AAAP Scientific Program

Keynote Address

The Changing Global Landscape of Highly Pathogenic Avian Influenza

David Swayne

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Virology

Isolation and Characterization of Adenoviruses Associated with Inclusion Body Hepatitis in the United States and Disease Control Through Breeder Vaccination

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Incidence of inclusion body hepatitis (IBH) has increased in broiler chickens in the United States over the past two years. Serotype 8b adenoviruses have been isolated from the majority of IBH cases, and these isolates are genetically highly conserved. Additionally, serotype 11 adenoviruses have been isolated from several cases of IBH. During propagation of the adenovirus isolates it was noted that not all serotype 8b isolates replicate equally in cell cultures, which has implications in isolate selection for autogenous vaccines. Autogenous vaccines containing serotype 8b adenovirus isolates have been used to mute vertical transmission of adenoviruses and to provide maternal immunity to progeny chicks. However, cases of IBH have continued to occur despite the vaccination. Adenovirus serotype 8b antibody titers were assessed in vaccinated breeder hens and day-old progeny chicks using a virus neutralization assay and showed that many breeder flocks had poor antibody titers post-vaccination and that many progeny chicks were adenovirus antibody-free at hatch, and therefore susceptible to infection at a young age. Additionally, serologic response post-vaccination with several serials of autogenous adenovirus vaccines was evaluated experimentally. It was found that antibody response varied among autogenous vaccine serials and among manufacturers of autogenous vaccines. Finally, little is known about pathogenicity differences among recent adenovirus isolates. Nineteen adenovirus isolates from different broiler producers, geographic regions, and replication rates were selected for pathogenicity evaluation. Pathogenicity of adenovirus isolates was evaluated in SPF chicks and susceptible broiler chicks, and findings will be presented.

Egg Drop Syndrome 76 Transmission in Broiler Chickens

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Egg Drop Syndrome 76 atadenovirus (EDSV) causes diminished egg quality and production in chickens. After being detected in commercial chickens showing clinical disease for the first time in the United States, EDSV became a trade issue. Further understanding the transmission potential of EDSV and the possible role of poultry meat to infect chickens is the aim of this study.An oral route challenge experiment was completed in SPF broiler chickens, and virus

could be detected in cloacal swabs and blood at 5 days post infection and occasionally after. All challenged birds seroconverted at the end of the study. EDSV contact transmission was evaluated between infected and naïve 1-day old SPF birds. Contact birds showed a delayed and sporadic detection of the virus in cloacal swabs and blood. Most of the birds seroconverted by the end of the experiment. To elucidate if tissue from infected birds could be a source for EDSV transmission by oral exposure, liver, spleen and muscle from infected birds was fed to 16-day old chickens. Infected muscle did not induce a marked infection or viremia when fed to chickens, while infected spleen and liver did.EDSV was detected in liver and spleen at the end of the experiment in most groups, with higher positivity and DNA levels in spleen. All muscle samples from all birds were negative for EDSV at the end of the experiment, as determined by qPCR. The spleen and liver samples 5 days after challenge, the peak of infection, could transmit EDSV to naïve birds through oral administration. However, no detectable virus was found in muscle tissue and was unlikely to spread infection at either peak infection or at 27 days post exposure suggesting an extremely low risk of this poultry product in transmission.

Double Trouble - Concurrent Egg Drops in Two Flocks of Turkey Breeder Hens

Jake Carlson, Ben Wileman, Marissa Studniski, Jewell Bremer

Select Genetics

In early December, a flock of turkey breeder hens began to experience an egg drop in two out of four barns. Blood and trachea swabs were collected and the flock was negative for influenza via PCR but serologically positive for PMV-3. Additional PCR samples along with entire oviducts were collected for PCR confirmation of PMV-3 along with virus isolation and Next Generation Sequencing. A couple of days later, a neighboring flock of turkey breeder hens tested PCR positive on routine sampling for avian influenza (low pathogenicity) to which an egg drop followed. This presentation follows the progression and resolution of both egg drops as well as outlines the clinical reasoning and sample collection methods used to rule out the major viral causes of egg drops. This case is still under investigation and all findings will be reported.

Reed Rumsey (Clinical Research) Award Winner

Detection, Surveillance and Mechanical Transmission of Egg Drop Syndrome

Ashley Hallowell[1], Eric Gingerich[2], Sherill Davison[3], Holly Sellers[1], Jenny Nicholds[1]

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Egg drop syndrome 1976 (EDS-76) is a viral disease caused by duck Atadenovirus A and was considered exotic to the United States; however in recent years and presently, the disease has been detected in domestic commercial layer and broiler breeder chickens resulting in significant economic losses. The disease is characterized by a decrease in egg production and soft or shell-less eggs in birds that otherwise appear healthy. Diagnostic tools for EDS-76 are limited in the U.S. In unvaccinated birds, detection of antibodies by the hemagglutination inhibition (HI) or enzyme-linked immunosorbent assay (ELISA) tests are the primary means of confirmatory diagnosis. Detection of EDS-76 DNA by PCR or real time PCR (qPCR) is utilized on suspect clinical cases. Virus

isolation (VI) is complicated, as primary isolation is performed in duck embryo fibroblasts (DEF). In the U.S., there are limited sources for specific pathogen free (SPF) duck eggs, so laboratories rely on serology and qPCR for confirmatory diagnosis. EDS-76 qPCR is not sufficient to detect viable virus load following cleaning and disinfection efforts nor is it capable of identifying the highest risk areas during and after an infection. Additionally, it is unknown if mechanical vectors such as rodents and insects play a role in transmission of EDS-76. The first objective of this study is to perform VI on EDS-76 qPCR positive clinical samples to evaluate utility of cultured cells for primary isolation. VI will also be used to test paired environmental samples to determine if cleaning and disinfection efforts are successful at reducing or eliminating live virus from infected premises. The second objective of this study is to conduct serological and viral shedding surveillance of flocks pre-vaccination (13 weeks-of-age), post-vaccination (16 weeks-of-age), and post-placement (35 weeks-of-age) on previously EDS-76 positive farms using EDS-76 HIs and ELISAs (BioChek, Netherlands). Cloacal swabs will be collected and tested by qPCR to determine viral load and VI will be performed on positive cloacal swab samples. The third objective of this study is to utilize EDS-76 qPCR to identify potential vectors such as rodents, beetles, and flies. The results obtained from these studies will provide the egg laying industry valuable information on how to prevent and mitigate EDS-76 in their operations. Preliminary data thus far has been collected for each of the three objectives. The EDS-76 strain Ad-127 (Charles River SPAFAS) readily replicates in primary and secondary specific pathogen free (SPF) duck embryo fibroblasts (DEF) obtained from the Avian Disease and Oncology Laboratory, USDA, East Lansing, MI. Cytopathic effect (CPE) in DEFs was observed as early as 72 hours post inoculation and is characterized by rounding and enlargement. The Ad-127 reference virus will be utilized to evaluate additional cells and cell lines for replication susceptibility. Additionally, a snapshot of HI geometric mean titers (GMT) and ELISA GMT and % coefficient of variation (CV) was captured across four houses on a single site at three time points, two weeks apart, where clinical signs of EDS-76 were first detected in house 4. Finally, a total of 22 sets of swab pools (191 total swabs) from live bird market ducks were tested using EDS-76 qPCR. Of the 22 cases submitted, 19 cases contained swabs positive for EDS-76. Additional diagnostic details and the outcome of further investigation of the objectives for this study will be presented.

Effect of Marek's Disease CVRM Vaccine on the Enhancement of Spontaneous ALV-like Tumors

John Dunn[1], Jody Mays[1], Cari Hearn[1], Kristen Roza-Sutherland[2]

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Spontaneous bursal lymphomas of unknown etiology have been reported in chicken lines and crosses that have been maintained in specific pathogen free (SPF) conditions that are free of exogenous retroviruses. The USDA-ARS Avian Disease & Oncology Laboratory maintains a genetic line of chickens, line ALV-6, that is prone to a small incidence of spontaneous tumors. We have previously shown that serotype 2 MDV strain SB-1 enhances the production of spontaneous tumors in this line. Given the recent increase in use of a new generation of Marek's disease vaccines and new MD-based vaccine vectors, there is renewed interest in confirming that these new MD vaccines do not similarly enhance spontaneous bursal lymphomas. One recently developed MD vaccine, CVRM, is a serotype 1 recombinant

vaccine based on CVI988 with insertion of reticuloendotheliosis virus LTR. In this study we evaluated whether either CVI988 or CVRM vaccines cause enhancement of spontaneous avian leukosis-like tumors in line ALV-6 chickens. Comparing 100 birds per group, we found no significant difference in the percent of spontaneous tumors from either vaccine compared to unvaccinated control birds.

Replication of Avian Orthoavulavirus 1 (AOAV1) and Induction of Innate Immune Response in TLR3 and MDA5 Knockout Quail (QT-35) and Chicken (DF-1) Cells

Chang-Won Lee

Southeast Poultry Research Laboratory, USDA-ARS

Chicken and quail share more than 90% sequence identity in Toll-like receptor 3 (TLR3) and Melanoma differentiation-associated gene 5 (MDA5) which serve as pathogen recognition receptors and are involved in the induction of innate immune responses. Utilizing CRISPR/Cas9 system, we generated TLR3 and MDA5 knockout quail (QT-35) and chicken (DF-1) fibroblast cells and studied the replication of AOAV-1 and its induction of innate immune response. We confirmed that the knockout of TLR3 and MDA5 affects the induction of type I IFNs and interferon-stimulated genes. However, the knockout effect on virus replication and level of induction in innate immune response was different between the AOAV-1 and previously tested influenza A virus. Our study shows that innate immune response can be host and viral pathogen specific and warrants further investigation in understanding the relevance of dsRNA receptor-mediated immune responses in viral replication and pathogenesis in different avian species.

NDV Vaccination in Backyard Unconventional Poultry Flocks

Rodrigo Gallardo[1], Theodore Derksen[1], Alejandra Figueroa[1], Marco Solis[2], Jose Garcia[2], Charlene Rivera[2]

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For the last 3 years we have been working with backyard unconventional flock owners in Southern California educating them on disease prevention, biosecurity, and management. Part of our program involves the recommendation of NDV live attenuated vaccine use every six months, allowing these birds to mount immune responses and in the case of an NDV velogenic outbreak decrease viral shedding and subsequently virus dissemination. This strategy is founded on the premise that NDV has a single serotype allowing lentogenic vaccines to protect against field strains. Throughout these three years, we have analyzed serology of unvaccinated and vaccinated flocks. Antibody titers in the average flock are un-uniform reflected by high variation coefficients and low to mid-level titers. Taking that into consideration we continued doing outreach to improve vaccination methodologies. Breeders complained about the amount of work that ocular vaccination required and that most of the time the vaccines were given via water. Taking that into consideration, we decided to design an experiment that compared Spray and ocular vaccination. Three backyard flocks were recruited, in one of them NDV vaccine was applied ocularly, in the second spray, using the right dose and droplet size and the third flock

was used as a control. Two weeks after a second live attenuated vaccine was applied. We will collect blood samples on the day of vaccination and every 10 days until 2 months after starting the experiment.

Field Evaluation of Two Different Vaccination Protocols for Label Rouge Broilers in France: from the Hatchery to the Processing Plant

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Newcastle disease (ND), caused by virulent strains of Avian orthoavulavirus-1 (AOaV-1), is a serious and notifiable disease in France and can cause severe damage to the poultry industry. Pigeon paramyxovirus -1 (PPMV) is an antigenic variant of AOaV-1. Although ND vaccination is not mandatory, except for PPMV1 in pigeons, it's highly recommended by the authorities for long-living birds. In addition to ND, Marek (MD), infectious bursal disease (IBD), and infectious bronchitis (IB) viruses are known to impact the performance of slow-growing broilers as well. Therefore, control of these 4 pathogens is one of the priorities of the slow-growing broiler industry in France, and biosecurity and vaccination are the main control measures applied. This study aimed at evaluating the impact of two different vaccination programs in the field, between November 2020 and June 2021. A total of 343 flocks, (a total of 1.45 million birds) were evaluated. The birds were in the same geographical area under the same producer organization, and 95% of flocks originated from the same hatchery. The two vaccination protocols tested are: Protocol A (=151 flocks), using a dual-construct rHVT-IBD-ND (Innovax®-ND-IBD, MSD Animal Health) combined with MD Rispens CVI 988 (Nobilis® Rismavac, MSD Animal Health) via in-ovo , followed by IB vaccination (Nobilis® Ma5 and Nobilis® 4-91 vaccination) at 1 day of age, and Protocol B (=192 flocks), using rHVT-IBD (company B) + MD Rispens CVI 988 (company B) via in-ovo, followed by live ND plus IB H120 and IB88 vaccination (company B) at day 1. All flocks were followed up to the slaughter age (average 86 days), when blood and cloacal swabs samples were collected on 20 of them for further IBD and ND serological analysis, along with IB PCR tests (average sampling age 85 days). Performance data at slaughter were also collected and analyzed using the Wilcoxon rank sum test with continuity correction or the Two-sample T-test, to compare the two vaccination protocols (A & B). The results indicated that there was a significant reduction of whole condemnations as well as a significant increase of class-A quality carcasses % ($p<0.05$) in the protocol A flocks. Economic average daily gain (eADG), performance index, and economic feed conversion ratio (eFCR) were also numerically better for the birds vaccinated with protocol A. Although there was no statistical significance, the economic analysis revealed a better total gross margin per 1000 birds for protocol A. The serological results showed adequate seroconversion after the vaccination for both IBD VP2 (protocol A&B) and ND F protein (protocol A). Whereas the flocks vaccinated with protocol A showed a better recovery of IB vaccine strains and no detection of any other IBV, a field IBV was detected from one of the flocks with protocol B vaccination based on IB PCR results. At 85 days of age, 40% of protocol B sampled flocks showed ND titers above 2900 [2918-6871] (ID Screen® ELISA), and lower average CVs. Considering that protocol B included only one live attenuated ND vaccine at 1 day of age, this finding might imply the circulation of low pathogenic AOaV-1, as there

was no clinical evidence of highly virulent AOaV-1 circulation during the study. Overall data showed the good performance of protocol A in terms of flock health and economic performance of slow-growing broilers in France.

Antigenic Relatedness of Field Isolates of Avian Reovirus Isolated at the Poultry Diagnostic and Research Center of the University of Georgia

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Avian reovirus (ARV) infections cause diseases of significant economic importance in commercial poultry flocks across the US and beyond. Seven genetic clusters (GCs) have been described for ARV. Due to the continued increase in reports of variant ARV infections in progeny from vaccinated flocks, there is a need to determine the antigenic characteristics of field isolates across the 7 GCs. In this study, selected field isolates representing the seven defined ARV GCs and subgroups within were plaque purified three times on primary chicken embryo liver cells (CELiC). Clonal isolates were subsequently administered in a series of live and inactivated inoculations to hyperimmunize 3-week-old specific pathogen free (SPF) chickens to produce antiserum. Next, 2-way cross neutralizations were done by utilizing homologous and heterologous antigen and serum to perform beta virus neutralizations (VNs). The VN titers were then used to calculate the Archetti and Horsfal antigenic relatedness (R) value. In keeping with the goal of working across the seven GCs, antiserum is being produced for GCs 6 and 7 while results have been obtained for GCs 1-5. In these results, homologous VN titers of 512 units were observed for all isolates except Reo 99846 in GC 3 which had 1024 units. The highest heterologous VN titer recorded was 32 units observed between subgroups within each of GCs 1 and 2; and between ARVs 107177 (GC 2) and 94826 (GC 5). The R values (expressed as percentages) ranged from 0.3% - 8.8% - the highest value was observed between 107177 (GC 2) and 108089 (GC 4). A similar percent relatedness of 1.6% was obtained for 2 subgroups within each of GCs 1 and 2. In conclusion, homologous VN titers show that the hyperimmunization regimen resulted in high titers while low heterologous VNs and R values suggest that GCs 1-5 are distinct serotypes and subgroups within these GCs are likely different serotypes.

Field Vaccination of Turkey Breeder Candidates with Oral Chicken Reovirus Primer and Injectable Killed Turkey Reovirus

Ben Wileman, Marissa Studniski, Jewell Bremer, Jake Carlson

Select Genetics

In a previously shared study the safety and shedding of oral chicken reovirus vaccine with and without injectable boost in turkey breeder candidates appeared to be promising. The decision was made to implement field vaccination in a high Reo risk geographic area where turkey breeders were being raised. Turkey breeders were vaccinated with a single dose of Chicken 1133 strain oral Reovirus vaccine and were then given the normal two doses of commercial autogenous killed and injected Reovirus vaccine in an attempt to better protect breeders and to improve maternally derived antibody transfer to poults. Vaccinated (receiving the oral vaccine) and non-vaccinated (only receiving the injectable autogenous vaccine) turkey flocks will be followed through

the production cycle and findings will be shared. Also maternally derived antibodies will also be analyzed and compared between vaccinated and non-vaccinated flocks.

Systemic Invasiveness and Pathogenicity of an Avian Reovirus Field Isolate Compared to a Reference Strain Following Oral Inoculation

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Avian reovirus (ARV) is associated with considerable economic losses in the global poultry industry, especially due to stunting, malabsorption, tenosynovitis, and arthritis as well as other lesions in broilers. However, the timeline of systemic invasiveness and viral dissemination from the intestine to other tissues after oral infection is poorly understood. In our current study, an ARV isolate was isolated from chickens exhibiting myocarditis (Alabama isolate). The isolate was plaque-purified using chicken embryo liver (CEL) cell culture. Specific-pathogen-free (SPF) embryonated chicken eggs were inoculated with a standard strain, S1133, or the plaque-purified isolate via the yolk-sac route. The infected embryos appeared stunted with profuse subcutaneous hemorrhages. The titers of these propagated viruses were determined using CEL cell culture.To determine the pathogenicity of the purified isolate in comparison with S1133, seven-day-old SPF chickens were orally inoculated with 10^4 or 10^6 TCID50 of either the ARV Alabama isolate or the S1133 strain. The chickens were weighed weekly and seven birds from each group were necropsied at 7-, 21-, and 35-days post-inoculation. Samples were collected from the jejunum, heart, flexor tendons, and hock joint cartilage for viral loads and histopathology. RNA extracted from the jejunal wall was investigated for expression of immune-related and tight junction genes. Additionally, samples from bursae and thymi were collected for histopathological analysis. Results will be presented and discussed.

Pathology

Unusual Outbreaks of Neoplastic Disease in Broiler Breeders

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In 2022, geographically distant flocks of 36- to 56-week-old broiler breeders (Ross708), experienced increase in mortality due to visceral tumors. Fresh and formalin-fixed tissues, and blood samples were submitted at the Poultry Diagnostic and Research Center, University of Georgia, for diagnostic investigation. Histologically, neoplasia included lymphoma (3/5 cases), fibrosarcoma-like (1/5 cases) and

adenocarcinoma (1/5 cases). Lymphomas were positive for Pax5 (B cell marker) and negative for CD3 (T cell marker) leading to the diagnosis of B cell lymphomas. Attempted virus isolation in chicken embryo fibroblasts (DF-1 and CEF) cells, ALV p27-antigen capture ELISA (IDEXX), PCR panel for ALVs A, B, C, D and J, MDV and REV were unsuccessful. Samples were positive against the subtype specific PCR primers for ALV-E, or endogenous ALV. Most neoplastic diseases in poultry have viral etiology, largely attributed to infection by herpesviruses (Marek's Disease, MD) or retroviruses (avian leukosis/sarcoma, AL/S and reticuloendotheliosis, RE). Among these, the AL/S virus group is mostly known for the development of B cell lymphomas (lymphoid leukosis), although it also includes various forms of hemopoietic neoplasia, sarcoma of soft tissues and bones, and more rarely carcinomas. Hereby, we present the details of the diagnostic investigation and discussion on the presumptive etiology.

Misdirection! A Case of Iatrogenic Head Tremors in Chicks

Kathryn McCullough, Reece Bowers, Jenny Nicholds, Susan Williams

Poultry Diagnostic and Research Center, Population Health Department, University of Georgia

Neurologic signs including tremors, paddling, variably rigid leg extension, and the inability to self-right were present in all fifteen, 8-day-old, live, commercial, broiler breeder chicks submitted to the Poultry Diagnostic and Research Center Diagnostic Laboratory in November 2022. At necropsy, one 3 mm-diameter, slightly raised, immobile, firm, yellow nodule was present within cervical musculature of one bird. In all histologic samples from a subset of three birds, regionally extensive granulomas disrupted cervical vertebral anatomy with variable effacement of epaxial musculature, extension between vertebra into the spinal canal, and compression of the spinal cord. Epithelioid macrophages and multinucleated giant cells contained periodic acid-Schiff (PAS)-positive intracellular material. PAS, Gomori's methenamine silver, and Giemsa stains were negative for protozoal, fungal, and bacterial elements. The lumbar spinal cord in two of three sections contained edema within ventrolateral white matter tracts. All four brain sections examined contained marked meningeal and intraparenchymal perivascular edema that was more severe within the brainstem and cerebellum than in the cerebrum. Four colonies of Escherichia coli were grown from one of three brains submitted for bacterial and fungal cultures. Despite an initial presentation mimicking the clinical signs that may be seen with an infectious disease, such as Avian encephalomyelitis virus or a fungal or bacterial encephalitis, histopathologic changes in this case suggest a misplaced injection as the cause for morbidity.

Characterizing Potential Physio Pathological Dynamics of a Woody Breast Myopathy in Modern Meat Broiler Lines

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The global broiler industry has evolved over the years with increasing selection pressure geared toward quantitative traits such as growth rate and lean mass. This has resulted in altered broiler physiological homeostasis to adapt towards increased rates of protein turnover in Pectoralis (P) major muscle. Physiological

stresses induced due to selection pressures in broilers have produced several muscle myopathies of unknown etiology including an emerging one called woody breast (WB) myopathy. A series of experimental broiler floor pen studies with birds reared from day(d) 0-56 were conducted and various metabolic tools were applied to assess various biomarkers involved in the manifestation of WB myopathy to understand potential etiopathology. Two high yielding modern strains of broilers were utilized. Our findings showed that these modern broilers were synthesizing P. major mixed muscle protein at the rate of 8 %/day (d) higher compared to broilers reared two decades earlier at d 42 when reared under primary breeder nutrition guidelines for the strain (3.17g dLys/Mcal). Furthermore, the myopathy affected broiler had higher differential expression ($P< 0.05$) of plasma 3-methylhistidine for both the strains that were reflected with higher %/d P. major muscle degradation rates. Myodegeneration in P. major were exhibited in histomicrographs as early as d 21 in WB myopathy affected bird accompanied by replacement of muscle-specific protein with (insoluble) collagenous tissue in perimysial and endomysial connective tissue spaces that significantly increased as bird aged ($P<0.05$). Targeted metabolomics performed in plasma samples in myopathy birds showed possible cardiovascular involvement and circulatory insufficiency in P. major myofibers. Differentially significant metabolites expressed ($P< 0.05$) in plasma were homocysteine, cyclic GMP, trimethylamine N-oxide, tyramine, carnitine and acetyl carnitine. While timeline transcriptomics analysis of P. major of WB affected broiler exhibited genes associated to hypoxia and oxidative stress, carbohydrate metabolism, muscle growth, calcium signaling, and cell membrane integrity were expressed differently (towards the adaptive physiological feedback responses to adverse cellular states) at d 56 than at earlier grow-out period at d 21 and d 42. Overall, pathophysiological and histochemical phenomena occurring in myopathy broiler have caused net loss of muscle protein quality, and quantity. Findings warrant future investigations be directed toward improving meat broiler lines that will deliver improved vascularity in P. major to alleviate this myopathy condition.

An Emerging Trend: Non-Inflammatory Spinal Cord Degenerative Myelopathy in Turkeys

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Non-inflammatory degenerative myelopathy of the central nervous system is known to be caused by various nutritional deficiencies or toxicities, resulting in neurologic clinical signs, poor flock uniformity, and variable mortality. This case report describes a recent atypical trend in lesion presentation involving spinal cord myelopathy in samples from 17 turkey flocks submitted to the Pennsylvania State University Animal Diagnostic Laboratory from 2019 through 2022. The afflicted flocks were between 6-12 weeks of age and were primarily raised organic or antibiotic free. Major clinical observations included ataxia, weakness, lameness, and lateral recumbency. Postmortem examination revealed no significant gross lesions of the brain, spinal cord, or peripheral nerves. Histologic examination of the central nervous system revealed spinal cord lesions including multifocal regions of non-inflammatory myelin sheath swelling, spheroid formation, axonal swelling, fragmentation, and degeneration. All other

histological regions of the central nervous system presented within normal limits. The inciting cause is thought to be vitamin E deficiency, but is currently not known. Differential etiologies may include botulinum toxicity, riboflavin deficiency, pantothenic acid deficiency, cobalamin deficiency, or organophosphate toxicity. Investigations are ongoing.

Welfare

Implementing the 5 Domains Welfare Framework into a Poultry Welfare Program

Kate Barger Weathers

Cobb-Vantress

The 5 Domains of Animal Welfare is a globally recognized philosophical framework that focuses on health, nutrition, environment, behavior and the mental state of the animal. Originally developed by Mellor, the 5 Domains Model was meant to facilitate welfare assessment from a structured and comprehensive perspective across a wide range of management systems and animal types. In addition to the ability to conduct an objective assessment on the functional states, the 5 Domains Model allows for subjective assessment of the experience (mental status) of the animal and the overall welfare state. While the 5 Domains Model has been widely incorporated into animal care and welfare assessments for zoos and aquariums, the operational details and practical application for poultry are not widely publicized. In this talk, practical tips and personal experiences will be provided with regards to integration and implementation of the 5 Domains within a poultry company. Specifically, examples will be provided to demonstrate how this welfare model can be:-introduced and incorporated into employee and farmer training and how the domains can be related to everyday management and production practices, -used within welfare documentation for corporate policies,-utilized to connect individual domains with existing audit metrics and then integrated into internal poultry welfare assessments, and-used to proactively evaluate innovative technology so that we understand what poultry want and need within their environment.In summary, this presentation will highlight how the 5 Domains welfare framework can be operationalized within a poultry company and poultry welfare programs. By focusing on animal-based outcomes, this holistic and science-based approach to welfare is a positive step forward for welfare assessments for the poultry industry. Rather than simply focusing on minimizing negative experiences, this framework can be used to provide poultry with opportunities to have positive experiences and to help ensure that we are providing a “life worth living” for the poultry that we care for in our poultry operations.

A Survey Detailing Perceived Importance of Welfare and the Practical Aspects of Poultry Welfare Measurements on Farm

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Past research has identified multiple potential markers of stress and welfare in avian species. However, not all markers may be practical, economical, or feasible on farm. To better understand applicability of some of these markers, a survey requesting opinions about poultry welfare and measurements of stress and welfare on farm was sent out through poultry

industry social media, newsletters, and mailings. There were 72 responses to the survey, 11 from those involved in daily bird care and the remaining 61 providing support for those providing daily care. Approximately 80% of respondents believe measuring on farm welfare is useful and approximately 90% reported using animal welfare most or all the time. The largest deterrent to measuring welfare was not having enough financial, labor, or time resources to complete those measurements. Thus, providing adequate resources may be crucial to the success of understanding and improving welfare on farm.Beyond general welfare viewpoints, respondents ranked a given set of welfare measurements based on usefulness, cost-effectiveness, and feasibility. Welfare measurements included blood draws, immune organ size, bird condition, and growth and production parameters. The most useful measures were blood draws for vaccine titer and corticosterone levels, immune organ size, feather coverage, and shank length. The least useful measures of welfare were body weight uniformity, feed conversion ratio, foot condition, and body weight. Immune organ size measurements and blood draws were ranked as the most feasible welfare measurements. The least feasible welfare measurements were feather coverage and shank length. The least cost-effective measures were feather coverage and blood collection while immune organ size and production measurements were considered some of the most cost-effective measures. When considered together, immune organ size ranked highly for usefulness, feasibility, and cost-effectiveness, blood draws for corticosterone and vaccine titers ranked highly for usefulness and feasibility, and shank length ranked highly for usefulness and cost-effectiveness. Although these parameters show promise for practicality of measurement on farm, the measurements themselves have not always been reliable indicators of stress and welfare across commodity groups, housing types, and other factors. Thus before widespread implementation of these parameters into a welfare program, further investigation for validity as a measure of stress and welfare should be evaluated.

Understanding Consumer Perceptions of Animal Welfare

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Encouraging fair portrayals of animal agriculture companies and practices requires a deep understanding of not only consumers' existing beliefs and attitudes about animal welfare but also the underlying bases for these beliefs and attitudes. As poultry consumption continues to grow, so do the opportunities to educate the public about the proactive industry practices that ensure proper poultry care, welfare, and food safety. Despite documented improvements in welfare and sustainability outcomes in the food animal industry, consumers remain concerned about how animals are raised and slaughtered. These concerns often translate into low levels of trust in producers and other members of the supply chain with regards to animal welfare. To address these issues, the primary objective of this project was to develop a better understanding of consumer perceptions

of animal welfare, with focus on the poultry industry, by examining (1) consumer welfare perceptions resulting from common poultry industry practices and communications, (2) consumer knowledge of, and trust in, various welfare information sources (including NGO/activist groups), and (3) the role of welfare considerations in consumers' purchase decisions.To achieve these objectives, we conducted a nationally representative survey of American consumers. The comprehensive welfare-focused survey questions pertained to four general areas, including (1) the importance of food/poultry attributes in purchase decisions, (2) sources of farm animal welfare knowledge, (3) beliefs about current farm practices and their impact on poultry welfare, and (4) beliefs about sources of welfare information and claims. Survey findings indicated that welfare considerations play an important role in food purchase decisions but are less important than other considerations (e.g., healthiness of product, price). The survey also illustrated that there are interesting and important differences in welfare perceptions depending on whether the farm practices require the use of humans or machines. Consumers acquire most of their knowledge about farm animal welfare from the Internet or social media sources but have little direct experience with farm animals. Consumers have little knowledge of welfare standards, and they believe that labeling that highlights products that are hormone, antibiotic, and steroid-free are the most important claims sellers can make on poultry products. Insight into consumer perceptions of animal welfare, specifically poultry welfare will be shared during this talk. These results can guide poultry producers, veterinarians and the supply chain as they attempt to develop effective marketing messages. Because welfare information is more trustworthy when it comes from veterinarians or scientists than from companies directly involved in the supply chain, this talk will emphasize key findings that may help poultry leaders improve their credibility with consumers, highlight specific packaging claims and welfare standards that resonate with consumers, and illustrate consumer beliefs about the impact of common farming practices on welfare. In summary, these findings can guide poultry companies and the supply chain as they attempt to build consumer trust.

Depopulation of Laying Hen Flocks Using Several CO2 Strategies

Mike Petrik

McKinley Hatchery

There has been a pressing need to have accessible, rapid methods of humanely killing large numbers of laying hens to help control the spread of Avian Influenza. The aim of this project was to develop practical, effective SOPs for the destruction of laying hen flocks in various housing styles. The goals were to develop procedures that were humane, effective, repeatable, reasonably priced, and safe for users. CO2 gassing is an acceptable method of euthanasia for poultry throughout North America, yet strategies for the practical administration of appropriate volumes of CO2 have not been well developed. Through collaboration with the laying hen industry in Canada, we have developed SOPs for the effective and humane destruction of laying hens using CO2 gas. Whole barn gassing strategies,

Modified Atmosphere Killing (MAK) carts, and medium group size destruction using transport containers have been used successfully to humanely and effectively kill commercial-sized groups of laying hens. This summary will discuss the practical aspects of using each of these modalities, the responses of the birds to this destruction method, overcoming predictable challenges, and a model to scale the techniques to various sizes of barns. With effective preparation, these methods are humane options for responding to disease outbreaks such as avian influenza, as well as removing spent laying hens at the end of their laying cycle.

Effects of Lighting on Poultry Welfare

Ian Rubinoff

Hy-Line North America

The basics of poultry management are often described as feed, light, air, and water (FLAW). Improvements in feed, air, and water have progressed slowly but steadily over the last 100 years as we have learned to adapt to modern genetics and housing systems. Lighting is different. Over the last 15 years, there has been a radical transformation in the types of bulbs utilized for artificial lighting in turkey, layer, and broiler production systems. Until around 2010, most poultry houses had a combination of incandescent and fluorescent bulbs. Since then, we have seen many poultry farms in the USA and around the world pivot to LED lights which have a far greater level of flexibility in light duration, wavelength, and intensity than the old-style bulbs and controllers. With this rapid increase in lighting options, we have not only a paradox of choice, but a sincere question of "what is best for the bird?" LED lights have clearly demonstrated an improvement in energy efficiency, wavelength adaptability, and production. Additionally, there is strong evidence of welfare improvements with the optimization of duration, spectrum, and intensity. We will explore the progress in welfare that LED lights have made in reducing in fear, stress, and adverse behaviors and what we still need to understand.

The Effect of Light Intensity on Grade and Performance in Regular Turkey Hens

Laura Tensa[1], Shawna Weimer[2], Brian Wooming[1]

[1]Cargill,
[2]University of Arkansas

There are many factors that influence the grade of regular turkey hens including density, light intensity, and day of hatch treatments. Grade is especially important in regular hens, as they are typically sold as whole birds, requiring a Grade A status. This study aims to investigate if different light intensities affect growing downgrade, livability, weight gain, and feed conversion in regular turkey hens from July 2022-June 2023 in two complexes. High light intensities have been associated with increased pecking/cannibalistic behavior in turkeys. A side-by-side trial was designed for multiple barns on the same farm. One barn was set to the normal light intensity, the other was randomly assigned to one of three treatment groups, 0.5, 1, or 2 foot candles. All

flocks received full light for the first two weeks, with four hours of darkness starting at 7 days, to meet welfare standards. Performance and grade data will be compared at every light intensity to determine if there is a significant difference in lighting programs. Preliminary data indicates that light intensity has a significant effect on livability, condemn, and grade.

Effects of Mirror and Coloured Balls as Environmental Enrichment Tools on Performance, Welfare and Meat Quality Traits of Commercial Broiler

Muhammad Shahid Zahoor, Sohail Ahmad, Muhammad Usman, Muhammad Dawood, Karim EL-Sabrout, Syed Ghulam, Ehsan Ullah Khan, Murrawat Hussain, Muhammad Adeel, Hafiz Rao Abdual Latif

University Of Veterinary and Animal Sciences Lahore

This study evaluated the effect of environmental enrichment on the performance, behaviour and welfare aspects; blood biochemistry; carcass and meat quality traits of broiler chickens. A total of 450 straight run broiler chicks (Ross-308) were divided into 5 treatment groups having 6 replicates of 15 birds each under a completely 14andomized design. Treatments were environmental enrichment (EE) tools and consisted of C = control group; R = red ball for EE; G = green ball for EE; B = blue ball for EE and M = mirror for EE. These environmental enrichment tools were provided throughout the experimental period (0 to 35 days). Mean feed intake per bird was higher in all treatment groups except the blue balls group; weight gain and feed conversion ratio were better in the green and blue ball groups. Broiler chickens reared under different environmental enrichment were more active and they exhibited maintenance behaviour (preening, dust bathing and wing stretching, or scratching) more frequently. Regarding welfare traits, lower incidence of toe damage, footpad dermatitis and hock burn was observed in birds having different environmental enrichment tools as compared to the control group. The birds reared with red balls as environmental enrichment showed the lowest values for glucose, cholesterol, total protein, albumin and globulin amongst all the treatment groups. Birds reared with green balls had the highest body weight at slaughter, dressed weight, carcass yield and liver weight. Breast meat of environmentally enriched treated groups was lighter and had lower ultimate pH. It was concluded that the addition of environmental enrichment tools (visual, structural and plastic) motivates the birds for physical activities and improves the performance of broiler chickens.

AAAP Symposium

Welcome/Introduction

Andrea Zedek
AWN Chair

Bridging Generations in the Workplace

Zahira Gonzalez
Boehringer Ingelheim

The Value of Personality Profiles

Gail Stickney
Cobb-Vantress

Unlocking Influence: Leverage Your Brain to Increase Your Impact

Jacquie Stephens

Neuroshift

Infectious Bronchitis Virus

Identifying Infectious Bronchitis Virus Vaccines by Vaccine Takes in Commercial Broilers: Not All Vaccines are Created Equal

Brian Jordan, Deborah Hilt, Sunny Cheng, David Wills

The University of Georgia

Infectious bronchitis virus (IBV) is an economically significant respiratory pathogen of commercial poultry causing the disease infectious bronchitis (IB). Infection with IBV most often causes an upper respiratory tract disease in commercial chickens, but some strains of the virus can also infect and damage the kidneys and oviduct. In an attempt to control IBV infection, nearly all commercial poultry produced worldwide are vaccinated with live-attenuated IBV vaccines. For broiler chickens in the United States, chicks are vaccinated on day of hatch in the hatchery by mass coarse spray with at least one serotype IBV vaccine, but more often with two or more serotypes to broaden the protection against variant IBV challenges. For most of the industry, this is the only vaccination against IBV. Despite widespread vaccination efforts, IBV is still a common disease issue year after year. Knowing this, my laboratory began analyzing vaccine takes more than 5 years ago, where we measure IBV vaccine viral load by real-time polymerase chain reaction (qRT-PCR) 7 days post-vaccination. This data revealed that many hatcheries were not applying vaccine correctly and that not all vaccines were as efficiently applied as others, both of which can lead to poor protection from challenge. After working extensively with the broiler industry on hatchery vaccination, we have seen IBV vaccine application improve but still see discrepancies between specific vaccines. For example, strains of the Massachusetts (Mass) type IBV deemed “mild Mass” do not infect and replicate in chicks to the same level or with the same efficiency as Mass type IBV vaccines known to be “strong”, with variances among manufacturer. In other cases, certain serotypes, such as Del072, GA98, or Conn, do infect and replicate to the same level as Mass or GA08 types regardless of manufacturer. Additionally, certain vaccines tend to infect, replicate, and clear very quickly while other vaccines persist in flocks for many weeks. This data is critical for the poultry industry to understand when choosing vaccines to combat IBV infection, as we can now see that there are clear differences between them that will influence efficacy.

Efficacy of IBVAR206 Vaccine Against Challenge with Infectious Bronchitis Virus Arkansas Strain in SPF Chickens

Dana Goldenberg, Udi Ashash, Avner Finger

Phibro Animal Health Corporation

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 only against related viruses and have little to no cross-protection against heterologous viruses. Knowledge of the circulating IBV types causing outbreaks in a specific geographic region is beneficial to select the appropriate vaccines and vaccination programs. Identification of IB strains in Mexico isolated from chickens with typical respiratory clinical signs revealed few circulating lineages, including GI-9, virulent field strains known as Arkansas (Ark). Our studies have shown that the IBVAR206 vaccine, belonging to the GI-23 lineage, can provide cross-protection against different IBV field variants for example, the IBVAR206 vaccine shows very strong cross-protection against the QX and 793B strains. Therefore, we conducted an animal experiment to evaluate the efficacy of IBVAR206 combined with different IB vaccines against the Infectious Bronchitis Virus Arkansas strain challenge in SPF chickens. The evaluation of the protection was based on a ciliostasis test and clinical signs post-challenge. All challenged control chickens showed cessation or extreme loss of vigor of ciliary activity. No chickens died, and no abnormal clinical symptoms were observed in the vaccinated groups. Among the vaccinated groups, the best protection (87.5%) was observed in birds vaccinated at one day of age with the combination of IBVAR206 & H120. In conclusion, the IBVAR206 vaccine can contribute to the control of the heterologous Ark IB variant and continuous monitoring of the IBV types circulating worldwide and evaluation of the protection induced by vaccines is necessary to control IBV infection.

Evaluation of Safety and Efficacy of a DMV 1639 Infectious Bronchitis Virus Hyperimmunization Program in Broiler Breeders

Alexandra Hovan[1], Blayne Mozisek[2]

[1]University of Georgia College of Veterinary Medicine,
[2]Merck Animal Health

Infectious bronchitis virus (IBV) is an RNA coronavirus that causes damage to the epithelial cells of the respiratory, digestive, and urogenital tracts. Infectious bronchitis virus can pose an economic threat to all facets of poultry production. Damage to the respiratory and reproductive tracts of broiler breeders can ultimately lead to decreased egg production and increases in mortality, thereby limiting broiler supply and magnifying the economic impact of the virus. Infectious bronchitis virus vaccination is commonly used to prevent disease and limit production losses in commercial poultry. However, vaccination of broiler breeders in the United States is often limited to pullet rearing and ignored once these flocks are moved into production. This study implemented a vaccination program in broiler breeder flocks that encompasses both the pullet rearing and production phases. A live commercial MA5 serotype vaccine and a modified live DMV 1639 vaccine, in combination with a killed commercial vaccine containing IBV antigen, were applied at respective time points through 49 weeks of age. The objective of this study was to 1) evaluate the safety of this hyperimmunization program and 2) determine if the late-stage challenges of infectious bronchitis virus that negatively impact production were limited. Samples were collected weekly to evaluate flock health and viral load. Serological samples were collected, and antibody titers of hyperimmunized versus conventionally vaccinated control flocks will be

presented. Performance parameters of the breeder flocks from placement throughout production will be shared.

Evaluation of IBV Protection Against Recent DMV1639 Isolates Using Molecular Techniques and Challenge Studies

Jose Linares, Robert Beckstead,
Matilde Alfonso, Mark Jackwood,
Marshall Putnam

Ceva Animal Health

Evaluation of IBV protection against recent DMV1639 isolates using molecular techniques and challenge studiesJose A. Linares, Robert Beckstead, Matilde Alfonso, Mark Jackwood, Marshall PutnamCeva Animal HealthInfectious bronchitis (IB) is a highly contagious upper-respiratory viral disease of chickens caused by infectious bronchitis virus (IBV), an Avian Coronavirus. It has worldwide distribution and multiple antigenic types have been identified. New IBV types continuously arise from the accumulation of mutations in the spike (S1) protein. While many types of IB are circulating globally, there is a limited number of live attenuated IBV vaccine types available. Thus, developing and evaluating vaccines with cross-protection potential and the application of those vaccines is critical for control. During the winter of 2014 mortality rates as high as 30% due to nephritis were documented in broilers in the Delmarva Peninsula of the United States due to infection with IBV DMV1639. From 2015 to present, evolution of the DMV1639 virus resulted in lesions shifting from severe nephritis to mostly airsacculitis. DMV1639 spread to most broiler producing states in the United States (US), becoming the most relevant IBV challenge. In 2015 a cross-protective variant GA08 vaccine was introduced with label claims against GA08, GA13 and DMV1639. In this presentation, we will provide data on the evaluation of IBV protection by the variant GA08 vaccine against recent DMV1639 isolates using molecular techniques and challenge studies.

Protection of Laying Chickens After Heterologous Live Vaccine Priming and Heterologous or Homologous Inactivated Vaccine Boosting Against the Canadian DMV/1639 Infectious Bronchitis Virus Infection

Mohamed Hassan, Ahmed Ali,
Shahnas M. Najimudeen, Danah Altakrouni,
Motamed E. Mahmoud,
Mohamed Faizal Abdul-Careem

Faculty of Veterinary Medicine, University of Calgary

The Canadian DMV/1639 strain of infectious bronchitis virus (IBV) was shown to have a devastating effect on egg production in chickens. The Canadian poultry vaccines market has a limited number of infectious bronchitis (IB) vaccines available. The aim of this study was to compare the efficacy of an inactivated heterologous commercial vaccine to an in-house prepared inactivated homologous oil-emulsion vaccine following heterologous priming. Thirty-six specific-pathogen-free (SPF) pullets were vaccinated with live vaccines of the Mass and Conn types at 2, 5, and 9 weeks of age. At 16 weeks of age, the vaccinated pullets were divided equally into two groups: the first group was vaccinated with a commercial inactivated vaccine of the Mass type (Mass-boosted) and the second group was vaccinated with an in-house prepared inactivated oil-emulsion vaccine of the DMV/1639 type (DMV1639-boosted). A third group of 18 SPF pullets was kept as a mock-vaccinated control. At 21 weeks of age, the Mass-boosted group demonstrated a low neutralization titer (> 8) compared to a relatively higher titer (>256) in the DMV1639-boosted

group against the DMV/1639 IBV strain. At 35 weeks of age, 9 hens from each group were challenged oculo-nasally with the Canadian DMV/1639 IBV strain, leaving the other 9 hens as their non-challenged controls; all groups were observed for 12 days post-infection (dpi) before euthanasia. The two vaccination programs provided protection against the significant drop in egg production observed in the non-vaccinated challenged group. Both vaccinated challenged groups showed a significant rise in anti-IBV antibodies (measured by ELISA) compared to all other groups at 12 dpi. Other protection parameters will be evaluated. The preliminary findings indicate that both vaccination programs are promisingly efficacious against the Canadian DMV/1639 IBV infection.

higher incidence of long-term reproductive lesions than early challenge, raising concerns about vaccination use within the first few days of life. Here, we attempt to prolong the protective effects of passive immunity by investigating various administration methods (spray, in ovo injection) of hyperimmune serum to day-of-age chicks. In addition, the half life of IBV maternal antibodies and the protection of supplemental antibodies against IBV challenge will be studied. It is expected, should supplemental maternal antibody use be effective against challenge, that these methods may be useful in prevention of chronic effects of IBV infection affecting production and could allow vaccination to be postponed avoiding detrimental long-term reproductive effects in layers.

Mimicking Maternally Derived Antibodies for Early Protection Against Infectious Bronchitis Virus in Chicks

Rachel Jude, Laura Flores, Rodrigo Gallardo

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Infectious bronchitis virus (IBV) is a gammacoronavirus causing acute respiratory disease and, occasionally, urogenital disorders. We have previously demonstrated that birds with maternal antibodies are protective against both acute and chronic effects of IBV challenge and live vaccination. Additionally, we observed that day of age vaccination was culpable for

The Effect of Stabilizers on Live Attenuated Infectious Bronchitis Virus (IBV) Vaccine Under Different Temperature and pH Conditions.

Kim Bouwman, Cory Yarborough, Brian J. Jordan

Poultry Diagnostic and Research Center, Department of Population Health, College of Veterinary Medicine, University of Georgia

Infectious Bronchitis Virus (IBV) live attenuated vaccines are widely used in the industry to protect chickens from infection with circulating field strains. When used in the hatchery, there is less opportunity for environmental factors (temperature, diluent source, pH) to influence vaccine efficacy, though it does happen. When applied in the field however, there are distinct

challenges for using these vaccines that can influence the vaccine's ability to be effective. Many factors play a role in the success of vaccinating flocks, with the diluent used and the preparation protocol being common to all vaccines applied, and they are essential to obtain the dosage and full potency stated by the manufacturer. Often temperature and pH of the diluent will vary as a consequence of transport or water source, and these variables are not routinely monitored. One way to protect the vaccine from losing potency is to add stabilizers, which protect the live virus from destruction or destabilization from these factors. Currently, more data is needed to show the efficacy of commercially available stabilizers. To test the effect of pH and temperature of the diluent on live attenuated IBV vaccine, we diluted IBV vaccine with different stabilizers and titrations were performed. Distilled water at different temperatures (55, 68 and 80F) or at different pH (4, 7 and 10) was mixed with stabilizers and titrations of vaccine dilutions were tested over a 24 hour time period. We also tested combinations of temperature and pH to see if there was an interactive effect of the two variables and if the stabilizers would counteract those effects. These results will increase our knowledge on the effect of stabilizers when pH and temperature of the diluent fluctuates and how to potentially reach full efficacy of the live attenuated IBV vaccine under simulated field vaccination conditions.

