) and *E. tenella* (7th wk), with 0.122, 0.106 and 0.086 points respectively. Lastly, in Brazil, the most prevalent coccidia and the most challenging period was *E. acervulina* (6th wk), followed by *E. maxima* (7th wk) and *E. tenella* (6th wk), with 0.078, 0.077 and 0.048 points respectively. The low levels of *Eimeria maxima* and *Eimeria acervulina* have contributed to the high I2 Index in South America. Furthermore, high average score of coccidiosis found in the postings from Brazil and high levels of other lesions like Cellular Sloughing, Excessive Fluid and Excessive Mucus contributed negatively to the Brazilian Index. Coccidiosis is a multifactorial disease and differences in management, nutrition, anticoccidial programs, weather and immunological status of birds (Chapman, 2014) directly impact the differences observed among different regions. Furthermore, it is important to highlight the greatest challenge of coccidiosis in the late stage of birds, with effective control being important throughout the bird's entire life due to the economic and performance impact of this disease. This review further highlights the importance of fully utilizing the data available to continue monitoring broiler health and identify ways to implement early intervention strategies to optimize health, welfare and performance.

### **A Live Look at the HTSi Database and I2 Index**

Francene Van Sambeek<sup>1</sup>

*Elanco Animal Health<sup>1</sup>*

Elanco's Health Tracking System (HTSi) is a comprehensive poultry health database integrating more than 20 years of data and an analysis of more than 6 million individual birds post-mortem. The database provides associated Intestinal Integrity (I2) scores, which give an overall picture of broiler gut health. When coupled with real-time information, the large data sets of historical information can help producers identify actionable insights for improving overall bird health and performance. Having a deeper understanding of the performance drivers through data helps producers optimize and identify when to intervene and select the right program or intervention that will deliver better results. Dr. Francene Van Sambeek will give a live view into the HTSi database, providing commentary behind today's flock health challenges and how the most common gut health concerns can be improved.

### **Sources of Viable Eimeria Oocysts to Young Chicks in Commercial Broiler Houses**

Mark Jenkins<sup>1</sup>

*Agricultural Research Service, USDA<sup>1</sup>*

A common practice in the poultry industry is to place auxiliary feed trays in broiler houses during the first 10 days after placement of chicks. The purpose of this practice is increase access of young chicks to feed in the early stages of growout. Unlike feed pans, chicks can freely enter the auxiliary feed trays. The objective of this study was to analyze fecal droppings in feed trays and in the surrounding litter for the presence of Eimeria oocysts and determine if these oocysts were capable of infecting immunologically naïve chicks. Our preliminary studies revealed Eimeria maxima and other Eimeria spp. oocysts in both feed lids and litter at about  $10^2 - 10^4$  oocysts/gram. Viable E. maxima oocysts were observed in 2/3 feed lids compared to 0/3 litter samples. In contrast, viable Eimeria spp. oocysts were observed in 3/3 feed lids and 3/3 litter samples. Studies are underway to determine the Eimeria species composition of these oocysts and if there is any relationship with the species used in the live Eimeria vaccine given at the hatchery. Moreover, similar studies are underway to determine if Eimeria oocysts are present in feed lids during a chemical anti-coccidial drug program. These findings may point to a potential source of Eimeria oocysts to young broiler chicks early in growout.

### **Serological Monitoring of a trivalent vector HVT-IBD-ILT vaccine in Layer Pullets under Field Conditions in Canada**

Kevin Amini<sup>1</sup>, Marina Gaimard<sup>2</sup>

*Boehringer Ingelheim Animal Health Canada Inc.<sup>1</sup>, Innovative Diagnostics (ID Vet) Lab, France<sup>2</sup>*

A field study was conducted in order to monitor the serological titers of a trivalent vector HVT-IBD-ILT vaccine in layer pullets. 23900 layer pullet chicks received the vaccine at the hatchery. Blood samples were collected randomly from 15 birds on 14, 21, 35, 49 and 63 days of age, and feather samples were collected from 15 birds on 14, 21, 28 and 35 days of age. For ILT titers, a newly developed indirect ELISA kit specific for detection of ILT Glycoprotein D antibodies was used, and the results proved it to be successful in detection of the antibodies and for monitoring of the trivalent vector HVT-IBD-ILT vaccine in the field. Serological monitoring of IBD titers was conducted using multiple different ELISA kits and the results were compared to each other. Singleplex PCR for HVT detected the vaccine virus in feathers. Overall, the trivalent vector HVT-IBD-ILT vaccine generated significant IBD and ILT titers in layer pullets, which were detected by different ELISA kits.

## **A Novel Application Procedure for Applying Vaccines Via Gel Droplets on the Farm at Day of Age in Commercial Poultry**

Kelli Jones<sup>1</sup>, Robert Beckstead<sup>1</sup>, James White<sup>1</sup>

*Ceva Animal Health*<sup>1</sup>

As tools used for combatting today's diseases of concern in commercial poultry continue to be taken away, maximizing the effectiveness of the tools that are available is essential for ensuring continued bird health. It is obvious that on farm day of age vaccination, especially in long lived poultry, is becoming more and more important in this role. Therefore, it is critical to ensure that vaccines are not only administered in a way that is appropriate and optimal, but that they are also user friendly to those that are tasked with the administration. As new technology enters the field, opportunities exist to explore options for its use in commercial poultry settings. One such new technology uses a handheld gel droplet application bar like the stationary gel application systems that have been used in commercial hatcheries for several years now. In this study, for the first time in the US, a handheld gel droplet application bar was used to vaccinate commercial broiler & layer breeder pullets at day of age in chick boxes on the farm with vaccines that are typically administered at this time. More specifically, this study included vaccination with commercially licensed coccidiosis, Salmonella, and Infectious Bronchitis Virus vaccines, but this method could encompass other products, as well. To help validate this method of application, vaccine "takes" were performed via tongue checks immediately following vaccination, as well as OPGs for coccidiosis, & tracheal swabs for molecular detection of IBV at 5-7d of age. Feedback from vaccinators in this study was very positive in that this method took much less time and effort compared to a traditional pump-up sprayer type gel or water vaccination set up. Chicks appeared to consume gel droplets immediately and did not become wet. This study is a good example of how existing equipment can be re-purposed and/or re-designed for use in the field to provide easier more efficient tools to ensure vaccination success and improved personnel compliance.

## **Development and characterization of HVT vector vaccines to control ND and IBD**

Blanca Lupiani<sup>1</sup>, Mohammad AL-Mahmood<sup>1</sup>, Owais Khan<sup>1</sup>, Sanjay M Reddy<sup>1</sup>

*Department of Veterinary Pathobiology, Texas A&M University*<sup>1</sup>

Turkey herpesvirus (HVT) is widely accepted as a vector for the development of vaccines for protection against economically important diseases in chickens. Here, we report the development and characterization of HVT vectors expressing the VP2 gene of infectious bursal disease virus (IBDV) and the fusion (F) gene of Newcastle disease virus (NDV) alone or in combination. All recombinant HVT vector vaccines (HVT-IBD, HVT-ND, and HVT-IBD/ND) were stable after in vitro propagation and continued to express the foreign genes after 5 passages. In addition, we evaluated different promoters and gene orientations for the F gene in the HVT-IBD/ND constructs. The levels of F expression in cell culture, as determined by immunofluorescence, varied depending on the promoter used and gene orientation, while the VP2 levels were the same for all constructs. All vaccine constructs were evaluated for protection against IBD and ND in SPF chickens inoculated subcutaneously at day of age. All animal experiments included uninoculated chickens and equivalent commercial vaccines as controls. To determine IBD protection, antibody levels, and