Development of a Multiplex PCR Combined with Nanopore Sequencing for Identification of IBV Strains in Mixed Infections

David Wills, Benjamin Jackwood, Brian Jordan

University of Georgia

The avian coronavirus Infectious Bronchitis Virus (IBV) is the causative agent of one of the most prevalent viral respiratory diseases in chickens. The virus is a highly contagious and economically significant upper respiratory pathogen causing one of the costliest diseases of commercial chickens worldwide. Because of the economic significance of IBV, nearly all poultry produced in the U. S. are vaccinated against IBV infection. IBV vaccines provide serotype-specific protection; therefore, a variant virus may still cause an infection and clinical disease. Detection of variant IBVs is difficult for multiple reasons, so novel diagnostic methods that can make this process more efficient and accurate are always needed. Critical to developing improved diagnostic methods for IBV variants is a rapid and accurate assay that can detect IBV in a sample and distinguish between IBVs in a co-infection, which is common in commercial poultry. Current molecular diagnostics for detection of IBV's are two-fold; direct detection via "real-time" RT-qPCR or "traditional" RT-PCR with Sanger sequencing. Real-time PCR can be performed directly from viral RNA isolated from field or clinical samples and is a rapid and sensitive assay for routine surveillance and vaccine virus detection. However, the assay is limited to only known IBV serotypes and is not designed to detect variant or emerging IBVs. Alternatively, RT-PCR followed by Sanger-sequencing will yield sequence information of any IBV present in a sample, but amplification of the Spike gene and sequencing this is not always successful, especially directly from field or clinical samples, and it only detects the dominant virus in a sample. Additionally, to achieve good sequence information, viruses are often propagated in embryonated chicken eggs prior to PCR and sequencing, which adds cost and time to the process. This is also problematic as true field variant IBVs often do not grow well in eggs. Therefore, novel methods that can produce sequence information of any IBV present and can be updated and standardized for high

throughput analysis for diagnostic laboratories are desperately needed. To this end, rapid, high throughput, and sequence intense methods for analysing genomes that fall in the category of "next generation sequencing" (NGS) have been developed for use in other industries. One of these assays is Nanopore-based sequencing from Oxford Nanopore Technologies that can be combined with real-time data analysis, which speeds results and enhances cost savings. Here we outline a new approach to identify all strains of IBV in co-infected field samples, including novel or unknown strains, using a multiplex PCR of the S1 gene followed by Nanopore sequencing. Novel, degenerate PCR primers were designed to amplify the entirety of the S1 gene, as well as the individual hypervariable regions simultaneously. These primers were designed against an alignment of 31 S1 sequences and were placed in relatively conserved regions flanking and inside the S1 gene so that they would detect as wide a variety of IBV strains as possible. These PCR products were then sequenced using Oxford Nanopore technology on the MinION platform to determine the type, number, and relative abundance of all IBV strains present in a sample. All sample reads are matched to known IBV S1 sequences using the map to reference function in the Geneious Prime software and counted. All remaining reads are then run through a BLAST search pipeline developed by our laboratory to identify additional IBV sequences that do not stringently match our known serotypes. These additional IBV reads are then de novo assembled using the Geneious Prime software to identify any potential variants. Unlike real-time PCR, this approach allows the determination of both expected and novel IBV strains with a single PCR reaction using common primers, making detection of IBV rapid, simple, and cost-effective.

Enteric Health

Environmental Factors Outweigh the Impact of Antibiotic Growth Promoters on Cecal Microbiota Composition of Commercial Broiler Chickens

Martine Boulianne[1], Eric Parent[1], Robert J. Moore[2], Thi Thu Hao Van[2], Marie Archambault[1]

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[2]RMIT University

The objective of the study was to investigate the association of various flock-level factors on the composition of the cecal microbiota in commercial broiler chickens at the end of the grow-out. The caecal content of chickens in 84 broiler chicken flocks was recovered at the end of grow-out (n = 1002) and total DNA was extracted to amplify and sequence the V3-V4 region of 16S rRNA to assess the composition of the microbiota. All factors evaluated significantly impacted the structure of the microbiota ($p< 0.002$). The farm (R-value = 0.239) and flock cycle (R-value = 0.374) showed the highest association with the microbiota composition, the feed provider (R-value = 0.118), type of antibiotic program (R-value = 0.039) and chick provider (R-value = 0.035) were considerably smaller. There were important microbiota differences between farms as taxa identified by LEfSe were highly variable, and determinant microbiota features in one farm could be partial to totally different from other farms. Numerous significant differences were identified between farms for Richness and Evenness as five significant pairwise comparisons were identified ($p< 0.05$) between the seven farms. No significant alpha diversity variation was detected between antibiotic programs ($p > 0.05$). In conclusion, this study highlights the importance of

environmental factors possibly influencing the microbiota in broiler chickens, and future strategies aimed at modulating the GIT microbiota of broiler chickens should involve a holistic approach by considering the multifactorial aspects of the microbiota development in commercial broiler chickens.

Microbiome Modulation by a Precision Biotic in Broilers Chickens: A Commercial Study Validation

Cristiano Bortoluzzi, Britt Blokker, Luis Valenzuela

DSM Nutritional Products

Precision biotics (PB) are glycans with specific glycosidic linkages that can redirect the functions of the microbiome towards increased beneficial outputs, such as higher propionate production and nitrogen utilization, regardless of the taxonomic composition of the microbial community. The objective of the present study was to evaluate the effect of the supplementation of PB on the growth performance, and cecal microbiome modulation of broiler chickens raised under field conditions. A field trial was carried out at a commercial farm in China. A total of 190,000-day-old Ross 308 straight-run broilers were randomly assigned to two dietary treatments. There were 5 houses per treatment with 19,000 birds per house. The two dietary treatments included a control diet (a commercial broiler diet) and a PB supplemented diet at 0.9 kg/MT. At 42 d of age, the bird weight (BW) and feed intake (FI) of each house were recorded, the feed conversion ratio (FCR) was calculated and corrected with the final body weight (cFCR). Additionally, 40 birds/experimental group (80 birds in total) were randomly selected and the cecal content was aseptically collected. The samples were then sent to the lab and frozen at -80oC until further processing (DNA isolation, sequencing, and bioinformatics). The supplementation of PB numerically improved BW of birds by 52 g on d 42, and significantly improved ($P< 0.05$) the cFCR by 2.2 points and the EPI by 13 points. The Local Fisher Discriminant Analysis (LFDA) of functional profiles showed a significant difference in the cecal microbiome metabolism between control vs PB supplemented birds. The abundance of pathways modulated by PB involve those associated with amino acid fermentation and putrefaction, particularly from lysine, arginine, proline, histidine and tryptophane, which led to a higher Microbiome Protein Metabolism Index (MPMI) in supplemented birds. Other pathways of importance related to purine, vitamins, carbohydrates, and ABC transporters were also modulated by the supplementation of PB. It was observed that the supplementation of PB significantly reduced the abundance of Escherichia coli and Salmonella enterica in the cecal microbiome. In conclusion, the results presented herein prove that the PB can efficiently modulate the intestinal microbiome of broiler chickens towards a beneficial metabolism related to protein metabolism and utilization with positive effects on the growth performance of the birds.

Effects of the Phytogenic Feed Additives on the Production Performance of NAE Broilers

Callie McQuain, Kowsigaraj Palanisamy, Ajay Bhoyar

EW Nutrition

Essential oil and saponin-based feed additives are widely used in No Antibiotic Ever (NAE) poultry operations to help with performance

parameters. This study aimed to compare the effects of three different phytogenic (saponin or phytomolecule-based) feed additives on the performance of NAE broilers. In this study, three consecutive pen trials with six treatment groups, each having 12 replicates of 40 male broilers, were conducted in a randomized block design. All the birds were vaccinated with a coccidiosis vaccine on day 1 and placed on used litter. The treatment groups consisted of Group 1: Negative control, no phytogenic additive; Group 2: Saponin-based gut health additive (A) at 0.50 lb./ton during the starter and grower phases; Group 3: saponin-based gut health additive (B) at 0.50 lb./ton during the starter and grower phases; Group 4: saponin-based gut health additive (B) at 0.50 lb./ton only during the grower phase; Group 5: Phytomolecules blend (C) at 0.25 lb./ton during the starter and grower phases; Group 6: saponin-based feed additive (B) at 0.50 lb./ton and Phytomolecule blend (C) at 0.25 lb./ton during the starter and grower phases. Live performance parameters, such as body weight and FCR, were evaluated on days 0, 14, 28, and 42.The meta-analysis of these three trials showed that the inclusion of these phytogenic feed additives supports improved production performance of the broilers. In these studies, saponin-based gut health additive B in the starter and grower phases enhanced the live performance of broilers similar to a comparable saponin-based feed supplement A ($P<0.0001$). Moreover, the birds receiving phytomolecules blend feed additive C or feed additives B + C combination significantly ($P<0.0001$) outperformed all the other trial groups regarding the FCR. It is concluded that phytogenic feed supplements support the production performance of broilers under the NAE production system.

Targeting Gut Neuroimmune Axis to Control Enterobacteriaceae in Chickens

Melha Mellata

Iowa State University

The gut microbiota is important in chicken health and performance but can also serve as a major reservoir for Enterobacteriaceae, which includes pathogens such as Salmonella and Escherichia coli. Emerging studies have shown that the interaction between gut neurochemicals and microbiota affect health and well-being in humans and animals, but little is known about this interaction in food-producing animals, such as chickens.Enterobacteriaceae are rapidly becoming resistant to the last resort antibiotics; however, they are considered emerging health and food safety concerns. My talk will present our recent studies on gut neurochemicals and their role in chickens. Our research elucidates the crosstalk between neurochemicals and the gut microbiota, and how the environment and live prophylactics impact this interaction. Finally, our research shows how some treatments can alter the intestinal immunological response via neurochemical and metabolic pathways and improve resistance to bacteria, especially against immunotolerant bacteria such as Salmonella. Overall, our studies show that targeting the neuroimmunological axis can be an effective strategy to minimize Salmonella persistence in poultry to improve food safety and should be further explored.

Induction of Protective Immunity Against Necrotic Enteritis by In Ovo Delivery of Oligodeoxynucleotides Containing CpG Motifs Prior to Vaccination of Broiler Chickens at Hatch with Clostridium Perfringens Antigens

Hemlata Gautam

University of Saskatchewan

Necrotic enteritis (NE) is a major economically important diseases in the broiler chicken industry and caused by Clostridium perfringens (CP) type A/G. Amidst withdrawal of prophylactic antimicrobials use in the broiler chicken industry, CP infections have led to a considerable increase. The objective of this study was to develop a novel vaccination strategy against CP by synergizing immune enrichment with immunomodulation in broiler chicks using synthetic oligodeoxynucleotides containing CpG-motifs (CpG-ODNs) prior to vaccination of broiler chickens with CP antigens at hatch by intrapulmonary (IPL) route and boosting with CP antigens at day 10 of age by the subcutaneous route. Birds were challenged with CP in feed (feed: media, 1:1) at the age of day 20 – 22 to study vaccine efficacy. Groups of broiler chickens (n=35) were vaccinated as: (1) No CpG-ODN delivery, no CP vaccination, and no CP challenge; (2) CP challenge only; (3) CpG-ODN + CP vaccination + CP challenge; (4) saline + CP vaccination + CP challenge. Blood, intestinal mucosal scrapings, and sections of intestines were collected at day 23 of age for histopathological and serological studies (IgY and IgA). Group of birds vaccinated with CP antigens by the IPL roue following in ovo delivery of CpG-ODN was protected against CP challenge at a significant level ($P<0.01$). Protection of birds against CP challenge was corelated with IgY and IgA levels.

<u>Richard Rimler Award Winner</u>

Focal Duodenal Necrosis: Identification of Potential Pathogens Contributing to the Disease in Layers

Yuyang (Jerry) Tsai, Monique Franca, Sonsiray Alvarez Narvaeza, Alvin C. Camus, Nicolle L. Barbieri, Catherine M. Logue

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Focal Duodenal Necrosis (FDN), an intestinal disease is among the top five concerns in table egg layers. The economic impact of FDN is associated with a decrease in egg case weight and a drop in egg production. In this research, we used bacteriological analysis to analyze the microbiological composition of FDN lesions. Fifteen duodenal samples with characteristic FDN lesions were examined bacteriologically and a total of 161 colonies were recovered on agar plates incubated aerobically and anaerobically. Through 16S rRNA gene PCR and Sanger sequencing, 89/161 colonies were identified as Escherichia coli. PCR for avian pathogenic E. coli (APEC) virulence genes found 18% of isolates are considered APEC-like. PCR panels examining for virulence genes associated with inflammatory bowel disease (IBD) showed 94% of E. coli isolates possessed multiple virulence genes.To understand the detailed genomic information of E. coli, 5 isolates that possessed multiple virulence genes associated with intestinal pathogenic strains were sent for whole genome sequencing. Genomic and phylogenetic analysis revealed that one isolate had a closer relationship to IBD associated E. coli while the others were closer to extraintestinal pathogenic E. coli (ExPEC) and APEC strains. Examination of the microbial composition of duodenal FDN lesions can provide insight about the roles of which organisms contribute to the disease. Ten frozen duodenal lesion samples were used for microbiome analysis. The Powersoil kit was used for DNA extraction and subjected to 16S rRNA sequencing using PacBio II Sequel. Clostridium perfringens, Enterococcus cecorum, E. coli, E. fergusonii, Lactobacillus aviaries and Tyzzerella/clostridium colinum were among the dominant flora detected in the lesions. These bacterial species can be considered as contributing to the pathogenesis of FDN.FDN

appears to be a multifactorial inflammatory intestinal disease associated with multiple bacterial species and E. coli isolated from the duodenal lesions is linked with other intestinal disease strains.

Dose- and Time-Dependent Effects of Campylobacter jejuni Challenge on Growth Performance and Campylobacter Colonization in Broilers

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The present study aimed to investigate the effect of the dose levels (0.5 ml of 10^4 and 10^8 CFU CJ per bird) and challenge time (d 0, d 7, and d 14) of Campylobacter jejuni on growth performance and Campylobacter colonization in the broiler. A total of 672 broiler chickens (Cobb 500, a day old, male) were completely randomly allocated in 48 cages (8 treatments with 6 replicates, 14 birds per cage). The treatments were comprised of T1 (non-challenge control), T2 (challenged with 104 CFU C. jejuni at d 0), T3 (challenged with 10^8 CFU C. jejuni at d 0), T4 (challenged with 10^4 CFU C. jejuni at d 7), T5 (challenged with 10^8 CFU C. jejuni at d 7), T6 (challenged with 10^4 CFU C. jejuni at d 14), T7 (challenged with 10^8 CFU C. jejuni at d 14), T8 (challenged with 10^4 CFU C. jejuni at d 0, d 7, and d 14). C. jejuni dose levels and time did not affect growth parameters. However, the highest mortality was observed (P = 0.099) in T2. Cecal campylobacter loads in T2, T3, and T8 were higher than that in T1 at d 7. The highest Campylobacter load of ceca was shown in T3 at d 14. At d 21, cecal Campylobacter loads in T3, T4, T5, T6, T7, and T8 were higher than that in T1. At d 28, the highest cecal Campylobacter load was observed in T3. Campylobacter loads of liver were increased in Campylobacter challenge groups as compared with T1 at d 7, and d 14, regardless of C. jejuni challenge dose levels and time. The lowest Campylobacter load of the liver was observed in T1 at d 21. However, C. jejuni challenge dose levels and time did not affect the Campylobacter load of the liver at d 28. Campylobacter loads of small intestinal digesta were increased in T2, T3, and T8 as compared with T1 at d 7. However, there were no significant differences in Campylobacter loads of small intestinal digesta at d 14, d 21, and d 28 among treatments. This study confirmed that C. jejuni challenge at d 0, d 7, and d 14, regardless of the dose levels within the range of 10^4-10^8 CFU, increased Campylobacter colonization of the ceca in the broiler. However, additional investigation for the reduction of cecal Campylobacter loads in the weekly low dose (10^4 CFU) challenge group.

Investigation into Increased Incidence of Gizzard Erosions in the Southeastern United States

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Caustic agents, mineral interactions, mycotoxins, adenoviruses, and bacterial induced ulcers have been documented to cause lesions in the gizzard. These gross lesions are tracked in the field at posting sessions across the industry. The incidence is typically low in prevalence and severity. Recently, an increased incidence in gizzard erosions has been observed. An Alpha toxin positive, NetB negative Clostridium perfringens was isolated from many of these field cases. There are few applied models which

evaluate erosions and a product's ability to resolve these lesions.A floor pen trial was conducted to evaluate whether gizzard erosions could be induced experimentally. One replicate pen of 25 male Ross broilers were given either non-medicated feed, feed containing 250 ppm of tribasic copper chloride (TBCC), or 250 ppm of copper sulfate. All groups were gavaged on day 21 and 22 with C. perfringens (~1.0 x 108 CFU/bird) cultured from a field sample containing a gizzard erosion. Gizzard lesions were standardized by gross appearance and size using a scale from 0 to 3 to increase the objectivity of the evaluation. Lesions were observed in each challenge at both day 23 and day 27. In a second trial an unchallenged control, a challenged control, and a challenge control plus TBCC (250 ppm) were evaluated. CP was orally gavaged on DOT 21 and 22 (~1.0 x 108 CFU/bird) in challenged groups and gizzard lesions were evaluated on day 23 and 28. Gizzard samples were collected for histology and ceca were also collected for CP enumeration on days 23 and 28. Birds were weighed on day 0, 14, 23 and 28. Gross gizzard lesions were greater in the treatment containing TBCC + CP. On histology there was greater incidence of koilin fusion defects in the treatment containing TBCC. Overall prevalence and enumeration of CP was significantly greater in the CP challenge relative to the unchallenged group or the group TBCC + CP group.These experiments validated a model design which creates appreciable gizzard lesions and CP colonization in the ceca. The results suggest dietary copper at high-normal inclusions may contribute to koilin irritation. Despite these observations, there was no evident loss of broiler performance due to these lesions in the controlled experiment.

Vaccinology

Protection Efficacy of Bivalent and Trivalent HVT Recombinant Vaccines Against Infectious Laryngotracheitis (ILT) in Broilers Chickens

Daniel Maekawa[1], Maricarmen García[2]

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Infectious laryngotracheitis (ILT) is an economic respiratory disease that affects the poultry industry worldwide. Biosecurity and vaccination are the tools used to control the disease. Vaccination with Infectious laryngotracheitis virus (ILTV) recombinant products have gained popularity because these are safer than live attenuated vaccines and are easy to administer. Recombinant vaccines that utilize the Herpesvirus of turkey (HVT) vector have become widely available. Currently, there are two bivalent (rHVT-LT gD-gI & rHVT-LT gB) and two trivalent (rHVT-ND-LT gD-gI & rHVT-IBD-LT gD) recombinant HVT-LT vaccines commercially available. The objective of this study was to evaluate the protective efficacy against ILT of the two bivalent and two trivalent HVT-LT vaccines. Preventing mortalities, decreasing disease (clinical signs), weight gain after challenge, and decreasing challenge virus replication in the trachea were adequate protection parameters. Results: Bivalent rHVT-LT gD-gI and trivalent vaccines HVT-ND-LT gD-gI and HVT-IBDV-LT gD significantly reduced clinical signs of the disease and virus replication in the trachea. At the same time, clinical signs and virus replication did not decrease for chickens vaccinated with the bivalent rHVT-LT gB. Percent survivability after the challenge for groups of chickens immunized with rHVT-LT gD-gI, rHVT-ND-LT gD-gI, rHVT-IBD-LT gD, and rHVT-LT gB were 91%, 87%, 84%, and 42% respectively. Lastly, the average weight gain one-week post-challenge was 528g, 469g, 457g, and 246g for rHVT-LT gD-gI, rHVT-ND-LT gD-gI, rHVT-IBD-LT gD, and rHVT-LT gB, vaccinated

groups of chickens, respectively. Overall results show that recombinant ILTV, bivalent or trivalent vaccines expressing either the viral glycoproteins D and I or only glycoprotein D, were more effective than the bivalent rHVT-LT vaccine expressing the glycoprotein B. The protection efficacy among the rHVT-LT vaccines that express gD-gI and gD was categorized as best for rHVT-LT gD-gI, good for rHVT-ND-LT gD-gI, and moderate for rHVT-IBDV-LT gI.

Reed Rumsey (Basic Research) Award Winner

Protection Efficacy of Recombinant HVT-ND-ILT and the Live Attenuated Tissue Culture Origin (TCO) Vaccines Against Infectious Laryngotracheitis Virus (ILTV) when Administered Individually or in Combination

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Infectious laryngotracheitis (ILT) is a respiratory disease that cause significant economic losses to the poultry industry. Control of the disease is achieved by vaccination and implementation of biosecurity measures. The use of herpesvirus of turkeys (HVT) and fowl pox virus (FPV) recombinant vector bivalent vaccines expressing exclusively infectious laryngotracheitis virus (ILTV) genes (HVT-LT, FPV-LT) or trivalent HVT vaccines expressing ILTV plus Infectious bursal disease (IBD) (HVT-IBD-LT) or Newcastle disease virus (HVT-ND-LT) genes have increased worldwide. In the United States (US) most vaccination programs of long-lived birds (broiler breeders and commercial layers) against LT include immunizations in ovo or at hatch with HVT recombinant vector vaccines followed by live attenuated vaccines, either with a chicken embryo origin (CEO) or tissue culture origin (TCO) vaccines administered via the drinking water or eye-drop, respectively. The efficacy of the HVT-LT + CEO vaccination program has been tested experimentally and found that rHVT-LT + CEO vaccination provided a more robust protection than vaccination with HVT-LT alone. The objective of this study was to evaluate the protective efficacy of the HVT-ND-LT + TCO vaccination strategy as compared to each vaccine given by themselves in long-lived birds. The HVT-ND-LT vaccine was administered subcutaneously at day of age, and the TCO vaccine was given at 10 weeks of age via eye drop. HVT-ND-LT + TCO, HVT-ND-LT, TCO, and non-vaccinated groups of chickens were challenge with a virulent ILTV strain at 15 weeks of age. After challenge, mortalities were only prevented in the group of chickens vaccinated with the HVT-ND-LT + TCO. Clinical signs of the disease and challenge virus replication in the trachea were significantly reduced for both HVT-ND-LT + TCO and TCO vaccinated groups of chickens but not for the HVT-ND-LT vaccinated group. After eight days post-introduction of contact naive chickens to challenged groups, infection was only prevented for contact naïve chickens introduced to the HVT-ND-LT + TCO vaccinated group of chickens. Overall, these results indicated that compared to HVT-ND-LT or TCO when administered alone, the HVT-ND-LT + TCO vaccination strategy maximized protection against disease and reduced shedding of the challenge virus. Therefore, this vaccination strategy provide a great tool to control ILTV in commercial layers and broiler breeders in endemic areas.

Vaccine Take Evaluation of Two Recombinant Vector HVT Vaccines Applied Simultaneously in

Commercial Layers

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Early and simultaneous field challenges with multiples pathogens are leading to the need of using more than one recombinant vector HVT (rHVT) vaccine applied simultaneously either in ovo or at day one of age. To verify the compatibility of two rHVT vaccines applied simultaneous at hatchery, a trial was conducted to evaluate vaccine take, serological immune response profile and productivity parameters. A rHVT vaccines carrying Newcastle virus F gene (ND), and another carrying the Infectious Laryngotracheitis virus gB gene (ILT) developed by the same pharmaceutical company were used. Both vaccines were administered together by subcutaneous route in one-day-old commercial layers (Isa Brown). Feather pulp and spleen samples of 30 birds were collected at 3 and 5 weeks post vaccination (pv) to evaluate vaccine take by qPCR using specific primers for the rHVT ND vaccine. At 0, 4, 7 and 14 weeks pv, sera of 20 birds were collected for ND and ILT antibody quantification. The rHVT ND vaccine virus replication was detected in 100% of sampled birds from 3 weeks pv. Higher positivity and virus yield was detected in spleen samples. The serological analysis using Idvet ELISA kits showed evident seroconversion to both applied vaccines. ND and ILT antibody levels were similar to those expected for the vaccination program used (100% of positivity from the seven week of age). Body weight and viability parameters were higher than the standard of the genetic lineage indicating the safety and protection conferred by the two vaccines applied simultaneously. Therefore, the results indicate that the rHVT viruses of the two vaccines replicated simultaneously in the vaccinated birds. However, it has to be taken into account that the results of compatibility are valid only for the vaccines used in the present study. Vaccine virus titers, vaccine virus replication kinetic, level of attenuation, rHVT vaccine construction and Master seed virus are all factors that can affect the compatibility of rHVT vaccines applied simultaneously.

Progress in Developing a Vaccine to Prevent/Decrease Colonization of Campylobacter jejuni in Poultry

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We used an immunogenic Salmonella Typhimurium UK-1 vaccine vector strain that displays regulated delayed attenuation, regulated delayed synthesis of protective antigens, and regulated delayed lysis phenotypes by inserting the C. jejuni 14-gene pgl operon that encodes enzymes to synthesize the 27mmune-protective N-glycan attached to C. jejuni surface proteins and inserted additional D/E-X-N-Y-T/S N-glycosylation sequences in genes encoding Salmonella surface antigens. Six challenge trials were conducted varying use of broiler and layer chicks, timing and dose of vaccine administration and the challenge dose of a mixture of 5 C. jejuni strains isolated from chickens. We monitored induction of antigen-specific antibody responses and C. jejuni cecal colonization levels 9 and 17 days after oral challenge. We observed that C. jejuni challenge

doses of 105 CFU overwhelmed immunity such the no significant reductions in C. jejuni colonization were observed. However, we observed significant reduction in levels of cecal colonization (up to 2 log reduction) with challenge doses of 103 CFU in chicks orally vaccinated at day-of-hatch with individual and mixtures of two vaccine strains delivering different C. jejuni antigens. Based on the collective results, we constructed pG8R410 encoding the most protective C. jejuni antigens CjaA, StiK, FlaA, PppA and ChpA coordinately expressed under Ptrc control and with different optimized type 2 secretion systems. We are in the process of testing S. Typhimurium ï‚„PmurA25::TT araC ParaBAD murA ï‚„asdA33 ï‚„(wza-wcaM)-8 ï‚„relA1123 ï‚„recF126 ï‚„sifA26 ï‚„wbaP45 ï‚„pagL14::TT araC ParaBAD wbaP ï‚„lpxR9 ï‚„pagP8 ï‚„cysG123::pgl-(gne-plgG)-12 ï‚„fliC180::cj1433 ompA132::cj1433 (pSTUK206 ï‚„[traM-traX]::araC ParaBAD lacI) with the pG8R410 plasmid to determine whether we observe even better levels of protective immunity. The research was supported by USDA/NIFA award 2017-67017-26179 to RC.

Studies Demonstrating the Potential to Enhance Immunity and Improve Overall Efficacy of Live Salmonella Typhimurium (ST) Vaccination by Parenteral Administration

Manuel Da Costa, Kalen Cookson, John Dickson, Jon Schaeffer

Zoetis

Live ST vaccines have been in use for over two decades to help control salmonella. A typical vaccination program includes 2-3 live ST vaccinations in the first several weeks of the pullet's life followed by 1-2 inactivated vaccinations prior to the point of lay. One common perception is that live salmonella vaccines only induce a limited duration of immunity and little, if any, humoral antibody response. Currently, live vaccines are only being applied by mucosal application—coarse spray or drinking water. However, because of the unique properties of the aro-A attenuated live ST vaccine, recent studies have been designed to see if this vaccine might provide additional benefits not currently being realized in today's vaccination programs. This paper will summarize three different studies—one exploring the efficacy of injecting live ST at day of hatch and two others where the ST vaccine is injected with or without a killed Salmonella Enteritidis (SE) bacterin at 10-12 weeks of age. The results will show that salmonella protection can be enhanced by applying live ST vaccine by injection.

Efficacy of a Trivalent Coryza Inactivated Vaccine Against Challenges with Wild Type Avibacterium paragallinarum Serovars A and C

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Avian Coryza is a poultry respiratory disease observed in some regions of the United States, that can be presented as an acute or complicated respiratory infection in commercial layers and broilers. Commercial and autogenous inactivated vaccines are available as a strategy to reduce or prevent clinical signs and to reduce bacterial shedding, in addition to biosecurity measures implementation. A commercially available trivalent inactivated vaccine, containing Avibacterium paragallinarum (A.P.) serovars A (083), B (Spross) and C (H-18) was

used to vaccinate commercial pullets under controlled conditions. One hundred and forty leghorn pullets were placed in BSL-2 temperature-controlled rooms on wood shavings and divided in 7 groups. Two groups received two doses at 7 and 10 weeks of age (woa), and two groups only one dose at 10 woa. The experiment contained two positive and one negative control. The vaccine (0.5ml) was applied subcutaneously (SC) in the leg fold.Challenges were performed at 14 woa via oculo/nasal route with a field isolate (200ul) with A and a C wild type serovars of A.P. obtained from clinical cases in different locations in the U.S. We evaluated i) protection based on: i) a clinical sign scoring system, ii) bacterial shedding reduction at 5- and 9-days post challenge. Clinical signs differences were noticed between the controls and vaccinated groups but, clear protection was noticed in all vaccinated groups. In addition, minor differences were noticed between groups with different challenge strains. Bacterial shedding values will allow us to discriminate between the protection provided by the one dose or two dose strategy.

Adenoviral Progeny Protection Results

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An increase in cases of inclusion body hepatitis (IBH) have been observed in broilers over the past 5 years. Aviadenoviruses isolated and detected from IBH cases primarily belong to FAV group E, FadV 8b. Many companies elect to utilize adenovirus isolates from their clinical case submissions in autogenous vaccines for use in pullet breeders to prevent vertical transmission and provide maternal antibodies to progeny. Virulence of FAVs varies by serotype and within serotypes. In this study, we evaluated the pathogenicity of an FAVE, FadV 8b IBH field isolate in specific pathogen free (SPF) chicks. Mortality and gross and microscopic liver lesions was observed in inoculated chicks. In a follow-up vaccination protection study, progeny from adenovirus FAVE/8a, FAVE/8b and FAVD/11 autogenous vaccinated breeders were challenged with this contemporary FAVE, FadV 8b field isolates, or FAV D, FadV 11 or FAV E, FadV 8b challenge viruses to determine whether antibodies generated from vaccination were sufficient to provide protection. No clinical disease, gross or microscopic lesions were observed in any of the challenged flocks suggesting that the autogenous vaccine provided protection.

Epidemiology

Interaction Among Variables Affecting Production Performance

Dave Fernandez

FSTAATS

There are several univariate approaches to evaluating multiple variables affecting production performance. Oftentimes, only a fifth of these variables are responsible for up to 80% of the variability in the model. The interaction among variables has largely been overlooked. In many cases, the model does not call for identifying possible interactions, as in interventions-based models and other solutions-based evaluations. Interactions are most important, especially when dealing with variables that have marginal effect on the outcome. The combined effect of two or more variables may contribute a much greater effect on the outcome. This presentation is geared towards identifying possible interactions and a presents a simple approach to testing whether the interaction effect is truly significant.

Outbreak Management of High Pathogenic Avian Influenza in the Most Densely Populated Poultry Production Area in Germany

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The third year of devastating outbreaks of HPAI H5N1 also in the summer months has led to a worldwide discussion about the use of vaccines in the control of avian influenza. Open questions about the effectiveness of vaccines in different target species, their availability and application, the monitoring of vaccinated flocks to prove freedom from field infections, but also the question of an exit strategy pose major challenges, especially for those countries that have so far followed a stamping-out policy and are dependent on international trade.The poultry industry in regions of Germany with the highest poultry density has made enormous efforts in recent years to minimize the introduction of HPAI, especially into highly susceptible turkey populations, by optimizing husbandry and management practices. This is only possible through close cooperation between animal owners, veterinary authorities, private and state laboratories as well as companies specialized for certain tasks in animal disease control. The following article is intended to describe this closely networked mechanism and the parameters contained therein for effective animal disease control from high sensitive surveillance, rapid diagnosis in both private and state laboratories, depopulation and disposal, but also necessary measures to prevent secondary outbreaks.

Evaluating the Risk of Moving HPAI-Vaccinated Turkey Breeder Hens to an Egg Production Farm or Other Premises

Mickey Leonard, Catherine Alexander, Benjamin Blair, Peter Bonney, Carol Cardona, Cesar Corzo, Marie Culhane, Timothy Goldsmith, Sasidhar Malladi, Rosemary Marusak, Miranda Medrano, Amos Ssematimba, Kaitlyn St. Charles, Margret Tavai-Tuisalo'o, Sylvia Wanzala Martin

University of Minnesota

The United States Department of Agriculture Animal and Plant Health Inspection Service Veterinary Services (USDA APHIS VS) response plan for foreign animal diseases (FAD), such as Highly Pathogenic Avian Influenza (HPAI), has vaccination as part of the response. However, the vaccination strategy is not thoroughly defined. For example, neither the full surveillance protocols to follow nor the biosecurity criteria that need to be met in order to confidently move vaccinated animals to production or slaughter sites are well-described. Furthermore, the risks of outbreak spread as a result of moving vaccinated groups have yet to be evaluated. To address the uncertainty regarding biosecurity and surveillance criteria and to evaluate the risk of further outbreak spread from moving vaccinated groups, the University of Minnesota's Secure Food System (UMN SFS) is conducting a risk assessment (RA) for the movement of HPAI-vaccinated turkey breeder hens to an egg production farm or other premises. The RA work is conducted as part of the larger Secure Poultry Supply (SPS) plan. The SPS plans provide science-based proactive RAs and permit guidances (PG) for permitted movement of poultry and poultry products from monitored premises out of and within control

areas (CA) during an HPAI outbreak. Evaluating the risk of the movement of vaccinated turkey breeder hens can reduce the uncertainty of vaccination as a control strategy for HPAI. Reducing the uncertainty involved with movement of HPAI-vaccinated breeder turkey hens would provide emergency disease managers with risk-based evidence that this continuity of business move is either reasonable/could be allowed or unreasonable/to be avoided. There is a possibility that an acceptable level of risk cannot be obtained while using vaccination as a control strategy.A workgroup, comprised of the UMN SFS team, turkey breeder industry representatives, state authorities, and federal agencies, was formed to evaluate the risk of HPAI spread from the movement of vaccinated turkey breeder hens. Working together with subject matter experts in the areas of turkey breeder production and management, HPAI vaccination strategies and development, and regulatory matters, such as permitting and trade, allows for a more comprehensive understanding of real practices and concerns surrounding this movement. This collaborative workgroup meets to identify and discuss gaps in industry and vaccination practices that impact the risk analysis. Potential mitigations to prevent infection and spread – including appropriate surveillance and targeted biosecurity – are also assessed in these discussions. Once these gaps are identified and scientifically evaluated with the workgroup, this RA will incorporate that information to reduce uncertainty around the use of vaccination as a control strategy for HPAI.

Evaluating the Risk of Moving Uninfected Poultry Industry Product from HPAI Positive Premises to Market

Rosemary Marusak, Carol Cardona, Michelle Leonard, Sasidhar Malladi, Margret Tavai-Tuisalo'o

University of Minnesota

During highly pathogenic avian influenza (HPAI) outbreaks, there is often a lot of uninfected/uncontaminated material remaining on positive premises. Some of this material is valuable poultry product that is destroyed during the depopulation process, resulting in significant lost revenue for the producer. Examples of these destroyed products are eggs and egg industry products. Three kinds of eggs are involved on positive premises: 1) eggs laid prior to HPAI infection and diagnosis; 2) eggs laid by PCR negative flocks during the depopulation process; and 3) eggs laid after a flock is known to be infected. While eggs laid by infected flocks will likely be contaminated and risky to move, it is worth investigating if eggs produced by not known to be infected hens (scenarios 1 and 2 above) that are washed, sanitized, stored, and segregated in coolers, have the potential to be moved off the premises to market. If this were possible, producers would capture this revenue, USDA-APHIS indemnification costs would be reduced, and perhaps, rising consumer prices for food/food security during an outbreak would be mitigated.Poultry product movement from monitored (i.e., not known to be infected) premises out of and within established control areas (CA) using the permitting process helps producers maintain continuity of business while reducing the risk of virus spread. The Secure Poultry Supply (SPS) Plans provide science-based

proactive risk assessments (RA) and permit guidances (PG) for these movements.1 Starting in 2009, guidances for movements of lowest risk - commercial layer eggs and egg industry products - were first published. These RAs indicate that pasteurized liquid egg has a negligible risk of containing HPAI virus,2 non-pasteurized liquid egg can be moved with negligible risk,3 and eggs from flocks testing negative can be safely moved.4 Since then, movements of increasingly higher risk have been assessed, published, and successfully implemented. Once considered too dangerous, highest risk movements involving the transfer of birds to sites for continued production were safely carried out using PG-defined mitigation measures during the 2022 HPAI outbreak.5 With increasing outbreak experiences, the poultry industry – both producers and allied partners- has continued to improve and refine biosecurity efforts and outbreak control. Company investments in advanced biosecurity measures, such as shower-in-out facilities, on-site vehicle wash stations, and permanent employee workforce, along with upgrades in biosecurity to allied industry facilities and services, indicate a culture shift to safer procedures and practices once thought impractical.5 With notable progress in these areas, we propose to consider assessing the risk of moving uninfected, eggs and egg industry products to market. 1. Securepoultrysupply.umn.edu/2. Goldsmith T, Halvorson D, Malladi S, McElroy K, Waters K, Clouse T. An Assessment of the Risk Associated with the Movement of Pasteurized Liquid Egg and Its Products Into, Within, and Outside of a Control Area During a Highly Pathogenic Avian Influenza Outbreak. Published online 2009.3. Goldsmith T, Funk J, Halvorson D, et al. An Assessment of the Risk Associated with the Movement of Nonpasteurized Liquid Egg (NPLE) and Its Products Into, Within, and Outside of a Control Area During a Highly Pathogenic Avian Influenza Outbreak. Published online 2009.4. Goldsmith T, Funk J, Halvorson D, et al. An Assessment of the Risk Associated with the Movement of Washed and Sanitized Shell Eggs Into, Within, and Outside of a Control Area During a Highly Pathogenic Avian Influenza Outbreak. Published online 2009.5. SPS Cross Commodity Workgroup, personal communication, 2022

Examination of Salmonella Livingstone and Salmonella Mbandaka Identified in Poultry Supply Chain to Determine the Source of Contamination Using Whole Genome Sequencing

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Although Salmonella spp. are repeatedly seen in the poultry supply chain, sources of introduction remain unclear. The goal of this project was to identify the source of environmental Salmonella strains (S. Livingstone and S. Mbandaka) detected in selected broiler breeder flocks with the final objective to reduce or eliminate the source of contamination with an appropriate intervention program. Several samples (feed fines, farm and hatchery environmental swabs, chicken broiler fluff and live beetles captured in parent stock barn) were cultured for Salmonella spp. using a MFHPB-20 or MFLP-06 Canadian standard isolation and detection methods with serotypes being identified using Check and Trace™ technology. Relatedness of Salmonella isolates was determined using whole genome multilocus sequence typing (wgMLST). In total, wgMLST of 5 S. Mbandaka and 4 S. Livingstone isolates were compared. As expected, the isolates were initially separated into two

different clusters corresponding to the specified serotypes. Within each serotype cluster, there were 0-3 wgMLST differences among the isolates indicating their close genetic relatedness. Based on the results, 3 S. Livingstone isolates from the breeder flock boot covers samples were related to 1 S. Livingstone isolates recovered from feed administrated to the breeder flock. Interestingly, 1 S. Mbandaka isolated from darkling beetle samples was identical to 2 isolates recovered from fluff and boot covers. This cluster was within 1 and 3 wgMLST difference to isolates recovered from the breeder flock environmental samples taken from different consecutive flocks housed on the same site. In conclusion, the wgMLST analysis is a useful tool for determining level of relatedness among Salmonella isolates tested in various number of samples collected on farm in order to identify sources of contamination.

Monte Carlo Simulations: A Solution for Improved Decision Making in the Poultry Industry

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Decisions regarding product selection or management practices aimed at enhancing poultry health and production outcomes should be based on both scientific trials and field trial results. Furthermore, statistical methods are used to draw inferences about the population based on a sample, while financial methods assess the risk of adopting new technologies. The analysis of field data in the poultry industry is challenging due to the absence of controlled experimental conditions. Despite being limited and unreliable, field trial data are often used to inform financial decisions. However, relying solely on statistical significance to evaluate such data may result in Type II errors, also known as false negative. Thus, P-values should not be used for risk analysis.In this study, 30 poultry houses were evenly and randomly assigned to one of three treatments (Control=C, treatment 1= T1, and treatment 2=T2). The mean ($\bar{x}$) and standard deviation (SD) of body weight for each treatment was calculated in relation to the control, as follows: C (100 ± 10), T1 (109 ± 4), and T2 (110 ± 12). The means alone indicated that the body weight of T2 was improved compared to both C and T1. However, when a Monte Carlo simulation, which involves repeated random sampling, was performed to predict the performance of various production strategies, the simulation showed that the chance of choosing a better treatment than the control was 81% for T1 and 75% for T2, due to the differing SD values. Thus, Monte Carlo simulations provided a comprehensive risk assessment for the implementation of new programs or technologies, and can be easily performed with a spreadsheet. In conclusion, Monte Carlo simulation serves as a valuable tool for understanding the potential performance of small trials in field situations and can assist veterinarians in making sound financial decisions in various production scenarios.

Broiler Breeder Pullet Salmonella Epidemiology Study: 2017-2020

Louise Dufour-Zavala[1], Kathleen Muro[1], Dave Fernandez[2], Douglas Waltman[1]

[1]GPLN,
[2]EZ Stats

In order to understand and explain the Salmonella prevalence results on meat type integrator pullet farms over a period of 4 years, several production, geographic and health parameters factors were taken into

consideration as part of an epidemiological analysis. The results of the study will be presented and show that although the original question that triggered this study was puzzling, the answers that were found went in a totally different direction!

Wealth of Knowledge

Online Poultry Medicine Certificate Course for Practicing Veterinarians

Richard Fulton[1], David Frame[2], Jaquie Jacob[3], Roberta Dwyer[3], Tony Pescatore[3]

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The demand for private practice veterinarians to treat individual chickens or entire flocks has increased dramatically recently due to the recent growth in small flocks and urban poultry flocks. Many private practice veterinarians have no experience with chickens and little knowledge about their production, disease, treatment, and prevention. It is with this conundrum that the authors developed an online poultry medicine course which upon successful completion, grants the candidate a certificate from the American Association of Avian Pathologists as well as 8 hours of RACE-approved continuing education.The course consists of 22 modules which can be taken at the practitioner's leisure. Modules vary in duration from 10 to 35 minutes. At the end of each module is a quiz. Successful completion of all quizzes for the course requires a score of 70 percent or higher score. Subjects included are: Home Poultry External anatomy of Poultry Chicken behavior Physical Exam Nutritional concepts Digestive system Reproductive system Respiratory system Skeletal system External parasites Internal parasites Respiratory diseases Digestive diseases Neurological diseases Cardiovascular diseases Tumor-causing diseases Performing a necropsy Disease prevention Poultry health conditions and miscellaneous abnormalities Poultry first aid Poultry medications and the Veterinary Feed Directive Zoonotic diseases

Mortality in Broiler Breeder Males: What Field Experience is Telling Us

Jose Bruzual

Aviagen

Mortality in broiler breeder males. What field experience is telling us.Over the last decade, male broiler breeder mortality appears to be increasing and 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 changed much over the years, their requirements have changed. Therefore, management of the male broiler breeder must be fine-tuned, requiring more precision and attention to prevent different health issues. To assess the impact of improved efficiency we utilize a routine group evaluation of male mortality to provide the technical staff with some guidance on how to improve the management of male broiler breeders. An evaluation of the most common findings from several integrations in the US will be presented along with suggestions on how to prevent issues and improve final production results. The purpose of the recommendations is to increase uniformity, livability, and fertility and to decrease the incidence of issues in production

like vent gleet, leg problems, and cardiopathies, among others that could affect male performance.

valuable information that allows us to adjust the health programs, management programs, and nutritional programs conducive to better performance and livability in the operations.

Broiler Breeder Mortality Surveys – The Importance of Systematic and Continuous Post-Mortem Evaluations

Harold Echeverry

Aviagen

A mortality survey consists in the post-mortem evaluation of all the mortality seen on a given day across a breeder complex. In an effort to understand the causes of the increase in hens' mortality in the USA industry seen in recent years, our team has been performing broiler breeder mortality surveys across the USA and systematically compiling these data for the last 3 years. The analysis of these data has allowed us to understand not just the particular conditions associated with elevated mortality in a particular farm, but also across a complex or company. Our findings showed peritonitis as the leading cause of mortality in broiler breeder hens, followed by peck-outs, sudden death syndrome, septicemia, and calcium tetany. All of these conditions seemed to be mainly associated with metabolic conditions and disorders rather than associated with primary pathogens due to the lack of additional lesions that could be related to primary pathogens, and the high incidence of secondary findings in all these cases such as over-fleshing, multiple yolk hierarchies, and the excessive number of follicles in the ovary. Routine post-mortem evaluations give us

Impact of Heat on Blood Chemistry and Heart of Turkeys

Rocio Crespo, Jesse Grimes

North Carolina State University

The exact etiology of spontaneous cardiomyopathy in turkeys is unknown. It has been hypostatized that overheating during brooding could increase the incidence of dilated cardiomyopathy. The purpose of this study was to evaluate how changes in the environmental temperature and humidity during brooding affects the blood parameters and its association with dilated cardiomyopathy and performance in meat type turkeys. For this study, a total of 2,240 female poults from a commercial hatchery were assigned to one of four treatments: T1 (85F-65%RH), T2 (90F-65%RH), T3 (95F-65%RH), and T1 (55F-80%RH). Blood was collected at on days 1, 3, 5, 7, 10, 14, 21, 28, 35. Blood was analyzed using an iSTAT 1 analyzer using the CG8+ cartridge, the VetScan2 using the Avian and Exotic rotor. In addition, cardiac troponin was measured in plasma by ELISA.Higher brooder temperatures resulted in a lower feed conversion and higher incidence of pendulous crop. Lower myocardial contractibility was noted by ultrasound of the hearts in turkeys from treatments T3 and T4. Brooding temperatures also had a significant effect on blood pH, partial CO2, CK, and cardiac troponin.In conclusion, high

brooding temperatures can affect negatively performance and heart function of poults, and predispose dilated cardiomyopathy. Analysis of blood may be used for evaluation heat stress in turkey poults and predisposition to cardiomyopathy.

Practical Considerations for Field Trial Design

Kabel Robbins

Butterball, LLC

Production veterinarians are presented with in vitro, benchtop, safety studies or at best pen trial research to support the inclusion of health, food safety, nutrition, disinfection, or pest control products in their routine tool belt. Field trials are important considerations for the further evaluation of veterinary and nutritional products in commercial poultry production. This presentation will review practical considerations when designing a field trial including the results of a commercial turkey study to calculate the inherent variation in commercial turkey field trials. By understanding this variation, we can then accurately calculate and design the sample sizes needed in field trials to achieve meaningful results.

Poult Performance Using Different Supplementation Procedures

Nisana Siman-Tov[1], Laura Tensa[2], Brian Wooming[2], Andrew Gomer[3]

[1]Hybrid Turkey LLC,
[2]Cargill LLC,
[3]NTE Global

Poult Performance Using Different Supplementation Procedures Nisana Siman-Tov(a), Laura Tensa(b) & Brian Wooming(b), Andrew Gomer(c)Hybrid Turkeys LLC(a), Cargill Turkey Production LLC(b), NTE Global(c)The ability of the newly hatched turkey poult to get off to a solid start is critical in the success of the flock. Many turkey poults are trucked long distances, held for next day placements, or otherwise delayed at the hatchery which presents another challenge for the grower. Companies have experimented with different ways to deliver both hydration and nutritional products to poults during this shipping period to minimize the stress to the neonate. This presentation will evaluate the use of nutritional supplementation for newly hatched poults that are shipped more than 8 hours on the road. Poults will be given product using either the standard gel drop method, or a new gavage system developed by NTE Global. Poults will be raised in pens and evaluated for mortality, feed conversion, weight gain & uniformity to 6 weeks.

Biosecurity in an Endemic Epornitic

Eric Gonder

Consultant

The strains of highly pathogenic avian influenza circulating in 2022 severely affect many more types of birds than seen previously and over a long period of time. They continue to evolve, affecting more species, with some spillover into mammalian species as well. There is significant risk that these strains are becoming endemic in areas where they have not previously. The threat is more generalized and long-lasting. This may require a more long-term approach than is currently pursued. Current efforts have focused almost exclusively on operational biosecurity - efforts to control daily movements of personnel, equipment and commercial poultry. These

efforts have been quite successful in limiting introduction into commercial premises given the presence of HPAI viruses in many more locations than seen previously. Without those efforts, the situation in North American during 2022 would have been even more catastrophic. However, given the extreme damage during the current outbreak, it is apparent that the current emphasis on operational biosecurity must be maintained, but is not adequate to a more widespread and probably endemic situation. Vaccination may be of some use, but is expensive, will take some time to implement and has significant trade and movement implications. If a significantly different antigenic variant arises, this approach will take some time to reconfigure. This begs for more emphasis on long-term solutions. Buildings and ventilation systems that are easier to secure; personnel policies that make operational biosecurity easier and more foolproof; buildings and equipment that are easier to clean and disinfect; remote and robotic systems that do not require as any incursions into populated flocks, etc. These are long-term solutions that will require capital investment and research rather than the training and minimal physical modifications required for operational biosecurity. Failure to pursue these necessary structural changes will continue to imperil the existing commercial industry, especially for the turkey industry. At some point, government and popular support for current depopulation and indemnity practices may erode.

Development of a National Veterinary Accreditation Module on "The Veterinarian's Role in Preharvest Food Safety"

Michelle Kromm[1], Charles Corsiglia[2], Ashley Peterson[3], Kathy Simmons[4], Jamie Jonker[5], Abbey Canon[6], Annette Jones[7], Russ Daly[8], Misa Robyn[9], Marta Zlotnick[9], Jane Rooney[10,12], John Korslund[10,12], Sherry Shaw[11,12]

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[6]American Association of Swine Veterinarians,
[7]National Assembly of State Animal Health Officials,
[8]National Assembly of State Public Health Veterinarians,
[9]Centers for Disease Control and Prevention,
[10]APHIS,
[11]FSIS,
[12]United States Department of Agriculture

The idea for a USDA-APHIS NVAP microbial food safety module for veterinarians grew from recent food safety challenges and from recent

industry-government collaborative preharvest efforts. The proposed module was developed as a collaborative effort between industry associations, veterinary associations, government agencies, and other allied entities. One goal of the module was to increase awareness among veterinarians that preharvest factors and practices could help prevent human foodborne illness. The presentation will walk through the approach taken by the very diverse working group to identify key "model practices" across all sectors as well as discuss module objectives.

Utilizing Dry Hydrogen Peroxide As A Replacement For Formaldehyde In Commercial Poultry Hatcheries

Kylie Bruce[1], Russ Stephens[2], Kirk Dawkins[3], Jeanna Wilson[4], Brian Jordan[4]

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The health of newly hatched chicks is severely impacted when hatched in a bacteria filled environment. Though formaldehyde is the industry standard for reducing microbial load in hatchers, a new hydrogen peroxide gas could serve as a replacement. Previous research has shown that using dry hydrogen peroxide (DHP) in common spaces of the hatchery resulted in increased hatch of fertile, decreased aspergillus positive chicks, and decreased 3-day mortality at the farm. Additional research has shown that using DHP in single stage incubators can achieve the same level of microbial reduction on eggshells as pre-fogging eggs with formaldehyde prior to set. The next step in the research process, and thus the purpose of this experiment, was to directly compare dry hydrogen peroxide to the traditional method of using formaldehyde for microbial reduction. The trial is set in a commercial broiler hatchery, where DHP units were installed throughout the egg room, inside incubators in one incubator hall, the transfer room, and in half of the hatchers. For the first three months of the trial, the DHP was used in addition to formaldehyde, then the formaldehyde will be turned off in the DHP treated hatchers for the next three months of the study. Eggs were followed throughout the hatchery and 3 & 7-day percent mortality, hatchability, HOF, fluff counts, egg and surface swabs, chick quality summaries, breakouts, and necropsy of 3-day mortality were parameters of interest, with hatchability and HOF tracked daily for analysis. After data collection, summary results indicate a significant increase in percent hatchability when eggs were treated with DHP in incubators and hatchers ($p=.0001$). When broken out by treatment group, there was a numerical difference but no significant difference when comparing hatchability for eggs treated in the incubator and hatcher, treated in the incubator but not in the hatcher, or not treated at all, where the treated groups were higher. There was a significant difference, however, in hatchability for eggs not treated in the incubator but treated in the hatcher when compared to 2022 baseline averages prior to the beginning of the trial ($p=.01$, one-way ANOVA). In conclusion, DHP, when used in addition to formaldehyde, may improve hatch throughout the hatchery.

Impact of a Feed Sanitizer on Broiler Breeder Mortality, Fertile Egg Contamination and Improvement of Chick Quality

Enrique Montiel[1], Luis P Avila[2], Kelly M Sweeney[3], Cheryl Schaffer[1], Nicole Holcombe[1], Callie Selby[1], Jeanna L. Wilson[2]

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The effect of a feed sanitizer in broiler breeder feed was evaluated. At 21 wk of age, Ross 708 breeder pullets (n = 256) were placed in 6 floor pens with 3 Yield Plus males and fed a common pre-lay diet until 25 wk when they were fed 1 of 2 laying diets: CTL = untreated control diet; or TRT = treated diet with a formaldehyde+propionic acid+terpene-based product (Termin-8® dry powder at 0.4% inclusion rate). The following parameters were evaluated: 1) hen mortality and reproduction traits; 2), feed and eggshell contamination; 3) incubation characteristics; 4) chick quality and 5) offspring early mortality. Contamination was tested bi-weekly in feed, and in eggshell when the hens were 31, 36, 44, 50, 54 and 59 weeks of age. Eggs were incubated 9 times by pen at weeks 29, 33, 37, 41, 45, 49, 54, 57, and 60. Data were analyzed using a GLM PROC with SAS v 9.4 at a significance of $P \leq 0.05$, and tendencies declared when $0.05< P \leq 0.10$. Hen groups consuming treated feed had approximately 50% lower cumulative mortality than the untreated group between 25 and 60 weeks of age. Egg production, hen reproduction or hatchability ($P \geq 0.222$) were not significantly different among groups, although TRT groups showed reduced live pip % at hatch during early lay (wk 27 to 45; $P = 0.042$) and improved chick quality ($P = 0.002$). TRT feed showed less presumptive aerobic bacteria, fungus, Enterobacteriaceae and C. perfringens compared to CTL feeds ($P< 0.001$). Similar to the feed analysis, the surface of nest eggs from hens consuming TRT-feed showed reduced presumptive aerobic bacteria ($P< 0.001$) and tended to have less fungi contamination ($P = 0.061$). Overall chick mortalities with signs of yolk-sac contamination were reduced when obtained from hens consuming TRT feed ($P = 0.099$), particularly from 60 wk-old hens ($P = 0.031$). Study results indicate that treating broiler breeder hen feed with a formaldehyde+propionic acid+terpene -based sanitizer reduced the microbial contamination of feed and eggshell surfaces, and positively impacted the quality and livability of the hatched chicks.

Utilizing UV Lights in Service Rooms as Part of a Cleaning and Disinfection Protocol in Commercial Turkey Brood Barns

Jolene Tourville

Jennie-O Turkey Store

Maintaining ideal biosecurity in a commercial turkey brood barn is of utmost importance to preventing disease in young poults. A stringent cleaning and disinfection protocol is the first step. A key component of a cleaning and disinfection program is a clean service room. Without continuous disinfection, the service room can serve as an environment favorable to pathogen growth which could be tracked into the barn by employees. Utilizing UV lights is an additional tool that could be included in an existing biosecurity protocol to decrease microbial growth on various surfaces in the service room. This study will cover the use of UV lights in commercial turkey brood barn service rooms and how the use of these lights could decrease poult exposure to pathogens.

Comparison of Two Different Pipelines to Analyze RNAseq Data from Chicken Organs

Ruediger Hauck, Andrea Pietruska, Jannis Nankemann

Auburn University

Analysis of the transcriptome in samples from chicken organs by sequencing the total messenger RNA (RNAseq) is an increasingly common and affordable method in animal experiments as well as in-vitro experiments. The advantages include no need to limit the analysis to certain pre-selected genes, so results and correlations that have not been anticipated are more likely. The downside is that analyzing the large amounts of data requires complex methods and carries the risk of false positive results. In addition, presenting the results in a way that they are intuitively understood can be a challenge. However, being able to understand the results and the underlying methods will only become more important for everyone interested in current research.RNAseq data from two independent experiments involving infection of commercial broilers infected with enteric pathogens and specific pathogen free (SPF) layer-type birds infected with Newcastle disease virus were analyzed with two different pipelines (tophat2 – Htseq – edgeR and HISAT2 – StringTie – Ballgown). The underlying differences of the methods will be described and the results including the differentially expressed genes and up- or down regulated pathways compared. In addition, some common methods to demonstrate results will be highlighted and explained.

Investigating Imidacloprid Toxicity in Chicks

Jenny Nicholds[1], Reece Bowers[2], Ashley Hallowell[2], Maurice Raccoursier[2], David French[2], Karen Grogan[2],

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Imidacloprid belongs to the class of neonicotinoid insecticides and is commonly applied to poultry houses prior to day-old chick placement for the control of darkling beetles (Alphatobious diapernus). The established LD50 for imidacloprid is 104.1 mg/kg body weight (Kammon et al., 2010). The established Effective Dose 50 (ED50), the dose at which 50% of the population displays neurobehavioral abnormalities, for imidacloprid is 4.62 ± 0.98mg/kg body weight/day (Franzen-Klein et.al., 2020). The study establishing the LD50 utilized layer type chickens that were fifty days old and the study establishing the ED50 utilized commercial chickens that were either 6 weeks or 9 weeks of age. There are currently no studies that we are aware of that examine the toxicity and ED50 of imidacloprid in one-day-old meat type chickens, even though day-old chicks are often times exposed to the highest concentrations of imidacloprid. There have been several presumptive imidacloprid toxicity cases in young, meat-type chickens submitted to the University of Georgia Poultry Diagnostic and Research Centers Diagnostic Lab. Toxicology screening for imidacloprid and its major metabolites has been performed on an assortment of samples; crop contents, liver and/or brain. Upon testing, imidacloprid levels are not as high as expected in submitted samples, and are often lower than the reported ED50, which can add confusion in the diagnosis. This confusion can lead to a final diagnosis being made via diagnosis of exclusion rather relying on the toxicology results solely. Investigating the effect of quantified imidacloprid exposure in day-old chicks and levels detected in tissues at specified time points post exposure will be

presented and can help diagnosticians better understand toxicology results in clinical cases with suspected imidacloprid toxicity in young meat-type chickens.

Key Elements to Ensure Effective Prevention and Control of Avian Influenza Outbreaks

Nathaniel Tablante

University of Maryland College Park

Despite the latest scientific and technological advances and enhanced biosecurity measures, outbreaks of avian influenza and other catastrophic poultry diseases continue to occur worldwide. Successful and effective prevention and control of avian influenza requires simple, "common-sense" methods. However, these methods must be clear, concise, practical, science-based, and adapted to each specific poultry operation and facility. Animal health authorities and poultry production personnel must focus on critical or key "elements" such as appropriate biosecurity practices, early detection, prompt response, and full cooperation between the poultry industry, government agencies, diagnostic laboratories, cooperative extension, and allied industries. As the AI virus mutates and tries to evade current prevention and control measures, we must also remain vigilant and relentless in adjusting our AI prevention and control programs as new challenges arise.

Bacteriology

Evaluation of a Mycoplasma synoviae Live Attenuated Vaccine Candidate in Chickens

Naola Ferguson-Noel,
Eniope Bamidele Oluwayinka

University of Georgia

One of the approaches to control Mycoplasma synoviae (MS) in commercial poultry production is vaccination programs with live attenuated or killed immunizing agents. An ideal Mycoplasma vaccine should be avirulent, induces long-lived (lifelong) protection, poorly transmissible, affordable, easy to administer and stable. In this research, three potential live attenuated vaccine candidates were evaluated in naïve chickens and one of them (K5885) successfully colonized the upper respiratory tract of inoculated chickens while producing mild lesions after inoculation that were not significantly different from the controls ($P< 0.05$). With respect to efficacy, there was a significant reduction in airsacculitis and footpad lesions in birds vaccinated with K5885 following virulent challenge compared to non-vaccinated controls ($P< 0.05$). This vaccine candidate (K5885) was determined to be safe and efficacious in these preliminary studies although further development and research is necessary.

The Detection of Avian Mycoplasma spp. in Fecal Matter from Poultry

Mattie Capehart, Michael Rose Davis,
Marianne Dos Santos, Naola Ferguson-Noel

University of Georgia

Mycoplasma synoviae and Mycoplasma gallisepticum are poultry pathogens of worldwide prevalence. The current approaches to control avian mycoplasmosis include continuous surveillance and quarantine, medication, vaccination and/or elimination of infected breeding flocks. The transmission of M. synoviae among flocks is generally more rapid than the transmission of M. gallisepticum. In previous research, M. synoviae was detected from various fomites in the environment of naturally infected broiler breeders; M. synoviae was detected in dust, litter, and feather samples

by real time PCR. In this research, the detection of M. synoviae and M. gallisepticum from cloacal swabs, fecal matter, and litter from infected broilers was compared using real time PCR and culture. A protocol to successfully facilitate the growth of Mycoplasma spp. in the presence of highly contaminated samples of feces was developed. The differences found in the level and duration of shed of M. synoviae compared to M. gallisepticum indicates that M. synoviae is more likely to be transmissible via fecal matter (as well as dust and litter) than M. gallisepticum.

Understanding the Length of Time Required for Campylobacter hepaticus to Infect or Cause Spotty Liver Disease in Chickens

Catherine Logue, Julia Ienes-Lima, Roel Becerra

University of Georgia

Spotty liver disease (SLD) has emerged as an important cause of loss in layer hens. The organism implicated, Campylobacter hepaticus, causes focal lesions on the livers, and decreased feed consumption resulting in reduced egg production, and increased mortality. The transmission route is not well understood, but research from our group and others suggests it is fecal-oral. The disease appears to be more prevalent in pasture raised or organic hens with outside access to the environment and water sources. Infection of layers with C. hepaticus appears to affect birds at peak production; however, there is limited data available on the length of time required for birds to show gross SLD lesions following exposure. Here, we assessed the "time to lesion" using model studies.In the first study we examined C. hepaticus transmission to chickens when finches were used as surrogate wild bird model. Finches were orally challenged with C. hepaticus at a dose of 106 cfu/ml from a cocktail of four C. hepaticus strains and then introduced into a naïve chicken group. All chickens were monitored to determine when they became infected. Chickens were sacrificed at weekly intervals and tested bacteriologically to recover the challenge strains and confirmed by PCR of the liver. In addition gross lesions were monitored and recorded and livers collected for histopathology. Spotty lesions developed in finches, challenged and naïve birds at differing intervals depending on the bird status (naïve vs challenged) with a range from 9 to 28 d.In the second study, SPF layers (17 weeks of age) were orally challenged with a 107 cfu/ml cocktail of four C. hepaticus strains at three day intervals over a week long period. Chickens were monitored and sacrificed at weekly intervals post challenge to measure C. hepaticus in the liver and gall bladder and to monitor for gross lesions. Also, naïve non-challenged SPF chickens were introduced into the challenge group to monitor transmission of C. hepaticus to naïve chickens and the interval required for the disease to transmit and develop. Gross SLD lesions were confirmed on necropsy and C. hepaticus was isolated from bile and PCR analysis the livers of naïve and challenged chickens. Some of the chickens developed gross spotty lesions 15 to 20 days post challenge. Overall, our lab has shown that under experimental settings, C. hepaticus can cause disease and develop spotty liver lesions in time frames as short as 9 d post exposure/ challenge

with a range of 9-28d. Further, research is ongoing to identify the pathophysiology of C. hepaticus causing SLD in affected chickens.

Use of a Molecular Monitoring Tool for Campylobacter Detection in a US Poultry Integrator

Ana Maria Villegas[1], A. Menconi[1], K. Reed[1], N. Kryzhevoy[2], M. Langenkaemper[2], E. I. Igwe[2]

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A digital monitoring tool based on qPCR testing and data analysis was used to detect Campylobacter jejuni during two consecutive broiler production cycles. The methodology involved the collection of boot swabs from a farm with four houses. Boot swabs samples were collected at placement, end of the flock, and post-windrow. The quantification was described as log10 DNA copies/ mL of booth wash. The results showed that Campylobacter jejuni values reached a maximum from placement (3.17) to the end of the flock (4.78) in the first cycle, remaining higher than the limit of detection (LOD). In the post-windrow, the levels dropped (3.61); in the second cycle, the levels remained stable, ranging from 2.53 to 2.72. The environmental screening method uses a non-invasive approach that enables poultry producers and veterinarians to detect Campylobacter jejuni throughout the entire flock, providing the opportunity to make adjustments in production interventions. The digital monitoring tool offers a quick and accurate way to monitor Campylobacter levels in broiler farms, enabling proactive management of potential risks associated with this pathogen.

Divergent Chicken Genetics Correlate with Shifts in Cecal Associated Antimicrobial Resistance Transfer Ex Vivo

Logan Ott, Melha Mellata

Iowa State University

The gut of chickens serves as a potentially significant source of antimicrobial resistance genes, which can be mobilized and spread between members of the poultry gut flora. The persistence and spread of these novel resistant and virulent bacteria are of utmost concern and are the target of significant efforts. However, little is known about the relationship between the host and the process of bacterial plasmid conjugation in vivo. Here we attempt to study the role of host genetics in the regulation of bacterial plasmid transfer in a host-associated explant model. Ceca tissues were harvested from inbred Line-8, M-15.2, and GHS-6 birds at both 73 and 363 days of life (DOL) for explant culture or storage for RNA extraction. Tissues for explant culture were serially washed with antibiotic media and sterile PBS and then placed in a complete growth medium supplemented with ~ 1.0 OD600nm of donor (E. coli APEC-O2-211) and recipient (E. coli HS-4). Tissue and suspensions were then incubated for six hours at 41˚C, serially diluted, and plated on selective and differential media. Furthermore, small RNA was extracted from cryo-homogenized content-free ceca tissue using the RNAzol RT extraction kit. In vitro broth conjugations were set up likewise using Luria Broth media supplemented with ~ 1.0 OD600nm of donor and recipient cultures. To conjugations, either 0 or 500 ng of small RNA was supplemented and incubated and enumerated as with explant cultures. Ceca tissue explants demonstrated a significant reduction in donor and recipient CFU/mL compared to the no tissue controls for all genetics at 73 days of life, and Line-8 and GHS-6 tissues at 363 DOL. Furthermore, transconjugant populations were significantly reduced in tissue explants. However, conjugation frequency demonstrated

an increase in conjugation in tissue explants. In vitro ceca RNA conjugations revealed a slight but insignificant increase in donor and recipient populations treated with small RNA isolated from birds of 363 days of life. Alternatively, small RNA from both 73 and 363 DOL significantly reduced transconjugants for all genetic lines. Interestingly, there was a significant difference between all transconjugant populations between ceca RNA from 73 and 363 days of life, with a lesser reduction observed in RNA from the older bird group. These results indicate that there is a variation in the response of avian ceca tissue to bacterial plasmid conjugation in explant culture, and that cecal small RNA may be integral in the regulation of bacterial plasmid conjugation in the poultry ceca. Additionally, these data indicate that the host's response wanes with host age. Further experimentation is required to determine the molecular mechanisms of action for the regulation of bacterial plasmid conjugation by host ceca small RNA.

Point-of-Care Biosensors for the Detection of Antimicrobial Resistance Genes

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As poultry veterinarians continue to be pressured to improve antimicrobial stewardship practices, new tools are required to facilitate rapid, improved treatment of common poultry bacterial pathogens. Antibiotic sensitivity testing is a critical aspect to antimicrobial stewardship, but it's often replaced by empirical treatment due to the prolonged length of time to received results. Empirical treatment is often initiated to reduce the impact of disease on the welfare of the sick flock. Balancing animal welfare and antibiotic stewardship is a difficult one for production veterinarians. Point-of-care diagnostics are needed to quickly and effectively identify antimicrobial resistant genes to decrease the tension between welfare and antimicrobial stewardship. Here we demonstrate the use of paper-based biosensors for characterizing genes in the field from animal samples. These biosensors use loop-mediated isothermal amplification for the detection of DNA/RNA and provide a visible response in the form of a color change within an hour. Our assays achieve high analytical sensitivity and specificity (>~90%) and can be conducted in the field, with farm-based results having high concordance with lab-based results (60-100%). The incorporation of all the reagents on paper-based devices simplifies the operation of biosensors, making them user-friendly. Currently, the biosensors are used for applications in animal health (bovine respiratory disease, African Swine Fever), food safety (fecal contamination of fresh produce), and human health (COVID-19). Since the approach is versatile, it can be adapted for applications in genes relevant for applications in poultry. This approach could help validate antibiotic-based interventions quickly and in a cost-effective manner.

Assessing the Relationship Between O Serogroups and Virulence Gene Carriage in Georgia Poultry Colibacillosis Cases

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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 180 E. coli serogroups and the most common types associated with colibacillosis include O1, O2, and O78. APEC harbor several virulence-associated genes (VAGs) including those linked with invasins, adhesins, iron acquisition systems, and toxins which influence bacterial pathogenicity and disease severity. Currently, there is limited information on APEC O-groups and VAGs in Georgia poultry. This study assessed the prevalence of O-types and VAGs in Georgia APEC. A total of 603 isolates collected between March 2021 – 2022 from submitted diagnostic cases were analyzed and screened for O-serogroups and VAGs using multiplex polymerase chain reaction (PCR). Overall, 309 isolates were identified as serogroups O78 (41%), O2 (19%), O25 (16%), O8 (9%), O1 (9%), O86, O18, and O15 (5%). Chi-square and odd ratios analysis were employed to identify the relationship between APEC O-serogroups, bird types, and VAGs. The odds of O25 were 1.16 higher in broilers than any other bird type, while in the broiler breeder, the odds of O1 were 9.31. Broiler breeder pullet and cockerel had an odds ratio for O78 of 1.81 and pet/hobby birds were more likely to harbor O8 (OR 2.94). The prevalence of VAGs among all isolates was ompT and hlyF (84.7%), iss (82.5%), iroN (82.2%), aerJ (73.4%) cvaC (42.7%), ireA (42.4%), etsB (24.6%) and papC (24.2%). The number of genes detected per isolate ranged from 0 (41 isolates, 13.27%) to 9 (3 isolates, 0.97%). The most common virulence gene patterns included cva-iroN-ompT-hlyF-iss-aerJ-ireA-papC (33 isolates, 10.68%), iroN-ompT-hlyF-etsB-iss-aerJ (29 isolates, 9.39%) and iroN-ompT-hlyF-iss-aerJ-ireA (28 isolates, 9.06%). The relationship between O-serogroups and VAGs for O2 was likely to have an odds ratio for cvaC, ireA and papC of 2.45, 1.87, and 2.48 higher than other O-serogroups. O8 tends to harbor aerJ and papC (OR 20 and 27). O1 had odd ratios of 1.94 for cvaC and 13.81 for papC. O25 tends to harbor etsB (OR 7.4), while O78 is likely to have ireA (OR 4.18). Lastly, O15 had an odds ratio for cvaC and etsB of 22.24 and 3.3. Further phenotypic characterization and molecular research will allow us to develop diagnostic tools for detecting and lessening APEC associated with colibacillosis in Georgia.

Quantification and Characterization of Avian Pathogenic E. coli in Commercial Turkey Toms

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Danisco Animal Nutrition & Health (IFF)

The avian gastrointestinal tract (GIT) is the major reservoir for potential Avian pathogenic E. coli (APEC). Opportunistic APEC infections are a significant burden on the poultry industry, especially in turkey production, resulting in early mortality and condemnations. The objective of the45urrentt study was to evaluate how potential intestinal APEC populations change with age in commercial turkeys, with a focus on how the gut microbiome changes during the move from grower to finisher (6 to 10 wk). Four commercial turkey flocks were selected and followed from placement to 120 d of age (17 wk). All birds were obtained from the same hatchery, fed the same diet and followed identical nutrition, health and husbandry practices. Farm 1 & 2 were placed a week apart from Farm 3 & 4 thus were from different hatches. Two farms

were tunnel ventilated (Farms 1 and 2) and two were curtain sided (Farms 3 and 4). At each sampling point, five entire GITs were collected from each house at placement, week 4, 6, 10 and 17 of production, prior to the birds moving to the withdrawal phase. The small intestine was excised, digesta removed, and mucosa lining enumerated for E. coli on selective media. Following enumeration, 5 distinct colonies from each bird were selected and evaluated via PCR for 10 APEC virulence genes. Any E. coli isolate with >3 genes was defined as APEC. At placement, average E. coli concentration in the GIT was 7.8 log CFU/g, with 85% of the E. coli isolates being APEC and 15% commensal. Of the 10 gene markers, 7 were present in more than 50% of the samples indicating a high reservoir of pathogenicity genes coming into the turkey gut at hatch. By d 28, total GIT E. coli was found to be reduced to 4.75 log CFU/g with 43% of the E. coli isolates being potential APEC. None of the 10 gene markers were found at a high prevalence (<50%). At 6 wk, around the time of move, total E. coli and pathogenicity was not different from 4 wk but found to increase to 5.28 log CFU/g at 10 wk with 65% of the E. coli isolates as potential pathogenic and an increased prevalence of virulence genes. This increase in E. coli pathogencity may be a result of moving turkeys into their finishing barns. E. coli is one of the first microbial species to colonise a chick's naïve GIT and this study illustrates the risk of potential early APEC infection as a result of a high APEC prevalence. This is aligned with the literature for broiler chicks. In summary, this study demonstrates that as toms are raised there are various opportunities for APEC to become opportunistic and early intervention, monitoring and intestinal health support as a strategy to reduce the residing reservoir could prove valuable tools in production.

Impact and Efficacy of Utilizing a Modified Live E. coli Vaccine in Broilers

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Zoetis

Escherichia coli (E. coli) is a major bacterium that has severe health and financial implications to poultry production worldwide. It is responsible for both mortality and morbidity throughout the life of an affected broiler flock. It is an opportunistic organism that is responsible for various disease manifestations, leading to increased mortality and compromised performance. E. coli is also involved in millions of dollars of processing condemnations if uncontrolled. The gram-negative organism is often resistant to antibiotics currently labeled for use in poultry in the United States. Because of its resistance to various antimicrobials, prevention and treatment practices are often difficult and unsuccessful. Over the years, we have conducted various field trials and controlled studies to examine the efficacy and impact of a live, attenuated e. coli vaccine for use in broilers. During these trials, we were able to repeatedly demonstrate reduction in E. coli associated lesions, as well as reduction in processing condemnations. One particular controlled study involved vaccinating commercial broilers with live E. coli vaccine via the spray or gel method, and challenged with APEC E. coli via the intra-tracheal route. Birds were later euthanized and the weight, sex, and lesions were recorded for statistical analysis. Overall, treatment with live E. coli vaccine demonstrated reductions in pericarditis, perihepatitis, airsacculitis, as well as overall mortality. In another study, we looked at the effects of live E. coli vaccine on the severity of cellulitis after birds were inoculated with APEC through the skin. Compared to the negative control group, birds that received the E. coli vaccine demonstrated a significant reduction in

cellulitis lesion. A similar study was conducted examining airsacculitis lesions in a vaccinated versus non-vaccinated flock after an intra-tracheal challenge. Again, we witnessed a reduction in the mean airsacculitis lesion in the vaccinated group compared to the non-vaccinated control. Furthermore, we examined the effects of E. coli vaccine In a commercial broiler flock with a rising respiratory challenge, where flocks were challenged with heavy airsacculitis and E. coli starting around 4 weeks of age. In this field study, flocks were vaccinated in alternating weeks for a total of 9 weeks. At the end of the trial, mortality, livability, body weight, FCR, live cost, and condemnations were analyzed. Overall, there was a consistent reduction in mortality, as well as improvement in FCR and ROI, in flocks that were vaccinated with the E. coli vaccine. Undoubtedly, E. coli is an ongoing stressor in broiler production, and results demonstrated during these field and controlled trials suggest that improvements in live performance and processing costs can offset the incremental cost of using a live E coli vaccine. With limited availability of tools to mitigate severe E. coli challenges, there are circumstances in which the addition of the vaccine may be highly beneficial, especially among operations that struggle with condemnations, or those that are NAE.

A Field Investigation of Positive Impact of a Live E.coli Vaccine on Performance of Selected Broiler Farms in BC, Canada

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The objective of this study was to evaluate the positive impact of the current available commercial live E.coli vaccine in broiler flocks with history of colibacillosis, and/or high condemnations and poor production performance. Series of 8 broiler farms were selected in BC, Canada. The first flock was used as a control flock and compared to the subsequent three placements that received E.coli vaccine via spray at the hatchery. The E.coli isolates from outbreaks in non-vaccinated flocks were sent for pathotyping and presence/absence of virulence genes. In conclusion, in comparison to non-vaccinated flocks, after third placement on each farm, 6 out of 8 vaccinated farms showed gradual improvement on consecutive flocks in one or more production parameters such as flock mortality, average daily gain, and condemnation. The positive impact of E.coli vaccination was not observed In 2 out of 8 farms due to multiple concurrent viral infections at various ages (e.g. IBH, IBV, Reo and IBD). It can be concluded that when Avian Pathogenic E.coli is the main primary source of recurrent colibacillosis or poor flock performance, application of live E.coli vaccine can significantly improve the performance of vaccinated flocks over time.

Contamination of Enterococcus faecalis at the Hatchery and Presentation of Septicemic Disease in Young Broilers

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Bacteria from the genus Enterococcus (E.) are considered normal inhabitants of the

gastrointestinal tract of chickens. These are facultatively anaerobic, gram-positive cocci. Among the most isolated from poultry are E. faecalis, E. faecium, E. hirae, E. durans, and E. cecorum. Although considered part of chickens' normal microbiota, over the past years, pathogenic strains of E. faecalis have been identified in cases of broilers from 1 to 7 days old causing yolk sac infections and septicemic disease. In recent submissions, E. faecalis has been repeatedly isolated from samples collected at the hatchery. The goal of the present study is to characterize Enterococcus spp isolates recovered at the Poultry Research and Diagnostic Laboratory of Mississippi State University (2020-2023), from hatchery samples and broilers 1 to 7 days old of vertical integrations located in the Southern US. Thirty-seven cases were identified from January 2020 to January 2023. From these, thirty-five isolates were identified as Enterococcus faecalis, and two as Enterococcus gallinarum using Vitek – MS -MALDI-TOF technology. These results were then sent to the RUO/SARAMIS database for analysis. In the chickens, most of the E. faecalis were isolated from samples of yolk sac. In a few cases, E. faecalis was isolated from organs (hearth and liver). At the hatchery, E. faecalis was isolated from newborn chickens, hatchery residues, and the hatchery environment. These results suggested that the presence of Enterococus spp in the hatchery could be associated with the yolk sac infection detected in broilers 1 week old. Further studies must be performed to identify the sources of these bacteria for the hatchery.

Optimization of Avibacterium paragallinarum Isolation Methods

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Avibacterium paragallinarum (AP) is a primary pathogen that causes Infectious coryza (IC), a respiratory disease in chickens. IC has a significant economic impact on laying hens, but it is particularly damaging to multiage layer complexes. Prevention and control of IC requires reliable diagnostic methods, but AP is a fastidious microorganism, difficult to isolate from clinical samples and often needs supplements and a nurse bacterium to enhance its growth. The objective of this study was to optimize the isolation conditions of AP to improve the detection of IC in chickens. Toward this goal, we tested different culture media and analyzed the effect of different nurse bacteria on the growth of AP clinical isolates and an ATCC strain. On chocolate agar, a common media used for AP isolation, Staphylococcus chromogenes performed better than Staphylococcus hyicus as nurse colony for AP growth, showing the typical satellitism phenomena. In the absence of a nurse bacterium, Mueller Hinton agar (MHA) plus 5% fetal bovine serum (FBS) and 0.0025% NAD+ demonstrated the best growth of AP as evidenced by appearance of bacterial colonies on the full plate. When we tested MHA plus 5% FBS and 5% filtered S. chromogenes supernatant as supplement, it showed similar results to using NAD+, suggesting that the supernatant of S. chromogenes is a good supplement for AP isolation. These results demonstrate the potential ways to optimize the culture media for AP growth, which will eventually increase the success of AP isolation from clinical samples and improve diagnosis of IC in chickens.

Necrotic Enteritis Lesions Are Not Always Correlated with the NetB Toxin Status of Clostridium Perfringens Strains

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With recent scientific advances, it is now easy to decipher genomes. Reverse vaccinology allows for the comparison of pathogenic and commensal bacterial genomes and the identification of potential vaccine candidates only harbored by pathogenic strains. However, such strategy requires accurate determination of strains pathogenicity status. Necrotic enteritis is a deadly intestinal disease affecting poultry and is caused by Clostridium perfringens. Commercial vaccines are available but clinical trials reveal variable efficacy. Even though necrotic enteritis is often correlated with the NetB toxin, there is mounting evidence that this toxin is not essential for lesions development. To determine the pathogenicity of NetB-positive and Net-B-negative C. perfringens strains, we used the intestinal ligated loop model as an in vivo model. Surgeries were performed using 14 to 15-week-old SPF hens (n=25) and 79 C. perfringens strains of various sources and NetB status were tested. A strain was deemed pathogenic based on a high lesion score and commensal with a minimum of two low lesion scores. Strains with inconsistent scoring were repeated a third time. Negative controls consisted sterile broth inoculated in ligated intestinal loops inserted between tested strains. Seven hours p.i., intestinal samples were collected and processed for histology and microbiology analyses. Lesion scoring (H&E staining) and immunohistochemistry using NetB and Cpa toxin antibodies were done, while toxinotyping was performed to confirm that inoculated strains were recovered. From the 79 tested strains, 69 were determined pathogenic, 9 were determined commensal and 1 was inconclusive. From the 69 pathogenic strains, 39 were NetB-positive and 30 were NetB-negative. For the 9 commensal strains, 5 were NetB-positive and 4 were NetB-negative. Immunohistochemistry results show that NetB-negative strains could cause severe NE-like lesions while some NetB-positive strains could not. The results of this study highlight the importance of using an in vivo model to accurately determine C. perfringens strains pathogenicity prior to the identification of potential vaccine candidates.

CpG-ODN-Induced Immune Cell Metabolism and Antimicrobial Trained Immunity in Chickens

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Cytosine phosphodiester guanine oligodeoxynucleotides (CpG-ODNs), a synthetic DNA molecule, promotes antimicrobial immunity by enhancing immune cell activity in chickens. Activated immune cells undergo profound metabolic changes to meet cellular biosynthesis and energy demands and facilitate the signaling processes. We hypothesize that the stimulation of immune cells by CpG-ODNs promotes profound metabolic programming to facilitate the antimicrobial activities of immune cells. CpG-ODN (two doses, at day 1 and day 3) or saline were administered to broiler chickens via the intramuscular route and longitudinally evaluated immuno-metabolic responses. Cellular mitochondrial activity [oxygen consumption rate (OCR)] and glycolysis rate [extracellular acidification rate (ECAR)] were

measured in peripheral blood (T and B cells) in real-time using Seahorse XFp Extracellular Flux Analyzer (Seahorse Bioscience). To identify whether T cells or B cells are responsible for CpG-ODN mediated protection, the PBMC layer was incubated with Pokeweed mitogen (PWM) and Phytohaemagglutinin (PHA-P) separately and ECAR and OCR were quantified. Immune cell populations in each sample were identified and quantified using the flow cytometry method (CytoPLEX, Beckman Coulter Life Sciences). The CpG-ODN injected group showed higher, statistically significant (p<0.001) mitochondrial respiration and glycolytic capacity than the saline group. Furthermore, PWM (mitogenic for both T and B cells) stimulated cells showed significantly higher (p<0.05) OCR than PHA-P (mitogenic only for T cells) stimulated cells in the CpG-ODN group indicating increased B cell activity. Flow cytometry analysis revealed that CpG-ODN has increased B and T cell population in blood. The data indicate a substantial elevation of immune cell metabolism, especially in mitochondrial respiration. The metabolic pathways highlighted in the present study can facilitate vaccine-developing studies to better understand immuno-metabolic interactions contributing to immunity in the chicken industry.

Diagnostics

The Use of ATP Bioluminescence Technology to Monitor Key Aspects of Your Biosecurity Program

Jantina De Vylder, Anne Calitz, Gwen Slacum

BioChek

Effective biosecurity procedures play an important role in preventing the introduction and spread of infectious diseases in poultry operations. One of the key components of biosecurity is sanitation / cleaning and disinfection. To verify, monitor and improve cleanliness procedures as part of routine biosecurity measures in an objective and timely manner, an on-site test tool would be useful. A study was set up to evaluate ATP bioluminescence as tool for assessing the cleanliness of surfaces in poultry production sites.ATP bioluminescence technology is extensively used in the food industry as a rapid, reliable and simple tool to monitor and maintain cleanliness of surfaces. Adenosine triphosphate (ATP) is the basic energy molecule found in all living cells that allow cellular metabolism to take place. Therefore, the detection of ATP on a surface can be used as an indicator for the presence of organic material. To evaluate whether ATP bioluminescence technology can be adopted by poultry producers as tool to assess cleanliness in hatcheries and on poultry farms, field trials were set up using the Ensure® Touch luminometer and VetAssure™ ATP Surface tests. Multiple surfaces (such as floors, walls, setters, hatchers, …) were tested including hands of personnel and visitors before and after washing during the trials. The amount of ATP measured by the luminometer and is expressed in RLUs (Relative Light Units). The higher the RLU number the more ATP present and the dirtier the surface tested. In one of the hatchery trials, hatchers were tested pre- and post-cleaning. Mean RLU decreased from 4.687 RLU pre-cleaning to 74 RLU post-cleaning (p<0,0001) showing clearly how ATP can be used to assess the cleanliness of surfaces. In another trial, employees were screened after handwashing

prior to entering the premises. Based on the results, employees were identified who required additional training on handwashing procedures. Once trained, significant improvement was achieved.A well designed and executed biosecurity program is one of the most effective and cost-efficient methods to prevent the introduction and spread of infectious diseases in poultry operations. Many poultry production sites, do have a well-designed program in place related to hygiene and sanitation. But due to the fact that no on-site, real-time verification test is in place to check if implemented sanitation and hygiene procedures are well executed, failure can happen with no recourse for correction. Results from the pilot trials showed that ATP bioluminescence increases awareness and compliance with biosecurity measures and it is a proven tool to verify, monitor and improve hygiene and sanitation procedures and interventions real time for a variety of surfaces including personnel/visitor screening before entering the premises. Because ATP testing can be done on-site and provide results within 10 seconds, it allows users to take corrective actions in case results are unsatisfactory.

Ct Values: Facts, Fallacies, and Best Practices for Use in Veterinary Research and Diagnostics

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Ceva Animal Health

Over the past quarter of a century, qPCR and RT-qPCR have become the gold standard methodologies for the detection of specific nucleic acid molecules in biological samples. The often reported, but sometimes misunderstood, Ct value plays a significant role in the interpretation of qPCR and RT-qPCR results. However, Ct values for different samples are commonly compared incorrectly and worse yet, inappropriately averaged when drawing conclusions from experimental and diagnostic results. In this work, we seek to take a practical approach to educate researchers and clinicians about the appropriate use, interpretation, and limitations of Ct values by using multiple real-world examples from avian health research studies and diagnostics.

Infectious Laryngotracheitis Virus (ILTV) Genotyping Assay by Multiplex PCR and High Throughput MinION Sequencing

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A three allele PCR- Sanger sequencing assay is currently used to genotype Infectious laryngotracheitis virus (ILTV) from clinical samples. This assay amplifies target regions of three ILTV genes (gB, gM and ORFA/B). Sanger sequencing of these alleles provides non-continuous coverage for approximately 4.0 (Kb) of the ILTV genome (150 to 155 Kb). This sequencing method allows for genotyping circulating ILTV in the United States into four main categories- vaccines and vaccine-like viruses (genotype II and IV), virulent vaccine revertants (genotypes III and V), virulent no-vaccine related virus (VI), and archived (80's & 90's) wild type viruses from backyard flocks (genotypes VII to IX). Though useful, the ability of the assay to differentiate between closely related genotype groups is built upon a limited number of informative single nucleotide polymorphism (SNP) sites (n = 41) which curtails our ability to identify the emergence of new variant viruses or novel recombinant strains. This study aims to develop a novel ILTV genotyping assay by expanding the viral genome region analyzed, consequently including more SNP

sites, and lastly establishing a protocol using Oxford Nanopore Technology's (ONT) MinION device to increase sequence coverage of amplified products. First, bioinformatic tools were used to screen for a continuous 15 to 20 Kbp within the ILTV genome containing SNP sites that maintain the discrimination among the genotype groups described above. One of these regions, is a 13.7Kbp corresponding to the ILTV genome Unique short (Us), which contains 173 SNP sites that are informative to discriminate at the same level than the full genome sequence of 38 North American viral strains available in GenBank. Sixteen overlapping primer pairs were designed for multiplex PCR. Each pair amplifies approximately 1Kb in order to obtain the complete sequence of the Us region. Secondly, a native ligation library protocol and pipeline analysis for MinION sequencing were developed. Initial assessment of the ILTV Us Multiplex PCR amplification and MinIon sequencing assay generated 90 to 93% of the Us region sequence length with at least 20x depth of coverage. Ongoing work is focused on optimizing primer sets for multiplex PCR to improve the coverage. The protocol will then be tested with clinical samples. This high throughput sequencing assay is much faster and provides a broader and deeper coverage of the ILTV genome that is unobtainable by Sanger sequencing.

<u>Dwight Schwartz Travel Scholarship Winner</u>

Infectious Laryngotracheitis: A New Assay for Characterization from Clinical Samples with Less Genetic Material

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Infectious laryngotracheitis (ILT) is a respiratory virus that affects chickens leading to significant economic losses. ILT standard diagnostic approach is through the use of quantitative real-time PCR (qPCR) and histopathology. However, characterization of positive samples is challenging. Current sequence-typing assay targets five large segments with a total size of 11,000 base pairs (bp) to classify ILT in United States into one of nine different genotypes. However, amplification of these large segments requires samples with a high copy numbers1, which is not usually the case in clinical samples. The objective of this study is to optimize the existing sequence-typing assay for clinical samples by targeting the minimum number and size of segments necessary for ILT characterization. In-Silico analysis of the 11,000 bp resulted in the identification of 3,000 bp that could capture the genotyping of ILT. The 3,000 bp were divided into five segments then primers were designed to amplify the identified segments. Samples were collected from isolates (n=7) and from clinical samples submitted to the ISU veterinary diagnostic laboratory (ISU-VDL) (n=11). Collected samples were tested for ILT using qPCR and tenfold serial dilutions of samples were performed. To determine the analytical sensitivity of the assay, amplification of the five segments was attempted on the last three qPCR positive dilutions from each sample (n=54). Results from this study showed that the new sequencing-typing assay was able to make an accurate characterization of ILT from samples with Ct value up to 30.5. This study will

allow for further characterization of ILT from clinical samples that typically do not have a high load of genetic material. This in turn will help to generate epidemiological data to monitor circulating strains of ILT.

Optimizing Protocols for Monitoring a Novel Marek's Disease Vaccine (CVI-LTR)

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Monitoring of Marek's disease vaccination is routinely done by evaluating load of vaccine DNA in feather pulp samples. If vaccination has been done correctly, vaccine will be detected in acceptable levels in an expected percentage of chickens (variable depending on the vaccine and protocol) at 7-10 days. A novel vaccine based on insertion of the LTR promotor of REV in the CVI988 strain has become commercially available (CVI-LTR). However, it has been difficult to detect this vaccine in the feather pulp at 7-10 days, even in chickens vaccinated properly. The objective of this study was to determine which tissues and time points were optimal to monitor vaccination with CVI-LTR. Also, we want to evaluate how CVI-LTR replicated in lymphoid organs and feather pulp when compared with two commercial CVI988 strains. Vaccine load was evaluated by qPCR in samples of bursa, thymus, and spleens collected at 3, 4, and 5 days of age and of feather pulp collected at 7 and 21 days. Our results demonstrated that CVI-LTR replicated strongly in the lymphoid organs at 3-5 days of age but could not be readily detected in the feather pulp. Furthermore, replication of CVI-LTR differ from the two CVI988 included in this study. CVI-LTR replicated in thymus and spleen earlier than CVI988 (as early as 3 days of age) but it could not be found in the feather pulp at later times. Further studies to understand CVI-LTR biology are mechanisms of protection are warranted.

Comparing Aviadenovirus Group Specific and Serotype Specific ELISAs to Virus Neutralization

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Over the past five years, an increase in cases of Inclusion Body Hepatitis (IBH) has been observed worldwide. The causative agents of IBH belong to the Aviadenovirus genus within the Adenoviridae family of viruses. Multiple species of Aviadenoviruses cause IBH, including Fowl Adenovirus (FAV) group D serotype 11 (D11), FAV group E serotype 8a (E8a) and FAV group E serotype 8b (E8b). No commercially licensed live or inactivated FAV vaccines are available in the US and Canada. Therefore, broiler companies elect to vaccinate source flocks with autogenous vaccines as a primary control and prevention measure for IBH in broilers. A majority of companies vaccinating for IBH include E8b and/or D11 isolates in their autogenous vaccines. Serologic evaluation of vaccine-induced antibodies is problematic as the only commercial ELISA kit licensed for use in the United States detects group specific antibodies. Since adenoviruses are ubiquitous in the environment, antibodies to any and all adenoviruses are detected by the group specific ELISA and may or may not represent the serotypes included in autogenous vaccines. Virus neutralization (VN) assays are considered the gold standard for measuring serotype specific antibodies; however, this is time consuming,

expensive and not adapted for high through-put testing. Several non-licensed ELISA kits have been produced for detection of E8a and E8b or D11antibodies. This study was designed to evaluate FAV antibody response using both commercial (FAV group I, BioChek) and non-commercial FAV ELISAs (TropBio IBH Adenovirus 8 & AsurDx FADV 8ab and D11), as well as serotype specific virus neutralizations in flocks vaccinated with FAV autogenous vaccines. Multiple broiler breeder flocks, vaccinated with an autogenous FAV 8b (with or without a D11), will be followed at pre-vaccination, 20 weeks, 30 weeks, and 50 weeks of age. In addition, serum from broiler chicks, hatched from eggs collected from hen flocks at 30 and 50 weeks of age, will be tested for FAV antibodies by ELISAs and VN. Serological tools for monitoring the immune response following vaccination are important for assessment of vaccination protocols, vaccines and efficacy. The use of a serotype specific ELISA to detect antibody response will provide companies with a high-throughput tool for companies to monitor their flocks.

Nanopore Sequencing: Promises and Perils For Fast Identification and Accurate Characterization of IBV Directly From Clinical Samples

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Infectious Bronchitis virus (IBV), a single-stranded, positive sense RNA virus, is responsible for various disease conditions in chickens leading to significant economic losses. Serotype-specific live-modified vaccines are a very effective and commonly used tool to control this disease. Therefore, the accurate identification and characterization of IBV are critical for properly selecting vaccines and vaccine combinations. However, IBV has a high mutation rate and a large amount of genetic diversity. This leads to frequent failure to characterize IBV and the need to update the sequencing primers continuously. Oxford Nanopore Technology (ONT), the most recent long-read sequencing platform, has been proven to improve the diagnostic process of multiple human, animal, and avian pathogens. The objective of this study is to adopt ONT in IBV diagnostics and compare different approaches to improve our ability to identify and characterize IBV directly from clinical samples. Improved characterization includes the determination of the IBV genotype (S1 characterization) and also improves the IBV whole genome recovery from clinical samples. Three methods were compared: metagenomics, sequence-independent single-primer-amplification and IBV multiplex PCR amplicon sequencing. Results from different trials emphasize ONT's promises to improve IBV diagnostics; however, much work is still required to overcome limitations associated with each workflow.