bursa atrophy (histological examination and bursa/body weight ratio) upon standard IBDV challenge were examined. For ND protection, antibody response as determined by virus neutralization (VN), hemagglutination inhibition (HI), and ELISA were determined. Our results show that all vaccine constructs containing the VP2 gene elicited protective antibody titers at all times tested and protected chickens from disease (bursa atrophy) upon challenge. Both HVT-ND constructs (F14 and F22) elicited protective antibody titers against NDV by both VN and HI. On the other hand, one of the HVT-IBD/ND constructs (VF01) did not elicit protective antibody levels at any of the times tested, and a second one (VF10) induced protective antibody levels at weeks 7, 9, and 11 but not week 5, while two others (VF08 and VF27) elicited protective antibodies at all times tested. All HVT vaccine constructs generated provided levels of protection against vMDV similar to HVT and commercial HVT-IBD, HVT-ND, and HVT-IBD/ND vaccines. We also examined if vaccination with a mixture of HVT-IBD (V45) and HVT-ND (F14) vaccines induced similar protection as the double HVT-IBD/ND (VF27) vaccine. Our results show no difference in antibody titers to VP2 or protection upon IBDV challenge for any vaccines tested, including commercial controls. However, significant differences in protective NDV antibody titers (as determined by VN and HI) were observed when a mixture of HVT-IBD (V45) and HVT-ND (F14) or commercial HVT-IBD and HVT-ND vaccines were used compared to the HVT-IBD/ND or HVT-ND constructs. Our results confirm that caution should be observed in how HVT vector vaccines are used since the mixture of two HVT vector vaccines can reduce vaccine efficacy.

### **Understanding the Impact of Backpack Sprayers on Field Vaccination**

Cory Yarbrough<sup>1</sup>, Brian J Jordan<sup>2</sup>

*Graduate Student - University of Georgia<sup>1</sup>, Associate Professor - University of Georgia<sup>2</sup>*

Infectious Bronchitis Virus (IBV) is one of the primary viral respiratory pathogens in chickens and practically all poultry raised in the U.S. are vaccinated with an IBV vaccine. Pullets are vaccinated via field application multiple times throughout rearing, yet testing of vaccine application repeatedly shows shockingly low or negative results after field spray vaccination. Previous research showed that modifications in application parameters improved hatchery spray application, therefore the same parameters need to be evaluated for field spray vaccination equipment. This study evaluated two commercial field spray applicators, a Stihl gas-powered, single-wand sprayer provided by Merck Animal Health and a SoloVac battery-powered, double-wand sprayer provided by Boehringer Ingelheim. The initial portion of this study measured several parameters including output volume, flow rate, and spray pattern. Weights of each machine were similar, but the SoloVac has a larger capacity tank which makes it heavier at full volume. The spray patterns of each machine were similar when using the conical distribution settings, but the Stihl sprayer also had a flat distribution setting that changed the pattern. The SoloVac dispersed diluent with a consistent spray pattern up to 15 feet with an average flow rate of 1.03 gal/min at 3 gallons, while the Stihl sprayer reaches 20 feet with a consistent spray pattern with an average flow rate of 0.68 gal/min at 3 gallons. At these rates with 3 gallons of diluent, it would take 2 minutes and 55 seconds to empty the SoloVac tank and 4 minutes and 24 seconds to empty the Stihl sprayer tank. With these parameters measured, the next step in this project is to understand vaccine loss through field spray application in terms of destroyed vaccine moving through the sprayers and vaccine lost to the environment during the spray. These findings will be discussed in this presentation, and this data will aid in improving the vaccination

success of IBV and other vaccines that are mass-applied to breeders and layers during rearing and lay in a field vaccination scenario.

### **Field safety of in ovo and subcutaneous administration of HVT-ILT-IBD vaccine in commercial Broilers**

Faris Jirjis<sup>1</sup>, Stephanie Cook<sup>1</sup>, Nicholas Finnegan<sup>1</sup>, Kayla Del Vechio<sup>1</sup>, Gabriela Beltran Garza<sup>1</sup>, Ivan Alvarado Ortiz<sup>1</sup>

*Merck Animal Health*<sup>1</sup>

Studies were conducted to establish the field safety of a Bursal Disease-Infectious Laryngotracheitis-Marek's Disease Vaccine, Serotype 3, Live Marek's Disease Vector (HVT-ILT-IBD), administered by the in-ovo route in 18- to 19-day-old chicken embryos or by the subcutaneous route in one-day-old chickens under field conditions. The field safety studies were conducted in three different states in the US with four site locations. All chicks were observed for mortality and signs of systemic and localized adverse events until 21 days of age. Mortality rates were within the acceptable range by each producer as were condemnation rates at slaughter for all sites. There were no systemic adverse events related to vaccination. There were no localized injection site reactions in any of the test subcutaneously vaccinated chicks when injection sites were evaluated following vaccination. These data provide support for the safety of HVT-ILT-IBD vaccine for use by the in-ovo and subcutaneous routes under field conditions.