Use Infectious Bronchitis Vaccines Takes at 1- and 5-Days Post Vaccination to Evaluate Vaccine Delivery and Vaccine Interactions

Robert Beckstead, James White, Sean Brimer, John Elattrache

Ceva Animal Health

Infectious bronchitis (IB) is a highly contagious gammacoronavirus that causes clinical outcomes in chickens varying from suboptimal performances to acute respiratory disease. Live-attenuated vaccines are widely used to control circulating IB field viruses. Hatchery application

of relevant vaccine strain(s) using an appropriate spray equipment is of paramount importance to reach this goal. To check proper vaccine delivery, IBV RT-qPCR is routinely used on RNA isolated from choanal-cleft swabs taken 5-7 days post vaccination (dpv); optimally, the generic 5'UTR protocol is complemented with a vaccine strain-specific detection protocol. The percentage of birds that are positive for the IBV vaccines is used to infer the quality and consistency of vaccine delivery. To compare 1 and 5 dpv sampling for vaccine takes, 100 chicks were placed at a stocking density of 0.35 ft2/bird with 50 birds vaccinated intraocularly with either vGA08 or Mass vaccines and 50 birds placed as contact controls. At 1 dpv only vaccinated birds were positive for IBV RNA at high viral load (Ct<23), while at 5 dpv 100% of the vGA08 and 94% of the Mass contact birds were positive (Ct<36). This data suggests that 1 dpv sampling provide an accurate assessment of vaccine application. To assess the spread of vGA08 strain when applied in concert with a Mass vaccine, the above trial was repeated with either 30% or 50% of the chicks being vaccinated for both vGA08 at 10e3.9 and Mass at either 10e3.4 or 10e4.7-EID50/dose. All vaccinated birds were positive for both vGA08 and Mass at 1 and 5 dpv (Ct<35). At 5 dpv, when 30% of the chicks were vaccinated, 100% and 80% of the contact birds were positive for vGA08 and 67% and 96% positive for Mass when the Mass vaccine was used at 10e3.4 and 10e4.7 respectively. Similarly, when 50% of the chicks were vaccinated, 98% and 88% of the contact birds were positive for vGA08 and 80% and 98% positive for Mass. For vGA08 and Mass vaccinated bird, the higher titer vaccine inhibited the spread of the paired IBV vaccine. However, if vaccine application was at 50%, which is a very poor application, both vaccines regardless of titer had vaccine takes of 80% or greater at 5 dpv. This data highlights the importance of proper application of vaccines to provide early vaccination of all birds in the flock and suggests reviewing vaccine take check's protocol (especially, its timing) in case of troubleshooting.

Case Reports

Detection of Avian Encephalomyelitis Virus in Turkey Poults

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Avian Encephalomyelitis virus (AE) is a member of the pircornaviridae family and typically affects one to two-week-old, immunologically naive chickens, turkeys, pheasants, and quail. Avian Encephalomyelitis infects parent flocks through the fecal-oral route and is vertically transmitted to the chicks/poults. The first known case of a naturally infected AE turkey flock was in 1970 and there have been very few clinical cases of AE in commercial turkeys described in the literature since. In October 2022 over a seven-day period, two different brooder farms, on different premises, with poults ranging in age from ten days to fifteen days, developed similar clinical signs. Clinical signs within the flocks included ataxia, dehydration, anorexia, and head shaking/tremors. Four different accessions were submitted to the Purdue University Animal Disease Diagnostic Laboratory (ADDL) representing the two flocks. Necropsy results of the two flocks provided similar findings of mild enteric lesions, with one flock showing signs of mild cerebellar congestion. Histopathologic examination was performed in all four accessions, focusing especially on the brain and spinal cord. The cerebrum and cerebellum, in both flocks, revealed lymphocytic perivascular

cuffing, one of the two flocks' brain stem contained central chromatolysis of the neurons with histiocytic perivascular cuffing, and the spinal cord, in both flocks, showed lymphocytic perivascular cuffing with neuronal degeneration, gliosis, and central chromatolysis. The lesions seen within the spinal cord strongly indicated an AE infection. Samples of frozen brain and formalin-fixed paraffin-embedded (FFPE) brains/spinal cords were sent to the University of Georgia Poultry Diagnostic Research Center (PDRC) for AE PCR for each accession. Results from the PDRC were negative for AE PCR on both FFPE brains/spinal cords and frozen brains in all accessions. Based on the strong histopathologic evidence indicating AE, a literature search was performed regarding the PCR primers used. The PDRC was using a published set of primers (Xie, et al., 2005), that while validated for U.S.-based strains, may not detect all strains, as determined by recently published work (Goto 2019). The FFPE brains/spinal cords and frozen brains were then re-tested using the Goto 2019 primers and were positive in two of the four accessions which encompassed both brooder farms. Follow-up with the primary breeder company revealed that the breeder hens that supplied eggs to the affected brooder sites had strong seroconversion to AE at 46 weeks of age. This case highlights that histopathology still has a major role in the diagnostician's tool chest, and that incongruous results should be questioned to ensure an accurate diagnosis. This case also highlights the necessity of accurate and sound vaccination of turkey breeder hens and the need for strict biosecurity.

The Impact of a Virulent Strain of Reovirus in a Heavy

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During the summer of 2022 it was discovered that a turkey production company had received poults from a breeder flock infected with reovirus despite being vaccinated. Based on serum antibody titer levels it was determined that the breeder flock was exposed to reovirus at 16 weeks of lay and continued to shed the virus until 32 weeks of lay. The production company received infected poults via vertical transmission for approximately 4 months. The strain of reovirus proved to be highly virulent, resulting in various clinical signs and pathologic lesions. Multiple flocks experienced elevated mortality from the starve out period through 2 weeks of age. Varying prevalence of torticollis was observed between 2 to 3 weeks of age and lameness and incoordination from 10 weeks to market age. Gross examination of poults revealed hepatitis within 2 weeks of age. Tendonitis and aortic ruptures were observed from 10 weeks until market age. Diagnosis of Reovirus was confirmed using virus isolation of livers, digital flexor tendons, and brains. Horizontal transmission was confirmed between brooder barns within the hub brooding system. Within the first 4 weeks of the initial diagnosis 73% of conventional turkeys placed within the company's system were either directly or indirectly impacted by the reovirus associated with the breeder flock. Due to tendonitis flocks experienced increased difficultly in ambulation which resulted in significant reductions in feed consumption and weight gain. Production records from flocks placed during the first 6 weeks of reovirus infection revealed significant differences in mortality, weight, feed conversion, and average daily gain compared to previous two-year averages for the respective farms. Flocks that received poults from the affected breeder flock weighed 14% less, feed conversion was 6.79% higher, condemnation was 0.87% higher, daily rate of gain was 13.5% lower, and mortality was 5% higher. Flocks that

were placed in brooder barns on the same premise as infected barns were suspected of horizontal transmission. These flocks were negatively impacted but not as drastically. Flocks weighed 4.65% less, feed conversion was 0.16% higher, condemnation was 0.05% higher, daily rate of gain was 4.07% lower, and mortality was 0.5% lower. The breeder flock was removed from production and the strain of reovirus was gene sequenced and incorporated into to the vaccine for use in subsequent breeder flocks.

A Case of Rickets in Commercial Poults

Katie Stumvoll

Jennie-O Turkey Store

A sudden onset of leg weakness and increase in daily mortality were detected at 4 days of age in a flock of 31,000 commercial poults. Affected poults attempted to walk with help of their wings and were unwilling to move more than a few steps at a time. Necropsy findings were normal at 6 days of age, except femurs would snap and bleed instead of dislocating femoral heads. Growth plates were not affected at this time. However, when growth plates were examined 7 days later, zone of hypertrophy was visibly enlarged indicating a phosphorus deficiency. Feed analysis revealed that both phosphorus and calcium were lower than expected. Deficiency developed because of lower levels of both calcium and phosphorus in the bone meal, which was used as the main source of calcium and phosphorus. The flock was treated supplemented with dicalcium phosphate and vitamin D which corrected the mortality problem. Immediately, starter was reformulated which resulted in only two similar cases with far less mortality. Examination of growth plates continued at weekly intervals in an attempt to determine how long it would take for bones to repair and calcify.

Egg Drop Syndrome Epidemic in Northeast Indiana 2021-2022: A Review of Disease Presentations and Containment/Control Strategies

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From September 2021, to November 2022, 53 cases of EDS-76 (Egg Drop Syndrome) were reported in the Northeast Indiana region. This epidemic affected over 1M birds and 46 unique premises. Over 500,000 birds from 20 premises were depopulated in an effort to control and mitigate the spread of the disease. Egg production drops of 10 to over 80% were documented in association with the disease. Increases of up to five-fold in shell-less, off-color, and mis-shaped eggs were reported and observed. Almost all disease outbreaks were confirmed through PCR identification. Thus far, the EDS-76 virus has not been isolated in association with any of the disease cases. From the initial index case, a number of disease control and eradication measures have been employed. This case report will document and discuss the geographic spread of the EDS epidemic. Disease symptoms and characteristics will be presented as well as an overview of several control and preventative measures employed to mitigate and eradicate the disease. Further discussion will be presented regarding the identification of risk factors associated with the spread of the disease. The initial utilization of biosecurity enhancement programs and the later implementation of vaccination of flocks

after March 2022 will be discussed. Further modifications in approval stipulations for the use of commercial EDS vaccine were granted by the state of Indiana in June, 2022 as the disease continued to plague the region. The apparent positive effect on EDS-76 control in the region utilizing the combination of enhanced biosecurity measures, industry cooperation and communication, and a comprehensive vaccination program will be presented.

Egg Drop Syndrome in Broiler Breeders in the United States

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Egg Drop Syndrome - 1976 (EDS-76) is a disease of commercial laying poultry characterized bya drastic decrease in egg production, shell abnormalities, and shell-less eggs. The causativeagent is Duck Adenovirus 1 (DAV-1). Egg Drop Syndrome is not reportable in the United States.This is the first case of EDS-76 known to the authors in broiler breeders in the US. A 35-week-old broiler breeder (708 x YP) flock in the United States was diagnosed with EDS-76 by PCR and HI. At the time of this case, there were a significant number of EDS cases in table egg layers in the general area. The broiler breeder flock experienced a significant egg production drop over a seven-day period, prompting the investigation. Upon farm investigation of the production drop, a significant number of shell-less and wrinkled eggs were noted. No increases in mortality nor respiratory signs were observed. The affected breeder farm consisted of 4 houses of approximately 10,000 hens a piece. Clinical signs of egg production drop and shell quality issues were noted first in house four at 34 weeks and the other houses followed at 36 (house 3), 39 (house 2), and 39 (house 1) weeks. Approximately one week after the first clinical signs, egg production reached its lowest point at 55% hen housed (HH) egg production compared to a target of 80%. Four weeks after initial clinical signs, production returned to 74% HH, approximately the breed standard egg production for the age. Hatching egg utilization was reduced for another 2 weeks after returning to standard egg production due to the number of thin shelled eggs. The other houses followed a similar pattern of egg production drop and return. Tracheas were submitted for detection of IBV and NDV; cecal tonsils and oviducts were submitted for IBV to rule out differential diagnosis that could cause shell abnormalities and egg production drops. Shell-less eggs were submitted for detection of EDS-76 by PCR. EDS-76 was detected by PCR at two separate laboratories. After PCR detection, serum was submitted for HI and was positive in the house with clinical signs but not the other houses. All houses seroconverted at approximately the time they returned to breed standard production. There were no other farms in the company's system that observed similar production drops or clinical signs.

Managing an Outbreak of Highly Pathogenic Avian Influenza in a Layer Breeder Flock

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Highly Pathogenic Avian Influenza (HPAI) has been devastating to the US poultry industry throughout 2022. All segments of commercial industry and non-commercial backyard flocks have been affected directly or indirectly. Commercial operations and NPIP participants have biosecurity policies to prevent disease

introduction into their premises. Primary breeding companies are at the top of the genetic supply chain, providing commercial day old chicks to producers. They maintain the highest level of biosecurity to protect their pedigree lines, grandparent, and breeding stock. Breeder flocks are not impervious to contracting HPAI, even with heightened biosecurity infrastructure, culture, policies, and procedures. This case study shares the experience of suspecting, confirming, quarantining, eliminating, and restocking a biosecure layer breeder facility challenged with HPAI. Planning, preparation, communication, and relationships were key to managing this stressful HPAI event.

This Better Not Be HPAI…Acute Elevated Mortality in Layer Pullets

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Ten 17-week-old brown layer pullets presented to the diagnostic lab in spring of 2022 with history of acute, elevated mortality (5, 13, 26, 77 dead over the last 4 days). Clinical signs of weakness/lethargy were reported from the flock owner. Pullets arrived live yet moribund upon presentation, huddling together with eyes closed and ruffled feathers. All ten birds are emaciated (BCS 0 based on scoring system by NG Gregory 1998). Gross necropsy findings include no feed in crop or gizzard in 9/10 birds and cecal cores in 2/10 birds. Differential diagnosis included enteric coccidiosis, salmonellosis, and histomoniasis. Oropharyngeal swabs were collected immediately for qPCR and tested for avian influenza, which was negative. Fresh samples were held off initially pending qPCR results for HPAI. Meanwhile, histopathologic sections revealed diffuse transmural necrosis of the mucosa with large aggregates of bacteria admixed with abundant, weakly eosinophilic protozoal trophozoites (histomonads) with scattered nematodes characterized by prominent lateral alae (Heterakis spp). Diagnosis is consistent with histomoniasis, although management issues contributed to emaciation and subsequent mortality.

A Case of Cystic Oviducts and Egg Quality Issues in a Commercial Layer Flock

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In February 2022, the California Animal Health and Safety (CAHFS) laboratory, Turlock branch received four case submissions from a 25 to 28-wk-old commercial brown layer flock for diagnostic evaluation due to reduced egg production and pecking issues. Submissions consisted of live birds, recent mortality and eggs. The most common gross findings included cystic left oviducts, bruising and signs of vent pecking. The eggs submitted were of abnormal shape with irregular deposits of calcium on the shell. Microscopically, there was glandular hypoplasia and atrophy of the oviducts and lymphocytic salpingitis. Infectious bronchitis virus was isolated and identified by reverse transcription quantitative PCR from cecal tonsils tissue pools and tracheal swab pools. Sequencing of the S1 hypervariable region was most similar to a local California variant, CA1737. Definitive proof of CA1737 strain causing cystic oviduct and oviduct abnormalities require a challenge study with fulfillment of Koch postulate.

AAAP History Lecture

Centenary of the First Infectious laryngotracheitis (ILT) Report in the United States and Ninety Years of Vaccination Control

Maricarmen Garcia

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Salmonella

Pre-Harvest Salmonella Monitoring to Develop Risk Profiles for Directed Processing

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Nearly one in five salmonellosis cases are attributed to broilers, with renewed efforts to improve Salmonella control during broiler production and processing. There is a need for improved efforts that target serovars which may survive antimicrobial interventions and cause illness, as well as to focus on lessening the amount of contamination entering the processing plant. Advances in molecular enumeration approaches allow for the rapid detection and quantification of Salmonella in pre- and postharvest samples, which can be combined with deep serotyping to properly assess the risk affiliated with a poultry flock. In this study, we collected a total of 160 bootsock samples from 20 broiler farms across four different broiler complexes with different antibiotic management programs. Overall, Salmonella was found in 85% (68/80) of the houses, with each farm having at least one Salmonella-positive house. The average Salmonella quantity across all four complexes was 3.6 log10 CFU/sample. Eleven different serovars were identified through deep serotyping, including all three USDA-FSIS Key Performance Indicators (KPIs; serovars Enteritidis, Infantis, Typhimurium). There were eight multidrug resistant isolates identified in this study, and seven which were serovar Infantis. We generated risk scores for each flock based on the presence or absence of KPIs, the relative abundance of each serovar as calculated with CRISPR-SeroSeq, and the quantity of Salmonella detected. The work presented here provides a framework to develop directed processing approaches, and highlights the limitations of conventional Salmonella sampling and culturing methods.

Effect of the use of a natural microbiocide for the control of Salmonella Infantis loads in the gut, and to improve performance in broiler chickens

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Alquermold is a natural antimicrobial (botanical origin), composed of Active Molecules against *Salmonella infantis* (AMSI) specifically developed

to control this strain of *Salmonella,* a zoonotic microorganism with negative impact in public health. A trial was conducted in broiler to evaluate the efficacy of the AMSI in the reduction *S. infantis* load and to improve performance, compared to formaldehyde, a common compound used for the same purpose. 224 male broilers were raised up to day 35 and distributed into 4 groups: "NC" non-challenged birds, without antimicrobials, "PC" challenged birds, without antimicrobials, "AMSI" challenged birds, with the plant-based antimicrobial; "FO" challenged birds, with a product containing 33% formaldehyde. Performance, mortality, homogeneity, and *S. infantis* presence in gut, were evaluated. Significance was considered if $P<0.05$. Challenge consisted of the administration of $2x10^8$ CFU of *S. infantis*/bird directly to the crop, in groups PC, AMSI and FO, on day 21 of age. *S. Infantis* was isolated and quantified in 6 birds/treatment through swabs from each intestinal portion. No presence of *S. infantis* was observed in AMSI and NC, in any intestinal portion. PC and FO showed high counts of *S. infantis*, specially in ileum, with counts of $3.3x10^6$ and $5.1x10^4$ respectively. These results are related to better gut health and food safety. Average weight was significantly better in AMSI compared to PC and FO, and numerically better compared to NC. FO obtained no significantly better results than PC. Feed conversion was numerically better in AMSI compared to all the groups during all the trial, and significantly better at weeks 14 and 18. Feed intake, was lower in almost all weeks in AMSI not affecting the performance. Homogeneity was significantly higher in AMSI during most weeks, compared to all groups. AMSI obtained the lowest mortality rates. In conclusion, the AMSI had the greatest positive effect on the control of *S. infantis*, as well as on the performance, mortality, and homogeneity. Therefore, the AMSI are a natural tool to improve productive performance and decrease the risk of contamination with *S. infantis*, with a positive impact for food safety and public health.

Salmonella Serotypes and Their Antimicrobial Resistance Profiles Characterizing Chicken Samples from USDA FSIS Regulated Poultry Establishments Based on Their Category Status

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The U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) publishes establishment microbiological data from their verification activities at chicken processing plants in the U.S. as part of the Agency's efforts to prevent pathogens from entering the food supply. This data includes sample origin, Salmonella isolation results, serotyping and genotyping results. Posting of data intends to help industry identify repetitive subtypes and implement control measures. In addition, FSIS posts the category status of individual establishments for Salmonella performance standards, and the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) reports antimicrobial resistance data for Salmonella isolates recovered from ceca samples collected at poultry processing plants within their antimicrobial resistance surveillance efforts in the U.S. The Salmonella whole genome sequencing data used by the FSIS and NARMS reports is deposited at the National Center for Biotechnology

Information (NCBI) website. This work focusses on determining associations between the reported FSIS categorization status for chicken processing plants in the U.S. and the presence of specific Salmonella serovars or subtypes and their antibiotic resistance profiles in poultry products and chicken cecal samples, using the Agencies' published information and whole genome sequencing data. This assessment will give an insight on the relationship between Salmonella carried in chickens from prior processing (ceca) and in poultry products. This is the first step towards the design of a prediction tool using Montecarlo simulation for quantitative risk analysis. The goal is to identify Salmonella monitoring targets that can be useful for the prediction of FSIS categorization status and therefore help guiding or adjusting Salmonella control interventions in the poultry industry.

In silico and PCR Screening for Live Attenuated Salmonella Typhimurium Vaccine Isolates

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The administration of live attenuated Salmonella Typhimurium vaccines in live production has significantly improved Salmonella control in the poultry industry. Since USDA-FSIS scores all isolated Salmonella as positive, regardless of serovar, attenuated vaccine strains that are identified at processing contribute negatively toward Salmonella performance standards. This study was designed to determine the incidence of a live attenuated Salmonella Typhimurium vaccine identified in broiler products by FSIS and to develop a PCR assay for screening of isolates. Salmonella Typhimurium short-read sequences from chicken samples uploaded to NCBI Pathogen Detection by FSIS from 2016-2022 were downloaded and assembled. These were analyzed using BLAST with a sequence unique to field strains, followed by a sequence unique to the vaccine strain. PCR assays were developed against field and vaccine strains and validated by screening isolates in our collection. Between 2016-2022, 1,682 Salmonella Typhimurium isolates were found on NCBI Pathogen Detection, corresponding to 7.9% of all Salmonella identified. Of all Typhimurium isolates, a total of 104 (6.1%) were identified as the vaccine strain. The PCR assay differentiated field strains from the vaccine strain when applied to isolates as well as to overnight mixed Salmonella enrichments. Live attenuated Salmonella vaccines are widely used in the industry as a critical preharvest tool for Salmonella control. With forthcoming regulations that will likely focus on Salmonella Typhimurium, along with other serovars, there is a need to distinguish between isolates belonging to the vaccine strain and those that are responsible for causing human illness.

Evaluation of a Live Salmonella Typhimurium Vaccine in a S. Infantis Challenge Model in Broilers

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Preharvest interventions for Salmonella have become increasingly important to reduce colonization of specific serotypes invasive to chickens and significant to human health. Vaccines can be effective tools to help achieve USDA objectives to lower prevalence of invasive serotypes. This study had 1,000 broilers in 40 floor pens with 20 pens per treatment. The treatments were 1) non-vaccinated, and 2)

Megan™ Vac1, a live, attenuated S. Typhimurium (S.T.) vaccine, administered on days 1 and 14. The challenge was 107 CFU/bird S. Infantis (S.I.) by oral gavage to 15 birds/pen (seeders) at 3 weeks (D21). Samples were collected at 4 weeks (D28) and 5 weeks (D36/37), including the ceca, liver/spleen and hot carcass rinse of both gavaged, direct challenged birds as well as horizontally exposed birds. Salmonella cultures were performed with tetrathionate, followed by XLT-4 microbiological plating, then most probable number (MPN) evaluation for ceca and carcasses. In pooled liver/spleen, vaccination reduced S.I. colonization significantly in direct challenged birds at 4 and 5 weeks, and horizontally challenged birds at 4 weeks. In the ceca, vaccine reduced S.I. in horizontally challenged birds at 4 and 5 weeks, and direct challenged birds at 5 weeks. Carcass S.I. prevalence was reduced significantly at 4 weeks in horizontally challenged birds. Quantitatively, vaccination reduced ceca and carcass MPN results of S.I. in direct challenged birds at 5 weeks, and in the ceca of horizontally challenged birds at weeks 4 and 5. All other comparisons of cultures and MPN values were not significantly different. Overall, in this study a live S.T. vaccine demonstrated cross protection against S.I. colonization in broilers in multiple comparisons. This study complements other research showing reduction of multiple harmful serotypes from live, attenuated Salmonella vaccines.

The Impact of Salmonella Typhimurium and Coccidiosis Vaccine on the Intestinal Integrity in Broiler Chickens in the Late Stage of Production

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Salmonellosis and coccidiosis are common intestinal diseases in the poultry industry. Antibiotic-free and organic production have increased the use of vaccines against both pathogens to minimize the zoonotic risk of salmonellosis for consumers and to reduce economic losses by coccidiosis. Previous research indicated that in broilers a systemic infection with Salmonella Typhimurium (ST) was more frequently found after vaccination against coccidiosis. In addition, the two vaccines showed interactions on their effect on the number of differentially expressed genes at 28 days of age. The aim of this study was to investigate the interaction of both vaccinations on performance and microbiome of broiler chickens after a Salmonella challenge on day 28. We used a 2 x 3 experimental design with different vaccination schedules on day 0 and 14 to further investigate the interactions between both vaccines. The six groups consisted of six floor pens each with 45 broiler chickens per pen. Stocking density was reduced to 30 birds per pen on day 14.On day 0 the chicks were tested to confirm that they were negative for vertically transmitted Salmonella. Environmental samples were taken every week to detect a potential spread of ST. Performance data was collected every other week. On day 28 all groups were challenged with a ST field strain. Cecal content for the microbiome analysis and organ samples for re-isolation of ST were taken on day 28, 35 and 42. A microbiome analysis was performed by Illumina sequencing of 16S rRNA. Results will be presented and discussed.

An Evaluation of Two Commercially Available Live Salmonella Vaccines in Turkey Toms

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A field trial was conducted to evaluate two commercially available live Salmonella vaccines in tom turkeys. Birds were placed onto single farms as split flocks, with houses designated as either vaccine A or vaccine B, and followed from day of placement through to ground sampling at processing. In addition to evaluating Salmonella prevalence and serotype, enumeration was evaluated across all sampling points as well. Across all flocks, Salmonella prevalence levels were similar but there was a significant difference in enumeration results. This trial demonstrates the importance of evaluating enumeration of Salmonella, in addition to prevalence and serotype, when conducting preharvest food safety trials.

Comparison of Commercial SE Whole Cell Bacterin and SRP Salmonella Vaccines in Chickens: Evaluation of Protection to Homologous and Multiple Heterologous Challenge Strains

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Salmonella control is one of the top priorities for poultry producers due to the potential of the bacteria to infect humans and cause severe disease. Salmonella in poultry is typically controlled through biosecurity and vaccination. Serotypes of Salmonella have been identified based on somatic O-antigens, and generally these antigens do not confer cross protective immunity when used in inactivated whole cell bacterin vaccines. Siderophore receptors and Porins (SRPs) are highly conserved pore proteins on the surface of gram-negative bacteria, including Salmonella, that transport essential nutrients and iron for bacterial growth. Antibodies produced against Salmonella SRP proteins have been shown to be cross-protective and not serotype-specific. Commercially licensed vaccines utilizing whole cell bacteria or SRP proteins from Salmonella Enteritidis as antigens were compared for protection against homologous and multiple heterologous challenge in chickens. Internal organ protection and intestinal carriage was evaluated following simultaneous intraperitoneal and oral challenge. Findings from these studies will be presented.

Effects of Salmonella Enteritidis and Mild Intestinal Inflammation on Broiler Performance and Health

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This study aimed to investigate the impact of mild intestinal challenge and Salmonella Enteritidis on the performance and health of broiler chickens. A total of 270 day-of-hatch Ross × Ross male broilers were randomly allotted to 2 treatments of 5 replicate pen with 27 birds/pen, in a randomized complete block design. All birds were cocci vaccinated at hatch. On d 0, fourteen seeder chicks per pen were tagged, and orally gavaged with 8 × 107 CFU/chick nalidixic acid-resistant SE. the remaining 13 birds were horizontally exposed (HE). Furthermore, birds were placed on either new litter (Treatment; T1) or re-used litter from a prior Necrotic Enteritis (NE) study (T2). All diets were pelleted and fed ad libitum in 3 phases: starter (1-14 days),

grower (14-28 days), and finisher (28-42 days). Performance, mortality, and NE lesion score (LSC) data were analyzed by ANOVA using the general linear procedures of STATISTIX, with comparison of means using LSD (t-test) at a significant level of 0.05. Boot sock and ceca SE prevalence was compared between T1 and T2 using Fisher's exact test and generalized estimating equations (GEE) logistic regression, respectively. Ceca Salmonella abundance (Log10 MPN/g) was compared using linear models and Tobit regression models in culture-positive and culture-negative ceca. There was no significant difference in LSC between T1 and T2 at d 19. However, T2 had numerically higher mean LSC than T1. Body weight gain from d 0-15 was significantly higher in T1 in comparison to T2. At d 42, no significant difference in performance was observed between both groups. However, total mortality and mortality due to Femoral head necrosis were lower in T2 in comparison to T1. There was no statistical difference in environmental bootsocks prevalence at d 15 indicating a uniform challenge between both groups. Ceca samples collected on d 42 showed higher prevalence in directly challenged birds (75%) in comparison to the HE birds (62%). Furthermore, HE birds in T2 had lower prevalence (50%) in comparison to those in T1 (65%). There was a treatment × challenge status interaction for ceca Salmonella abundance (Log10 MPN/g) at d 42, with a lower MPN in T2 vs. T1 for the direct challenge and HE birds. Overall, this study showed that re-used litter in combination with mild intestinal inflammation negatively affect early growth performance, while lowering Salmonella prevalence and abundance, and mortality at d 42.

Novel Adjuvant Salmonella Strains to Enhance Food Safety and Animal Health

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We have recently developed novel self-destructing attenuated adjuvant Salmonella strains (SDAAS) to be safely delivered in ovo and induce an early and protective innate immune response in newly hatched chicks against pathogens of public and animal health importance. These strains express the delayed or regulated lysis phenotype based of deletion or deletion-insertion mutations in the asdA, alr and dadB genes. After in ovo inoculation, SDAAS strains can successfully colonize and invade lymphoid tissues, such as the mucosa associated lymphoid tissues (MALT), gut associated lymphoid tissues (GALT), bursa of fabricius and spleen, delivering a bolus of pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) such as DNA, RNA, ATP, peptidoglycan, LPS, which have already been extensively shown to activate the innate immune system through interaction with pattern recognition receptors (PRRs) present in different cell types. In addition, these live SDAAS display complete biological containment with no persistence in vivo and no survival if excreted and have been derived from the highly virulent S. Typhimurium UK-1 strain. Fertilized novogen brown eggs are incubated and hatched in our poultry research facility. At 18 days of incubation embryonated eggs are injected in the amniotic fluid with different doses of our SDAAS strains. Hatchability is measured and birds are subsequently challenged with 1x103 CFU of wild-type S. Typhimurium UK-1 (□3761), S. Enteritidis (□3550) or S. Heidelberg (□3749). Five birds are necropsied at 7, 14, 28 and 42 days after challenge to quantify titers of Salmonella in various tissues. Animals were also challenged in the thoracic caudal air sac with 1x103 CFU of APEC strain □7122 and mortality was recorded up to 7 days after challenge. In these studies we observed the ability of our SDAAS strains to

reduce Salmonella colonization and decrease mortality caused by APEC. This research was supported by the USDA-NIFA.

Pullet Rearing Conditions and Susceptibility to Experimental Salmonella Enteritidis Infection

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The dissemination and prevalence of the egg-associated pathogen Salmonella Enteritidis in laying flocks can be influenced by the poultry housing environment. The egg industry is shifting toward cage-free housing, but the food safety implications of this transition are unresolved. In the present study, internal organ colonization by S. Enteritidis was assessed in egg-type pullets after rearing in different housing conditions. In each of two similarly designed experiments, 16-wk-old pullets were transferred from a rearing facility to a containment facility with 4 isolation rooms simulating commercial cage-free barns with perches and nest boxes (72 birds/room). 24 pullets in each of 2 rooms were orally inoculated with S. Enteritidis immediately after placement in this facility and an identical proportion of pullets in the other 2 rooms were similarly infected at 19 wk of age. At 1-2 wk after each inoculation, samples of liver, spleen, and intestinal tract were collected from all birds for bacteriologic culturing. The 1st trial compared the consequences of rearing pullets in conventional cages to rearing in a cage-free system: S. Enteritidis was isolated significantly more often from spleens (56% v. 47%) and intestines (90% v. 83%) of cage-reared pullets than from birds reared in cage-free housing, especially among birds infected at 16 wk of age (1 day after placement in the containment facility). The 2nd trial compared the consequences of rearing pullets at 2 different bird stocking densities in cage-free housing: the frequency of S. Enteritidis recovery was not affected by stocking density during rearing, but S. Enteritidis was found significantly more often in livers (39% v. 29%) and spleens (40% v. 28%) from birds infected at 19 wk of age than from those infected at 16 wk. This study documents the importance of attentive pathogen risk reduction at a critical phase (at or just before sexual maturity) in the productive life of egg-laying flocks.

Salmonella Pullorum and Gallinarum Testing - A Review and Look Forward

Elana Huong

Hendrix Genetics

Salmonella pullorum and Salmonella gallinarum are host-specific bacteria that can have devastating consequences in poultry flocks. Flocks enrolled in the NPIP Program in the USA and the OH&FSP in Ontario, Canada must confirm Salmonella negative status through approved tests at nationally accredited facilities, such a microagglutination and tube agglutination tests at the Animal Health Lab (AHL) in Guelph, Ontario. These tests have been utilized, albeit modified, since the early 1930s. Given the known knowledge about the limitations of these tests, such as the antigen cross-reacting with other bacteria which can result in false-positives, new laboratory methods for rapid and accurate detection of S. pullorum and S. gallinarum should be employed.

Polymerase chain reaction (PCR) has shown much promise, providing high diagnostic sensitivity and specificity as well as the ability to detect multiple serotypes in minimal time. In this presentation, we will delve into the literature to re-learn the established tests for Salmonella testing, and to consider why we need an upgrade.

Overview of Salmonella Programs within NPIP

Elena Behnke

USDA NPIP

In 2022, there was a strong desire to have a more in depth discussion surrounding the Salmonella Programs provided through the National Poultry Improvement Plan. The 2022 presentation was a general update of the NPIP program and we received input that the audience would like more information in 2023 about the Pullorum Typhoid, Salmonella Monitoring, and Salmonella Enteritidis programs.

Case Reports

Neurologic Signs in Turkey Poults: A Case of Avian Encephalomyelitis

Ashley Anderson[1], Yuko Sato[1], Elizabeth Beilke[2], Mohamed El-Gazzar[3],

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Neurologic signs in turkey poults: a case of Avian EncephalomyelitisAshley Anderson1, Yuko Sato1, Elizabeth Beilke2, Mohamed El-Gazzar11Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames IA 500112West Liberty Foods, West Liberty, IA 52776Eight 14-day-old turkey poults presented to the Iowa State University diagnostic lab in August of 2022 with a history of neurologic signs affecting about 10% of the flock. Clinical signs of tremors and ataxia were reported. Poults arrived freshly dead along with 5 swabs and 1 bone sample. Gross pathology for the poults with tremors (Group A, 4 poults) include dehydration in 4 out of 4 birds, marginal bone strength in 4 out of 4 birds, and joint deformity in 1 out of 4 birds. Gross pathology for the poults with ataxia (Group B, 4 poults) include: mild dehydration in 2 out of 4 birds, and mild tracheitis in 2 out of 4 birds. Differentials diagnosis for both groups included viral encephalomyelitis and bacterial meningoencephalitis. Histopathologic findings from both groups revealed gliosis and focal lymphocytic infiltration and perivascular cuffing in the cerebrum. One brain from Group A also revealed there are 2 sections where there is edema in the meninges and moderate to severe heterophilic infiltration with visible bacteria in the meninges. Brain from Group B had a few degenerating neurons with central chromatolysis in 1 out of 4 sections. Bacterial culture findings revealed, there were high amounts of E. coli in one brain swab and moderate amounts of Staphylococcus cohnii, Staphylococcus aureus, and Enterococcus faecalis in the other 3 brain swabs. Bone ash testing revealed no significant findings. There were no significant findings in the spinal cord, peripheral nerves, and tibiotarsus growth plate in Group A, nor in spinal cords, tracheas, eye, nasal cavity, peripheral nerves, and tibiotarsus growth plate in Group B. Based on history, clinical signs, bacteriology and histopathologic findings in group B, diagnosis is consistent with Avian Encephalomyelitis viral infection. Avian Encephalomyelitis has been previously reported in turkeys; however, it still is considered uncommon. This presentation is to bring the

attention of diagnosticians and clinicians to AE as a potential differential in neurological cases of young turkeys. Keywords: Avian Encephalomyelitis, turkey poults.

Highly Pathogenic Avian Influenza: The Mississippi Experience

Natalie Armour Manginsay[1], Martha Pulido-Landinez[1], Danny Magee[1], Heidi Rose[2]

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Since 2021, an unprecedented outbreak of highly pathogenic avian influenza (HPAI) has threatened the poultry industry globally. Mississippi is one of 47 U.S. states which have reported HPAI in commercial or backyard poultry since February of 2022. HPAI has been detected in two commercial poultry flocks and in three backyard flocks (1 poultry and 2 WOAH non-poultry) in Mississippi since November 2022. The commercial poultry cases involved a 52-week-old broiler breeder flock on a 4-house farm in Lawrence county in November 2022, followed by 28-day-old broiler flock on a 6-house farm in Leake county in February 2023. Weakness, trembling, sudden death, and increased mortality were reported in the breeder flock, while rapidly increasing mortality was reported in the broiler flock. In both cases, only one house on the farm was reported to be affected. Gross and histologic lesions included dark discoloration of the legs and feet, heterophilic and lymphocytic tracheitis, and pulmonary congestion and hemorrhage with lymphocytic interstitial pneumonia. Multifocal necrotizing splenitis was marked in both cases, and several birds in each case had pinpoint hepatic necrosis. Cyanosis of combs and wattles and petechial to ecchymotic visceral hemorrhage was prominent in the breeders, and several broilers had marked periorbital edema with rhinitis. Influenza A matrix, H5 and Eurasian H5 clade 2.3.4.4.b specific rRT-PCR tests performed on pooled tracheal/oropharyngeal swabs collected in BHI broth yielded non-negative results, with low CT values. These results were confirmed by the National Veterinary Services Laboratories, which reported the detection of Eurasian goose/Guangdong lineage H5N1 clade 2.3.4.4b highly pathogenic avian influenza. A total of 1,492 Avian Influenza rRT-PCR tests were performed by the Mississippi Veterinary Research and Diagnostic Laboratory on surveillance samples from the two commercial and one backyard poultry control zones. No additional infected flocks were detected within the control zones, and to date, no epidemiologic link has been found between the HPAI cases in Mississippi poultry.

A Case of the FluDETECT or Fluke-DETECT?

Jewell Bremer, Ben Wileman, Jake Carlson, Marissa Studniski

Select Genetics

In a recent case study done in Minnesota, semen from a turkey tom stud farm was identified as a highly likely method of transmission for highly pathogenic avian influenza (HPAI) to four naïve turkey hen breeder farms. In the article the FluDETECT Avian Influenza Virus Type A Antigen Test Kit by Zoetis was presented as an off-label option to use for pen-side testing and early detection of influenza A virus (IAV) in pooled semen. This method was adopted on a stud farm as an added layer of protection against HPAI to prevent dispersing positive semen from subclinical toms to naïve hens. Semen from one

particular barn of young toms began to present with consistent and strong positives on the FluDETECT. Toms were negative for AI PCR on tracheal swabs as well as cloacal swabs and semen for three consecutive days although semen tested on the FluDETECT remained positive. Multiple potential causes of the false positives on the FluDETECT were investigated and will be presented.

Cecal Core and Necrotic Foci in Turkey Liver: What is Your Diagnosis?

Vijay Durairaj, Steven Clark, Emily Barber, Ryan Vander Veen

Huvepharma Inc.

In August 2022, histomoniasis was suspected in a flock of 9 week-old turkeys in Midwest, USA. On gross pathology investigation, typhilitis, cecal core and necrotic foci on the liver were observed. A presumptive diagnosis of histomoniasis was made based on the gross pathology lesions. Sequencing of the Histomonas meleagridis 18S rRNA gene confirmed the presence of a genotype-1 isolate. Microscopic investigation of cecal scrapings revealed Eimeria spp oocysts. Gene targeted sequencing of cytochrome c oxidase subunit I gene (COI) of Eimeria confirmed E. meleagridis. Cecal and liver lesions of turkeys are considered hallmark and distinctive manifestations of histomoniasis. Histomoniasis is not the only disease in turkeys that induces lesions in the ceca and liver. Several other pathogens induce lesions both in the ceca and liver or in either one of these organs. E. meleagridis, considered as a nonpathogenic species, is a cecal inhabitant that induces mild damage to the ceca and also induces cecal core formation. Although E. meleagridis is considered nonpathogenic, it adversely affects the cecal integrity providing a suitable niche for entry of H. meleagridis.

Emerging Reovirus Infections in Turkeys in Quebec, Canada

Louise Mercier[1], Sonia Chenier[2], Monique Dore[3], Antony Bastin[2], Carl Gagnon[4]

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Since 2011, new reovirus strains emerged in commercial turkey in USA and other countries, mostly associated with tenosynovitis but more recently, with necrotizing hepatitis in younger birds. In 2020, those reovirus infections were diagnosed in turkey flocks for the first time in Québec, Canada. These infections led to important economical losses due to the death of young poults around 14 days of age, and culling or sudden death of older turkeys around 80 days of age. In older birds, typical lymphoplasmacytic tenosynovitis lesions were observed. In younger poults, the lesions were rather observed in the liver, and were characterized by randomly-distributed necrotic foci with syncytial degenerated hepatocytes. Poults that survived the hepatitis did not develop tenosynovitis later in life. Also, a reovirus-associated non-suppurative encephalitis located in the cerebellum and pons, never described before, was rarely detected in some flocks, leading to ataxia and tremors in 31 to 69-day-old poults, with no concurrent hepatitis or tenosynovitis lesions. Poults with hepatitis had the highest viral load (average ct of 11.56 with the RT-qPCR) compared to tenosynovitis (23.54) and encephalitis cases (27.75). Viral culture and sequencing of the sigma-C (σC) gene were performed to determine the homology of these reoviruses with known strains and preliminary results identified two clusters that were not

associated with a type of lesion in particular (hepatitis, encephalitis or tenosynovitis) and had a 82.5 to 99.1 % homology with reference turkey reovirus strains. For the first time, an immunoperoxydase protocol was developed and successfully demonstrated the virus within the liver lesions, but not in other tissues. In conclusion, different reovirus strains are spreading in turkey flocks in North America, associated with multiple diseases syndromes, such as tenosynovitis and hepatitis but also with a newly described encephalitis.

Case Report Part 2: Clostridium Septicum Dermatitis and Septicemia in Commercial Egg Layers. Interventions, Diagnostics, and Unknowns

Mark Mouw, Daniel Wilson, Michaela Olson

Wilson Veterinary Co.

At the 2022 AAAP Scientific Meeting our practice presented an unusual case presentation of Clostridium septicum septicemia and dermatitis in cage layers. The remainder of 2022 led us down different diagnostic and intervention paths with many of these options leading to dead ends. This case report will serve as an update to this interesting presentation in specific cage layer facilities and flocks. Actual flock data will be presented to demonstrate the livability impacts of this ongoing challenge. Specific discussions on the role of feed overconsumption and timing of feed delivery will be addressed. If any interventions are successful as of the 2023 AAAP meeting those field observations will also be presented. Most importantly if no interventions have worked at this time we will propose additional areas to review in relation to dermatitis and septicemia in conventional cage-housed egg layers.Tags: Avian, Bacteriology, Diagnosis, Poultry Learning Objectives: Layer flocks with Clostridium septicum dermatitis present with a variety of lesions including serosanguinous subcutaneous fluid and internal lesions consistent with ante-mortem gas pockets in the liver and spleen. There is the possibility that cases of Clostridium septicum dermatitis may be being overlooked by commercial egg layer field veterinarians and diagnosticians that easily write mortality causes off as post mortem decomposition. Clostridium septicum dermatitis and septicemia can cause sharp spikes in mortality rates and directly affect flock health and welfare by impacting livability. With these effects the condition ultimately dramatically affects flock profitability.Provide other poultry field diagnosticians and opportunity to learn the diagnostics and interventions we have attempted in these flocks.

Lameness in Young Broilers: The Case of the Rocky Chicks

Peter O'Kane[1], Allan Ball[1], Carlo Bianco[2]

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Elevated mortality and culling was reported from a farm of seven-day old broilers in the United Kingdom. Two of the eight houses were affected. Chicks in affected houses showed clinical signs of weakness and lameness; litter was wetter than usual. Same day necropsy findings from dead and culled lame chicks included: minimally widened tibiotarsal growth plates; superficial lesions on the gizzard koilin; and mildly congested kidneys in several birds. Antibiotic treatment and vitamin D3 supplementation were commenced and the feed was replaced. The mortality and culling rate remained elevated for several more days but remained confined to the two affected houses. Bacteriological examination yielded no significant findings.Four days later, undersized birds were submitted for necropsy to investigate deteriorating uniformity.

Grossly widened tibiotarsal growth plates were apparent, with osteomyelitis or thickened proventriculi in several birds. Histological examination of tibiotarsal growth plates revealed mild osteodystrophy and tibial dyschondroplasia Mineralisation and/or calcium deposits were identified histologically and histochemically within the kolin layer of the gizzard, the kidneys and the proventriculus. No significant findings were noted in brain, skeletal muscle and peripheral nerves. Feed analysis from the initial starter ration revealed calcium and phosphorous levels were respectively approximately three and four times in excess of target levels, with a reduced calcium: phosphorous ratio. The culling rate for lameness and undersizing remained elevated for the duration of the crop, with an overall mortality of approximately 15%.

Hypophosphatemic Rickets in Breeder Pullets

Alexander Strauch

Alexander Strauch Consulting

A flock of ~1000, eight-week old black australorp breeder pullets located in the Midwest U.S. alerted Veterinarian of "wobbly and weak birds" of 1.5 weeks duration and a slightly increased mortality rate. Upon examination of the flock, approximately 10% of the mixed-sex flock showed an incoordinated gait, hock-sitting, and falling-over to the point of being stuck on their backs. Size-wise, the flock was "behind" in weight gain and demostrated poor uniformity when compared to historical growth averages. Post-mortem examination of affected pullets showed numerous coarse calcium particles in the gizzards and rubbery shank bones. Coarse calcium particles were also evident in the feed troughs. Review of the the most recent feed analysis report available on farm showed a 4.04% DM calcium inclusion rate with a Ca:K ratio of 5.9:1 - which was inapproriate for growing pullets and more appropriate for a laying hen. A field diagnosis of rickets due to calcium:phosphorus imbalance was made for these pullets. Secondary consultation sought from nutritionist via phone call provided further confirmation of preliminary diagnosis. Managmenent elected to act upon field evaluation and agreed to remove all on-farm feed within 24 hours, replace with pullet-appropriate rations, and run a phosphorus-based mineral supplement through the waterlines for at least 1 week. Flock metrics improved over the following 2 weeks and the surviving portion of the flock began their breeder lay cycle around the normally-scheduled timeframe. Further investigation into the root-cause of the nutritional management error revealed that the contract farmer who was sourcing his own feed had consulted the same service provider that analyzed his dairy cattle rations - a potential source of the formulation oversight.

Hatchery

Understanding the Recent Increase in Chick Mortality in Commercial Broiler Operations in the USA

Matilde Alfonso, Donna Hill, Isabella Hannay, Fred Hoerr

Ceva Animal Health

In the last 10 years there has been a steady increase in broiler mortality in the USA (Agristats) despite all the nutritional, genetics, housing and equipment advances. This increase

has also been noticed during the chicken's first week of life. 7-day chick mortality in 2022 has almost doubled compared to 2012. In the same time frame, overall hatchability in commercial broiler hatcheries in the USA has decreased by 5%. This hatchability decrease has been dramatic in the last 2 years when chick mortality has been the worst. During that period, chick mortality surveys performed across broiler integrators in the USA are revealing an increase in unthrifty chicks with impaired growth, that contribute to high chick mortality around day 4. Those chicks, in many cases, have failed to utilize their yolk effectively. This presentation will introduce a summary of personal field observations accumulated during the last 10 years, comparing different chick mortality patterns. It will also present chick length as a diagnostic tool for chick mortality troubleshooting. The information presented will serve as an introduction to Dr. Donna Hill's presentation "The Impact of Current Incubation Conditions on Embryo Temperature Profiles in Multistage and Single Stage Incubation in the US and the Outcomes in Chick Quality" and Dr. Fred Hoerr's presentation "Histopathology in Chicks of Normal and Short Body Length at Hatch" intended to understand the increase in chick mortality observed in USA broiler operations recently.

The Impact of Current Incubation Conditions on Embryo Temperature Profiles in Multistage and Single Stage Incubation in the US and the Outcomes in Chick Quality

Donna Hill, Marshall Putnam, Matilde Alfonso, Fred Hoerr, Isabella Hannay

Donna Hill Consulting

The concept of applying embryo temperature to determine the optimum incubation conditions first occurred in the field in approximately 1996. With the advent of yield breeds, the embryo was producing more heat on the end of incubation. Hatchers were run at wet and dry bulb temperatures higher than they are today. Therefore, the most obvious issue was overheating on the end of incubation, especially in the hatcher. Much research was done in the years 2000-2016. This research usually used an ideal embryo temperature of 100°F until a specific day of incubation when it was increased. The research showed that there were developmental issues related to high embryo temperature that caused hatchability, chick quality, and field performance losses in the chicks (relative to the normal which were keep at 100°F throughout incubation). Unfortunately, this research does not reflect the actual embryo temperatures in multistage or single stage incubation in the US today. The problem that I see in the field today has emerged during the COVID era, when problems with infertility arose and companies installed new controllers on multistage setters and hatchers that allowed managers to change the set points. The impact on the hatchers has been positive. The impact of lowering the set point in the setter to prevent overheating on the end of incubation, results in low embryo temperatures in the first 16 days of incubation. Like overheated chicks, early cold incubated chicks also are poorly developed and result in chick quality, hatchability and field performance issues. There are four ways to create incubation quality problems in chicks:a.Cold for the first 16 days b.Hot for the first 16 daysc.Hot on the last 3-5 daysd.Cold on the last 3 daysIn this talk, I will show field evaluations of embryo temperature to contrast to the research embryo temperature profiles in the various machine types in the US. I will also discuss evaluations of embryo length, chick length and hatchability that are related to the various embryo temperature profiles.