### **Replication and early immune responses induced by various MD vaccines when administered in ovo in meat type chickens**

Abdelhamid Fares<sup>1</sup>, Carissa Gaghan<sup>2</sup>, Matthew Browning<sup>3</sup>, Allison Boone<sup>4</sup>, Raveendra Kulkarni<sup>5</sup>, Isabel M Gimeno<sup>6</sup>

*North Carolina State University, College of Veterinary Medicine*<sup>1</sup>, *North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27607*<sup>2</sup>, *North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27607*<sup>3</sup>, *Experimental Pathology Laboratories, Morrisville, NC 27560*<sup>4</sup>, *North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27607*<sup>5</sup>, *North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27607*<sup>6</sup>

It has been shown that in ovo administration of Herpesvirus of Turkey (HVT) hastens the immunocompetence of newly hatched chickens. HVT enhances both innate and cellular immune responses evaluated by the immunophenotypes present in the spleens at one day of age, ability of T cells to proliferate ex vivo when exposed to concanavalin A, and the skin test with Phytohemagglutinin-L. In previous work, we demonstrated that in ovo administration of other Marek's Disease (MD) vaccines (CVI988 and SB-1) had a very different effect on the splenocytes population at day of age when compared to HVT. However, when those vaccines were administered with HVT the effects were identical to HVT. In the present study, we evaluated replication of MD vaccines, when administered alone (HVT, SB-1, or CVI-988) or in combination (HVT+SB-1 or HVT+CVI988), in the embryonic spleen, thymus, and bursa. In addition, we evaluated the effect of those vaccination programs on the cytokine gene expression in the spleen of one-day-old meat type chickens. Our results demonstrated that HVT is the vaccine that most readily replicates in embryonic tissues whether it is administered alone or in combination. However, the vaccine program that had the



strongest effect on cytokine responses at day of age was the combined administration of HVT and CVI988. This group had a significant increase of interferon (IFN)-beta and IFN-gamma compared to all other vaccinated groups. No differences in the expression of Toll-like receptor (TLR)-3, TLR-21, interleukin (IL)-10, or IL-12 were detected among groups. Although no protective synergism has been found between HVT and CVI98 when evaluated for protection against MD, the poultry industry typically administers the two vaccines together. Our results suggest that such combination has a positive effect on the early immune responses elicited by the vaccines. Additional studies are warranted to further evaluate how HVT enhances the immune responses elicited by CVI988.

### **“Staph” Problems Again: Synovitis and Broiler Breeder Livability**

Maggie Thompson<sup>1</sup>

*AMT Poultry Health Consulting, LLC<sup>1</sup>*

Current broiler industry trends indicate a decrease in both breeder male livability and fertility. As a result, focus is gradually shifting to more precise male management. Among the primary concerns surrounding breeder male livability includes the prevalence of synovitis, not only during the rear phase but also following move to the hen farm. Many cases of synovitis are diagnosed, either presumptively or with culture confirmation, as Staphylococcal-induced synovitis. This presentation will highlight the importance of astute diagnostic lab personnel and the subsequent identification of a new bacterial organism linked to synovitis in broiler breeders. Additionally, results from an industry survey on the prevalence and overall concern for synovitis in broiler breeders will be presented and discussed.