Histopathology in Chicks of Normal and Short Body Length at Hatch

Frederic Hoerr[1], Donna Hill[2], [3]Isabella Hannay, [4]Matilde Alfonso, [4]Marshall Putnam

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[4]Ceva Animal Health

Preliminary histopathologic comparisons of chicks with either normal or short body lengths led to a focus on the liver (cytoplasmic lipid vacuoles) and intestinal morphology to better understand the pathogenesis of reduced embryonic growth and failure to thrive after hatch. The histopathology protocol that evolved uses the livers and intestines from hatch mates in groups of five normal- and five short-body-length chicks selected on the day of hatch before placement. In liver, hepatocyte vacuoles (lipid) are analyzed on routine histologic sections using ImageJ (National Institutes of Health, public domain, https://imagej.nih.gov/ij/download.html), using Bandpass filter, Thresholding, Watershed, and Particle Analysis (method available upon request). Five villi (V) and 5 corresponding crypts (C) are measured for each circular section of intestine and the V:C is calculated. All organs are examined for histopathology. In three surveys conducted with this protocol, the observed trend was for small chicks to have more numerous but smaller liver vacuoles (lipid), shorter intestinal villi, and a reduction in the V:C, often with significant differences. In one survey, small chicks had ingested egg fluids laden with bacteria in the ileum and cecum, variably accompanied by mucosal lesions. The histologic findings are consistent with the short and normal chicks differing in hepatic lipid metabolism and in intestinal villus development. These have implications for thermoregulation and digestive efficiency, respectively. Ingested egg fluids and bacteria in the lower gut at hatch could be indicators of embryonic stress and initial impairment of gut health.

Updating Hatchery Quality Assurance to Fit Emerging Challenges

Isabella Hannay[1], Donna Hill[1], Fred Hoerr[2], Matilde Alfonso[3]

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A common hatchery monitoring technique is 21-day residue evaluation. The current, widely used criteria don't necessarily identify the cause of hatch loss. These criteria need adjustment to improve the value of routine residue evaluation. Incubation loss is not categorized appropriately by the industry-wide breakout model. Embryonic development and growth are restricted with suboptimal incubation. Poor incubation conditions can result in both embryo loss and small chicks. Embryo length, when compared to established guidelines, determines when embryo loss occurred. A concerning, nationwide trend is reduced hatchability, increased abnormalities, and small chicks at hatch due to early cold incubation. One common misconception is the perceived risk of overheating embryos in early incubation. Whilst this is possible, this has only been documented once by the authors and is associated with high fertility in multistage machines. Dead embryos observed with overheating will appear vastly different to those associated with cold incubation. Our suggestion is to update current breakout evaluation categories whilst maintaining an easy-to-follow protocol. To maximize the usefulness of hatchery quality assurance programs it is advised that breakout analysis is combined with both embryo and chick length evaluations. Finally, data related to

incubation losses should be analyzed over time by breeder flock age group, machine, and season. This will highlight trends, enable pinpointing of where hatch loss occurred and anticipate future problems. This information will provide background to Dr. Donna Hill's, Dr. Matilde Alfonso's and Dr. Fred Hoerr's presentations.

AAAP Wellness Talk

I Have Friends in Poult Places: Connecting Mentorship and Wellbeing

Valerie Marcano

Pawsibilities Vet Med

Avian Influenza

Analysis of Available Vaccines for Highly Pathogenic Avian Influenza in the U.S.

David Suarez, Erica Spackman,
Mary Pantin-Jackwood, Darrell Kapczynski,

Southeast Poultry Research Laboratory

The interest in vaccination for highly pathogenic avian influenza has increased as the current outbreak of H5 2.3.4.4b avian influenza continues for a second year. Currently no vaccines are being used in the United States because of the concern about the effect it would have on international trade of poultry and poultry products. In the United States, several vaccines are licensed for use for H5 avian influenza, but most are not in active production. Because of the continued antigenic drift of the avian influenza virus, there is concern that the currently licensed vaccines will have reduced protection if vaccination were allowed. Vaccine challenges are being conducted to determine the efficacy of the available or likely available vaccine strains in the U.S. Consideration or analysis of other vaccines on the new horizon or available in other countries will also be made. An update on the role of DIVA surveillance strategies will also be reviewed in relation to how they might facilitate trade.

Antigenic Map of the Hemagglutinin of Influenza A Virus of the H9 Subtype

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Low pathogenic avian influenza virus (LPAIV) of the H9N2 subtype is enzootic in poultry in Asia, the Middle East, and Africa, causing significant economic damage to the poultry industry due to high morbidity and associated mortality. Due to their zoonotic potential, the World Health Organization (WHO) places H9N2 AIV among those with pandemic concern. To determine molecular signatures of antibody recognition of the hemagglutinin (HA) of the H9 subtype, phylogenetics, and antigenic cartography were combined. Analyzing the HA1 portion of H9 AIV, 10 consensus sequences were produced to capture the potential antigenic diversity of these viruses on a global scale. We created 10 chimeric HA sequences containing the HA1 of these consensus sequences on a constant HA2 portion from a prototypic H9 strain. Eight chimeric HAs were successfully rescued by reverse genetics,

and the resulting viruses were used to generate antibodies in quails. Antigenic maps were generated by plotting the cross-hemagglutination inhibition (HI) data from the panels of sera generated against the chimeric constructs as well as 50 H9 field isolates from different parts of the world. Furthermore, mutagenesis experiments showed that few amino acid positions allowed two-way complete antigenic cluster transitions. Although mutations at amino acid positions 150, 180, and 217 (H9 HA numbering) had a relatively significant impact on HI activity, only the mutations E180A and R131K/E180A led to complete cluster transitions. These studies suggest that a combination of a few amino acid residues modulates HI activity in the HA of H9 AIVs. Our studies provide significant insights into the antigenic profile of H9 AIVs with essential implications for understanding antigenic drift and improving vaccine development.

Development of a Modified Live Attenuated Influenza Virus Vaccine Against H9N2 for Poultry

Flavio Cargnin Faccin[1], C. Joaquin Caceres[1], L. Claire Gay[1], Brittany Seibert[1], Ginger Geiger[1], Matias Cardenas[1], Daniela S. Rajao[1], Silvia Carnaccini[1], Darrell R. Kapczynski[2], Daniel R. Perez[1]

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Influenza A virus (FLUAV) of the H9N2 subtype is enzootic in poultry in parts of Asia, the Middle East, Europe, and Africa. Despite being classified as low pathogenicity avian influenza (LPAI), H9N2 viruses cause significant economic losses to the poultry industry. In addition, H9N2 viruses have acted as donors of internal gene segments of zoonotic strains of various subtypes. Vaccination is widely used to prevent disease and control virus spread. Inactivated whole virus vaccines are being used extensively, though they confer limited protection. Live attenuated influenza virus (LAIV) vaccines mimic a natural infection and therefore have the potential to induce broader immune responses through a combination of humoral, mucosal, and cellular immunity. We aimed to generate efficacious and safe LAIVs carrying molecular markers and immunomodulators. We used reverse genetics to generate LAIVs based on genome rearrangement where the open reading frame of M2 was introduced downstream PB1. Additionally, multiple stop codons were introduced in the M segment to prevent the expression of M2 from segment 7. Unique molecular markers in the HA segment and immunomodulators in the NA segment were incorporated. The stability and growth properties of the viruses were analyzed in vitro whereas safety, immunogenicity, and efficacy were evaluated in two-week-old chickens. LAIV candidates were stable and grew to similar levels compared to wild-type viruses. Studies in vivo showed that LAIVs are safe and immunogenic generating neutralizing antibodies predictive of protection as well as antibodies against other viral proteins. The inclusion of

immunomodulators enhanced the generation of neutralizing antibodies, suggesting a role in the host immune response. The decrease in viral loads post-challenge demonstrated the protective effect of LAIV vaccines. The inclusion of immunomodulators improved the protection compared to the LAIV without immunomodulators. This work provides novel insight into the development of vaccines against FLUAV-carrying molecular markers and immunomodulators.

Transgenic Approaches for Control of Highly Pathogenic Avian Influenza Virus in Poultry

Kelsey Briggs, Ryan Sweeney, Karen Segovia, Klaudia Chrzastek

USDA-ARS-USNPRC

Avian influenza virus (AIV) is a highly contagious and lethal disease that can have major impacts on the global poultry industry and food supply. While more frequent in Asia and Europe, highly pathogenic avian influenza virus (HPAIV) outbreaks have traditionally been rare in the U.S. However, in recent years, the U.S. has seen an increase in the incidence of HPAIV outbreaks in wild birds and commercial poultry. In 2014/2015 and in 2021/2022 outbreaks of HPAIV subtype H5NX clade 2.3.4.4 resulted in the death and destruction of over 50 million birds each costing billions of dollars to the U.S. economy. Vaccines are not currently approved for use in the U.S. so control strategies for HPAIV are dependent on biosecurity and culling of infected flocks. New strategies for HPAIV control based of gene editing of poultry species could offer solutions for disease control. Here we demonstrate two different strategies for improving disease resistance to HPAIV based on enhancing innate immunity or targeting the viral RNA genome. First, editing innate immune modulators, including myxovirus resistance gene-1 (Mx) and retinoic acid gene-I (RIG-I), will improve specificity to recognize AIV infection at the cellular level. Their roles have been well established as initiators of the anti-viral response. Our in vitro results demonstrate reduced virus titers following HPAIV infection. The second strategy utilizes CRISPR Cas13a to degrade viral RNA with guides that target the AIV genome. When introduced in chicken DF-1 cell line, we observed knock-down of AIV growth up to 99% and enhanced cell viability. Taken together, we demonstrate that gene editing can be a feasible strategy in the control of AIV.

Evaluating the Impact of IBDV-Mediated Immunosuppression on the Shedding and Evolution of a Mallard Avian Influenza Virus (AIV) in Chickens

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Immunosuppression in birds reduces vaccine efficacy, and affects the shedding and pathogenicity of avian influenza viruses (AIVs). However, the role immunosuppression plays in the spillover of AIVs from aquatic waterfowl to domestic birds is poorly understood. To address this, we conducted a superinfection study to better understand the consequence of infectious bursal disease virus (IBDV), a globally endemic immunosuppressive disease of chickens, on the transmission and evolution of AIV of mallard origin. Two groups of 66 two-day old chicks each were inoculated with either PBS, or the classical IBDV strain F52-70. Thirty-six birds from each group were bled to confirm immunosuppression, housed in isolators, and intranasally inoculated

with influenza strain A/mallard/Alberta/156/2001 (H3N8) virus at 14 days post-IBDV inoculation (dpi). To examine transmission, 6 mock or IBDV- infected sentinels (S) from the remaining birds were added to each isolator at 15 dpi, making a total of six groups: mock/mock, IBDV/mock, mock/AIV+Smock, mock/AIV+SIBDV, IBDV/AIV+Smock, and IBDV/AIV+SIBDV. Buccal and cloacal swabs were taken from AIV-inoculated birds 14-24 dpi, tissues were harvested from 6 birds per group at 17 and 21 dpi, and remaining birds were culled at 28 dpi. Birds infected with IBDV alone had a reduction in the number of B cells in PBMCs, spleen, bursa, lung and cecal tonsils compared to mock controls, but an elevation in the number of CD4+ and CD8+ T cells, whereas the opposite was true of AIV infection alone. Chickens with no IBDV exposure cleared the mallard AIV in 5 days, however, some birds pre-exposed to IBDV had prolonged AIV shedding, and did not clear infection until 7 days, suggesting that IBDV-immune dysregulation may influence the spread of mallard AIV in chickens, although, in our experiment, the sentinel birds did not shed AIV. We are currently evaluating the effect of IBDV on the evolution of AIV, using next-generation-sequencing.

Comparative Pathology of Chickens Naturally Infected with Highly Pathogenic Avian Influenza Viruses H5N2 clade 2.3.4.4c in 2014 and H5N1 Clade 2.3.4.4b in 2022

Cheng-Shun Hsueh

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Highly pathogenic avian influenza viruses (HPAIV) of clade 2.3.4.4, causing an outbreak for the first time in North America in 2014, have reemerged and caused losses to the commercial and backyard flocks in the U.S. in 2022. In this report, we describe 1 case of 2014 H5N2 clade 2.3.4.4c HPAIV infection in a backyard flock and 2 cases of 2022 N5N1 clade 2.3.4.4b in commercial flocks in Iowa. Clinically, all three flocks were reported to have sudden increased mortalities with inapparent clinical signs. Grossly, chickens infected with H5N2 had edema and hemorrhage of the comb, catarrhal exudate in the oropharynx, and multifocal tracheal hemorrhage, while chickens infected with H5N1 had petechia on the fat pad, cloudy air sac, mottled spleen and red conjunctival and tracheal mucosa. Histologically, both H5N2 and H5N1 infected chickens had pulmonary congestion and edema, while lesions in the brain, trachea, pancreas, spleen, liver and skin varied in severity and distribution, but all characterized by acute necrosis and hemorrhage +/- lymphohistiocytic inflammation, with no histologic lesions in pancreas and liver in H5N2 infected chickens. Influenza A virus nucleoprotein, detected by immunohistochemistry, was located in neurons, respiratory epithelium and macrophages in the lung, cardiomyocytes, endothelial cells of blood vessels, hepatocytes and Kupffer cells in the liver, and epidermal keratinocytes in both H5N2 and H5N1 infected chickens. Nevertheless, viral nucleoprotein was only detected in pancreatic acinar cells, feather follicles, renal tubular epithelium, and tracheal respiratory epithelium in H5N1-infected chickens. The varied pattern of pathological findings and viral tissue tropism may be due to the AIV strain, viral load, and pathogenicity subtype.

Identification and Genome Characterization of Highly Pathogenic H5N1 Avian Influenza A from Wild Birds in Peru

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Wild birds are the main reservoirs for Influenza A viruses, representing the major source for viral spread worldwide. Currently, we are witnessing an unprecedented emergence of highly pathogenic avian Influenza A in Europe, Asia, and America. Thus, our objective was to describe the wild bird species involved in viral circulation in Peru, while characterizing the viral genome. Hence, our study consisted of molecular detection, isolation and genomic characterization of the circulating Influenza A virus in fecal samples from wild birds in Peru. The samples were collected during November 2022 and January 2023, we assessed the presence of highly pathogenic avian Influenza A in multiple species showing clinical signs compatible with influenza infections such as depression, respiratory distress, and neurological disorders. We evaluated 20 samples of wild bird species including hawks, Peruvian gulls, kestrels, Guanay Cormorants, Peruvian booby, Common Barn-owl, Humboldt penguin, Peregrin falcon and Peruvian Pelicans. Out of those 20 samples, 11 resulted positives for Influenza A by RT-PCR, that allowed obtaining 07 viral isolates following inoculation in embryonated chicken eggs. These 07 isolates were processed by next generation sequencing for genome characterization and reconstruct their evolutive origin among those isolates described to date. Our results showed that most species studied are infected by this virus and highly pathogenic virus can be detected and isolated from a wide range of avian hosts. Moreover, genetic analysis identified that our isolates correspond to the H5N1 highly pathogenic avian Influenza A clade 2.3.4.4b. Eventually, this study represents the first report to characterize this virus in the current outbreak in multiple wild birds from a South America country.

Characterizing the interface between wild birds and poultry in a high-risk area for avian influenza introduction: camera traps in poultry farms and virological surveillance in waterfowl

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Avian influenza (AI) is a viral epidemic disease for which interactions at the wild bird-poultry interface determine the risk of new introductions into poultry holdings. To date, the identification of bridge hosts between low pathogenic AI reservoirs, the waterfowl, and poultry farms is still limited. Furthermore, recent detections of highly pathogenic avian influenza viruses (HPAIV) in migratory birds inhabiting the Atlantic and Eurasian flyways raised concerns on

virus circulation in wild hosts. Therefore, three commercial layer farms with an history of AI outbreaks, located in a densely populated poultry area in Northern Italy, were monitored through ten camera traps for a one-year period (February 2021 to January 2022). Concomitantly, a molecular survey in wild birds inhabiting nearby wetlands was carried out by collecting cloacal swabs (CS) from hunted waterfowls and fecal droppings (FD) from roosting sites. Sampling activities targeted different species belonging to the Anseriformes and Charadriiformes orders. Results from camera trap observations showed a conspicuous birdlife diversity occurring nearby poultry houses. The most frequent visitors were corvids (Eurasian magpie), passerines (Eurasian blackbird and grey wagtail), wild galliforms (ring-necked pheasant), doves (European turtle dove and Eurasian collared dove), and wild ducks (mallard). Time series analysis revealed a higher abundance of visits in early spring and winter. The molecular survey in waterfowl detected 11 positives out of 534 collected samples. Different co-circulating subtypes were identified in mallards, Eurasian teals, greylag goose and common snipe, including H5N3 LPAI, H1N1, H6N1, and H9N2 viruses. Overall, this study disclosed a conspicuous diversity of wild birds in proximity of poultry houses, identifying the main species that could be prioritized for future epidemiological surveys. Furthermore, the viral diversity observed highlights the importance of combining different sampling methods to identify AIVs concurrently circulating in wild bird populations nearby poultry farms.

A Lesson in Acronyms

Emily Pittman

Georgia Poultry Laboratory Network

In the current outbreak of highly pathogenic avian influenza (HPAI), both of Georgia's detections were unique situations. In August of 2022, HPAI was detected in domestic ducks on the premise designated as Henry County 01. This effort called for coordination of multiple governmental agencies for planning, biosecurity, waste removal, and decontamination planning. This talk will discuss lessons learned, the vulture connection, the cooperation between agencies (GPLN, GDA, USDA, DNR, WS, DPH, PD), biosecurity and disposal planning, and the role of the avian veterinarian to guide event decisions.

Nanopore Sequencing: Evaluation of Enrichment Methods for Avian Influenza Virus Recovery from Clinical Samples

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Highly Pathogenic Avian Influenza (HPAI) is a Foreign Animal Disease (FAD) considered one of the most devastating poultry diseases, causing significant economic losses to the poultry industry. Accurate and timely diagnosis is essential; however, it is often a major bottleneck for successful outbreak control efforts. Moreover, Avian Influenza virus (AIV) sequencing directly from clinical samples is

challenging due to low virus abundance compared to the host and other non-target sequences. Our hypothesis is that combining metagenomic Next Generation Sequencing (mNGS) using Oxford Nanopore Technology (ONT) with target-independent enrichment strategies allows the fast detection and further genetic characterization of AIV when facing outbreaks. In this study, we evaluated different enrichment strategies to increase the ability to identify and characterize the AIV directly from clinical samples using the ONT sequencing platform. Viral RNA extraction of eight known HPAI-positive clinical samples was performed using MagMAX™ Pathogen RNA/DNA Kit (Thermo Fisher Scientific). Real-time RT-PCR targeting the Influenza Virus Matrix gene was run to estimate the samples' viral copies. Host nucleic acid depletion was performed, including a DNA depletion step using TURBO DNA-free™ Kit (Thermo Fisher Scientific), followed by purification using RNA Clean & Concentrator™-5 (Zymo Research). Additionally, rRNA depletion was implemented using custom-designed probes. Treated and untreated RNA extracts from each sample were amplified via sequence-independent, single-primer amplification (SISPA) to increase the percentage of viral reads. Sequencing libraries were prepared from purified SISPA PCR products using Rapid sequencing gDNA - barcoding (SQK-RBK110.96, ONT) and loaded onto a MIN106 R9.4.1 flow cell (ONT) using the MinION device. Base-calling was performed using MinKNOW, and the FASTQ files were analyzed using EP2ME and Genome detective. Enrichment and depletion strategies were efficiently able to decrease the concentration of non-target DNA and RNA concentration, whereas SISPA significantly increased nucleic acid concentration to start the library preparation of the samples. Samples were sequenced for six hours, and 747 Mb of estimated bases were generated. Additionally, Avian Influenza virus (AIV) reads were detected from all samples, and there was a positive correlation between the number of viral copies and the number of AIV reads. Furthermore, treated samples showed a higher count of AIV reads than the original extracts. In conclusion, enrichment strategies for NGS can significantly improve our ability to identifying and characterizing IAV from clinical samples.

Coccidiosis

Concepts Concerning Coccidiosis: Host and Strain Specificity, Immunity, and Viable Control Programs

Gregory Mathis, Chuck Hofacre

Southern Poultry Research, Inc.

Coccidiosis is a major disease in the poultry industry. Immunologically distinct, developmental site specific, and host specific Eimeria species multiply in the intestinal tract of chickens. Efforts to control coccidiosis has long been one of the major objectives of research. Lack of coccidiosis in certain avian species hybrids demonstrated host specificity. Eimeria PCR diagnostics from broiler complexes have shown that all species are often present. Many studies have shown there is no cross-species immunity. However, there are species interactions. Studies suggests that E. acervulina can interfere with colonization or development of E. maxima. This interaction was useful in developing an NE model using one coccidia species (E. maxima) + clostridium prefringens. Understanding coccidiosis epidemiology was expended by using oocysts shedding patterns. In 1939, drugs were proven to treat coccidiosis. Since then, over 30 drugs have been researched and developed for prevention. Currently due to production, toxicity, and resistance issues only 12 FDA approved drugs are available.

Anticoccidial sensitivity tests (ASTs) are used to determine drug use patterns to prolong their sensitivity. Another approach to control is live coccidia vaccines. Research studies demonstrated the importance of vaccine application, storage, self-life, immunity development, attenuation, and drug sensitivity. The demand for poultry 'Raised Without Antibiotics', greatly increased the interest and knowledge of alternative feed additive products such as probiotics, prebiotics, essential oils, organic acids, saponins, and tannins. There continues to be a demand for research studies to give us more information on how to manage and control this widespread disease.

3 Years of Field Data of US Broiler Performance Under Different Coccidiosis Control Programs

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Poultry producers face an increasing challenge to control coccidiosis as the anticoccidial treatments are getting less effective while available treatment options are more limited with the recent societal pressure to reduce antimicrobial products. The true cost of coccidiosis is from subclinical coccidiosis, which does not accompany distinct clinical symptoms but reduces feed efficiency in chickens by disrupting feed digestion/absorption in the gut. Both chemical compounds and ionophores have been widely used in the poultry industry as a feed additive to prevent coccidiosis, however, increasing resistances for chemical anticoccidial drugs have been reported globally and effectiveness of the chemical treatment in the field is quickly eroding. In the US, ionophores are classified as antibiotics, and more than 50% of the total US broiler production has moved to antibiotic-free nowadays. In our previous study, we analyzed one year of US field data from 2020 for broiler performance under three different coccidiosis control programs. Chemical-only program, Ionophore-only program, and vaccine-only program. For the in-feed program, only starter and grower feed were considered. The production data represented approximately 70% of the US broilers produced during 2020. The results indicated the comparable performance between the three programs in terms of feed efficiency, while vaccine only program had significantly lower total mortality as well as lower total production cost compared to the chemical-only program. Due to the relatively lower number of farms utilizing vaccines during the winter season, it was difficult to look at seasonal changes using 1-year data. Now we have compiled the performance data for 3 years (2020-2022), we have looked at the multiple performance indexes and examined to see the impact of seasonal changes in performance under the different programs.

Evaluation of the Effects of Litter Moisture on Cycling of Coccidiosis Vaccine: Part II

Nicholas Brown

Huvepharma, Inc.

High litter moisture has been associated with increased severity of coccidiosis as well as prolonged oocyst survival. However, litter moisture can be difficult to objectively quantify on the farm due to the requirement for oven-drying of samples to obtain an accurate result. In a previous study, a method for controlling litter moisture and measuring it on-farm was determined. However, the precise moisture levels needed to promote or inhibit coccidial cycling are not well known. The purpose of this study was to to assess if measured differences in litter moisture cause any detectable changes in

vaccine cycling during a 5 week grow-out. To accomplish this, broiler chicks were inoculated with coccidiosis vaccine and reared for 5 weeks in floor pens maintained to have two distinct moisture levels (<20%, and ~30-40%). To assess coccidial cycling, lesion scoring was conducted throughout the trial. Litter samples were taken at several timepoints to determine which devices correlate best to the traditional oven method. The results of this study are pending.

Effects of Different Oocyst Doses of Turkey Coccidia Eimeria meleagrimitis and E. adenoeides on Growth Performance, Intestinal Health, and Bone Quality in Turkeys

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The aim of the current study was to investigate the effects of different oocyst doses of turkey coccidia Eimeria meleagrimitis (EM) and E. adenoeides (EA) mixture on growth performance, intestinal health, and bone quality in turkeys. A total of 384 0-d-old male Aviagen Nicholas turkeys were allotted to 12 treatments (2 challenge point with 6 different doses) with 4 replicates. Six different treatments were consisted with NC, a treatment with non-challenged; T2K, a treatment challenged with 2,000 EM and 2,000 EA oocysts; T4K, a treatment challenged with double oocysts dose of T2K; T8K, a treatment challenged with double oocysts dose of T4K; T16K, a treatment challenged with double oocysts dose of T8K; T32K, a treatment challenged with double oocysts dose of T16K. All treatments were divided into 2 different inoculation groups (d 8 or 15), and were orally administered EM and EA mixture on each day. The experiment lasted until d 28, and 28% crude protein diet and water provided ad libitum. Body weight (BW), body weight gain (BWG), feed intake (FI), and feed efficiency (FE) were recorded on d 8, 14, 15, and 22. On d 14 (6 dpi, days post infection; of d 8 challenged group, C8) and d 22 (7 dpi of d 15 challenged group, C15), 4 chickens per replicate were euthanized for the analysis of intestinal permeability, coccidial lesion scores, villus height:crypt depth ratio (VH:CD), bone mineral density (BMD), and bone mineral contents (BMC). All data were analyzed using R software, and statistical difference between treatments was determined by Tukey's HSD test if P value< 0.05, and an orthogonal polynomial contrast test also performed. The result showed that BW, BWG, FI, and FE decreased with increasing doses of EM and EA in C8 and C15 groups (linear and quadratic, P< 0.001), and all challenged treatment in C8 group showed significant differences on BW, BWG, and FI compared to NC, but T2K challenged treatment in C15 group showed no differences in all growth performance results compared to NC (P< 0.001). Daily FI result showed that FI of challenged treatments decreased the most at 6 dpi in both C8 and C15, up to 86% decrease in C8 group, and 91% decrease in C15 group compared to NC (P< 0.001). Intestinal permeability increased with increasing doses of EM and EA in both groups (linear, P< 0.001), and only T32K challenged treatment in both groups showed statistically significant compared to NC (P< 0.05). Duodenal and cecal lesion score increased with increasing doses of EM and EA in both groups (C8, linear and quadratic, P< 0.001; C15, linear, P< 0.001), and T2K challenged treatment in C8 group showed no differences of lesion scores, but in C15 group, only T16K and T32K challenged treatments showed significant differences compared to NC (P< 0.001). Duodenal, jejunal, and ileal VH:CD decreased with increasing doses of EM and EA in both groups (linear and quadratic, P< 0.001), and groups in C8, all

challenged treatments showed significantly decreased ileal VH:CD compared to NC, but T2K, T4K, and T8K challenged treatments showed no differences compared to NC in C15 groups (P< 0.001). BMD and BMC measured by DEXA decreased with increasing doses of EM and EA in C8 and C15 groups (linear, P< 0.001), all bone parameters showed significantly differences in T8K, T16K and T32K challenged treatments compared to NC in both groups. In conclusion, increased doses of EM and EA mixture negatively affects turkeys, including reduced growth performance, intestinal health, and bone parameters. Based on these parameters, we conclude that the early stages of turkey (d 8 challenged) may be more susceptible to turkey coccidia EM and EA than later stages (d 15).

Using Infrared Camera Technology to Predict Coccidiosis Oocyst Survival During Brood

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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 coccidiosis vaccines are 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 allowed to re-cycle in the poultry house environment in a way that stimulates adequate and proper immunity. As new technology enters the field, opportunities exist to explore options for its use in commercial poultry settings. One relatively new technology uses infrared cameras which have been used in hatcheries looking at heat loss and temperature variation around setters/hatchers, egg packs and chicks, and in commercial poultry barns to examine hot and cold zones to help optimize ventilation and bird comfort. In this study, infrared camera technology was used to predict coccidiosis oocyst survival in different areas of the poultry house during brood. The viability of oocysts in the environment is important to ensure that enough infective oocysts are available for recycling in vaccinated birds for proper development of immunity. Freshly shed oocysts are not infective until they sporulate in the environment. This process typically occurs over 1-2 days when optimal temperature conditions (70°–90°F [21°–32°C]) and adequate moisture and oxygen are present. Using infrared camera technology, we examined the environment for temperature zones most conducive to oocyst survival and thus re-cycling in cocci-vaccinated flocks. By predicting where the sporulated oocysts are most likely to be available, the poultry manager can adjust the brood set up accordingly to ensure proper exposure occurs regardless of what type of housing is available. This study is a good example of how modern equipment can be used to provide data for making critical management decisions regarding coccidiosis vaccination success.

Direct, Prolonged Exposure to Various Doses of Essential Oils Does Not Impact the Integrity of Coccidiosis Species Derived from Live Vaccines

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Phytochemicals are secondary metabolites derived from medicinal plants. A group of

phytochemicals known as essential oils (EO) have been used in poultry diets for years to decrease disease susceptibility and the need for veterinary interventions. Initial research on EO was based on in vitro models aiming to determine the minimum inhibitory concentrations against known pathogens; however, doses used in vivo are much lower and do not directly impact pathogen survival. Rather, at low doses, EO elicit host-mediated effects in the animal that can decrease inflammation and increase resistance to environmental stressors, including disease. Still, it is important to understand the interactions of EO with coccidiosis vaccine, since vaccination is a critical management strategy in many poultry production systems. In addition, attenuated coccidiosis vaccines are known to be relatively sensitive to disruption and need to be applied with care. The objective of this study was to investigate the effects of prolonged direct exposure of coccidiosis species to a formulated blend containing EO of cinnamon, clove, and oregano (CCO). Eimeria oocysts were sourced from a commercial coccidiosis vaccine and were exposed for 156h in vitro to one of the following treatments (12 reps/trt): distilled water (NEG), 50% citric acid (POS), and 1, 10, 100, or 1000 ppm of CCO. The three lowest doses of CCO are included within the recommended dose range; 100 ppm corresponds to the levels that would be used in feed. E. acervulina, E. tenella, and E. maxima species were identified, and oocysts were observed using a hemocytometer for enumeration and integrity at 0, 72, and 156h after exposure to treatment media. Intact oocysts were characterized by a distinct rounded mass known as a sporoblast (oocysts in an earlier stage of development), also defined structures of sporocysts and sporozoites (oocysts in a later stage of development). Loss of integrity was defined by malformation, with thinning or damage to the inner and/or outer cell wall, missing or scattered sporoblast, thinning of the sporocyst wall, and deconstruction of the sporocyst and sporozoite structures. Two-way ANOVA was used to determine the significance of treatment effects on integrity. Oocyst integrity of NEG at 0h was 75% intact and did not change over time ($P > 0.40$); integrity by treatment was made relative to NEG at the same timepoint. At 72h, 90.9% of the POS oocysts were degraded relative to NEG ($P < 0.001$), and none of CCO doses elicited loss of integrity ($P > 0.50$). By 156h, POS oocysts reached a 100% reduction in integrity, whereas CCO treatments still showed no loss of integrity relative to NEG (mean of 0% across all doses). We conclude that direct, prolonged exposure to CCO does not impact the integrity of Eimeria oocysts derived from a live attenuated vaccine. These results indicate that supplementing birds with CCO should not negatively impact the efficacy of live coccidiosis vaccine.

Anticoccidial Efficacy Evaluation

Hector Cervantes

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Over the past 7 decades, many formulas and indexes have been developed to assess the efficacy of anticoccidial drugs. A variety of parameters and measurements ranging from average daily weight gain and mortality to intestinal lesion scores and OPGs have been used. This presentation will review and attempt to identify the most useful indicators of anticoccidial drug efficacy in research settings and in the field.

Isolation of an Eimeria Maxima Immunovariant Late in Growout from Broiler Chickens Immunized at Hatch with an Eimeria Oocyst Vaccine

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Vaccination against avian coccidiosis is increasingly being used by the broiler and layer industries. This approach is based on the assumption that parasite antigens stimulating protective immunity are conserved among all strains of a single species. In this study it was shown that immunovariable strains of E. maxima may appear later in growout (~ 35 d) and affect chick performance. Litter from 5-week-old commercial broilers experiencing signs of coccidiosis was processed for total Eimeria oocysts. The predominant Eimeria species in this litter was E. maxima as judged by microscopy and corroborated by PCR. Eimeria maxima oocysts were isolated using a limiting dilution, microtiter plate technique and were inoculated into naïve chicks to obtain large numbers of oocysts, termed E. maxima APU3. Cross-protection studies revealed no protective immunity was elicited against E. maxima APU3 challenge infection by immunization with E. maxima APU2, which was isolated from broilers given a commercial vaccine. Immunization with another laboratory strain E. maxima APU1 provided complete protection against E. maxima APU3, whereas immunization with a commercial vaccine led to partial protection against E. maxima APU3. It is unknown at present if E. maxima APU3 is an immunovariant of E. maxima in the commercial vaccine or if it was present at low levels in litter at chick placement and its numbers increased slowly over time due to a lack of immunity to it. Nevertheless, these findings suggest that immunological E. maxima variants exist and may increase in numbers later in growout in chicks immunized at the hatchery with a coccidiosis vaccine.

Administering Coccidiosis Vaccine in the Drinking Water Improves E. maxima Uptake in Broiler Chickens

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Live coccidiosis vaccines are commonly administered to newly hatched broiler chicks via spray cabinets at the hatchery. Especially as it relates to Eimeria maxima, our research has shown that spray administration leads to poor vaccine uptake and a high percentage of unvaccinated chicks predisposed to necrotic enteritis getting placed into broiler houses. The purpose of this study was to optimize methods for administering Eimeria oocysts to broiler chicks through the drinking water system. Day of hatch spray vaccination was compared to vaccine administered in the drinking water to 3-day old chicks in commercial broiler houses. Post-vaccination, chicks (16/treatment) were randomly selected, housed individually, and feces were collected from each chick on days 5-

8 post-vaccination. The oocyst content of each composite day 5-8 fecal sample was determined using McMaster chamber methodology. In three studies, chicks receiving commercial vaccine by spray at the hatchery failed to excrete detectable numbers of Eimeria maxima oocysts. In contrast, 81-94% of chicks shed E. maxima oocysts when receiving the same commercial vaccine serial only administered through the water on day 3. In a fourth study, vaccine uptake by spray administration improved to just over 60% when a laboratory E. maxima strain was added to the commercial vaccine; however, in this same study, 100% of the chicks receiving this same vaccine combination in the water on day 3 demonstrated uptake and an unvaccinated group given access to the broiler house litter through day 4 demonstrated 19% uptake. Optimal variables to achieve a high level of E. maxima oocyst uptake with day 3 water administration were identified, and the methodology has been used across a myriad of poultry house designs with both center or end-house brooding. With some refinement, this approach might also be applied to other live vaccines, both viral and bacterial, that are difficult to administer effectively via day of hatch spray.

Infectious Bursal Disease

IBD Study Comparing rHVT-IBD Vaccine Protection Against AL2 Virus in Broilers Challenged at 25 Days of Age

Kalen Cookson, Manuel Da Costa, John Dickson, Jon Schaeffer

Zoetis

AL2 viruses are the most prevalent IBDV type in the broiler industry, having been recovered from almost half of all flocks sampled over the past decade. Unlike Del-E viruses which are more likely to be recovered from younger flocks, surveys show that AL2 viruses are just as likely to be recovered from younger flocks as from older ones. In the past year we've presented four different AL2 studies conducted in broilers challenged between 14-18 days of age. The protection levels achieved by the one recombinant HVT-IBD vaccine common to each of those studies ranged between ~70-90%. This paper will present an AL2 challenge study conducted in commercial broilers that were vaccinated with one of three different rHVT-IBD vaccines and challenged at 25 days of age. Bursal protection from gross atrophy and histological lesions as well as viral loads (qPCR analysis) will be summarized and discussed.

Efficacy Evaluation of Three Infectious Bursal Disease Vaccines by Quantifying the Viral Replication in Bursal Tissues in a Vaccination/Challenge Model

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Infectious bursal disease (IBD) is an important factor in chickens' susceptibility to pathogens. Although, in most cases, IBD is under control, there is evidence of its involvement as a predisposing factor for some common diseases in commercial poultry, such as coccidiosis. The aim of this study was to evaluate the efficacy of three IBD recombinant vaccines by the quantification of the viral replication in bursal tissues during a vaccination and challenge the experimental model. One hundred and twenty fertile eggs were obtained from a commercial broiler operation and assigned to five

experimental groups. At 18th day of embryonic development, three groups were vaccinated in ovo with three different recombinant HVT- IBD vaccines. One group remained as an un-vaccinated/un-challenged group, and another group as an un-vaccinated/challenged group. After hatching, the birds were housed in Horsfall Bauer-type isolators. At 28 days of age, the birds were challenged by eye drop with a Delaware E strain at a dose of 2.2 log10 EID50. Ten days after challenge, the birds were euthanized, and fresh bursal samples were collected for IBD virus (V) detection and quantification. An RT-qPCR method targeting the VP1 gene was used to quantify the IBDV challenge strain replication in bursal tissues. The highest viral replication was observed in the unvaccinated and challenged birds, and a reduction in the viral replication was observed in the vaccinated and challenged groups. The differences in the viral replication in the birds vaccinated with the three different vaccines will be discussed.

Evaluation of Protection Against T1-Like Variant Infectious Bursal Disease Viruses Using Dual Recombinant HVT-ND-IBD Vaccine Alone and in Combination with Live Intermediate Strain and 89/03 Strain of IBDV Vaccine

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Infectious bursal disease (IBD) is a highly contagious disease of young chickens caused by infectious bursal disease virus, characterized by immunosuppression and mortality generally at 3 to 6 weeks of age. IBDV is ubiquitous in commercial chicken operations. IBDV causes a prolonged B-lymphocyte immunodeficiency and increased susceptibility to various viruses and parasites. Both classic and variant strains of IBDV had been isolated in the southeastern United States. T1 is a variant strain of IBD that has been a problem in the southern states of United States. The T1 viruses are more similar to the AL2 family of viruses but are also genetically different from the Delaware-E variants. T1 variant had been reported to be the second most predominant IBDV variant in the USA present in broilers chickens causing production performance losses and consistently showed moderate to severe bursal atrophy. The objective of the study was to evaluate the protection provided by a dual recombinant HVT-ND-IBD alone or in combination with a 89/03 strain vaccine at day of age and the addition of an oral Live Intermediate Strain vaccine at 14 days of age in SPF chickens challenged at 26 days of age with IBDV variant T1 like isolated from a field case in broiler chickens. A total of 120 SPF chicks were placed and housed in negative pressure isolation units and had unrestricted access to feed and water and were divided in 6 different groups. A subcutaneous injection was used for injection of the chicks for each treatment group at hatch except for the positive and negative control groups. At 14 days of age two treatment groups received via spray a Live intermediate strain vaccine. The challenge viruses used in this study was a IBDV variant T1 from field submissions case 144843 to PDRC. The virus was expanded in 3-week-old SPF chickens prior to the start of the study, then titrated in chicken embryos. The virus was diluted to in tryptose phosphate broth (TPB) to a target dose of 103.0 EID50/0.05mls. On day 26 birds were challenged and at day 32 the study was terminated. Birds and bursae weighed data for Bursa /Body Weight (Bu/BW) ratio were collected and bursas also were collected for histopathology to determine mean bursal lesion scores to determine atrophy. Results showed protection in all the vaccinated groups when

they were compared with the Non-vaccinated/Challenged group. The Bu/BW ratios for all vaccinated/ T1 challenged groups were significantly higher, compared to the NoVAXT1 group, providing evidence that all vaccine combinations and the dual recombinant HVT-ND-IBDV prevented significant bursal atrophy following T1 like variant IBDV challenge. The bursal lesions scores for all vaccinated/T1-challenged groups were significantly lower compared to the NoVAXT1 group suggesting that all vaccine combinations provided protection against significant lymphocytic depletion in the bursa. Statistical difference was observed between the no vax/challenge group when compared to the vaccinated groups. An evaluation of the protection against T1 variant using a dual recombinant HVT-ND-IBD vaccine alone or in combination with a live intermediate standard strain and 89/03 strain of IBDV vaccine showed, based on bursa/body weight ratio and histopathology, protection in all the vaccinated groups when it was compared with the non-vaccinated/challenged group. The dual construct rHVT-ND-IBD vaccine alone or in combination with a live IBD vaccine or a Live Intermediate Strain vaccine at 14 days of age protected against T1 like variant IBD virus when challenged at 26 days of age.

Amino Acid Sequence and Phylogenetic Analyses of Segments A and B of Recent Infectious Bursal Disease Field Strains in Southern United States.

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Infectious bursal disease (IBD) is an acute, highly contagious viral disease of young birds characterized mainly by severe lesions in the bursa of Fabricius followed by immunosuppression. This disease is produced by induced by an Avibirnavirus also known as infectious bursal disease virus (IBDV) The subclinical and immunosuppressive form of the disease is prevalent in the United States. The aim of this study is to determine the characteristics of the amino acid sequences of genetic segments A and B of recent infectious bursal disease viruses circulating in Southern United States and to demonstrate their genetic relationships through phylogenetic analyses. Bursal tissue samples were collected from commercial broiler flocks. The ages of the birds analyzed ranged from two to five weeks of age. Two RT-PCR methods were conducted to partially amplify both segments, A and B. Phylogenetic analyses for both segments were performed by Neighbor-Joining method with 1000 bootstrap replicates using the MEGA software. The RT-PCR methods yielded amplicons of 698 bp for segment A and of 722 bp of segment B. The sequence analysis of the hypervariable region of the VP2 gene showed that all the viruses were genetically related to Delaware E (genotype 2a) and T1 strain (genotype 2b), with genetic similarities ranging from 94% to 98%. Phylogenetic analysis of the segment A showed more sequence diversity in comparison with segment B. The generation of different branches in the phylogenetic tree of segment A indicate that current IBDV field strain had underwent significant genetic drift from the original Delaware E subtype detected in the mid 1980's. Antigenic index profiles of the hypervariable regions of VP2 were generated by the Jameson and Wolf algorithm and these profiles were similar to those observed with Delaware E and T1 strains.

Molecular Characterization of Infectious Bursal Disease Virus (IBDV) Strains Circulating in the Delmarva (DMV) Peninsula from 2019- 2023

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Infectious Bursal Disease Virus (IBDV) is a major cause of immunosuppression in chickens that can exacerbate secondary infections and lead to production losses. While the control of IBDV is based on diverse vaccination schedules, vaccination does not induce sterilizing immunity, meaning vaccinated flocks may harbor subclinical infections with field strains that can evolve by antigenic drift and/or reassortment, generating variants that hinder flock protection. An effective prevention strategy should, therefore, include molecular surveillance of contemporary field strains, to optimize antigen selection for use in vaccines. The Delmarva (DMV) Peninsula is a major poultry producing area of the USA, however, to our knowledge, the last published molecular epidemiological survey isolated viruses sixteen years ago, in 2007. In the present study, we aimed to update the molecular characterization of IBDV strains circulating in the DMV. To achieve this, bursal samples were obtained from broiler farms between 2019 and 2023, and subject to reverse-transcription polymerase chain reaction (RT-PCR) to amplify the IBDV hypervariable region (HVR), encoded by Segment A, and the polymerase, encoded by Segment B. Sanger sequencing was conducted on positive samples, and the sequences of Segment A were analyzed for the presence of antigenic drift mutations, while the sequences of Segment B were used to evaluate if reassortment had occurred. We observed no evidence of very virulent strains, or reassortant strains circulating in the DMV, or evidence of co-infection with multiple strains. We did, however, identify HVR mutations that were consistent with antigenic drift. Moreover, some of these mutations appeared to have increased in frequency in the viral population since 2007. However, these observations are based on a limited number of samples, and the results of a larger dataset will be presented, with more robust conclusions.

Introduction of Genogroup-Specific RT-qPCR Methodology for Rapid Biomolecular Typing of IBDV

Martin Liman, Henning Bischoff, Jennifer Haneke, Theresa Menke, Diana Petzoldt

AniCon Labor GmbH

Gumboro infections that are of clinical and economic relevance are constantlychallenging worldwide chicken production. Thus, screening and direct pathotyping viaRT-qPCR as well as sequencing and phylogenetic analysis are daily routinemethodologies applied in veterinary services. This step-by-step approach gives basisfor reassessment of choice of live or recombinant vaccines applied while ensuring mosteconomic service.However, the RT-qPCR for direct pathotyping, i.e. differentiation of “very virulent vs.avirulent vaccine strains”, resulted in increasing cases of unsatisfying outcomes. Thismay among other things be explained by regional evolution of IBDV. We decided toovercome this by implementation of RT-qPCRs that specifically detect IBDVgenogroups in accordance with the classification as suggested by ISLAM et al. 2021.So far, we established methods for

detection of VP2 genogroup A1 that includes thevast majority of live attenuated, registered vaccines applied, as well as of genogroupA3 that comprises the "very virulent" strains.Experiences and data derived from these new methods will be presented anddiscussed in context of detection and typing of live and also recombinant vaccinestrains as well as field strains including reassortants.Literature:Islam, M.R., M. Nooruzzaman, Md.T. Rahman, T.T. Mumu, M.M. Rahman, E.H.Chowdhury, N. Eterradossi and H. Müller. A unified genotypic classification ofinfectious bursal disease virus based on both genome segments. Avian Pathol. 50(2):190-206. 2021

Let's Talk About IBDV in Layers

Fernando Ruiz-Jimenez, Manuel Da Costa, Kalen Cookson, John Brown, Therese Anderson, Jon Schaeffer

Zoetis

Infectious Bursal Disease (IBD) is a highly infectious viral disease that affects young chickens and can cause significant immunosuppression. Routine monitoring programs are key to the control of this disease since they help determine which IBDV subtypes are most prevalent in each poultry-producing area, and upon that information, the best vaccination strategy can be implemented. Due to the nature of the layer industry, most of the egg-producing companies in the US do not own parent stock birds so they have limited control over the breeder IBDV program. This underscores the importance of a well-designed and targeted vaccination program for commercial pullets, which can rely on recombinant vaccines, live vaccines, or a combination of both. The objectives of this presentation are to share some of the latest protection studies of a recombinant HVT-IBD vaccine against an early IBDV challenge in Leghorn birds, the methodology used to perform bursal surveys in commercial pullets, and a preliminary summary of the different strains of wild-type IBDV that have been found in layer surveys across the country.

Early Infection by Strains of Infectious Bursal Disease Virus Belonging to Genogroup 4 in Commercial Broiler Flocks in Brazil

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Infectious Bursal Disease Virus (IBDV) is a Birnavirus with worldwide prevalence throughout areas with high poultry density. It could be related to clinical signs and subclinical immune suppression that greatly affects welfare and productivity. In the last decade, several studies have demonstrated a high prevalence of strains from a distinct lineage of IBDV (dIBDV), belonging to Genogroup 4, even in vaccinated commercial flocks. The aim of this study was to carry out an epidemiological analysis in commercial broiler flocks in Brazil, with the objective of observing early infections by viral strains not represented in commercially available vaccines. Samples of Bursa of Fabricius (BF) were collected in thirty commercial broiler flocks of three different companies in Paraná State (the main broiler producing state in Brazil, responsible for 35% of national broiler stock and 40% of national broiler exports). Ten flocks of broilers aged between 18 and 23 days of age were randomly chosen from each of the three companies. Five BF samples were collected per flock, totaling 150. The samples were assessed in pools of five BF (one pool per flock). The samples were assessed by nested RT-PCR method

followed by RFLP based on a fragment of the hypervariable region of the vp2 protein. Samples were thus sorted into molecular groups according to their enzymatic digestion pattern. The three companies used vaccines applied in the hatchery but belonging to different technologies: rHVT-IBD vector vaccine (company A); immune complex W2512 strain (company B); and MB strain live vaccine (company C). From thirty samples, ten (33.3%) were negative for IBDV; ten (33.3%) were positive for dIBDV; in seven (23.3%) the MB vaccine strain was detected and three (10%) were positive for the vector vaccine strain. Evaluation by company, showed that the samples from company A had six (60%) negative samples for IBDV and four (40%) positive for dIBDV. In company B, five (50%) samples were positive for dIBDV, three (30%) were positive for vector vaccine strain and two (20%) were negative for IBDV. In company C, seven (70%) samples were positive for the MB vaccine strain, two (20%) samples were negative, and in one sample (10%) dIBDV strain was detected. These results suggest the capacity for early infection by viral strains of Genogroup 4 (dIBDV) present in Paraná, Brazil, possibly due to the immune gap between the decline of maternal immunity and the onset of active immunity. However, further studies are needed to confirm this hypothesis, blocking other variables that may interfere with the results, such as infection pressure, heterologous protection levels and maternal immunity.

Effect of Seroconversion to Infectious Bursal Disease on Physiological and Biochemical Paramaters of Broilers

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A database containing serological and biochemical data was datamined to see the effect of seroconversion to infectious bursal disease on physiological parameters. Data from broilers older than 21 days of age to avoid the interference from maternal antibodies were divided into two groups according to their infectious bursal disease titers on the Iddex IBD system. The negative group for birds with titers< 500 and positive birds with titers > than 500. The birds were divided into three age groups. Group 1 (21-24 days), groupe 2 (25-30 days) and group 3 (31-42days). Birds with antibody levels >500 were more likely to be in acute respiratory acidosis than birds with titers< 500 that were more likely to be in compensated respiratory acidosis. Significant differences were observed for Na, Cl, TCO2, Hct, Anion Gap, pH pO2, Beecf, HCO3, SO2, Lactate, AST, Uric acid, total calcium total protein, Globulins, and albumin to globulin ratios. Birds with titers >500 for IBD also had significantly higher titers for Reovirus and Bronchitis. This analysis indicates that seroconversion against classical VP3 Elisa can have an effect on biochemical markers. Many of these markers are associated with muscle deffects. Some of these markers are associated with the ability to compensate against respiratory acidosis. Co infections with reovirus and bronchitis could also play a role. Methods to aleviate these changes through sanitation and les reactive vaccines should be investigated.

AAAP Posters

Antimicrobial

Safety of a Fixed Antimicrobial Coating Alone or in Combination with Other Antimicrobials on Commercial Fertile Eggs.

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In this study we evaluated the safety of spraying commercial broiler eggs with a fixed antimicrobial (Armatrex™) alone or in combination with antimicrobials commonly used in commercial hatcheries. A total of 3,240 fertile eggs were equally divided in 6 treatment groups. Five treatment groups were electrostatically sprayed with Armatrex, Armatrex/QUAT 200 ppm, Armatrex/QUAT 400 ppm, Armatrex/Hydrogen Peroxide 3% and Armatrex/Hydrogen Peroxide 3%/Peracetic Acid 96 ppm. The remaining group remained as a non-treated control group.Soon after electrostatic administration, trays with eggs from all the treatment groups were randomly distributed and incubated following standard commercial procedures. Twenty percent of the eggs were numbered (mixed across all treatments) and weighed at day 1 of incubation and at transfer to determine the percentage moisture loss. Egg shell water vapor conductance was evaluated in three eggs per treatment. Egg residues were analyzed to determine hatchability, early/middle/late dead, pips and culls.At hatch, average chick weight, yolk weight and yolk free body mass were determined. When compared with the control group, no adverse effects on water vapor conductance, moisture loss, hatchability or chick quality were observed. Armatrex alone or in combination with other antimicrobials can be safely used in the presence of commercial breeder eggs.

Equisul-SDT Dose Variance Study in Turkeys

Adam Mueller[1], Nathan Winkelman[1], Grant Weaver[2], Nolan Wester[1], Travis Sobania[1]

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The purpose of the study was threefold: 1.Evaluate plasma sulfadiazine/trimethoprim levels resulting from varying oral doses of Equisul-SDT® (sulfadiazine/trimethoprim) in turkeys.2.Provide information that shows the dosage necessary to achieve plasma levels equal to or above the MIC for different poultry pathogens.3.Monitor the period post-treatment at which the drug was no longer present in plasma.

TiaGard (tiamulin) Dose Variance Study in Turkeys

Adam Mueller[1], Nathan Winkelman[1], Grant Weaver[2], Nolan Wester[1], Travis Sobania[1]

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The purpose of the study was threefold:1. Evaluate plasma tiamulin levels resulting from varying oral doses of TiaGardTM 12.5% (tiamulin hydrogen fumarate) in turkeys.2. Provide information that shows the dosage necessary to achieve plasma levels equal to or above the MIC for different poultry pathogens.3. Monitor the period post-treatment at which the drug was no longer present in plasma.

Antimicrobial Usage in Broiler Chicken Production in the United States, 2013 – 2021

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Although efforts to improve antimicrobial stewardship should include the collection of antimicrobial use data, most antimicrobial datasets collected at the national level consist of antimicrobial sales data which cannot inform stewardship. These data lack context, such as information regarding target species, disease indication, and regimen specifics like dose, route and duration. Therefore, the goal of this study was to develop a system for collecting data on the use of antimicrobials in the U.S. broiler chicken industry. This study utilized a public-private partnership to enable collection and protection of sensitive data from an extremely large industry while releasing deidentified and aggregated information regarding the details of antimicrobial use on U.S. broiler chicken farms over time. Participation was voluntary. Data were collected for the period 2013 through 2021 and are reported on a calendar year basis. Using production statistics from USDA:NASS as a denominator, the data supplied by participating companies represented approximately 82.1% of broiler chicken production in the U.S. in 2013, approximately 88.6% in 2017, and approximately 85.0% in 2021. The data that were submitted for 2021 are based on approximately 7,826,121,178 chickens slaughtered and 50,550,817,859 pounds liveweight produced. Granular flock-level treatment records were available for 75-90% of the birds represented in the 2018-2021 dataset. There was no use of antimicrobials in the hatchery for the years 2020 and 2021. Medically important in-feed antimicrobial use decreased substantially, with all in-feed tetracycline use being eliminated by 2020, and the use of virginiamycin being reduced by more than 97% since 2013. Medically important water-soluble antimicrobials are used for the treatment of disease in broiler production. Use decreased substantially for most water-soluble antimicrobials. The most important diseases necessitating treatment were necrotic enteritis and gangrenous dermatitis as well as E. coli-related disease. A focus on reducing the incidence of these diseases would reduce the need for antimicrobial therapy but will require an investment in research to find efficacious and cost-effective interventions for these diseases.

On-Farm Monitoring of Antimicrobial Use and Resistance in U.S. Broiler Production: 2020-2022

Randall Singer, Jennifer Staffenhagen, Iteeshree Mohapatra

University of Minnesota

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; between one and five complexes were selected, with the number roughly proportional to company size. During each 3-month interval, each complex selected 4-8 farms for sampling, with one house on each farm being sampled. Litter samples were cultured for Salmonella, Campylobacter, E. coli and Enterococcus. Salmonella isolates were serotyped, Campylobacter and Enterococcus isolates were speciated, and antimicrobial susceptibility testing was performed with microbroth dilution. AMU data were recorded for every sampled flock. Even with COVID-19 and

HPAI constraints, 346, 356 and 364 farms were sampled in 2020, 2021 and 2022, respectively. Prevalence of farms raising animals without antimicrobials was 48.6, 55.3 and 58.2% in 2020, 2021 and 2022, respectively. Farm level prevalence of Salmonella and Campylobacter was 323/964 (33.5%) and 330/964 (34.2%), respectively, with Salmonella-positive flocks being associated with Campylobacter-positive flocks (OR: 1.80, 95% CI: 1.2-2.7). S. Kentucky and S. Infantis were the most common serotypes identified. Most S. Kentucky isolates were pan-susceptible or resistant to tetracycline (TET) whereas most S. Infantis isolates were multidrug resistant. Most Campylobacter isolates were C. jejuni, and most were pan-susceptible or resistant to TET only; approximately one-third had resistance to ciprofloxacin. This program was designed for sustainable monitoring of on-farm AMU and AMR in the U.S. broiler chicken industry. Based on industry feedback, the program does not require an excessive time commitment, but changes have been made to the program over time to reduce the burden. To capture long-term associations between AMU and AMR, these datasets need to be collected in parallel at the farm level.

Minimum Inhibitory Concentration (MIC) of Mycoplasma spp Isolates Obtained from the South Indian Poultry Production Region

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Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) are two pathogens relevant to poultry production. They can be strategically controlled by biosecurity, vaccination, and treatment. Antibiotic stewardship principles demand careful observation of antimicrobial resistance development in food producing animals. To evaluate resistance profiles of poultry Mycoplasma, Minimum inhibitory concentration values (MIC) were obtained from MG and MS isolates from suspected poultry flocks in the region of South India. Eighteen isolates of MG (4) and MS (12) from commercial layers, breeders, broilers, and country chicken were evaluated for their susceptibility (MIC) to commonly used antibiotics against mycoplasma in poultry. MATERIALS AND METHOD Choanal cleft swabs were collected from suspected country birds, commercial layer, commercial broiler and broiler breeder birds which showed respiratory signs, eggshell apex abnormalities and leg weakness in 3 states of South India. For MIC evaluation the method described by Peter Hannan in 2000 with some modifications was used. RESULTSResults will be presented in table form.CONCLUSION The incidence of MS is higher (10.68%) than the incidence of MG (3.81%). It is concluded that in South India, Tylvalosin tartrate has the lowest MIC value for MG and MS when compared to other antimicrobials used to treat Mycoplasma infections in India. This is in accordance with the findings of other researchers globally that report

similar observations concerning the lowest MIC values for Tylvalosin tartrate (Hussein et al., 2017, Tavio et al., 2017; Taiyari et al., 2021).

Avian Influenza

Protective Efficacy of Inactivated Trivalent-H5 Vaccine Against Challenge with Both HPAIV-H5N1 and -H5N8 Viruses in Turkey Poults Under Both Laboratory and Field Conditions

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The highly pathogenic Avian Influenza (HPAI) virus belonging to subtype H5 assumed endemicity in Egypt since its first appearance in 2006, leading to severe economic losses. Turkey is one of the most susceptible birds to HPAI virus which may lead up to 100% mortality. The aim of this work was to evaluate the protection efficacy of a commercial inactivated oil emulsion H5 vaccine (MEFLUVAC H5 plus8 vaccine at 7th days old) against challenge with HPAI-H5N1 clade 2.2.1.2 and -H5N8 clade 2.3.4.4b in turkeys raised under field and experimental conditions. The study involved 10,000 white turkey poults (1-day old) that were imported from France (free from H5 antibodies since their breeder flocks did not receive any H5 vaccine nor got exposed to infection). The birds were divided into 8 groups(G), all birds in G1, 3, 4, 6, 7, and 8 contained 15 poults each and were similarly kept under BL-3 conditions. Birds in G1, G2, G4 and G5 served as vaccinated challenged groups, while G3 and G6 were non-vaccinated challenged. Birds in G7 as vaccinated nonchallenged and G8, maintained as non-vaccinated non-challenged group. The challenge was applied at 28-days post vaccination (35 day of life) with either H5N1 or H5N8. The remaining 9910 vaccinated turkeys raised in a commercial farm, but at 32 days of age, 30 birds moved to laboratory and divided into G 2, 5 and monitored for 3-days, then infected with H5N1 and H5N8, respectively. Serum was collected from all birds at 14,21, 28, and 35 days old to determine the antibody levels using hemagglutination-inhibition (HI) test. Regarding mortality rates, the results indicated that lab vaccinated G1 and G4 showed 100% protection after both H5N1 and H5N8 challenge, while farm vaccinated G2 and G5 had 100% and 93.3% protection after H5N1 and H5N8 challenge, respectively. The non-vaccinated challenged birds showed 100% mortality within 3 DPC in both H5N1 or H5N8 challenged G3 and G6, respectively. in conclusion, The Trivalent H5 vaccine (MEFLUVAC H5) (containing H5N1 clade 2.2.1.1, H5N1 clade 2.2.1.2 and H5N8 clade 2.3.4.4) was Immunogenic by 2-3 weeks post-vaccination and develop detectable level of humeral immune response and the vaccine significantly reduce virus shedding on level of shedders and amount of shedding virus in both commercial kept and BSL-3 kept birds in addition to providing 100% protection against mortalities for birds kept under BSL-3 condition and 93.3% for birds kept under commercial condition and moved to BSL-3 on 72 hrs. prior to challenge. Future work still required to evaluate the protection at older ages as turkey kept for 90-120-day age.

Efficacy of Inactivated Multivalent AIV-H5 Vaccine Against Highly Pathogenic Avian Influenza Clade 2.3.4.4 Subtype H5N8 And H5N6

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The highly pathogenic avian influenza virus (HPAIV) is endemic in some south Asian countries include Vietnam and associated withs severe losses, recently the HPAIV-H5Nx clade 2.3.4.4 emerged with different subtypes as H5N8 and H5N6. The Vaccination strategy became the primary control measure used to minimize losses in the endemic countries. The aim of this work is to evaluate the efficacy of MEFLUVACTM H5 PLUS 8 vaccine (a trivalent inactivated Avian influenza vaccine; contain clade H5N1 2.2.1.1, H5N1 clade 2.2.1.2 and H5N8 clade 2.3.4.4) in both single and prime-boost vaccination strategy in protection commercial chicks following challenge with either HPAIV-H5N6 or H5N8 (H5N8 clade 2.3.4.4b virus (A/ck/VN/HoaBinh/NCVD-21AD192/2021 and H5N6 clade 2.3.4.4g virus (A/ck/VN/BaRia-VungTau/NCVD-19A158/2019) under laboratory conditions. A total 150 of 3-week-old local chickens (Vietnam) were purchased from a farm with no Avian influenza (AI) vaccination history. Birds were checked for H5 antibodies and Avian influenza virus (AIV) (by Realtime RT-PCR tests randomly 10% birds) prior to the trial start with negative results for both tests. Birds were vaccinated the first shot at the age of 3 weeks-old with MEFLUVACTM H5 PLUS 8 following manufacturer instruction, at the age of 6 weeks-old (3 weeks after the 1st shot of the vaccine), all vaccinated birds were tested for H5 antibody titer with vaccine homologous antigens. Selected birds to the first challenged grouped randomly and the remaining chickens were given a 2nd shot of vaccine and kept for next challenge at 9-weeks-old (3 weeks post 2nd shot). HPAI challenge viruses were propagated in embryonated eggs and titrated in chick embryo fibroblasts cell culture (CEF). Viruses were prepared at a dose of 107 TCID50/ml and inoculated at 0.1 ml/bird through nasal route. Sera collected on weekly basis and tested with challenge-virus antigens; Oro-pharyngeal swabs were collected at day 3 and 10 or day-of-death after challenge for H5 RT-qPCR for detection virus shedding. The serological results showed that showed that MEFLUVACTM H5 PLUS 8 inactivated vaccine stimulated a strong immune response with geometric mean titer (GMT) of 6.6 log2 in single shot groups and 8.9 log2 in double shots groups. The results of survival rate showed that the single shoot provide 90% protection against mortalities when challenged 3-weeks post vaccination with either H5N6 or H5N8 while applying double shots give 90% and 100% against challenge with H5N8 and H5N6 respectively with significant reduction of virus shedding on term of number of shedders, amount of shedding virus or shedding interval. In conclusion applying single shot or double vaccination strategy with clade homologous vaccine can provide optimum protection in endemic areas and significantly reduce virus shedding thus support the effort to control the HPAIV with vaccination strategy in endemic areas.

Feather Tropism of H5 Highly Pathogenic Avian Influenza Viruses in Naturally and Experimentally Infected Domestic Waterfowl

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The spread of highly pathogenic avian influenza viruses (HPAIV) clade 2.3.4.4b over the past few years has significantly damaged the poultry industry and wild bird's populations worldwide, following the culling of positive flocks and massive die-offs of endangered species. In addition, viral detection in mammals has started to be reported with an alarmingly increased frequency. The well-known pantropism of H5 HPAIVs, together with the host susceptibility, allows viral replication in a wide variety of tissues, influencing the clinico-pathological presentation and mortality rates. Over the course of the infection, large amounts of infectious viral particles can be released into the environment, following the involvement of the mucosal and glandular compartments of the oculo-nasal, respiratory and digestive systems. Feathers are the most abundant cutaneous annexes of birds, involved in a variety of physiological functions, such as flight, insulation and communication. It is known that, following hematogenous spread, H5 HPAIVs are able to access and replicate in well-vascularized, growing feathers of domestic waterfowl and Galliformes. However, whether feathers represent an innocent bystander or actively contribute to viral environmental spread, transmission and infection dynamics is still unclear. The present study investigates tissue tropism of HPAIVs in growing feathers obtained from naturally and experimentally-infected domestic waterfowl. For this purpose, a multidisciplinary approach, combining histological and ultrastructural pathology, in situ detection and molecular techniques, was used. In domestic ducks naturally infected with H5 HPAIVs, necro-inflammatory lesions of growing feathers were associated with widespread and highly frequent epithelial viral antigenic detection in barbs, barbules and supportive cells. In experimental infection, H5 HPAIV were detected in feathers by PCR, as early as 2 days post-challenge (dpc) and up to 14 dpc, with a peak of detection at 4 dpc. Ultrastructural analysis was consistent with the accumulation of viral particles on barbs and barbules and within inter-cellular spaces. In addition, dust obtained from H5 HPAIV positive farms and analyzed with double immunofluorescence, revealed colocalization of viral and feather antigens. Overall, these preliminary results suggest that H5 HPAIV infection in domestic ducks is characterized by a strong feather epitheliotropism and potential release of viral particles into the environment.

Highly Pathogenic Avian Influenza Outbreak, What to Expect

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The current HPAI outbreak is challenging good industry biosecurity measures, migrating birds pose a constant menace poultry owners. We collected environmental and oropharyngeal swabs in an infected turkey premise for four days after HPAI was diagnosed, the idea was to track viral decay and / or persistence. Viral load was assessed by RT-qPCR and virus viability by embryo inoculation. Higher viral loads by RT-qPCR were detected in birds either composted or non-composted, when these samples were assessed for viral viability, we found live virus in composted and non-composted birds until 3 days of diagnostic confirmation. In the environment live virus was only found 1 day after diagnostic confirmation. It is very important to act quickly in HPAI positive flocks, HPAI can remain alive in dead birds, reason why biosecurity remains important even after depopulation. While the virus can be detected in the environment usually gets inactivate quickly after eliminating.

Active Surveillance of Avian Influenza A Virus in Wild Birds from Poultry Feed Mills and Water Bodies from the Peruvian Coast

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Universidad Nacional Mayor de San Marcos

Highly pathogenic avian Influenza is a major threat for poultry worldwide. Here, our objective was to evaluate the presence of avian Influenza A virus in fecal samples from wild birds living in the proximity of poultry feed mill plants, and water samples from natural water bodies nearby commercial poultry farms. Between November 2022 and January 2023, we collected 342 fecal samples from wild birds, 200 fecal samples from Columbiformes, and 05 samples from water bodies from Lima and Arequipa, the major cities for poultry production. To date, 27 samples have resulted positive for avian influenza virus including species such as Peruvian Pelican (Pelecanus thagus), Franklin's gull (Leucophaeus pipixcan) and Oystercatcher (Haematopus ostralegus). Out of 05 water samples tested, 04 resulted positive for Influenza A. In the mean time, twelve fecal samples from Columbiformes have resulted negative for avian Influenza A. All isolates were processed for complete genome characterization to define the genetic background circulating in Peru and to reconstruct their genetic relationship with other isolates from America. Our results indicate that despite virus circulation in wild birds in the coast and water bodies, our preliminary results in Columbiformes and poultry feed mill might suggest that they do not constitute a major source of virus spread into the poultry farms. The authors would like to express their deepest gratitude to the Peruvian Poultry Association (APA) for its financial support.

Genome Characterization of Influenza A Virus from Clinical Cases of Domestic Birds Received at the Faculty of Veterinary Medicine in the Universidad Nacional Mayor de San Marcos, Peru

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Gina Castro-Sanguinetti, Juan More-Bayona,
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As part of diagnostic service, we received birds showing clinical signs compatible with highly pathogenic avian influenza A infection from 06 poultry farms (F1-F6), located in Lima and Ancash in the Peruvian coast. These samples were submitted to the Laboratory of Avian Pathology between November 2022 and January 2023. In total, we received seven 20-weeks-old laying hens (F1), six 33-weeks-old laying hens (F2), four 70-days-old death ducks (F3), fifteen nine-month-old quail (F4), eleven six-days-old and six five-months-old quails (F5) and a death hen received from a small farm (F6). A high mortality was reported in all cases. Necropsy findings ranged from lung edema and general congestion which were the most common lesions within the animals evaluated. We did not find hemorrhagic lesions in intestinal tract lymphoid aggregates. Lungs, trachea, and brains were tested by real time RT-PCR, then, positive samples were inoculated into embryonated chicken eggs for viral isolation. Isolates were processed for sequencing of eight complete genome segments using a metagenomics approach. Our findings reveal the circulation of highly pathogenic avian influenza A virus in the Peruvian coast and representing a threat for the poultry industry in Peru.

Serological Cross Reactivity from Experimentally Vaccinated Birds Against the Clade 2.3.4.4b Highly Pathogenic Avian Influenza Virus

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Avian influenza (AI) is a highly contagious, agriculturally relevant disease that has spread globally to most countries. In the U.S. during 2014-2015 and again in 2021-2022, outbreaks of highly pathogenic AI virus (HPAIV) have resulted in the death and culling of over 50 million birds each, costing billions of dollars to the industry and economy. Currently, control strategies for poultry against HPAIV are dependent on biosecurity and vaccine application, which is not currently approved for use in U.S. During 2014-215, the H5Nx HPAIV belonged to 2.3.4.4c clade of the A/goose/Guangdong/1/1996 (Gs/Gd) H5N1 clade 0 lineage, while in 2021-current, the viruses belong to clade 2.3.4.4b. At the amino acid level, the 2.3.4.4b and c viruses share approximately 98 % sequence similarity of hemagglutinin (HA) protein. Compared to the original Gs/Gd virus, the clade 2.3.4.4 viruses share approximately 95% sequence similarity. In these studies, we examined the antigenic relatedness based on serological cross reactivity using the hemagglutinin-inhibition (HI) assay. Birds were vaccinated with various H5-based inactivated or recombinant vaccines (85-98% relatedness to HA) and challenged against the 2.3.4.4c lineage viruses. Serum was tested against homologous vaccine virus or the 2.3.4.4b and c viruses. As expected, birds vaccinated with inactivated 2.3.4.4c viruses maintained the highest level of cross reactive HI titers to the 2.3.4.4b viruses, with an average of 1log2 drop in titer using prechallenge serum. When birds in these groups were challenged with 2.3.4.4b

HPAIV, the serological cross reactivity was near 100% in titer. Conversely, when birds received non-Gs/Gd lineage H5 vaccine, the serum titers were generally 7log2 lower against either of the clade 2.3.4.4 viruses. Serum from birds that received vaccination with a clade 2.3.2 vaccine demonstrated reduced titers of approximately 4-5log2 titers. Taken together there appears to be limited serum cross reactivity from birds not vaccinated with 2.3.4.4 antigen, further strengthening the concept of matching the vaccine antigen to the field strain.

Nanopore Sequencing as a Rapid Tool for Identification and Pathotyping of Avian Influenza Viruses

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Oxford Nanopore Technologies

Several publications (Yip et. al., 2020; Crossley et. al. 2021; and King et al., 2022) have demonstrated the ability to use Nanopore sequencing for the detection of Avian Influenza. Moreover, the ability to obtain the read-out of Nanopore sequencing in real-time shortens the turnaround time to 24-48 hours including the initial PCR.The portability and use of Nanopore devices in the field have been demonstrated including Dr. Meagan Dewar's avian influenza surveillance in Antarctica. Several other field-deployable applications will be reviewed including using skim-seq for assessing genomic breeding values and microbiome analysis to determine herd fertility.Lastly, we will review the last capabilities of Nanopore sequencing including Q20+ chemistry and the ability to target specific sequences in real time and sample recovery and reuse.

Characterization of Immune Escape Mutants of H7 LPAIV Selected by Homologous and Heterologous Sera.

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Vaccination is an effective way of preventing a AIV outbreak. However, the use of vaccine cannot guaran tee complete protection. The incomplete protection can generate immune escape mutants, which leads to an emergence of variants. Therefore, understanding the mutations derived by immune pressure is crucial in preventing future variants and designing a vaccine program. In this study, to model the antibody escape mutants in laboratory, A/turkey/New York/4450/1994 H7N2 low pathogenic avian influenza virus was treated with homologous and heterologous polyclonal sera, and passaged in embryonated chicken eggs for several passages. Heterologous polyclonal sera were collected from chickens vaccinated with other H7 isolates. The titers of the sera were determined to make 1000-fold drops in the viral titer. To keep the serum pressure constant, serum titers were periodically increased to give 1000-fold reduction in viral titer. Viral RNA was extracted and sequenced to observe mutations in nucleotides and amino acids of HA genes. The antigenic distances between parental virus and escape mutants were determined using hemagglutination inhibition assay. This method of selecting escaping mutants using homologous and heterologous sera represents the immune

escape mutations made by vaccination-induced immunity. Thus, it helps predict mutations derived by vaccination and demonstrate different mutations made by homologous and heterologous vaccination.

Blue-Winged Teals in Guatemala and Their Potential Role in the Ecology of H14 Subtype Influenza A Viruses

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Influenza A viruses (FLUAVs) are divided into subtypes based on the antigenic properties of the two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Wild aquatic birds are considered the natural hosts of 16 HA (H1-H16) and 9 NA (N1-N9) subtypes found in different combinations. The H14 subtype was initially detected in 1982 in the former Soviet Union. After no further detections for almost three decades, H14 viruses surprisingly appeared in the US in 2010. Since 2011, H14 FLUAVs have been consistently detected in Guatemala, leading to the largest collection of this subtype from a single country. Globally, 62 H14 FLUAVs have been reported, including viruses from Guatemala (n=40), North America (n=14), and Eurasia (n=8). All H14 FLUAVs in Guatemala were detected from blue-winged teals and were mostly associated with the N3 subtype (n=25) whereas the rest were paired with either N4 (n=7), N5 (n=4), N6 (n=1), and mixed infections (N3/N5 n=2, and N2/N3 n=1). Using whole-genome sequence data from 17 new H14 FLUAVs from Guatemala that were identified from 2014 to 2019, we demonstrate that the H14 FLUAVs in Guatemala are not monophyletic and belong to a distinct H14 lineage in the Americas that is evolving independently from the Eurasian H14 lineage, including in the HA cleavage site. How H14 viruses persist in the Western hemisphere remains unknown, but it appears that Guatemala has an outsized role in the subtype's persistence in the Americas. Of note, the ORF of the H14 HA segment showed three distinct motifs at the cleavage site, two of these containing Arginine instead of Lysine in the first and fourth positions, not previously described in other H14 isolates. The effects of these mutations on virus replication, virulence, and/or transmission remain unknown and warrant further studies.

Pathogenicity of four genetically distinct H5N1 HPAI clade 2.3.4.4b viruses in chickens

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Highly pathogenic avian influenza (HPAI) subtype H5N1 Gs/GD lineage clade 2.3.4.4b viruses are a major threat to poultry and are spread by wild birds. In December 2021, the virus was detected in the United States in wild waterfowl, and since then, the virus has spread to many wild and domestic birds. The initial H5N1 HPAI viruses reassorted many times with North American avian influenza viruses found in wild birds. The reassortment in the PB2, PB1, PA, NP, and NS genes generated more than 20 gene constellations, of which the initial H5N1 virus (G1) and the resulting three major reassortants (G2-G4) caused most of the cases in wild birds and poultry through at least mid-2022. To determine if genetic changes influence disease presentation, we evaluated the infectivity, pathogenicity, and transmission of one of the early H5N1 HPAI viruses (G-1) and three of the

most common reassortant viruses (G2, G3 and G4). Birds were inoculated with a low, medium, or high dose of each the viruses and non-inoculated hatch mates were added to each dose group after 24hr to examine transmission. Although inoculated birds died in less than 2 days, we found that the mean bird infective dose (BID50) differed among the genotypes: 2.8, 3.3, 5.0 and 3.0 log10 50% egg infectious doses (EID50). Transmission to direct contact birds was observed only with G-1 and G-4 when given at the high dose. These results are important for understanding the pathobiology of these H5N1 HPAI viruses for surveillance and control purposes.

Mapping HPAI: An Illustration of the 2022 Outbreak in the United States

Nicki Smith

Georgia Poultry Laboratory Network

The 2022 outbreak of HPAI in wildlife and poultry has had an unprecedented impact on the US, affecting nearly every state. Throughout 2022, GPLN routinely produced maps of HPAI detections for Georgia's poultry industry and other stakeholders for the purposes of educational outreach. Now over a year into the outbreak, this project seeks to illustrate the scale and geographic distribution of detections through thematic mapping.

A Field Perspective on the Impact of Low Pathogenic Avian Influenza Virus on Turkey Breeders and Vaccine Approaches

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Avian influenza virus infections have a long history of causing disease in commercial poultry operations. Highly pathogenic avian influenza (HPAI) has been at the forefront of attention after causing devastating losses to the US poultry industry in 2014/15 and again recently in the 2022 outbreak. With most of the focus on HPAI, one can easily dismiss the impacts of low pathogenic avian influenza (LPAI) viruses on turkeys. LPAI viruses such as H1N2 and H6N1 cause significant egg production losses and in some instances increased mortality in flocks. The impacts of LPAI viruses will be described from previous field outbreaks along with a review of the current vaccine techniques used in turkey breeders. In addition, a novel DNA vaccine platform is currently being researched as a potential solution to some of the challenges with current vaccine options against LPAI viruses.

Bacteriology

Deconstruction of a Multi-strain Bacillus-Based Probiotic Used for Poultry: an in Vitro Assessment of its Individual Components Against C. perfringens

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Probiotics have been used in poultry production to improve the performance and health of chickens in the antibiotic-free production era. The combination of different probiotic strains has been used with the hope of conferring multiple benefits to the host. However, the inclusion of several strains does not necessarily boost benefits, and few studies have compared the efficacy of multi-strain probiotics with their individual components. The objectives of this preliminary study were to evaluate the effects of a multi-strain Bacillus-based probiotic product containing B. coagulans (BC), B. licheniformis (BL), B. pumilus (BP), and B. subtilis (BS) against Clostridium perfringens (CP) in vitro, as well as the individual strains and different combinations of the strains used in the product. A co-culture method was used to determine the effect of the probiotics on C. perfringens counts. Three co-cultures were conducted in triplicates: 1. Probiotic mix (BS, BC, BL, and BP) + C. perfringens; 2. Individual Bacillus spp. strains (BS, BC, BL or BP) + C. perfringens; 3. B. subtilis + Bacillus spp. (BC, BL or BP) + C. perfringens. The probiotic product mix (Co-culture 1) tested in this study did not reduce C. perfringens concentration (Co-culture: 2.5 x 107 cfu/mL; Control (CP only): 1.2 x 107 cfu/mL) (P = 0.499). When tested individually (Co-culture 2), B. subtilis was the most efficient strain to decrease C. perfringens concentration (Co-culture: 6 x 102 cfu/mL; Control (CP only): 5 x 108 cfu/mL) (P ≤ 0.01). The combination of the other Bacillus spp. strains with B. subtilis (Co-culture 3) significantly decreased the efficacy against C. perfringens (BS only: 5 x 102 cfu/mL; BS+BL: 2 x 104 cfu/mL; BS+BP: 1.1 x 107 cfu/mL; BS+BC: 4.3 x 106 cfu/mL; Control (CP only): 4.3 x 107 cfu/mL). We concluded that the combination of B. coagulans, B. licheniformis, B. pumilus and B subtilis is not effective in decreasing C. perfringens concentrations in vitro. B. subtilis alone and B. subtilis combined with B. licheniformis had the highest efficacy against C. perfringens. In vivo experiments need to be conducted to evaluate the efficacy of the proposed combinations of Bacillus spp. in controlling C. perfringens.

Core Genome Multilocus Sequence Typing (cgMLST) for Poultry Bacterial Pathogens Typing: The Future is Now

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Next Generation Sequencing (NGS) has changed many aspects of our lives, and veterinary diagnostics is no exception. Whole genome sequences (WGS) and metagenomics sequencing data is becoming readily available and accessible, even for routine veterinary diagnostics. However, the challenge often is making sense of the massive data generated by these new technologies. Standardized genomic typing methods are needed to address the challenge assessing the relatedness of bacterial isolates based on WGS. Core Genome Multilocus Sequence typing (cgMLST) is a standardized, scalable, sharable and reproducible sequence

typing assay that can be used to compare and determine the relatedness between bacterial WGS. Currently, as little as one to a few genes are used for bacterial sequence typing. Contrarily, and in order to increase the discriminatory power, cgMLST utilizes 50% – 70% of the total number of a bacterial species genes to more accurately reflect the evolutionary relatedness, especially between closely related isolates. However, these tests require development and validation for each bacterial species independently. This research team has developed and validated cgMLST assay for a number of poultry bacterial pathogens, including Mycoplasma gallisepticum, M. synoviae, M. iowae, Ornithobacterium rhinotracheale and Pasteurella multocida. Furthermore, cgMLST assays for additional bacterial poultry pathogens are currently under development. Databases that can be used for the analysis of cgMLST results are available online, which renders these typing assays accessible worldwide. The availability and accessibility of these assays will allow for a much better understanding of these pathogens' source of infection and transmission patterns, which in turn will allow for more effective disease prevention, control and eradication. The purpose of this presentation is to introduce these assays and describe examples of their use and application in poultry medicine and to empower poultry veterinarians to benefit from current and future technological breakthroughs.

Bacterial Diversity and Characterization of Staphylococcus xylosus Isolated from Layer Chicken Barn Bioaerosols in Alberta, Canada

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The quality of poultry barn bioaerosols determines the health of birds and poultry workers. Poultry barn bioaerosols contain bacterial components in addition to other microbes which could induce an inflammatory response in trachea. The study objective is to investigate the taxonomical classification of bacterial pathogens and characterize\Staphylococcus xyloses from layer barns in Alberta. A total of 18 barns in Alberta will be sampled. The XMX-CV air sampler will be used to collect air samples. Samples will be plated on tryptic soy agar to quantify total viable count and plated on mannitol salt agar for isolation of S. xylosus. Three presumptive colonies will be randomly chosen from the mannitol salt agar and further identified by Staph API strips. Samples will be submitted to University of Montreal for whole genome sequencing and 16S community analysis targeting all hypervariable regions. Bioinformatics and comparative genomics will be used to assess antimicrobial resistance genes and bacterial diversity within and between different types of layer management systems. Two barns have been sampled thus far, and S. xylosus were isolated and confirmed from both. We expect that bacterial diversity will vary based on type of housing and management system.

Antimicrobial Susceptibility Profile and Genetic Characterization of Ornithobacterium rhinotracheale Isolates from Commercial Poultry in the United States

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Ornithobacterium rhinotracheale (ORT) infections are a major concern in poultry worldwide, causing respiratory diseases, growth retardation, and high mortality. Despite the increasing trend of antimicrobial-resistant ORT isolates reported in the United States, there remains a knowledge gap on the current levels of resistance and the relationship between resistance phenotype and genetic predictions using whole genome sequence. This study aimed to fill this gap by determining the antimicrobial resistance profile and genetic relationship of 28 ORT isolates collected from commercial poultry in the United States between 1997 and 2020. The whole genome sequence of the isolates was generated and their susceptibility to 11 antimicrobials was tested. Results showed that all isolates were multi-drug resistant, with resistance to aminoglycosides, chloramphenicol, and colistin, and some isolates showed susceptibility to ciprofloxacin, nalidixic acid, tetracycline, and tylosin. The isolates were classified into three Sequence Types (STs) and all carried the tetQ gene, though this gene was found in tetracycline-susceptible isolates as well. Only three virulence genes were detected in all isolates. Phylogenomic analysis using the maximum likelihood method revealed close relationships among isolates belonging to the same ST, with four independent clades identified when compared to available ORT genomes from GenBank. Our results indicate a significant increase in antimicrobial-resistant ORT isolates from commercial poultry in four states in the United States, and highlight the need for further investigations to improve the accuracy of genotypic predictions of resistance profiles in ORT.

A New Solution for Managing Fowl Cholera

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Fowl cholera can present as an acute or chronic bacterial infection of poultry. The causative organism for fowl cholera is Pasteurella multocida, a gram-negative bacterium. The disease is of economic significance for most poultry types, with control mainly reliant on biosecurity measures and vaccination programs. Current methods of preventing the disease in broiler breeders is either by live attenuated vaccines or inactivated vaccines. The current study evaluated two autogenous antigen presentations ('Standard' and EASE©) combined with one of three adjuvant formulations to assess protection against a serotype 1 (X-73) homologous challenge and generated humoral responses. The study consisted of 7 different treatment groups utilizing 50 birds per group. Birds were administered two vaccinations at a 0.5ml/dose via intramuscular (IM) injection into the right side of the breast with 14-day intervals. Birds were challenged with 2000 CFU per bird of Pasteurella multocida serotype 1 (X-73) via IM inoculation 21-days post 2nd vaccination, with termination 14 days post challenge. Positive birds were determined by isolation from collected swabs during the study. The results of this study showed all vaccine formulations reduced the number of positive birds when compared to the control group. These results indicated a greater reduction in the number of positive birds (by culture) for vaccine formulations with EASE© compared to 'standard' produced antigen formulations. This reduction is further enhanced when EASE© antigens are formulated with specific adjuvants.

In conclusion, EASE© produced antigen in combination with polymer-based adjuvants show a significant reduction of fowl cholera in broilers breeders. Key words:Fowl Cholera, Pasteurella multocida Serotype-1 (X-73), Autogenous antigen, EASE©, Polymer-based adjuvants, broiler breeders

Extracellular Membrane Vesicles from Clostridium perfringens as a Vaccine Strategy Against Necrotic Enteritis

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Necrotic enteritis in broiler chickens is characterized by increased mortality and severe necrosis of the intestinal mucosa. This economically significant disease is caused by the Gram-positive bacteria Clostridium perfringens and is commonly controlled by antimicrobial feed additives. However, because of the reduction in antimicrobial use observed worldwide in the broiler industry, new alternatives must be found to prevent necrotic enteritis. Over the past few years, it has been shown that most Gram-positive bacteria can shed extracellular vesicles (EVs) mostly during exponential phase cell growth. Observed EVs are spherical structures with an average diameter of 20 to 400 nm. Their membranes contain phospholipids mixed with proteins, and the vesicular lumen may contain various compounds such as proteins, RNA, DNA, and peptidoglycans. These EVs have also been demonstrated to be immunogenic and protective against various bacterial diseases. However, very little is known regarding the composition of Gram-positive Clostridium perfringens EVs and their interactions with the innate and mucosal immune systems in chickens. Here, EVs from Clostridium perfringens have been produced, biochemically and proteomically characterized, and their immunomodulatory effects on chickens will be explored in a necrotic enteritis challenge as our final objective. Interestingly, EVs from a pathogenic Clostridium perfringens strain are released into the medium as 100 to 200 nm nanoparticles containing many cytosolic, membrane and surface-associated proteins. Our findings identified proteins with a cytoplasmic origin, primarily oxidoreductases, precursors for various metabolic process, ligases, as predominant in the EVs content. Interestingly, the presence of a toxin such as perfringolysin O could be demonstrated, an observation which confirms that these vesicles can also act as carriers for virulence factors. Overall, this study provides insights into the biology of an important Gram-positive pathogen and the role of extracellular vesicles as a preventive tool.

Diagnostic Approach for Identifying Avian Pathogenic E. coli (APEC) based on Molecular Serology and Virulence Characterization from the State of Georgia Isolates

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Avian pathogenic E. coli (APEC) has been identified and described as the primary etiologic agent of avian colibacillosis causing localized and systemic infections. Though, there are diverse strains and isolates of E. coli which remain a problem for poultry producers and consumers including public health professionals. However, because of the high diversity of APEC strains, verifying the accuracy of these predictors is difficult, which impedes the proper diagnosis, treatment, and prevention of E. coli infections in poultry. In this study, we identified the O-types (serogroups) pattern, and the prevalence of

virulence genes that typify the APEC pathotype. A total of 135 isolates were tested from poultry submitted for analysis of virulence associated genes and molecular serology. These isolates originated from birds identified as broilers, broiler breeders, pet/hobby birds and broiler breeder pullet/cockerel. Approximately 38% of APEC isolates could not be O-typed; of the remaining 62% (typeable) the most prevalent O types detected included O25 (32%), O62, O68 (24%), O78 (20%), followed by O86 (13%), O1 (10%), O88 (8%), O8 (6%), O65 (5%), O91 (5%) and O152 (3%). The serogroups, O104 and O155 were detected in less than 2% of isolates. As a result, many different serogroups such as O78, O25 and O62, O68 might include pathogenic strains harboring virulence genes associated with pathogenicity. This explains why these serogroups are more prevalent in studies investigating APEC serogroups. When examined by organ/tissue source, for broiler, pet hobby birds and broiler breeder the most common sources of APEC were the liver, body cavity, yolk sac and heart. A lower prevalence (2%) was associated with lungs, air sac, joints and peritoneum. A multiplex PCR panel targeting nine virulence genes was used to screen the 135 avian E. coli isolates. From this analysis 6 genes are plasmid associated, while two other genes are associated with the chromosome. Our data found APEC virulence genes ≥4 in 70% of all isolates and were most commonly found in broiler breeder pullet/cockerel birds and broilers, while the pet hobby birds (5%) were less associated with pathogenic APEC strains. Among virulence genes, iroN, iutA, iss, ompT, and hlyF had the highest prevalence. Identifying highly virulent APEC is critical in reducing the threshold for for opportunistic disease to occur in the bird and use of molecular characterization can be valuable in determining the presence of these strains. This data demonstrates the diversity of APEC causing significant losses to the poultry industry in Georgia and also remains an important concern for public health professionals. These findings provide a reference basis for future research on the pathogenesis of APEC as well as to develop effective intervention strategies in the prevention and control of APEC in the state of Georgia.

Complete Genome Sequences of Two Non-typical Avibacterium Paragallinarum Strains Isolated from Clinically Normal Chicken Flocks

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Avibacterium paragallinarum (AP) is the etiology of infectious coryza (IC), which is an economically significant bacterial respiratory disease in chickens. Recently, multiple US states reported a sharp increase in the incidence of IC. Concurrently, positive real-time PCR results were reported from several layer flocks without any clinical signs, leading to notable confusion in diagnosis. The objective of this study was to sequence and annotate the complete genome sequence of two AP isolates obtained from two clinically normal layer flocks (referred to as non-typical AP) and make it available on NCBI Database (GenBank). The complete closed genomes of two isolates were generated using the Illumina-Nanopore sequencing approach. General genomic features of the genomes, including GC%, ANI score and dDDH, confirmed their classification as AP. However, the genomic analysis showed meaningful differences from typical AP. These differences included the absence of capsular polysaccharides D (ctrD) virulence gene, major differences in the

sequences of hemagglutinin antigen (hmtp210) gene and the presence of a catalase (katA) gene only in isolate 2. The generation of complete genome sequences from these isolates will contribute to a better understanding of this avian pathogen and its pathogenicity to chickens as well as enhance IC diagnostics.

Genome Comparison of Campylobacter Hepaticus Isolated in Georgia, USA

Julia Ienes-Lima, Roel Becerra, Catherine M. Logue

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Campylobacter hepaticus has been identified as a causative agent of Spotty Liver Disease (SLD), characterized by multiple hepatic lesions, mortality, and reduced egg production. C. hepaticus has many properties of the other members of the genus, which allow it to colonize any tissue besides the liver and gall bladder. However, since C. hepaticus was recently identified, there remains a lack of information regarding virulence and pathogenicity of this bacterial species. This study aimed to characterize five C. hepaticus genomes isolated from the bile of laying birds in Georgia, USA. Methods: All isolates sequenced were recovered from the bile of Lohman Brown hens diagnosed with SLD from two different flocks and farms. The bile samples were plated on blood agar and incubated at 37C and 42 for seven days, and the isolates were confirmed as C. hepaticus using PCR targeting the glycerol kinase gene. Bacterial DNA was extracted using the MagAttract kit and sequenced on a PacBio Sequel II after genomic library construction. Reads were assembled with Canu, trimmed and circularized with Circlator, followed by polish using Pilon. The circularized genomes were rotated to the default starting gene, dnaA. Annotation used the NCBI Prokaryotic Annotation Pipeline and RAST. Virulence genes were identified using the Virulence Factor Database and ABRicate. Phylogenetic analysis were performed with CSI Phylogeny. Results: Genomic analysis found genome sizes of C. hepaticus had an average of 1,548,924 Mb with a GC content of 28.04% and approximately 293 sub-systems, 1,520 coding sequences, and 54-60 RNAs identified. No plasmids were detected in 4 genomes, while a pTet type plasmid was detected in one. The plasmid harbors genes related to the tetracycline-resistance gene and cytotoxin-associated gene- pathogenicity island (cagPAI), which encodes the Type IV Secretion System. Genes related to bacteriocin production and multidrug resistance efflux pump (CmeABC operon) were identified in the chromosome of C. hepaticus isolates. In addition, genes involved in resistance to fluoroquinolones and heavy metals were also present in the genomes. In comparison with other Campylobacter species, C. hepaticus has a significantly reduced number of genes related to iron acquisition and virulence. Comparative analysis of the five genomes with other C. hepaticus genomes found >99.9% identity with the reference strain HV10 from Australia and two other genomes from the USA. In addition, the C. hepaticus genome size is about 0.17 Mb smaller than C. jejuni, with 144 less genes. The phylogenetic analysis indicated that C. hepaticus is closely related to C. fetus and C. bilis. Conclusion: Genome reduction is associated with loss of genes linked with iron and carbohydrate metabolism. The presence of the multidrug efflux pump CmeABC in all genomes may function as a means to negate the presence of bile salts in the poultry host, enhancing survival. These findings suggest that host and niche adaptation might be an evolutionary strategy of C. hepaticus. Our results provide new information regarding C. hepaticus pathogenicity and help to build an efficient

control and prevention strategy for his emerging pathogen.

The Isolation of Salmonella and the Corresponding Serotypes from Poultry

Doug Waltman

Georgia Poultry Laboratory Network

Monitoring for Salmonella, except for Pullorum and Gallinarum, has been done using environmental sampling and testing. Substantial data exists on the incidence of Salmonella and the serotypes present in poultry environmental samples. Occasionally, individual birds may be euthanized and sampled to confirm the environmental testing results (for example Enteritidis). There is much less data on Salmonella in birds than from the environment.This is a retrospective study of the isolation of Salmonella from birds and the respective serotypes from 2000 to through 2022. We analyzed 3 groups of birds: Pullorum reactors, birds from Salmonella Enteritidis positive environments, and diagnostic cases.