### **The Life of an Extension Poultry Veterinarian: Lessons Learned from My Personal Journey**

Nathaniel Tablante<sup>1</sup>

*University of Maryland College Park<sup>1</sup>*

After spending several years as a live poultry production veterinarian in the Philippines and Canada, I decided to switch to a career in academia by joining the University of Maryland in 1997 as an Extension Poultry Veterinarian. In keeping with the mission of a land grant university, I was given a 3-way appointment (75% Extension, 15% Research, and 10% Teaching) which meant that, in addition to my main Extension responsibility, i.e. developing and conducting non-formal educational programs and disseminating science-based information to help poultry farmers keep their flocks healthy, I also had to teach courses, secure research grants, and publish articles in peer-reviewed journals. With a little bit of trepidation, I decided to face the challenge and forge ahead. After 27 years, I am proud to say, I think I did quite well (got tenured and worked my way up from Assistant Professor to Full Professor) and now I'm officially retired! To my colleagues who have been down this road before, you know what I'm talking about. To those who are thinking about pursuing or just starting a similar career, have no fear. While there is no easy way or a "one size fits all" approach, the keys to success are understanding your role and the needs of your clientele and stakeholders. Of course, as an Extension specialist, you must also have strong communication skills and the ability to work with a diverse group of people. While methods of communicating scientific information and delivering Extension services to farmers, poultry producers, and the general public have changed over the last century due to technological advances, the mission of the Extension Poultry

Veterinarian (and other Extension specialists). i.e. being relevant and effective in "taking the university to the people" will never change.

### **Experiences with Artificial Intelligence in Turkey Barns in Winter**

Brian Wooming<sup>1</sup>

*Cargill Turkey Production LLC<sup>1</sup>*

Regular turkey hens were raised in an all-in, all-out production system during the winter of 2023/2024. Two of three barns were managed by a commercially available system that controlled fan and stove run times. Parameter constraints included temperature, carbon dioxide and relative humidity. Bin and bird scales were also in use during rearing. The third barn was controlled by the manager and was used as a control for comparison on energy usage and performance. The presentation will focus on environmental control and final bird performance.

### **AAAP Diversity and Inclusion (D&I) committee: Evolving to meet the changing demographics of AAAP members**

Eliza Ripplinger<sup>1</sup>, Ashley Hallowell<sup>2</sup>, Valerie Marcano<sup>3</sup>, Susan M Williams<sup>4</sup>, Jessica K Hockaday<sup>5</sup>

*NCSU<sup>1</sup>, Phibro Animal Health Corporation<sup>2</sup>, ValCri Consulting, LLC<sup>3</sup>, PDRC-UGA<sup>4</sup>, Hockaday Consulting<sup>5</sup>*

The first AAAP Diversity and Inclusion (D&I) demographic survey was conducted in 2023. The purpose was to assess the everchanging demographics of the AAAP and evaluate the level of AAAP support to its members. A total of 214 participants, consisting of 19.8% of AAAP members, completed the survey. The AAAP's D&I conducted an anonymous demographics and climate survey to evaluate the diversity climate of the AAAP. Diversity is broadly defined as the practice of including persons from a range of backgrounds including race, ethnicity, gender, age, sexual orientation, class, physical and mental ability, family composition, social economic status, and spiritual practice which all relate to individual differences among individuals in our organization. As the AAAP membership changes, the D&I committee will also be adapting to ensure all members are represented and respected in the organization. In addition to the survey results, the D&I committee will present plans for organization-wide support to create a more inclusive space for all members. Upcoming committee events will be highlighted, as will plans on improving committee member participation, expanding membership, and support groups within AAAP (committees, BOD, student group, etc.). Focusing on a major goal of the D&I committee, "adapt as the AAAP membership evolves", the initial survey will be modified as needed and sent out every five years to AAAP members. Upcoming changes will be outlined in the presentation/poster. In addition, the D&I committee will provide an overview on focusing the committee's efforts to meet the needs of the AAAP members.

### **An overview on the AVMA Working Group on the Psychological Impacts of Humane Endings**

Michelle Kromm<sup>1</sup>, Cia Johnson<sup>2</sup>

*Food Forward LLC<sup>1</sup>, AVMA<sup>2</sup>*

The psychological impacts of humane endings on veterinarians, animal health and production professionals, and support staff are a growing concern within the veterinary community. The COVID-

19 pandemic has further highlighted the need for resources, understanding, and compassion for our colleagues involved in the care and wellbeing of animals. Whether humane endings activities are related to euthanasia of individual animals, slaughter of animals for human consumption, or emergency situations warranting depopulation of a large group, it is often the veterinarian's responsibility to make and oversee these determinations. The AVMA has historically and continues to lead the charge on humane endings and the various issues and difficulties surrounding this delicate topic and includes their three humane ending's documents (AVMA Guidelines for the Euthanasia of Animals, AVMA Guidelines for the Humane Slaughter of Animals, and AVMA Guidelines for the Depopulation of Animals). Recently, the AVMA formed a working group on the psychological impacts of humane endings, to address the concerns of its members, veterinary medicine, and animal health professionals at home and abroad. This working group is using end-of-life decisions information to lay the foundation and rationale for improved preparation, establishment of sustainable mental health resources, and to offer recommendations on pragmatic solutions to support and prepare veterinary professionals as leaders impacted by depopulation events. While there are inevitable mental health challenges shared by all people involved in end-of-life decisions, it is crucial to recognize that there are stressors unique to veterinarians working in settings outside private practice. Through ongoing leadership from the AVMA, these efforts will keep expanding, through continued enhancements on humane endings guidance documents, creation of better tools and resources, and taking the necessary steps of acknowledging and alleviating the associated psychological impacts that occur on the veterinary, research, and production animal community.

#### **AAAP Research priorities committee survey results**

Stephen Williams<sup>1</sup>

*AAAP Research Priorities Committee*<sup>1</sup>

2024 AAAP Research priorities committee survey results and findings by commodities group.

#### **Is male grading a proper management tool to improve male uniformity and livability as well as production performance?**

Jose Bruzual<sup>1</sup>, Yndira Vilorio<sup>2</sup>, Javier Alvarez<sup>2</sup>

*Aviagen*<sup>1</sup>, *Bachoco/OK Foods*<sup>2</sup>

Over the last decade, the male broiler breeder mortality appears to be increasing and the fertility decreasing. During this time, the broiler feed conversion ratio (FCR) has improved by approximately 2 to 3 points per year resulting in increased efficiency. The gains in feed efficiency are also expressed at the breeder level. While the body weight (BW) standards for male broiler breeders have not been modified significantly over the years, their requirements have changed. Therefore, management practices of the male broiler breeder must be fine-tuned and focused on the current requirements to prevent different health issues. The objective of this presentation is to introduce the concept of grading males as a tool for improving bird uniformity and livability during the grow-out period with better outcomes for livability and fertility in production. In this experiment, 5,368 males were subjected to a 2-way grading (sorting) system. The males were divided into two groups with an equal number of birds according to BW (Small-Medium and Medium-Large). The cut-off point between groups was the average BW for the whole flock. Birds were fed to reach standard BW during the grow-

out period with the goal for both groups to have similar weights at the time of transfer to the breeder house. Grading was performed at 4 and 11 weeks of age (WOA). To understand the growth profile at different ages, three groups of birds with different BW were followed throughout this study. Each group consisted of 50 birds each as follows: ~ 1lb./small, ~ 1.50 lb./average, and ~2 lbs./heavy, and were individually wing banded at 4 weeks of age. Birds in the small and average groups were grown on the small-medium bird side and those in the heavy group were grown on the medium-heavy bird side. BW and Shank Length (SL) were recorded at 4, 11, 18, and 27 WOA. In addition, testicular weight and microscopic evaluation of the testicles for Sertoli cell counts from 5-7 birds per group were done at 27 WOA in the stud house before the males were used as spike males. Results from this experiment showed promising results. When small birds were separated from their bigger counterparts and given proper housing conditions, they caught up to the BW standard. Similarly, when heavy birds were separated from the smaller groups, it became easier to control their BW and maintain it close to the standard. Furthermore, SL was significantly different at 4 WOA among the three BW groups; however, by 27 WOA, there were no major differences among the three groups of birds in shank length and testicular size. In summary, a 2-way grading system is a tool that helped improve male uniformity with a positive effect on livability in the grow-out period and also improved the male quality of the birds coming into production. A follow-up in the breeder house is planned to see the effect on livability, numbers of spikes, and fertility.