Case Report

First Report of Avian Encephalomyelitis Virus in Broiler Chicks in Morocco: A Case Report

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Avian encephalomyelitis (AE) is an infectious and contagious disease that affects poultry worldwide and is responsible for severe economic losses. The disease is caused by Tremovirus (AEV) of the picornaviridae family and affects several species in particular chickens, pheasants, quails, pigeons and turkeys. It is manifested in young birds by ataxia and rapid tremors and in adults by spawning losses. Clinically, after an incubation period of 2 to 7 days, symptoms appear in young birds, in case of vertical transmission, the embryonic mortality is noted during the last days of incubation of the eggs. After hatching, a high percentage of chicks show nervous clinical signs such as ataxia, incoordination, head tremor, flaccid paralysis and the mortality rate can reach 50%. In addition, the hatching rate also drops from 4 to 5%. Although vaccines are routinely used to control this disease, the problem still persists almost all over the world. In this presentation a typical case of AEV infection is reported for the first time in Moroccan broiler chickens according to clinical course, and results of histopathological and molecular investigations. Experimental design and methods. Two broiler flocks showed a very severe nervous clinical signs including tremors, drowsiness, locomotor incoordination, unstable gait (animals lose their

balance when trying to move), ataxia and paresis (lateral recumbency with abnormal position of the legs), associated with a drastic decrease in feed consumption and water intake. The age of the birds was 14 and 24 days and morbidity rates were up to 90% and mortality was very high and reached a rate over 25% in affected flocks. At necropsy of affected birds, no obvious macroscopic changes could be detected and samples including from the brain, cerebellum, and brain stem, myocardium, proventriculus, duodenum and pancreas were collected for molecular analyses, and also fixed in 10% buffered formalin (NBF) for histpathological examination. 150 µL of sample were used for RNA extraction using the NucleoSpin RNA Virus kit for viral RNA isolation from cell-free fluids (Macherey-Nagel). The conserved sequences located in the 5'-UTR gene were detected by one step RT-PCR. The PCR products were purified using a Nucleo Spin gel and PCR clean-Up kit (Macherey-Nagel). Sanger sequencing was then performed using the PCR primers (Eurofins, GATC, Germany). Assembly, analysis of sequence data and phylogenetic analyses were conducted using the BioEdit and Mega Softwares. Ten % NBF-fixed tissues were processed according to standard histological methods. Briefly, the tissues were dehydrated in alcohol and embedded in paraffin wax. Then, five µm thick sections were stained with hematoxylin & eosin and examined under light microscope. Results and conclusions. Histpathological examination of the brain showed typical and characteristic changes of AE which included severe, multifocal and diffuse gliosis, neuronal degeneration and necrosis with neurons showing central chromatolysis, neuronal satellitosis, axonal degeneration and demyelization, and mononuclear peri-vascular cuffing. In the cerebellum, multifocal clusters of glial cells were arising in molecular layer and appearing as "flame-like structures" projecting from the granular layer and very obvious central chromatolysis of Purkinje cells. Encephalitic changes were variably present in birds from the both flocks with a varying degree of severity and distribution. Other histopathological features included moderate to heavy lympho-plasmocytic infiltrates in the muscularis layer of the proventriculus, and interstitial tissue of the pancreas. Mild infiltrates were also found in the muscularis layer of duodenum and in the myocardium. The nucleotide sequences of the fragments amplified from both cases brain samples were determined to be identical, and this sequence was compared by multiple alignment with other AEV sequences available in the sequence databases. The Moroccan strains and Hungarian isolate pf-HK1formed a common branch within the group B. Consequently, this presentation is, to our knowledge, the first report of pathological and molecular diagnosis of AE among poultry flocks in Morocco and where factors of its occurrence will be discussed. Key words: Avian encephalomyelitis virus, broilers chicks, Genotype B, Morocco

The Devil is in the Details: How to Effectively Reduce Turkey Respiratory Disease through Grower Communication Strategies and On-Farm Management

Elizabeth Beilke

West Liberty Foods, LLC.

In 2020, a regional investigation began among several turkey operations facing major performance losses due to a turkey respiratory disease. This investigation has persisted as the underlying primary infectious agent or cause remains unknown to date. However, in the interim the ongoing focus has been to reduce the secondary effects of bacterial-related as well as managerial-related contributing factors. Amid the lingering challenge and unknown nature of the disease condition in question this case study

has highlighted the full extent to which various management factors can contribute to on-farm respiratory challenges. In the field of veterinary medicine a lot of focus goes into understanding the disease and transmission dynamics to prevent and/or control disease. However, as production veterinarians, while we often understand the value of the technical aspects of on-farm management to flock health we may not always get the chance to delve into the inner mechanics of them ourselves. So, in this review of a persistent and challenging regional respiratory investigation, I will discuss how a deep dive into these technical management aspects aided a field clinician in moving closer to resolution of the disease condition experienced. This proved to not only be a valuable exercise for the veterinarian but also the farmers enduring this complicated and challenging condition. Therefore, in summary, this presentation will review the strategies that were utilized to delve further into the different factors that appeared to play a role to varying degrees into the severity of the regional disease condition faced. We will also discuss strategies used to communicate and promote education of growers in order to further reduce the overall effects of the condition. This case study hopefully in its entirety will additionally highlight the importance of understanding of the details of managerial areas by field clinicians, so as to not only effectively be able to improve flock health, but also better support field technicians and/or farm staff/management.

Reproductive Disorders in Captive Houbara Bustards

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Good reproductive health and breeding performances are particularly important for endangered species. The African Houbara bustard (Chlamydotis undulata) is a terrestrial bird inhabiting the semi desert regions of the Canary Islands and North Africa and classified as Vulnerable. Since 1995, this species is the focus of a conservation breeding program in East Morocco, the Emirates Center for Wildlife Propagation (International Fund for Houbara Conservation) where captive breeding operations rely exclusively on artificial insemination of females and artificial incubation of eggs. Since 2018, an increased incidence of morbidity and mortality associated with reproductive disorders, has been reported in captive female breeders. To characterize the problem and identify potential etiological factors involved, a diagnostic investigation was conducted on selected cases. Affected birds ranged between 1 and 22 years of age and exhibited non-specific clinical signs, including sudden death, prolonged anorexia, reduced activity and lack of oviposition. Coelomic distension and locomotory issues were rarely observed. Gross and histopathological lesions ranged from acute to chronic salpingitis and/or peritonitis, oviductal impaction and septicemia, with Escherichia coli being commonly isolated from affected organs. Careful examination of the

oviductal mucosa, at necropsy, frequently revealed a cystic appearance, corresponding to areas of cystic hyperplasia at histopathology. Similar cystic changes, of unclear etiology, have been occasionally identified in poultry, pet and aviary birds. In addition, a condition known as cystic endometrial hyperplasia - pyometra complex, likely of multifactorial etiology, has been described in dogs and cats. This is the first case report providing an overview of reproductive disorders in captive Houbara bustards, discussing diagnostic findings and potential predisposing factors involved.

Budgerigar (Melopsittacus undulatus) Ventriculitis Associated with Millet

Richard Fulton

Michigan State University

Millet (Pennisetum glaucum) is a common seed fed to captive pet birds. Over a 5 year period, numerous budgerigars, Melopsittacus undulatus, have died due to ventriculitis. Within the ventriculus there was ulceration and dissection of the koilin layer by myriads of round refractile granules. Often found within the ventriculus and other gastroenteric organs were cross sections of seeds containing large number of those granules. Grossly, those seeds appear to be millet. In addition to budgies, the author has had numerous large psittacines that died soon after consuming millet from a sprig. This communication will report 26 such cases of ventriculitis in budgerigars.

Clostridial Infection of the Proventriculus and Ventriculus in Broiler Breeder Pullets

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Several broiler breeder pullet flocks of varying ages at a single company had increased mortality. The service technicians noticed a correlation between mortality and significant gross gizzard lesions. The technicians noted that copper sulfate administration in the water reduced the mortality. A flock of 18 week old 308AP pullets with elevated mortality was identified to have lesions consistent with the reports of the field technicians. On gross examination, the gizzard lesions were similar to lesions reported by the technicians; severe ulcerations were noted at the junction of the proventriculus and ventriculus. Gizzards were collected for histopathology and anaerobic microbiology. Clostridium perfringens was isolated from the gizzard, and gram positive and negative bacteria were noted in and around the lesions utilizing Brown and Hopps stain. The C. perfringens was alpha toxin positive and net B negative by PCR. Meat and bone meal was a suspected cause of contamination, therefore meat and bone meal samples from the feed mill were submitted for anaerobic culture. Several of the samples were positive for C. perfringens and C. bifermentans. The C. perfringens was positive for alpha toxin and negative for net B. Changes were made to meat and bone meal processing and delivery; the incidence of the lesions was significantly reduced in the complex.

The Salt Was Normal

Seiche Genger

Elanco Animal Health

In late June of 2022, a case of rapidly increased mortality and morbidity in a flock of 33-week-old laying hens with complete production halt was seen in one house of a multi-age, conventional caged egg layer facility in the South East. Hens in the affected house were anorexic, lethargic, and moribund. Birds were reported to be clinically normal and in good condition Saturday morning. New feed was augured into the house via onsite feed mill that evening. Mortality was normal Sunday morning, however, production begin to drop off sharply and a dramatic decline in feed and water consumption was noted along with subsequent flushing. Monday morning mortality was dramatically increased at 3000 birds collected. Production in the house halted entirely and mortality increased sharply throughout the week with no improvement. Wednesday, all feed was removed from the house and new feed brought in. By Friday morning exact mortality was unknown as full counts were not completed due to labor constraints. At that point the number of birds lost daily was estimated to be in the thousands with at least 15,000 birds collected from the 1st floor of the building. The 2nd floor of the building had not been counted yet but was assumed to be similar in mortality. Production parameters never recovered (feed consumption, water consumption, egg production), and the flock was depopulated the following week. No other houses on the facility were affected. A full diagnostic work up was completed and results will be shared in full as to the outcome and final diagnosis.

Infections Involved in Respiratory Diseases in Broiler at Different Ages

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Edmilson Freitas, Fábio Cristiano Vieira,
Gabrielle Nellis Bragaglia,
Adriane Holtz Tirabassi

Vaxxinova Brasil

Control of infectious diseases is essential for the production of healthy poultry flocks. Vaccination programs associated with good management practices, including biosecurity measures, are adopted to reduce the risk of infections. Any infection can cause clinical or subclinical disease resulting in immunosuppressive conditions, include Marek's disease vírus (MDV), Aviadenovirus (FAdV), reovirus (REO), avian pneumovirus (APV), chicken infectious anemia virus (CIAV), infectious bronchitis virus (IBV), among others. The objective of this study was to evaluate the main infections involved in flocks of broiler chickens with respiratory symptoms from two companies in Brazil. Thirty-four flocks of broiler with symptoms of sneezing, rales and coryza and with macroscopic changes of aerosacculitis, tracheitis and presence of mucus in the trachea were investigated for 13 infections (MDV, FAdV, REO, APV, CIAV, IBV, Laryngotracheitis (ILT), Reticuloendotheliosis (REV), hemorrhagic enteritis (HE), micoplasmosis – MS and MG, Infectious Coryza), that can cause respiratory diseases or be a gateway for other agents due to immunosuppression. The flocks were divided into three main production ages: 1 - 14, 15 - 28 and 29 - 46 days of age. Diagnoses were performed by molecular methods (PCR or RT-PCR). Of the eight flocks analyzed aged 1 to 14 days, 7 were positive for CIAV, 4 for IBV, and 5 for REO. Of the 16 flocks analyzed from 15 to 28 days, 11 were positive for CIAV, 14 for MDV, 3 for IBV, 1 for APV and 8 for REO. As for flocks between 29 and 46 days (10 flocks), 9 were positive for CIAV, 10 for MDV, 3 for IBV, 5 for APV

and 3 for FAdV. The typification of IBV showed predominance for the new variant found in Brazil belonging to the lineage GI-23. Diagnoses for CAV and MDV were from field variants. APV typing was for subtype B. Chicken flocks were positive for the immunosuppressive diseases MDV, CIAV, REO and FAdV and respiratory infections IBV and APV that result in a significant loss of production and economic performance. The key to preventing infections is maintaining adequate environmental conditions and vaccination programs, reducing nutritional stress and maintaining high standards of biosecurity at all ages. With the right procedures, diseases can be minimized or eradicated, and broiler performance and profitability will be improved.

Riboflavin Deficiency in Multiple Ontario Broiler Flocks

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In the fall of 2022, multiple broiler flocks presented with sudden onset of lameness. Clinical signs included walking on hocks and medial curling of toes. There were no observable postmortem lesions and histologic lesions were limited to peripheral nerves. Clinical presentation and histologic lesions raised suspicions for Riboflavin deficiency. Riboflavin deficiency is not a common diagnosis and can be difficult to confirm as there is no diagnostic testing that can be performed on samples from affected birds to confirm the diagnosis. The condition must be suspected and feed testing must be pursued. The clinical presentation, histologic lesions, and feed testing results will be presented.

Riemerella anatipestifer Associated to Polyserositis in Caged Laying Hens : A Case Report

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Riemerella Anatipestifer (RA) is the etiologic agent of infectious serositis, the most economically significant disease of domestic ducks. Less importantly of geese and turkeys. Although RA has been been isolated from the chicken (Gallus Gallus), the species has been recognized as refractory to the infection. In this study, we report the isolation and sequencing of Riemerella Anatipestifer from the lungs of caged laying hens. The flock involved consists of 128 000 layers aged 38 weeks, with respiratory signs and an elevated mortality rate up to 0,04%. Upon necropsy, birds exhibited fibrinous oophoritis, pericarditis, and perihepatitis associated to airsacculitis. Fibrinous pneumonia was also noted in two birds out of the 10 necropsied specimens. Based on these lesions Mycoplasma Gallisepticum (MG) and M. Synoviae (MS) were strongly suspected, Ornithobacterium Rhinotracheale (ORT) was also on the differential for the observed lung lesions. For laboratory investigations, samples of the liver, spleen, lungs, air sacs, and ovarian follicles were taken for bacteriological testing. Furthermore, tracheal and lung swabs, as well as samples of lung and tracheal tissues were taken for molecular confirmation of MG, MS, and OTR. Bacteriology yielded pure growth of E.Coli serotype O1K1 from all sampled tissues. In addition, the birds were positive for both MG and MS as confirmed by quantitative real-time PCR. On the other hand, Amplification of the ORT

specific 16S rRNA gene fragment (784 bp) was performed. The rpoB gene fragment (538 bp) was amplified using primers and protocol described by Veiga et al. 2019. 16S rRNA and rpoB PCR products were gel purified (Qiagen, Germany) and Sanger sequenced in both directions at Eurofins, Germany, using PeakTrace™ Basecaller and the PHRED 20 quality score. The identity of bacterial species was confirmed using BLAST search against the GenBank. Overall, the isolate was found genetically highly close from known Riemerella Anatipestifer based on partial sequences of the 16S rRNA and rpoB genes. Nucleotide sequence identities and amino acid sequence comparison reached 98.79% to 83.10% among Riemerella sp. IPDH 98/90 and RA strain WJ4 isolated in China from the duck and chicken respectively in 2000 and 2015. Pathological lesions in this case, although similar to RA outbreaks in waterfowl, can be a direct result of co-infection with Mycoplsma. Further studies notably of virulence factors of this strain need to be carried out in order to determine its pathogenicity.

Misapplied Insecticide: Why Chemicals and Eyes Do Not Mix

Emily Pittman

Georgia Poultry Laboratory Network

A Georgia bobwhite quail plantation grower experienced high losses of bobwhite quail over the course of several days. The birds presented with thin body condition, crusted eyelids, and corneal opacity as the only gross findings. Diagnostic investigations were conducted to rule out high mortality disease, such as Highly Pathogenic Avian Influenza, and other respiratory diseases such as Quail Bronchitis and Mycoplasma spp. No infectious agents were found. Upon re-interview with the grower, it was found that the pesticide containing lambda-cyhalothrin was applied shortly prior to the bird placement without an approved application label for the pesticide inside poultry grow out houses, as well as without appropriate ventilation in the house for the birds. This misapplication is theorized to have led to corneal irritation progressing to ulceration and blindness. The quail were then unable to find feed and water, thus leading to the elevated mortality.

The Importance of Compliance – A Case Study of Multiple Blackhead Flocks On A WI Farm

Ashley Poissant

Jennie-O Turkey Store

Blackhead, caused by the parasite Histomonas meleagridis, can cause high mortality in turkey flocks. Once clinical signs develop, the disease may spread quickly through the flock. This can lead to high mortality and low market weights. If sufficient cleaning and preventative measures aren't taken, flocks placed on the farm afterwards may also break with blackhead. Some evidence suggests that enteric issues such as coccidiosis and going off feed may cause intestinal disruption increasing susceptibility for infection with H. meleagridis. Working with farmers to develop a sufficient cleaning and disinfection protocol between flocks is important. Additionally, working with farmers to develop a program which includes keeping birds on feed, daily necropsies to watch for lesions, and coccidiosis monitoring may help reduce the incidence of blackhead. This presentation will use a case study to discuss prevention strategies between flocks, what we did to reduce mortality during active infection, and what we are currently trying to implement to monitor when or if infection occurs.

Case Study of Avian Nephritis in UK Broiler Flocks

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Increased mortality with reports of wet litter and poor uniformity was investigated in three broiler farms. The range of findings from post-mortem examinations included dehydration, pale enlarged kidneys, urolithiasis, and articular and visceral gout. Initial histopathological assessment raised the potential of a viral challenge, which could include two ubiquitous avian astroviruses (AAstV) of chickens, avian nephritis virus (ANV) and chicken astrovirus (CAstV). Certain strains of infectious bronchitis virus (IBV) also needed to be considered. There is little known about the pathogenicity of AAstV strains in circulation, and it has been speculated that some can cause subclinical disease. Therefore, differential testing is necessary and should include quantification of viral load and identification of AAstV strains present.Methods: Kidney samples from each farm were collected in buffered formalin saline and submitted to Animal and Plant Health Agency, where they were processed according to standard protocols and stained with haematoxylin and eosin (H/E). Following histopathological investigation, six kidney samples from these farms were tested by Agri-Food and Biosciences Institute with real time, reverse transcription quantitative PCR (RT-qPCR) for ANV, CAstV, and IBV. Typing of the ANV capsid gene by RT-PCR and DNA sequencing of the amplicon was performed on RNA from one of these samples. The partially typed ANV strain was isolated via yolk sac inoculation into SPF embryonated eggs and used to determine the reactivity of a monoclonal antibody (mAB) against the ANV serotype 1 (ANV-1) reference strain, G4260, via indirect immunofluorescence antibody (IFAT) testing. An immunohistochemistry (IHC) test for ANV-1 was developed using this mAB and used to detect the presence of ANV within kidney lesions. Results: Histopathology revealed multifocal lymphoplasmacytic interstitial nephritis in all kidney samples collected from three farms. ANV was then detected in follow-up kidney samples from all farms. It was present at very high levels in three samples (21 to 27-days-old), a high level in a fourth sample (21-days old), and a moderate level in a fifth sample (22-days-old). It was absent from a sixth sample, which was a later sampling from one farm (34-days-old). CAstV was present in all samples at lower levels than ANV, while IBV was detected at weak levels in three of the samples. The hypervariable capsid gene in one ANV isolate was typed. It matched >91% by amino acid with the G4260 reference strain of ANV-1. The IFAT testing showed strong avidity of the G4260 mAB with the ANV isolate from the embryo. It was then successfully validated in an IHC test using samples from the affected kidney samples. Discussion: ANV was detected in all farms with the same disease presentation and histopathological findings. It was present at high or very high loads in most kidney samples from these farms. Initial microscopic assessment of a viral challenge was later supported by IHC using the G4260 mAB, which demonstrated cell associated positive staining to ANV at the sites of lesions within kidney samples.

Managing Two Canadian Broiler Breeder Flocks with Histomoniasis Diagnosed at the Onset of Lay: Review of their Clinical Symptoms and Intervention Strategies

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Histomoniasis (or Blackhead disease) is more often reported in turkeys than in broiler breeders causing obvious clinical signs with listless birds, sulfur yellow droppings, and sudden death. There were only occasional cases of broiler breeders diagnosed with histomoniasis in the province of Ontario (Canada) by the Animal Health Laboratory as part of the Ontario Animal Health Network, 2022. This case report presents two distinct broiler breeder flocks in Ontario diagnosed with histomoniasis, their clinical symptoms, performance parameters, and intervention strategies. Two broiler breeder flocks were diagnosed with blackhead disease at the onset of lay (aged 27 and 28 weeks) during the late summer and fall of 2022. Upon reporting of consistent histopathologic lesions, an intervention program based on the review from Clark and Kimminau (2017) was presented to the producers and adjusted over time. Flocks were visited once per week for follow-up necropsy, sampling when required, and establishing next steps. Initial clinical signs included increasing mortality slightly over standard and egg production either stalling or decreasing. As birds progressed into production, weekly pullet mortality worsened recording between 0.77% to 2.7% and one flock peaking at 4.5%, despite medication and other interventions. Standard mortality resumed several weeks later once past peak production. For both cases, the source of the parasite remains unknown, although one flock has a history of manure re-introduction in the barn at transfer. The most successful approach was to medicate early with a broad-spectrum antimicrobial, deworming, and implementing a supportive intestinal care program as birds are also managing the stress of egg production.

Clinical Experiences with Treating Inclusion Body Hepatitis

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Inclusion Body Hepatitis is an emerging challenge for broiler producers in the United States. This presentation will discuss clinical experiences with treating repetitive cases of Inclusion Body Hepatitis in broiler chickens. Multiple modalities were used, and theoretical mechanisms of action as well as results will be discussed.

Case Study: Turkey Cellulitis Control

Brian Wooming

Cargill

Turkey Cellulitis is a major disease confronting the industry, and is the major obstacle to producing an antibiotic-free flock. The disease is usually associated with Clostridia septicum, and mortality is believed to be caused by the effects of toxin production. Antibiotics are the main form of control and therapy for an affected flock.This case study will describe one intervention that has been used on a turkey farm in Missouri. This intervention has been duplicated on other farms in the complex. Success of any intervention may be objectively evaluated using antibiotic volume from one year

to the next over the same set of farms. Results comparing 2022 to 2023 will be presented.

Diagnostics

Validation of Pooled Samples in BHI for Mycoplasma Testing

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Pooled swab samples in BHI (Brain Heart Infusion) are not a validated sample for Mycoplasma PCR for any of the kits or methods being used by laboratories and are not part of the NPIP procedures. A comparison will be made between BHI and saline in the extraction procedure and between single and pooled samples coming from the field dry or in BHI.

The Importance of Day Old Chicks Detection for Salmonella spp. with Rapid On-Site PCR Test, A Case Study in Taiwan

Keat Fu[1], Wei-Fen Tsai[2], Ping-Han Chung[2], Simon Chung[2]

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Salmonella infections, such as Salmonella Typhimurium and Salmonella Enteritidis are zoonotic and also related to poultry industries. It is important to identify the source of Salmonella contamination for disease prevention and control especially from the upper most of the poultry supply which is day old chicks from the parent stocks. 2 farm sites with 8 batches of day old chicks were evaluated with rapid on-site PCR system. 8 of 30 houses were PCR positive for Salmonella spp. (26.67%). 7 of 8 were cultured positive and 6 of 7 were S. Enteritidis (85.71%). The 14 days old chicks in 7 houses were PCR positive for Salmonella spp. and 5 of 7 were cultured positive with 4 of 5 houses were the same serotype as above mentioned day old chicks (80%). This report indicated the importance of day old chicks as Salmonella screening vertical transmission to the chicks. This rapid on-site PCR system can facilitate the process of Salmonella detection, more sensitive and enables prompt response when day old chicks are tested positive.

Using On-Site PCR for Epidemiological Investigation of Salmonella Spp. in Taiwanese Broiler Farm

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Salmonella Spp. screening are often labor-intensive, time-consuming and under diagnosed due to the limitation by bacterial culture method. In this study, we use on-site PCR test for Salmonella Spp. epidemiological investigation in Taiwanese broiler farm. We collected the samples from two broiler farms, farm A and farm B. There were three cycles with a total of 200,000 chickens in each cycle in the study. The samples were collected before introducing new flocks, day old chicks (DOC), during the rearing period and after the chicken were harvested. The detection items included a drinking nipple, feed plate, boot socks, cloacal swabs, and feces from the transport basket. The total positive rates of DOC in the two farms were 25%. The environment samples collected before introducing the new flocks are all negative. During the rearing period, the positive rate of drinking nipples was 3%, the feed plates was 10%, the boot socks was 100%, and the weak

chickens was 72%. After harvest, the positive rate of drinking nipples was 9%, the feed plates were 16%, and the boot socks were 65%. Of the total 380 samples, there were 134 positive samples detected by PCR. We confirmed the PCR positive samples by culture methods and found only 76 samples were able to be bacterial cultured as positive Salmonella.On-site PCR can get the results earlier than traditional methods and better sensitivity. In this study, the vertical contamination of Salmonella Spp. was identified. The environment cross-contamination and infection in the farm were also significant. Therefore, on-site PCR test in the farm level to evaluate the biosecurity is beneficial. The above data makes it worth further analyzing the serotyping and antimicrobial resistance in this farm.

Unfit Birds: Samples Unsuitable for Testing in the Necropsy Department

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Birds submitted for necropsy must be fit to test according to AAVLD standards to ensure quality test results. In some cases, deviations are required from established procedures due to poor sample quality. Examples of unfit samples may include decomposed or improperly stored specimens. This project explores how GPLN manages these issues from a Quality program perspective.

Development of an Enzyme Linked Immunosorbent Assay for Detection of Entercoccus Cecorum in Chickens

Roxanne Lopardo

AVS Bio

Pathogenic strains of Enterococcus cecorum are becoming a major issue in the broiler industry, causing high morbidity and mortality. The current method for screening chickens with a suspected Enterococcus cecorum infection is to isolate the bacteria from bone, joint, or spinal lesions, or from cecal content of necropsied birds, to grow a culture. The bacterial growth is then identified. This method requires a minimum 24 hours of incubation to grow a culture that would be ready for identification. To screen more chickens and have results within a few hours, an ELISA has been developed to screen serum samples from chicken flocks. This method will be more efficient due to being able to screen multiple birds using one test plate and in much less time than the current method of bacterial isolation.

Turkey Enteric Rotavirus PCR Test Lacks Correlation with Clinical Enteritis

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A state diagnostic laboratory provides a turkey enteric multiplex PCR panel to test for various enteric viruses of turkeys, including turkey coronavirus type N, turkey coronavirus type S, rotavirus type A, rotavirus type D, reovirus, and astrovirus. The presence of turkey coronavirus is known to be clinically important, but the clinical importance of the other viruses is uncertain. In an effort to determine correlation between the presence of enteric virus and intestinal disease, 45 tom turkeys 2-8 weeks of age were randomly collected from 12 different brooder houses (generally 4 turkeys per house) on 8 different farms. These turkeys were necropsied and examined for intestinal disease, and cecal samples from each bird were tested for the presence of enteric virus with the turkey enteric multiplex PCR. Of these 45 birds, 23 had normal (formed) cecal contents, 13 had loose (runny) cecal contents, and 9 had thin watery cecal

contents. For analysis purposes, the loose and watery cecal contents were collectively considered to have enteritis. 23 birds were positive for both rotavirus-A and rotavirus-D, 12 were positive for rotavirus-A only, and 10 were negative for both rotaviruses. None of the birds were positive for coronavirus, reovirus, or astrovirus. Both rotaviruses were highly prevalent in this population, but their presence was equally distributed between the normal and the enteritis birds, and chi-square analysis found no correlation (P=0.936) between the presence of rotavirus and clinical enteritis. A CT value was recorded for all birds that tested positive, and the average CT value of rota-positive normal birds was compared to the average CT value of rota-positive enteritis birds. Rota-A positive normal birds had an average CT value of 26.3, which was not significantly different (P=0.44) from the enteritis birds which averaged 28.0. Rota-D positive normal birds had an average CT value of 25.3 which was not significantly different (P=0.75) from the enteritis birds which averaged 24.4. In summary, in this population of tom turkeys, we found no correlation between their PCR rotavirus status and their clinical enteritis.

Enteric Health

Evaluating the Efficacy of Dietary Bacitracin Methylene Disalicylate and Sodium Butyrate on Intestinal Physiology of Broilers During a Subclinical Necrotic Enteritis Challenge

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For several decades, subtherapeutic levels of antibiotic growth promotors (AGPs) have been used as a poultry feed additive to promote intestinal function and enhance production efficiency. However, the ban on the use of dietary AGPs has increased the incidence of Clostridium perfringens (CP)-induced subclinical necrotic enteritis (SNE), which leads to poor growth performance in broilers. Mechanisms of how AGPs improve production efficiency are unclear, though they may regulate host intestinal immunity and inflammatory status, thereby improving intestinal function. This study investigated the role of a dietary AGP, bacitracin methylene disalicylate (BMD; 50 g/ton feed), and an AGP alternative, sodium butyrate encapsulated with sodium salt of coconut fatty acids (SB; 700 g/ton feed) on intestinal physiology during a SNE challenge in broilers. Day (D)-old male Ross broiler chicks were grouped into one of six treatments (n=7 pens/treatment): unchallenged control, challenged control, unchallenged with BMD supplementation, challenged with BMD supplementation, unchallenged with SB supplementation, and challenged with SB supplementation. Birds in challenged groups were orally gavaged with approximately 1,500 Eimeria maxima oocytes on D14, followed by oral doses of 108 CFU/mL/bird CP from D19-D21. Jejunal mucosa samples were collected from one bird per pen at 0 (D21), 24 (D22), and 48 (D23) hours post final infection (HPFI) for measurement of mRNA expression for inflammatory and jejunal tight junction (TJ) genes by RT-qPCR. At 24 (D22) and 48 (D23) HPFI, one additional bird per pen was orally gavaged with fluorescein isothiocyanate dextran (FITC-d; 5 mg/Kg body weight/bird) and blood was collected 2h later to assess intestinal integrity by measuring levels of FITC-d in plasma by fluorometry. Data were analyzed by 3-way ANOVA with diet, challenge, and HPFI as model effects, followed by Fisher's Least Significant Difference test when ANOVA indicated significance ($P \leq 0.05$). No diet-by-challenge-by-HPFI 3-way interactions were observed for FITC-d levels or any genes measured, though 2-way interactions were observed for several cytokines

and barrier proteins. Plasma levels of FITC-d approached a diet x challenge interaction (p=0.1018), where challenged control birds had higher levels compared to unchallenged control while plasma levels in BMD and SB supplemented birds, irrespective of challenge, did not differ from each other and the control groups. The mRNA expression of interferons (IFN) α and γ, which are involved in activation of macrophages and regulate immunity, had a significant challenge x HPFI interaction, where IFN-γ and IFN-α were higher in challenged birds compared to unchallenged birds at 0 and 48 HPFI, respectively (P< 0.05). Furthermore, IFN-α had a diet x HPFI interaction, where BMD or SB supplemented birds had higher mRNA levels compared to non-supplemented birds at 48 HPFI (P< 0.05). There was a main effect of challenge on the mRNA expression of pro-inflammatory and anti-inflammatory cytokines, interleukin (IL) 1β and tumor-like growth factor (TGF) β1, respectively, both of which were significantly higher in challenged birds compared to unchallenged birds (P< 0.05). The mRNA expression of TJ genes claudin (CLDN) 5 and zonula occludens (ZO) 1 had a challenge x HPFI interaction, where challenged birds had higher mRNA expression compared to unchallenged birds at 48 HPFI (P< 0.05). In addition, CLDN-5 showed a diet x HPFI interaction, and at 0 HPFI BMD supplemented birds had lower mRNA expression compared to birds fed the control diet (P< 0.05) and SB supplemented birds had intermediate levels that did not differ from basal or BMD supplemented birds (P > 0.05). These data provide evidence that SNE-challenged broilers could lead to higher inflammation in jejunum that could compromise intestinal barrier and integrity. Irrespective of challenge, dietary supplementation of BMD or SB showed a similar effect in regulating inflammatory signals and barrier proteins in jejunum, thus potentially protecting jejunal integrity. This could lead to improved intestinal function that support improved nutrient absorption and increased growth in broilers. Keywords: bacitracin methylene disalicylate, immunity, inflammation, sodium butyrate, tight junction

Comparison of the Effects Obtained from Eubiotics and Antibiotics Programs Used In-Feed in Different Real-Life Scenarios with a Moderate Environmental Challenge in Coccidia-Vaccinated Broilers

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A floor pen study was conducted in Cobb 500 broilers to compare the effects of eubiotic and antibiotic feed additives in improving live and processing performance parameters and reducing the incidence of coccidia lesions, fecal Clostridium perfringens (CP), and fecal Salmonella in an environmental challenge scenario. Birds were exposed to a moderate disease challenge involving used litter that was known to contain Eimeria acervulina and Eimeria maxima oocysts, and the spores of CP. Dividers were used between pens to limit the cross contamination of pathogens from one to another. A randomized block design was used to allocate chicks to pens, and pens into 9 treatment groups. Broilers were randomly distributed separately into blocks. The treatments were: T1, negative control; T2, positive control; T3, Magni-Phi® Ultra (MPu) in step down mode 250/126 grams per ton with feed change at 10 days; T4, MPu in step down mode 250/126 grams per ton with feed change at 18 days; T5, Bacitracin (BMD) at 50 g/t; T6, Flavomycin (FLV) at 2 g/t; T7, Salinomycin combined with MPu at 125 g/t; T8, Salinomycin

combined with Provia® Prime (PP), a 4-way direct-fed microbial; and T9, a Phibro blend of eubiotics (PBE) at 454 g/t. Each product was fed for the duration of the 42-day study. All broilers were vaccinated for coccidiosis at the hatchery (day-of-age). Zootechnical parameters including feed conversion ratio (FCR), body weight (BW) and weight uniformity (WU) at 42 days of age were obtained and analyzed. All treatments had significantly better FCR than both controls. Both MPu step-down programs (1.84f and 1.85f) and PBE (1.83f) had significantly lower FCR than the combination of SAL with eubiotics (1.89e and 1.91de), BMD (1.92cd), and FLV (1.94c). BW and WU followed the same behavior as the FCR. Coccidia lesion scores were assessed, and fresh fecal samples were collected for CP and Salmonella enumeration on day 18 and 42. On lesion score of the intestines, all groups containing a eubiotic had the lowest values compared to the positive control and the in-feed antibiotic treatment groups in all segments at 10, 18 and 21 days of age. Ileum morphometrics were assessed from histopathological sections of the intestine collected at 18 days of age. All treatment groups containing a eubiotic and BMD (800ab mm) had statistically similar villi height and no difference to the negative control. The Salinomycin+MPu group had the highest villi height (808a mm). FLV had lower villi height (787b mm) but higher than the positive control (732c mm). Whole carcass yield as percentage of the weight was obtained post-chill. The negative control had the lowest value (71.2d%) and it was statistically significant different than the rest. The positive control had statistically similar values to BMD and FLV (73.3c, 73.2c, 73.5c %, respectively). The treatment groups with Salinomycin and MPu, Salinomycin and PP or the PBE alone had the highest yield values (76.6a, 76.3a and 75.8a %, respectively). The MPu stepdown programs were statistically similar but numerically lower. This type of environmental challenge is helping us understand the role that some eubiotics and combinations play within modern poultry production practices.

Intestinal Histopathology Related to Fecal Parameters in Broiler Chickens

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This study involves broiler gut histopathology and fecal characteristics during early grow out. Four genetic strains of broiler chickens were raised under production conditions to processing ages for the strains (49 to 66 days of age). Fecal moisture, protein, and fat were measured at 3- to 7-day intervals through the duration of the study. Fecal minerals (Ca, P, Mg, K, S, Fe, Mn, Zn, Na, Cu) were measured on days 1, 3, 7, 10 and 14. Jejunum was collected for histopathology from 3 randomly selected chickens from each strain at 3- to 7-day intervals through the study. Jejunum was semiquantitatively scored for coccidia and other histologic lesions (0, normal; 1, minimal through 5, severe). Five villi and 5 crypts were measured for each intestine, and the villus:crypt ratio was calculated. Intestinal lesions comprised coccidia (Eimeria maxima), heterophil inflammation, cystic dilated crypts, misshaped villus tips, and increases in gut associated lymphoid tissue and intraepithelial lymphocytes. The villus:crypt ratio declined through 10 days of age, due primarily to increased crypt depth. Fecal moisture increased about 10% from 10 to 17 days of age for each strain in the study. Fecal fat and protein increased about 2-fold from 7 to 10 days of age. Fecal sodium concentration increased by 3- to 4-fold from 3 to 10 days of age. The increase in fecal moisture coincided with increases in crypt depth and fecal sodium concentration. This is suggestive that hyperplastic crypt cells increase

the secretion of water in the small intestine and contribute to intestinal water imbalance.

Effect of Quillaja and Yucca Saponins and Polyphenol Combination on Broiler Performance, Health and Welfare and Potential to Reduce the Use of Antibiotics

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Antibiotic growth promoters (AGPs) had been routinely used to improve performance and intestinal health of broiler chickens. However, due to the constant threat of occurrence of antimicrobial resistance, compromising the therapeutic value of different antibiotics in human and/or veterinary medicine, this practice is under scrutiny with strict regulation varying from complete ban of AGPs use in some countries to their restricted and limited use due to regulatory and/or consumer pressure in others. Various feed additives have been evaluated as alternatives to AGPs use instead. The current study aims to assess the effect of a phytogenic product, based on Quillaja saponaria and Yucca schidigera saponins and polyphenols with a minimum content of 3.5% triterpenoid saponins (QYP) in a setting mimicking intensive poultry production. Broiler performance: body weight (BW) and average daily weight gain (ADWG); feed conversion ratio (FCR); mortality+culling (M+C); European Production Efficiency Factor (EPEF), food pad dermatitis (FPD) at 32 days of age (Berg, 1998) and intestinal health: coccidiosis scoring (Johnson and Reid, 1970) and dysbacteriosis (DB) scoring (Teirlynck et al. 2011) at 21 and 32 days of age, have been compared to non-supplemented control (NC), the polypeptide antimicrobial compound bacitracin methylene disalicylate (BMD) and the quinoline antibacterial compound halquinol (HAL), also known as chlorohydroxyquinoline, as well as to a phytogenic product based on thyme oil, synthetic star anise oil and quillaja bark powder containing 2-4mg/g thymol and 40-50mg/g anethole (TAQ).A total of 5400 Ross 308, broilers were randomly allocated to 60 floor pens in 5 treatments with 12 replicates each and 90 (45 male and 45 female) birds per replicate in a complete randomized block design (RCBD). Floor pens were allocated in a closed environmentally controlled house and covered with 5cm fresh rice hulls litter. Stocking density was 15 birds/m2. Birds were provided ad-libitum with 3-phase diet (0-10, 11-21 and 22-32 days) formulated as per the genetics specs and supplemented with in-feed anticoccidial. The treatments were: 1) NC – non-supplemented standard diet; 2) BMD at 100ppm 0-10 days and 50 ppm 12-32 days of age; 3) HAL at 60ppm from 0-32 days of age; 4) TAQ at 150g/t 0-32 days of age; and 5) QYP at 250g/t 0-32 days of age. Trial results were analysed statistically: ANOVA and Tukey's HSD as post hoc analysis. Statistically significant differences were considered at $P< 0.05$.Overall birds' performance was meeting or exceeding genetic guidelines with ADWG of 66.79g average for the study. Average BW at 32 days was 2179g (NC 2166g; BAC 2194g; HAL 2179g; TAQ 2179g and QYP 2174g) with no significant difference between treatments. Homogeneity of the birds expressed by the CV was: NC 2.15; BAC 2.97; HAL 2.58; TAQ 2.17 and QYP 1.90. Average not corrected for mortality FCR 0-32 days was: 1.380 (NC 1.384 ab; BAC 1.391 a; HAL 1.376 ab; TAQ 1.376 ab and QYP 1.370 b). FCR of QYP was significantly better than BMD at $P<0.05$. M+C was on average 2.14% (NC

2.02%; BAC 2.77%; HAL 2.22%; TAQ 2.12% and QYP 1.54%) without significant difference between treatments and lowest numerical value for QYP. Calculated average EPEF was 490 (NC 486; BAC 487; HAL 490; TAQ 491 and QYP 494). Footpad dermatitis was not severe: average FPD score of 0.50 (NC 0.46; BAC. 0.58; HAL 0.47; TAQ 0.48 and QYP 0.51) with no significant difference between treatments.Intestinal health of the birds was good and there was no significant difference among different treatments. Average E. acervulina (duodenal lesion score) at 21 and 32 days were 0.00 and 0.07 respectively. Average E. maxima (ileal lesion score) at 21 and 32 days were 0.68 and 0.55. Average E. tenella (caecal lesion score) at 21 and 32 days were 0.25 and 0.15. Average DB score at 21 and 32 days were 2.40 and 3.35 respectively.The results demonstrated that QYP provided similar or superior performance, intestinal health, and welfare (FPD scores) compared to BMD, halquinol and another phytogenic product, thus it is a valid alternative to AGPs and can be used in both organic, but also conventional production systems to limit the use of antibiotics and thus reduce the risk of antimicrobial resistance, without compromise on birds' performance, health, and welfare.Note: Values without common letters differed significantly at $p<0.05$.

Effect of a Quillaja-Yucca Combination When Fed at Different Age Duration on Broiler Chicken Fecal Salmonella Shedding, Live Performance, and Breast Yield When Reared in a Typical Poultry Industry Disease Challenge Model

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A study was conducted to determine the effects of a quillaja-yucca combination when fed at 3 different doses (0, 250 and 500 ppm) on fecal Salmonella spp shed as well as several performance parameters for different periods of time during a 42-day growth period. Periods for the quillaja-yucca combination feeding were from 0 to 28 days, 29 to 42 days and 0 to 42 days at each dose level shown above. A quillaja-yucca combination step-down and a step-up program were also evaluated. All treatments, when fed at either 250 or 500 ppm at any age, obtained at least numerically (when statistically significance was at $P<0.05$ or better) improved live performance (body weight gain, feed conversion or feed:gain and mortality), as compared to the Negative Control Challenged group (with no test material and challenged). These responses indicate that a quillaja-yucca combination, even at lower dietary inclusions, performed well against the mild stress test model. The maximum live performance (body weight gain, FCR and mortality) was obtained by feeding 500 ppm of a quillaja-yucca combination throughout the bird's life. This level was also effective against fecal Clostridium perfringens, and Salmonella spp. ($P<0.05$). Feeding 500 ppm of a quillaja-yucca combination resulted in a statistical ($P<0.05$) reduction of fecal Salmonella spp. Feeding either 250 ppm of a quillaja-yucca combination (continuously throughout the life-cycle Days 0-

42), 500 ppm of a quillaja-yucca combination (0-28 days of age) or 500 ppm of a quillaja-yucca combination (0-28 days of age) and 250 ppm of a quillaja-yucca combination (29-42 days of age) appeared to be statistically ($P<0.05$) effective in improving live performance. The quillaja-yucca combination (500 ppm) was found to improve dry carcass yield, breast meat yield and both Pectoralis major and minor Breast Yield ($P<0.05$). Conversely, the quillaja-yucca combination (250 ppm) improved yield measurements when fed for the duration of the growth period. When fed for only 28 days, 250 ppm had no effect on yield measurements. These percentage improvements led to additional carcass meat yield, as well as significant improvements in total breast meat (both Pectoralis major and minor Breast Meat).

Effect of the Addition of a Concentrated Combination of Quillaja and Yucca Biomass on Production Parameters, Intestinal Morphometrics and Skin Pigmentation of Broilers

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A floor pen study was carried out to evaluate the effect of the inclusion in the diet of a natural additive based on concentrated combination of Quillaja and Yucca biomass (cQY) containing a minimum of 8% triterpenoid saponins, approximately 1% polyphenols on zootechnical parameters, duodenum and ileum morphometrics and skin pigmentation in broilers using 2 levels of in-feed pigment inclusion. A 2x2 factorial experimental design with 2,800 Ross 308 day-old male broilers was used, considering the inclusion of each of the feed additives and the concentration of the yellow pigment in the ration as variables. Four treatments with 9 replicates of 40 birds each were used until 42 days of age. The treatments included a negative control group (a basal diet without the inclusion of any additive), and a treatment with the addition of the cQY combination at 125 g per metric ton of feed. For each treatment, 2 levels of inclusion of yellow pigment were used, a conventional concentration of 60/80 ppm from 22 to 35 d and from 36 to 42 d, respectively and a low concentration of 50/70 ppm, respectively. The chickens in the cQY treatment group had higher body weight (BW) than the controls at 42 days of age. Their feed conversion ratio (FCR) was lower at 35 and 42 days of age. The birds of the cQY treatment group had increased villi width and decreased crypt depth in the duodenum and increased villi height in the ileum, which is consistent with larger surface of absorption. Regarding the skin pigmentation levels, there were no differences with standard (or higher) levels of pigment (44.33 vs 43.73) when no feed additive was used. The birds in the cQY treatment group fed a standard level of yellow pigment had significantly higher skin pigmentation than those chickens fed the lower level of yellow pigment (45.05 vs 42.07), which suggests that the use of this eubiotic could decrease the level of inclusion of the pigment without affecting the skin pigmentation of the birds, and this can be explained by better intestinal health resulting from the action of cQY. In summary, the addition of eubiotics to the feed strategy in broiler diets is becoming clearer but more research is needed. We used the lowest possible inclusion rate of the cQY in this trial. Further research will include the addition of higher doses either in a full program or a step-down program to simulate real life poultry production conditions.

Infectious Bronchitis Virus

Comparative Pathogenesis of California 1737/04 (CA1737/04) and Massachusetts (Mass) Infectious Bronchitis Virus Infections in Laying Hens

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The California 1737/04 (CA1737/04) strain of Infectious bronchitis virus (IBV) was first isolated in 2004 from poultry flocks in California, USA and later found in Ontario, Canada in 2012. Although CA1737/04 has been known to cause respiratory and renal diseases during the growing stages among chickens, few studies have been conducted to evaluate whether CA1737/04 can induce pathology in the reproductive tissues during the laying phase. Therefore, this study aimed to provide insights on the pathogenicity of CA1737/04-type IBV in comparison with an already known Canadian Massachusetts (Mass)-type IBV on different body systems in laying hens. Two groups of specific-pathogen-free (30-week-old) layers were infected either with CA1737/04 IBV or Mass IBV, whereas there was a mock-infected group (control). Six hens were euthanized from each group at 9 days post-infection (dpi), whereas the rest were euthanized at 19 dpi. Our findings showed that both CA1737/04 and Mass IBV infected groups exhibited significant decline in egg production with a maximum drop of 35.4 % and 37.1% between 10-12 and 13-15 dpi, respectively. Although both CA1737/04 and Mass IBV infected hens were shedding quantifiable amounts of virus from the cloacal route, the Mass IBV infected hens were shedding more IBV genome loads via the oropharyngeal route than that observed in CA1737/04 infected hens. The CA1737/04 IBV infected group had significantly higher IBV genome loads and histopathological lesion scores in different portions of oviduct, large intestine (colorectum), and kidney compared to the Mass IBV infected group, particularly at 19 dpi. Overall, the CA1737/04 IBV induced more pathology in renal, reproductive, and enteric tissues of hens than that observed in the Mass IBV infected hens. These results add to the existing knowledge about the pathological changes in different segments of various body systems during the laying period, which could provide a better understanding of IBV pathogenicity.

Serologic and Zootechnical Monitoring of Two Broiler Breeder Flocks Vaccinated with Multivalent Inactivated Vaccines Containing MASS and IBV Variant Antigenic Fractions

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Avian Coronavirus, the causative agent of Infectious Bronchitis (IB) is a pathogen than can affect industrial poultry and cause severe economic losses and hinder animal welfare. This

virus can infect a variety of organs depending on it's tropism and the sanitary status of the birds. In long living birds, such as layers and breeders, the reproductive organs can be greatly affected. Leading to drops in production, embryo mortality, decreased eggshell quality, infertility, among other problems. IB challenge in broiler breeders can ultimately have a negative impact in chick quality. In Peru, the detection of Q1-like variants of Avian Coronavirus since 2009 and it's dissemination to all the poultry producing regions in the following years, represents a great risk. Field evidence shows that vaccination with MASS-type live attenuated or inactivated vaccines fail to provide full protection. Biosecurity measures are no longer effective to control the clinical signs associated with the disease, therefore a new approach is needed. Since no attenuated live IBV variant vaccine is available in the country, this study proposed the use of a multivalent inactivated vaccine containing the IBV Mass and QX strains, as part of an heterologous approach to decrease production losses caused by the disease. A long-term field trial was carried out in a broiler breeder farm with recurrent IBV Q1 and 793/B viral detections. Two flocks were monitored. Flock 1 received Cevac Megamune (containing NDV, AMPV, EDS, IBV-MASS and IBV-QX) and Flock 2 received an inactivated vaccine containing NDV, AMPV, EDS, IBV-MASS and IBV-D274. Blood samples were taken the day before vaccination, 3 weeks post vaccination and at 30 weeks of age. Elisa tests (Kit Idexx®) were used to detect antibodies against Newcastle Disease, Infectious Bronchitis and APV. Production parameters were recorded weekly up to 50 weeks of age. For the statistical data analysis and data visualization the Python programming language coupled with Numpy/Scipy modules was used. Results show that 3 weeks after vaccination, Flock 1 generated a higher and more homogeneous ($p<0.05$) seroconversion for all 3 pathogens evaluated. In addition, at 50 weeks of age, Flock 1 presented 1.25% less mortality, 6 more eggs per caged bird and 9 more hatching eggs per caged bird. In conclusion, a polivalent inactivated vaccine containing Mass and QX IBV strains was able to induce a strong serologic immune response against Newcastle disease, Infectious Bronchitis and Avian Metapneumovirus, improving the sanitary status of the flock.

Economic Impact Reduction from IBV Q1 Variant Infection in Layers Using a Multivalent Inactivated Vaccine with Mass and QX Antigenic Fractions

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In Peru the first detection of Avian Coronavirus, Infectious Bronchitis (IB)-related clinical signs in poultry was done in the late 1960s. Since then, the presence of this pathogen has been a constant in the poultry industry. Biosecurity measures implemented at the time were effective in controlling the disease. Experimental and field evidence has long shown that IBV cannot be effectively controlled in long-living birds by vaccination with live Mass-type vaccines only and that it is necessary a booster with an inactivated vaccine. This inactivated vaccine will not be effective against a variant IBV if it contains the Mass strain. Considering the circulation of IBV variants such as Q1, QX and BR in the Peruvian territory, a new approach is needed. In commercial layers, the main clinical signs seen is a decrease in eggshell quality and drop in egg production. In addition, no variant live IBV vaccine is available in the country and therefore a strategy of using a multivalent inactivated

vaccine containing the IBV Mass and QX strains was proposed. A field trial was carried out in a farm where the Q1 strain had previously been detected in clinically affected flocks between 18-25 weeks of age. A flock of 30 thousand Hy-line layers was equally divided into two groups. At 13 weeks of age, one group (Group A) received the inactivated vaccine Cevac Megamune (containing NDV, AMPV, EDS, IBV-MASS and IBV-QX) and a control group (Group B) maintained the actual vaccination protocol of the farm (inactivated vaccine containing NDV, AMPV, EDS, IBV-MASS and IBV-D274). Serologic monitoring was done before vaccination (13 weeks) and at 16, 22 and 30 weeks of age. Molecular diagnostic for IBV was conducted when deemed needed. Elisa serology (Idexx) was used for monitoring circulating antibodies Newcastle, IB and Avian Metapneumovirus. Production and clinical parameters were recorded every week. For the statistical data analysis and data visualization the Python programming language coupled with Numpy/Scipy modules was used. Molecular monitoring indicated that the flocks were naturally infected at 19 weeks of age with the IBV variant Q1. Results show that 3 weeks after vaccination, Group A generated a higher ($p<0.05$) seroconversion for all 3 pathogens evaluated. In addition, Group A produced 150 more high quality eggs per thousand birds, compared to Group B (during the 30 weeks of the monitoring). Which is equivalent to a gross income of 14.99 € per thousand birds in sales of first-class eggs. More importantly, during the Q1 outbreak, Group A produced an additional 2.357 first-class eggs per bird, equivalent to a gross income of 236.00 € per thousand birds. In conclusion, an inactivated vaccine containing Mass and QX IBV strains was able to significantly decrease production losses caused by the infection from the field IBV variant Q1 in commercial layers.

Effects of Mixing of Commercial Probiotic Products with Infectious Bronchitis Vaccine Solution

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Probiotics are applied to day old chicks for establishment of a beneficial gastro-intestinal microbiota which, besides generating several general benefits, will also help in controlling early Salmonella infections. To save time and management procedures, many companies are applying probiotics mixed with Infectious Bronchitis (IB) vaccine solutions at the hatchery by spray route. IB virus is very sensitive to many physical and chemical factors that can affect the viral survival, and, consequently, vaccine take and protection. A study was conducted to evaluate the effect of adding different probiotic products into IB vaccine solution containing the Massachusetts and BR-I vaccine strains. Five commercial probiotics (four in powder and one in liquid presentations) were used. All probiotics and vaccine solutions were prepared strictly according to manufacturer´s recommendations. Physical effects on final vaccine drop was evaluated by measurement of aspect, quantity and distribution of drops. Vaccine was applied by a Desvac Duo in Line spray vaccinator (Ceva Animal Health). The effect of each probiotic on the vaccine virus was measured by titration in SPF embryonated eggs. Vaccine solutions mixed with probiotics presented pH values between 4.3 and 6.9 before pH stabilizer addition. Only one probiotic product did not affect the shape, distribution, and quantity of drops (probiotic 1). Probiotics 2 and 3 affected tremendously the physical aspect of drops since they could not be counted. As for probiotics 4 and 5, the number and distribution of drops were different of that

in the control solution (vaccine without probiotic). The virus titers of the two vaccine antigens were affected after the mixing of all probiotics tested. Reductions on vaccine titers ranged from 0.15 to 1 log 10EID50 per dose. The study clearly demonstrated a negative effect of commercial probiotic products on the IB vaccine titer and on the physical aspect of drops when applied mixed by spray route.

Pathogenicity of Infectious Bronchitis Virus Massachusetts and Delmarva (DMV) 1639 Strains: A Comparative Study

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This experiment was designed to compare the pathogenicity of two infectious bronchitis virus (IBV) strains belonging to Massachusetts (Mass) and Delmarva (DMV) 1639 genotypes. Specific pathogen-free laying hens were challenged during the peak of production keeping an uninfected control group. For 3 weeks observation period, a significant drop in egg production was observed in the DMV/1639 infected group only. The DMV/1639 infected group showed prolonged oropharyngeal and cloacal viral shedding compared to the Mass infected group. The viral genome loads in the respiratory, urogenital, and immune tissues were significantly higher in the DMV/1639 infected group compared to the Mass infected group. Moreover, microscopic lesion scores were significantly higher in the lung, kidney, cecal tonsils, and oviduct of the DMV/1639 infected group compared to the Mass infected group. This study elucidates the pathogenicity of two highly prevalent IBV strains impacting the layer industry in North America.

Prevalence of Infectious Bronchitis Virus (IBV) in Broilers During 2021 and 2022 in Paraná – Brazil

Edmilson Freitas, Gustavo Schaefer, Priscila Diniz Lopes, Fábio Cristiano Vieira, Gabrielle Nellis Bragaglia, Adriane Holtz Tirabassi

Vaxxinova

In Brazil, Infectious Bronchitis (IB) is one of the most frequent respiratory diseases in broiler chickens. Until mid-2021, GI 11 (BR-1) and Massachusetts were the predominant genotypes in the country. Ever since, a new variant, genotype GI 23 (VAR2), has been introduced into Brazilian poultry flocks, especially in the southern region, which has three states: Paraná, Santa Catarina and Rio Grande do Sul. Of all of them, Paraná has stood out due the high incidence of infection with GI 23 variant. The aim of this study was to demonstrate the prevalence of infection by the three genotypes of the infectious bronchitis virus (IBV) in broiler chickens in outbreaks that occurred in 2021 e 2022 in Paraná – Brazil. To carry out this study, field data results from 49 samples positive for IBV by molecular biology test (RT-qPCR) were used. These samples were also submitted to genotyping to detect the genotype involved in the outbreak. For the diagnosis, tracheas were collected from broiler chickens aged 18 to 47 days from different poultry companies in Paraná. Of all the positive samples from the two years evaluated, GI 23 genotype predominated (53%), followed by GI 11 genotype (35%). Mixed infections involving GI 23 and GI 11 genotypes were observed in 10% of the samples, while 2% had mixed infection for GI 23 and Massachusetts genotypes. During the two years evaluated there was no infection only by Massachusetts genotype. In 2021, there was a greater number of positive samples for GI 11 (67%) and GI 23 was detected in 33% of cases. Of

the 34 samples evaluated in 2022, 62% were positive for GI 23, 20% for GI 11, 15% had mixed infection with GI 23 and GI 11, and 3% of the samples showed mixed infection with GI 23 and Massachusetts. The 2022 results showed that the GI 23 genotype was detected in 80% of the analyzed samples. The results of this study showed a significant increase in infection of broiler chickens by GI 23 and its establishment in 2022 in Paraná – Brazil.

Revaccination of Broiler Chickens in the Field in a Region with High Infection Pressure by GI 23 Genotype

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After the introduction of a new variant, genotype GI 23 (VAR2), in poultry flocks in southern Brazil in mid-2021, there was a marked increase in the frequency of Infectious Bronchitis (IB), increasing productive losses due to reduced zootechnical performance and partial and total condemnations of broiler chickens slaughtered. Vaccination is the most efficient way to prevent infection by the infectious bronchitis virus (IBV). In the most cases, a single dose on the first day (in the hatchery) has been sufficient for chickens. In several countries, revaccination of broiler chickens in the field is frequently used, especially in places with high infection pressure. The aim of this study was to evaluate the revaccination of broiler chickens in the field in a region with high infection pressure by the GI 23 genotype. In this study, broiler flocks from three farms (A, B and C) that previously had a positive diagnosis and that had partial and total condemnations at slaughter were selected. The farms were in a region of high infection pressure. Initially, spray vaccination of chicks was carried out on the first day of life in the hatchery. Two vaccines were used for vaccination: one containing the BR-1 (GI 11) genotype and the other containing the Massachusetts (GI 1) genotype. At 18 days, these batches of broiler chickens were revaccinated in the field with the same vaccines. This procedure was performed in three cycles per farm. In the first cycle, vaccination was carried out by drinking water and the other cycles by spray vaccination. At slaughter age, the broilers were slaughtered and data on partial condemnations, for airsacculitis, and total condemnations were compiled. The average percentage of total condemnations in the last flocks of broiler chickens from farms A, B and C that were diagnosed with GI 23 before vaccination was 0.02%. From the first vaccination cycle to the last, total condemnations decreased to 0.0% on farms A, B e C. Regarding partial condemnations, the average of condemnations of the three farms was 1.1% before vaccination. In the first cycle, this rate decreased to 0.8%, in the second cycle it dropped to 0.7% and in the last cycle to 0.0%. On the other hand, farms A and B didn´t present partial condemnations from the first to the last vaccination cycle. On farm C, there was an increase in the partial condemnation rate in the first cycle from 1.28% to 2.45%. In the second cycle, this percentage decreased to 2.15% and in the last cycle, broilers didn´t show partial condemnation. This study demonstrated a significant decrease in partial and total condemnations in broilers at the end of the three revaccination cycles with the GI 11 and Massachusetts genotypes vaccines.

Protective Efficacy of Live Attenuated QX Type Infectious Bronchitis Virus Vaccine Against Variant QX Type IBV Circulating in South Korea

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QX like Infectious bronchitis virus (IBV) has become the predominant genotype worldwide in recent years. In South Korea, the QX like IBV was introduced around 2002-2003, and recently variant QX type IBV appeared and became a prevailing strain. Here, we examined protective efficacy of commercially available live attenuated QX type IB vaccine (PoulShot® QX-IB, CAVAC, South Korea) against variant QX type IBV recently circulating in South Korea. 7-day-old SPF chickens were vaccinated via drinking water method and challenged via ocular route with variant QX type IBV strain at 14 days post vaccination (21 days of age). To confirm Protective efficacy of vaccine, ciliostasis score of trachea and viral loads of choanal swab and kidney tissue were measured on 5-days post challenge. PoulShot® QX-IB vaccinated group showed significantly lower ciliostasis score than non-vaccinated group. The viral loads in the PoulShot® QX-IB vaccinated group were also significantly lower than that in the non-vaccinated group. Our results demonstrate that PoulShot® QX-IB could protect against variant QX type IBV circulating in South Korea effectively.

Analysis of Gel-Pac® as a Diluent for Combined Infectious Bronchitis Virus and Coccidia Vaccine Application via Gel-Drop

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Infectious Bronchitis Virus (IBV) and coccidia are two of the most economically significant diseases seen throughout the poultry industry. Multiple methods of control are employed to combat these pathogens, with live vaccination being primary. Vaccination occurs in the hatchery on day of hatch in a setting where application variables can be controlled. While being applied in the same setting, these vaccines have historically not been applied together for a variety of reasons. But with a new understanding of how application volumes and droplets sizes can influence IBV vaccination success, and new formulations of coccidia vaccines that make mixing possible, it can be desirable to mix these products to maximize efficiency. Novel administration methods are also being evaluated to optimize vaccine coverage and minimize stress. This study evaluated the stability of vaccines against infectious bronchitis virus and coccidiosis in commercial poultry when combined and administered via aerosolized water spray or gel-drop diluents, both in-vitro and in-vivo. Diluents were compared for their impact on IBV vaccine thermal stability, IBV vaccine titer stability, coccidiosis vaccine positional stability throughout the application process, hatchling chick body temperature, and coccidia vaccine cycling pattern uniformity. Diluents did not differ in effect on chick thermal response or IBV vaccine stability. Gel-drop diluent provided more stable coccidia oocyst suspension without agitation during vaccination, and improved vaccine oocyst uniformity during post-vaccination cycling. Gel-Pac® proved at least as effective as traditional water spray for

delivering the IBV and coccidia vaccines used in this study, both alone and together in a single vaccine suspension. This data validates another method of vaccine delivery that can be used in commercial poultry hatcheries.

Unique Variants of Avian Coronaviruses from Backyard Chickens in Kenya

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The avian gamma-coronavirus infectious bronchitis virus (AvCoV, IBV; Coronaviridae family) causes upper respiratory disease associated with severe economic losses in the poultry industry worldwide. Here, we report for the first time in Kenya and the East and Central African regions comprehensive analysis of two novel AvCoVs designated IBV/ck/KE/1920/A374/2017 and AvCoV/ck/KE/1922/A376/2017 inadvertently discovered using nontargeted NGS of clinical samples collected from backyard chickens during a 2016-2018 surveillance of NDVs in backyard poultry and wild birds in Kenya. Both isolates have genome organization (5'UTR-[Rep1a/1ab-S-3a-3b-E-M-4b-4c-5a-5b-N-6b]-3'UTR), canonical conservation of essential genes and size (~ 27.6 kb) typical of IBVs. However, excluding the spike gene, genome sequences of A374/17 and A376/17 are only 93.1% similar to each other and 86.7 – 91.4% identical to genomes of other AvCoVs. All six non-spike genes of the two Kenyan isolates phylogenetically cluster together but distinctly from all other established IBV lineages and turkey coronaviruses (TCoVs), including the indigenous African lineage GI-26 viruses, suggesting a common origin of the genome backbone of the Kenyan isolates. Whereas isolate A376/17 contains a TCoV-like spike protein coding sequence most similar to Asian TCoVs (84.5 – 85.1%) compared to other TCoVs (75.6 – 78.5%), isolate A374/17 contains an S1 subunit sequence most similar to the globally distributed lineage GI-16 (78.4 – 79.5%) and the Middle Eastern lineage GI-23 (79.8 – 80.2%) viruses. Unanswered questions include the actual origin of the Kenyan AvCoVs, the potential pathobiological significance of their genomic variations, whether they have indeed established themselves as independent variants and spread in Kenyan and to the neighboring east/central African countries that have porous borders in terms of trade of live poultry, and whether the live-attenuated Mass-type (lineage GI-1)-based vaccines currently used in Kenya and most of the African countries provide protection against these genetically divergent field variants.

Complete Genome Sequencing of Novel Recombinant Infectious Bronchitis Viruses in Korea

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Infectious bronchitis virus (IBV) has evolved through various mutation mechanisms, including antigenic drift and recombination. In Korea, four genotypic lineages of IBVs including GI-15, GI-16, GI-19, and GVI-1 have been reported. In a previous study, we isolated two IBVs from chicken farms, designated IBV/Korea/289/2019 and IBV/Korea/163/2021.

These isolates were two distinct natural recombinant viruses most likely produced by genetic reassortment between the S1 gene of K40/09 strain (GI-19 lineage) and IBV/Korea/48/2020 (GI-15 lineage) by co-infection in commercial chickens. Pathobiological evaluation of IBV/Korea/289/2019 and IBV/Korea/163/2021 in chickens did not correlate with that of the progenitor virus which contributed to the S1 gene. To uncover the implications of the rest of the IBV genome and its effect on the pathology of the viruses, the recombination distribution patterns of four IBVs including the progeny and progenitor viruses (K40/09, K289/19, K48/20, and K163/21) were analyzed using whole genome sequencing. Recombination analyses of whole IBV genome sequence revealed mosaicism of various IBV lineages resulting from multiple recombination events. These data show that whole genome sequencing of IBV will enable a deeper understanding of globally circulating strains and recombination events between strains.

IBV Molecular Surveillance using Oxford Nanopore MinION Sequencing

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Avian infectious bronchitis is a difficult poultry pathogen to control and surveillance is key to understanding the field challenges and plan preventive measures such as vaccination. Surveillance is an ongoing effort in California broilers, consisting of diagnostic RT-qPCR and isolation from positive samples followed by RT-PCR (S1 HVR) and Sanger sequencing. We have been investigating the use of MinION for surveillance strategies, mainly to obtain full S1 gene sequences to supplement our sequencing RT-PCR and to obtain full genomes for further investigation, phylogeny studies and recombination detection. Through this process we have detected and characterized numerous IBV strains including CA1737, Conn, CA 3099 and GA/13046. The strategy has allowed us to characterize new strains e.g., CA/2228 as a product from the evolution of resident strains, in this case CA/3099. Though it provides more in-depth data, the current tedium, cost, and requirement for high quality samples for MinION sequencing is not comparable to traditional Sanger sequencing for it to be considered for routine surveillance use and it is likely to be reserved for WGS and characterization of novel strains.Financial support: FAPESP (20/07292-0 and 17/50334-3)

Genome Variability of Infectious Bronchitis Virus from Mexico: High Diversity and Recurrent Recombination

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Infectious bronchitis virus (IBV) is the causative agent of a highly contagious disease that results in severe economic losses to the poultry industry worldwide. IBV is classified in genotypes (G) and lineages using the full-length S1 sequence. Five lineages of genotype 1 (GI-1, GI-9, GI-13, GI-17, and GI-30) and two novel genotypes (GVIII and GIX) have been reported in Mexico. The present study described the full-length genomes of 17 Mexican strains, including the first genomes of the newly described GI-30, GVIII, and GIX. Genomes were obtained using Illumina high-throughput sequencing.The GI-3 and GI-9 genomes obtained here have notorious genomic differences with previously published Mexican genomes, but the GI-13 exhibits low variability. The GVIII and GIX have unique genomic characteristics that support local differentiation in the country. Our findings evidence that Mexican lineages are also highly variable in their entire genomes. This extraordinary genetic diversity may result from the co-circulation of indigenous and introduced lineages and genotypes in the same territory over long periods, the extension of the poultry industry in Mexico, the use of vaccines with different strains, and the backyard industry and its relationship with wild birds.

Review of the Detection of Infectious Bronchitis Virus Strains in Industrial Poultry in Ecuador from 2013 to 2022

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Infectious Bronchitis virus (IBV) is an important poultry pathogen that generates significant economic losses to the industry worldwide. In South America, the first reports of IBV isolations date from the 1950s when all isolates were serotyped as Massachusetts strains. In the Ecuadorian poultry industry, most vaccination programs have historically been based on live vaccines containing the Massachusetts strain. Since 2013, clinical respiratory problems in broiler farms suggesting an IBV infection have been frequently reported in flocks well vaccinated against the Massachusetts strain. Attempts to control the clinical situation with improvements in biosecurity procedures did not yield significant beneficial results. Molecular diagnostic work on affected farms produced several detections of variant strains genotyped as 793/B and BR-1. During the first 5 years (2013-2017) the diagnostic effort focused almost exclusively on broilers farms. However, from 2019, several detections were also made in commercial layers and broiler breeders farms as well which included the variant strains 793/B, BR-1 and Q1. Those detections were made in all poultry production regions in Ecuador. Either samples from clinical organs (trachea, intestinal tract, kidneys and cecal tonsils) or FTA card

imprints from the same organs were used for the molecular detection and genotyping of the IBV variant strains. This presentation will detail the molecular diagnostic history of IBV in the poultry industry of Ecuador.

Identification of Infectious Bronchitis Virus Variant Strains in Peruvian Broiler and Layer Farms During the Period 2018-2022

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MSD Animal Health

Clinical evidence and lesions compatible with Infectious Bronchitis associated with variant strains have been reported in Peru during the last 5 years (2018-2022). We have monitored many of these reported cases with respiratory signs during this period. Animals demonstrating clinical signs and IBV-related lesions were sample with cloacal and tracheal swabs for molecular diagnostic. Samples were conserved in FTA cards were immediately submitted to IBV confirmation and sequencing of positive samples at X-OvO laboratory in UK. During the 5-year period, out of a total of 65 samples collected, a total of 26 samples (40%) were positive and sequenced, of which, 9 samples (35%) were identify belonging to 793B serotype and the remaining 17 samples (65%) to the Q1 genotype. Looking at sample origin, 13 samples (93%) from broiler farms were positive to Q1 and only 1 (7%) had 793B, while layer farms 5 samples (38%) were identified as Q1 type and the remaining 8 (62%) to serotype 793B. It's important to continue monitoring of these cases to better understand the field dynamics and the potential introduction of new serotypes in our country.

Genetic Typing of Infection Bronchitis Virus in Mexico in 2021 and 2022

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Phibro Animal Health Corporation

Infectious Bronchitis is one of the most important respiratory diseases in Mexico, however it is unknown what the prevalent genotypes are today. In order to determine what genotypes are present in Mexico during 2021 and 2022, genetic detection and sequencing studies were carried out from 86 samples of birds with clinical signs of Avian Infectious Bronchitis. 57 trachea and lung swabs samples were from broiler chicken, 27 trachea, lung and oviduct samples were from laying hens and 2 trachea, lung and oviduct samples were from breeding hens. 78 samples were sent to the Poultry Diagnostic & Research Center at the University of Georgia, USA and 10 samples were sent to the Clinic for Poultry and Fish Medicine, Clinical Unit for Poultry Medicine, at the University of Veterinary Medicine, Vienna, Austria. The conventional method of PCR testing based on 3 UTR and the conventional method of PCR testing based on the S1 gene were conducted. Out of 86 samples, 75 were positive for IB, 3 were suspicious samples and 8 were negative samples. The results of the genetic sequencing found the following IB genotypes: 3 GI-1 Massachusetts, 1 GI-25 CA/1737/04 variant, 1 GI-27 GA 66972b/2008 c1, 3 GI-13 Group 793 B viruses and 14 GI-9 Arkansas genotype viruses. 53 IB viruses only had small amounts of genetic material detected to be identified. These are probably the result of late infections. In broilers, the Arkansas genotype was identified 39.1% of the time while in laying hens 70.3% of the viruses could not be identified.The results of the present study indicate that the types of Avian Infectious Bronchitis virus circulating among commercially produced birds in Mexico have likely changed,

while the predominant strains are still unknown. Further work that includes egg passages for virus isolation is needed to establish the IB epizootiology in Mexico and to understand how to best protect the poultry industry from IB damages.

Infectious Bursal Disease

Flow Cytometric Evaluation of Bursal Immune Cell Profiles Post Infectious Bursal Disease Virus Challenge

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Six lines of MHC-B congenic SPF chickens and two lines of SPF chickens with similar B haplotype but differing non-MHC genes were utilized to investigate the effect of MHC-B haplotype on infectious bursal disease (IBD) incidence. Chickens were challenged at 28 days of age with STC strain of infectious bursal disease virus (IBDV). IBD severity was evaluated throughout a seven-day course of infection based on mortality rates, bursal lesion scoring from H&E bursal sections, and qRT-PCR analysis of bursal viral load. Two unique multicolor flow cytometry panels were created to assess the bursal immune response to IBDV infection by analyzing the phenotypes of bursal immune cell infiltrates seven days post infection.

Efficacy Evaluation of Commercial Immune-Complex Vaccine Against the Experimental Challenge by Brazilian Very Virulent G11 Strain of Infectious Bursal Disease Virus

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Vaxxinova Brazil

Gumboro disease is a very important issue in poultry production. The efficacy of a commercial immune-complex vaccine was evaluated against the challenge by the Brazilian G11 strain of Infectious bursal disease virus (IBDV), and by the serological response. 4 groups were used, 1 vaccinated and challenged (GT1), 1 vaccinated and non-challenged (GT2), 1 non-vaccinated and challenged (GCP), and 1 non-vaccinated and non-challenged. 1-day-old chicks were vaccinated with Vaxxon IBD Imc, subcutaneously, with 0.2 mL and the groups were challenged at 21 days-old, by the ocular route. ELISA results demonstrated a high serological titer response in vaccinated groups before the challenge. After the challenge, chicks were evaluated for 5 days, but 100% of GCP chicks died before that, 3 days after the challenge, and all vaccinated and challenged birds (GT1) stayed healthy and alive. There was a high reduction in the bursa weight for the vaccinated groups (0.62 and 0.45 g) compared with the negative control (1.88 g) and positive control (1.12 g). This reduction was expected for immune-complex vaccines. Statistical difference was seen in bursal histologic analysis, with lymphoid depletion score mean of 4.6 for GCP, 2.5 for GT1, 2.7 for GT2, and 1.0 for GCN. Was observed many bursae of GCP with hemorrhagic points, characteristic of challenge with G11 strain. Bursae were evaluated by PCR and results demonstrated 100% positivity for the G11 strain of GCP samples, with 1.42 x 107 DNA copies, and 80% positivity for genotype GM3-W2512 in GT1, with 3.93 x 103 DNA copies. The results

demonstrated high effectiveness of the Vaxxon IBD Imc vaccine, with a survival rate of 100% of vaccinated and challenged chicks against 0% of non-vaccinated chicks and a reduction in the PCR positivity, with the predominance of the vaccine strain.

Antigenic Cartography Analysis of Chimeric Recombinant IBDV Strains Can Be Used to Identify Hypervariable Region Sequences that Induce Broadly Cross-Neutralizing Antibody Responses Against Diverse Strains

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Eight infectious bursal disease virus (IBDV) genogroups have been identified based on the sequence of the hypervariable region (HVR) of the VP2 capsid, encoded by segment A (A1-8). However, there is a lack of information regarding how antigenically related the different IBDV genogroups are to one another. Such knowledge is important for informing vaccine design, as a vaccine that induces broadly cross-protective immune responses against a greater diversity of strains would be valuable for disease control. Previously, we used the chicken B-cell line DT40 to rescue a panel of recombinant chimeric IBDVs containing HVRs from diverse field strains in the backbone of a lab-adapted PBG98 strain. The HVRs were from around the world (USA, UK, Europe, China, Australia, and Mexico), and belonged to six different genogroups (A1, 2, 3, 4, 6, and 8). We previously used this panel to evaluate the breadth of neutralizing antibody responses elicited by genogroup A1 vaccine and field strains, which demonstrated our method could be used to screen vaccine candidates for the breadth of antibody responses they induce. In the present study, we extended these observations by conducting a cross-neutralization experiment, comparing the breadth of antibody responses elicited by all the strains in our panel, against each other. Following antigenic cartography analysis, we observed the Chinese strain SGH-19 and the US strain DEL-E (both genogroup A2) clustered closer together than the other strains, and induced the greatest breadth of neutralizing antibody responses against all the other genogroups tested. Approaches to determine the molecular basis underpinning this observation, and the consequences to vaccine design will be discussed.

Comparison of Bursal Lesions in Vaccinated Commercial Broiler Flocks Infected by Three Different Strains of Infectious Bursal Disease Virus

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Infectious Bursal Disease (IBD) is a well-known disease in the poultry industry worldwide. However, year after year, it is possible to observe new discoveries about the Infectious Bursal Disease virus (IBDV) and its evolution. In the last decade, several studies have demonstrated the high prevalence of strains from a distinct lineage of IBDV (dIBDV), belonging to Genogroup 4, even in vaccinated commercial flocks. The aim of this study was to compare the lesions found in the Bursa of Fabricius (BF) of birds infected by three different strains: Genogroup 4 (dIBDV) field strain; MB vaccine strain and W2512 vaccine strain in commercial flocks. BF sampling were carried out

in commercial broiler flocks of three different companies in Paraná State (the main broiler producing state in Brazil). The flocks were randomly chosen and the vaccination program against IBD was either an immune complex vaccine W2512 strain or a live vaccine MB strain, both applied via in ovo in the hatchery. 105 BF were collected from birds aged between 20 and 28 days old. The samples were assessed by nested RT-PCR method followed by RFLP based on a fragment of the hypervariable region of the vp2 protein. Samples were thus sorted into molecular groups according to their enzymatic digestion pattern. They were also submitted to histopathology analysis, following the European Pharmacopoeia standard of lymphoid depletion scores (0 to 5) and scores from 0 to 3 were attributed to the parameters of necrosis; inflammatory infiltrate; epithelial hyperplasia; hyperemia; hemorrhage; edema and cystic follicles. Histopathology data were submitted to descriptive statistical analysis, followed by the Kruskal-Wallis method and mean comparison by the DSCF method using the Jamovi software. BF samples were grouped into 3 groups after molecular biology analysis: MB vaccine strain; W2512 vaccine strain and dIBDV (field strain), with 35 samples for each group. In the BF where the MB vaccine strain was recovered, the lesions scores were significantly lower ($p<0.05$) than the other two groups on the parameters of lymphoid depletion (2.14); necrosis (0.66); inflammatory infiltrate (0.91); epithelial hyperplasia (0.63); edema (1.00) and cystic follicles (0.00). In BF infected by dIBDV strains and in BF where the W2512 vaccine strain were recovered, the findings were, respectively, lymphoid depletion (2.80 and 2.83); necrosis (1.09 and 1.14); inflammatory infiltrate (1.51 and 1.43); epithelial hyperplasia (0.9 and 0.97); edema (1.57 and 1.71) and cystic follicles (0.34 and 0.54), and no significant differences were detected between the two groups of the dIBDV field strain and the W2512 vaccine strain. Hyperemia and hemorrhage parameters were similar between the evaluated groups. These results suggest that dIBDV strains present in Paraná, Brazil, have the potential to cause BF damage, higher than those induced by commercial vaccines this could lead to immune suppression that greatly affects welfare and productivity. In Brazil, and other countries, new studies are needed for a better understanding of the full pathological effects of the dIBDV strains and the possible protection with available vaccines.

Characterization of the Variant A IBDV in Commercial Broilers in Peru

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In the past few years, in all main poultry producing regions in Peru (northern, center and southern areas), several molecular detections of a variant Infectious Bursal Disease virus (IBDV) have been made. Most of these detections were genetically characterized as Delaware A-like field IBDV, mostly the Ecuador_220 strain (GenBank accession number MF142513.1). All companies, besides being subclinically infected with this strain, also had different IBD vaccination programs, type of broiler facilities and policies of reuse of litter. An epidemiological/diagnostic investigation was set up in order to ascertain a possible effect on health and productivity of broilers infected with the Ecuador_220 IBDV strain. A total population of 570,580 broilers, housed in three different farms, was investigated. All broilers were vaccinated against IBD at day 1 with an immune complex vaccine. Blood sampling protocol for IBD Elisa serology

(Idexx) included the ages of 1, 7, 10, 14, 17, 21, 28 and 35 days. Histopathology of bursa, thymus and spleen will be carried out at 7, 10, 14, 17, 21, 28 and 35 days of age. Molecular detection and characterization (by RT-PCR/sequencing) of IBDV in bursa of Fabricius tissue taken from broilers at 7, 10, 14, 17, 21, 28 and 35days of age. This study will help us to understand the epidemiology of this variant and its possible impact in the health of commercial broilers.

Full Length Sequence Analysis of Both Genome Segments of Two Infectious Bursal Disease Viruses Isolated from California

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Infectious bursal disease virus (IBDV) remains a major problem for poultry farmers globally, causing huge economic losses. IBDV-induced immunosuppression results in vaccine failure, making the host susceptible to other diseases. IBDV is a birnavirus with a bi-segmented double-strand RNA genome. The 2 segments are designated as segment A and B. The larger segment A encodes the structural proteins while segment B encodes the viral polymerase. The two infectious bursal disease viruses designated rA and rB reported earlier were isolated from an outbreak in two California layer flocks. RNA was isolated from the viruses and reverse transcription was carried out by using primers against the 3' and 5' end of IBDV sequences to produce the full-length cDNA copies of both the segments. The 3262 base pairs (bp) of segment A and 2827 bp of segment B were amplified by LongAmp polymerase and the final PCR product was successfully cloned into a TA vector and sequenced. rA and rB genome assemblies were obtained using Geneious and subsequently used to determine their phylogenetic relatedness with 143 segment A and 131 segment B sequences from GenBank. Segment alignments were performed using Clustal and two subsequent phylogenetic trees (one per segment) were generated using RAxML. In both trees, rA and rB cluster together indicating high genetic proximity. The segment A tree shows two main clusters and the isolates rA and rB appeared in a monophyletic clade that also contained the A segments of isolates SD10LY01, HN, LJ-5, and CAHFS_K669. The topology of the segment B tree also separates the strains in two main clusters but with higher genetic diversity. As observed in the segment B tree, isolates rA and rB were also in a monophyletic clade but with segments from isolates YS07, SD-2020-1, and SD10LY01.

Analysis of Field Samples Collected from Farms Under Different Vaccines Programs Against IBDV Between 2015 and 2021 in Argentina

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Infectious Bursal Disease (IBD) is a viral morbid entity that affects the poultry industry around the world. The etiological agent is the IBD virus (IBDV), a double strand RNA virus belonging to the Birnaviridae family. IBDV presents viral variants that have been organized into 7 genogroups. An extensive study of characterization performed in 2015 showed that the genogroup (G) 4 has been highly prevalent in Argentina. For years, the prevention of IBD in this country has been mainly performed by attenuated, inactivated, and occasionally by virus-vectored vaccines, all of them based on strains (or viral antigens) belonging to G1 strains. In 2015, other types of vaccines were licensed in

Argentina to control the disease; immune-complex vaccines (based on G1) and vaccines developed with the G3 variant MB. Interestingly, the proportion of the different IBDV genogroups detected in the field seems to have changed with the use of the new vaccines. In order to evaluate that we analyzed if the prevalence of G4 is associated with the use of any particular type of vaccine. To achieve this aim we evaluated 300 field samples (Bursa of Fabricius) taken during a seven-year period from flocks vaccinated with different vaccine types. In the first place, we detected that between 2015 and 2020 the prevalence of G4 has decreased to a rate of 15.9 % per year. However, the analysis showed higher frequency of detection of G4 in those flocks vaccinated with viral-vectored (0.32143) and immune-complex vaccines (0.32143), than in those which received G1- or G3-based attenuated vaccines (0.2143 and 0.14286 respectively). We also, observed that the frequency of detection of G1 was highest in flocks vaccinated with immune-complex vaccines (0.500) and lowest in flocks vaccinated with attenuated G3-based vaccine (0.0714). In addition, G3 was detected only in samples from flocks that received MB vaccines. Moreover, the sequence analysis indicated that all the G3 viruses detected were related to the MB vaccine. In this study, we have identified some associations between IBDV genogroups and vaccines. The detection of G4 variant in flocks vaccinated with the immune-complex as well as viral-vectored vaccines suggest a low efficiency of those types of vaccines to control variants belonging to that genogroup. On the other hand, the presence of G3 (but not G4) in flocks vaccinated with MB suggests that the vaccine virus delivered prevent the G4 spread to the chicken population.

A Rapid and Cost-Effective Amplicon-Based Method for Next-Generation Genome Sequencing of the Infectious Bursal Disease Virus

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Infectious bursal disease virus (IBDV) causes one of the most significant immunosuppressive disorders responsible for severe economic losses in the global poultry industry. IBDV has a viral genome with two segments of double-stranded RNA (segment A and B) and is classified into seven genogroups (G1-G7) based on the hypervariable region of the capsid protein (hvVP2). A more comprehensive characterization, including the detection of recombinants and reassortments, requires the sequencing of the complete IBDV segments. The present study reports the development and standardization of a tiled amplicon protocol for the direct and cost-effective genome sequencing of global IBDV strains. Primers were designed to amplify regions between 200 and 300 bp. Amplicons were partially overlapped to obtain the complete genome sequence of IBDV and the sequence of the Illumina adapter was added to the 5' end of each primer. This procedure reduces cost and streamlines sequencing library preparation. Primers for multiplex-PCR were separated into two pools (pool 1 and 2) to avoid amplifying overlapping regions. Fourteen IBDV strains from different genogroups (G1-G2-G3-

G4) were used to standardize both multiplex PCR reactions. Pool 1 and 2 were then combined, indexed and sequenced with Illumina MiniSeq. With this methodology, we obtained the full-length genome sequences of the fourteen samples used in this work. The phylogenetic analyses carried out with the coding sequences of segments A and B confirmed the previous classification and show that the strains are not reassortants. The method here presented is robust, fast, and economical. In addition, it provides in-depth knowledge of this virus's genetic and evolutionary characteristics. We anticipate that this methodology will help IBDV genomic surveillance and provide relevant information for the poultry sector.

Genetic Characterization of Argentine Infectious Bursal Disease Virus Strains

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The infectious bursal disease virus (IBDV) is one of the most important pathogens affecting the poultry industry worldwide. Since its first detection in the late 1950s, IBDV has acquired significant levels of genetic variability, leading to the emergence of antigenically and pathogenically divergent strains. The IBDV classification scheme based on analyzing the hypervariable region of the VP2 gene (hvVP2) identifies seven genogroups (G), with the historically known classic, variant, and very virulent strains corresponding to G1, G2, and G3, respectively. In Argentina, the most prevalent IBDV field strain belongs to the G4, an interesting strain with distinct antigenic and pathogenic behavior. Recently, a novel G2 strain has emerged in Argentina and started to replace previous circulating strains. In the present study, we obtained and characterized the genome sequences of G2 emergent strains in the Argentine poultry industry. Employing a multiplex PCR-next-generation sequencing approach, we sequenced the complete genome of twelve Argentine G2 IBDVs directly from bursal tissues collected during 2021. The identity percentage of these strains ranges between 99.4%–99.8% for segment A and 99.2%–99.9% for segment B. The most similar strain found in the GenBank database was the Chinese strain SHG19 (seg A: 98.8%–99.1%; seg B: 98.9%–99.1%), which belongs to a recently described group of IBDVs denominated “novel variants”. The phylogenetic analyses of the partial and full-length sequences and the presence of characteristic amino acid residues confirmed that the Argentine strains share a recent common ancestor with the Chinese novel variant strains. Continuous analysis of the IBDV field strains circulating is fundamental to the early detection of epidemiological changes, allowing the adaptation of control strategies to the current circulating strains. Therefore, the emergence of novel IBDV variant strains in Argentina has clinical and evolutionary relevance and must be further evaluated.

Mycoplasma

The Detection of Mycoplasma synoviae in Fecal Matter from Poultry

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Mycoplasma synoviae is a poultry pathogen of worldwide prevalence. The current approaches to control avian mycoplasmosis include continuous surveillance and quarantine, medication, vaccination and/or elimination of infected breeding flocks. M. synoviae strains have been shown to vary widely in their virulence, tissue distribution and rate of transmission. In previous research, M. synoviae was detected from various fomites in the environment of naturally infected broiler breeders; M. synoviae was detected in dust, litter, and feather samples by real time PCR. However, M. synoviae was not isolated from any of the environmental samples in that study. In this research, a methodology was developed to successfully isolate M. synoviae in highly contaminated samples (i.e., fecal matter) and compare the detection of different M. synoviae strains using real time PCR and culture. The detection of living M. synoviae organisms is a valuable indicator of the importance of biosecurity (especially litter management) in the control of M. synoviae.

Molecular Epidemiologic Analysis of Mycoplasma Synoviae Cases from Alabama

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Thompson Bishop Sparks State Diagnostic Lab

Mycoplasma synoviae (MS) subtle infection is considered as a state reportable disease in the state of Alabama, which is ranked the second in broiler production. The economic losses due to MS infection have been underestimated compared to Mycoplasma gallisepticum and it is essential to study this organism further, especially in a state with high number of breeder facilities. In this study, MS positive cases submitted to the molecular section of Thompson Bishop Sparks State Diagnostic Laboratory from May 2018 to January 2023 were sequenced to better understand the possible correlations between the cases, identify genetic similarities and differences, and evaluate the efficacy of the biosecurity programs. To do so, backyard and commercial cases were selected based on the sampling region in Alabama, the severity of clinical signs and lesions mentioned in the history of the cases, and Ct values of the purified extracts. Then, several genes and regions (e.g., vlhA, nanA, and ugpA) were sequenced and compared with the publicly available genomes, including WVU 1853 as Mycoplasma synoviae reference genome, to identify and differentiate the strains. The strains were eventually classified using a phylogenetic tree.

A Longitudinal Study of the Mycoplasma Vaccine (MSH and ts-11) Populations and Serological Responses in Two Unchallenged Vaccinated Layer Flocks in Australia

Chris Morrow, Robin Achari

Bioproperties

Two commercial layer flocks were sampled after simultaneous eyedrop vaccination at 7 weeks with MSH and ts-11 until depletion. They were tested for colonization of the cloanal cleft by PAN-PCRs and serologically by ELISA (20 birds per sampling). The DIVA PCRs used were designed by comparison of the sequences of the vaccine and parent strains utilizing SNPs that were in genes not under immunological or in vivo fitness pressure. Their sensistivity was one hundredth of the PAN-PCRs. No evidence of wild strains was found during this study. The rearing site was remote and biosecurity was of a high standard. MSH colonization rapidly reached 100% and stayed very high till depletion (despite two brief treatments with OTC in feed for Fowl Cholera in one flock). In contrast ts-11 colonization detection decreased over four weeks and remained low till the flocks came into production. Seroconversion did not occur for MS or MG til end of rearing and was not complete until peak of lay in both flocks with MS seroconversion occuring first. It is suggested that these sort of field studies are needed before considering tests as validated.

Molecular Detection and Characterization of Mixed Mycoplasma gallisepticum and Mycoplasma synoviae Infections on Poultry Farms in Nigeria

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Avian mycoplasmosis, caused mostly by Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS), is an economically important disease that affects the respiratory tract and joints of poultry birds. We detected and characterized MG and MS in chicken carcasses from thirty-seven farms having history of respiratory disease submitted to diagnostic laboratories in three south-western states of Nigeria in this study. Trachea scrapings from the carcasses were collected on FTA cards and real-time PCR was carried out to identify MG and MS positive samples. Two MG (mgc2S and igsr) and three MS (vlhA, nanA and ugpA) genes of the positive samples were sequenced. Alignment of the concatenated two MG and three MS genes sequences of the Nigeria strains and other reference strains were constructed by the Clustal-W method with the use of the MegAlign program (DNASTAR Lasergene). A total of 36.8% of the farms were MGMS (MG and MS) positive, 7.89% were MG positive only while 13.1% were MS positive only. The Nigeria MG strains shared about 98.71 - 99.84% identity with strains from Egypt, Israel and Jordan strains while the MS strains were 93.8 – 96.2% with strains from the Great Britain. Six farms had 6/85 live vaccine-like strains but none of the farms was positive for MS-H vaccine. This study sheds light on the

molecular characteristics of MG and MS in Nigeria, provides important information that can be used to better understand the epidemiology of these diseases in the Nigeria and will help in developing control programs against these diseases in Nigeria.

Chick Quality in Vaxsafe MS Vaccinated Breeder Flocks

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Mycoplasma Sinoviae (MS) is increasingly predominant in layers, broiler breeder's and broiler farms. It is responsible for aerosaculitis, synovitis and shell abnormalities, affecting the production and quality of the hatching egg. The effect of MS in chick quality is often underestimated and covered by the presence of other field pathogens or the metaphylactic use of antibiotics. Eradication is the best way to keep the birds free from the disease; 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 on progeny of vaccinated breeder flocks was evaluated and compared with unvaccinated breeder flocks treated with antibiotics reared in the same farm. Before vaccination (7 weeks) rapid agglutination tests, ELISA and tracheal swabs PCR were performed to demonstrate MS negativity. The initial results (35 weeks) show significant differences favoring vaccinated vs unvaccinated flocks in terms of percentage of late embryo mortality (3,88 vs 4,88), hatching (89,49 vs 88,89), first week chick mortality (0,93 vs 1,49), cost of antibiotics used in breeders (0 vs 90 cents per bird) and the level of post vaccine reactions and chick quality complains from the field that lower for the vaccinated flock chicks suggesting the suitability of MsH vaccination for optimal MS control.

Pathology and Antibiotic Sensitivity Associated with Outbreaks of Respiratory Disease in British Game Birds that Include Mycoplasma gallisepticum (2016 to 2019)

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Between 2016 and 2019, a targeted survey was planned by the British Poultry Veterinary Association to investigate causes about respiratory disease in British game birds. Emphasis was placed on Mycoplasma gallisepticum, but testing was completed for other potential viral and bacterial challenges. Sixty-nine outbreaks of respiratory disease were investigated, which included 55 in pheasants, 13 in red-legged partridges, and one in grey partridges. M. gallisepticum was detected by RT-PCR at the University of Liverpool, who also sequenced the mgc2 genes and reported the possibility of a dominant strain in circulation from 2016 to 2017. Viable cultures from all cases were sent to the specialist lab to determine MIC values for common antibiotic treatments. Post-mortem investigations were completed by the Animal and Plant Health Agency for 41 of these outbreaks, in 37 of which M. gallisepticum was detected by RT-PCR at the University of Liverpool from pooled tracheal and conjunctival swabs. When PCR results from each outbreak were compared histopathological findings in individual birds selected for pooled sampling, the diagnosis of mycoplasmosis was confirmed in 56 of 101 birds where M. gallisepticum was detected. The cellular immune response in the infraorbital sinuses was the most important

variable for reaching this diagnosis, which was lymphocytic infiltration of the mucosa. These microscopic findings tended to be in birds with typical post-mortem findings, such as mucoid to caseous sinus exudate with facial swelling. Histopathology was also helpful to rule-out other conditions such as cryptosporidiosis and discriminate between those susceptible to mycoplasmosis and potential carriers, which potentially included 28 of the examined birds. Other challenges by aMPV, IBV, and ORT were detected during this survey, even if there was no definitive correlation with pathological findings or response to treatment. There were also no correlations with the MIC values for M. gallisepticum isolates, although these were significantly higher than the fully susceptible reference strain. This survey determined that the most frequently identified pathogen involved in respiratory disease outbreaks in game birds was M. gallisepticum, and histopathology was a valuable tool for diagnosing mycoplasmosis in PCR-positive birds. It also highlights the multifactorial nature of these outbreaks, as detected other pathogens could have synergistic and opportunistic roles.

Newcastle Disease Virus

Molecular Studies on Newcastle Disease Virus Isolates in Relation to Field Vaccine Strains in Egypt (2012-2015)

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Newcastle disease (ND) is a highly contagious viral disease of avian species and represents a major threat to the poultry industry worldwide. Regardless of which type of vaccine is used, birds are still able to become infected by NDV and can transmit the disease to others. This study aimed to obtain improved understanding of the variety and interrelationships of NDV isolates. Materials and Methods: A total of 116 tissue/organ samples were subjected to virus isolation and pathogenicity assessment in vivo by intracerebral pathogenicity index (ICPI) determination. Molecular characterization was performed by one-step RT-PCR to obtain a 535 bp fragment, including the fusion gene cleavage site. The purified PCR products of 12 isolates were selected for DNA sequencing. Results: Nucleotide and deduced amino acid sequence analysis of the cleavage site of the F gene of all field isolates revealed the motif 112R-R-Q-R-R-F117 at the C-terminus of the F2 protein and F (phenylalanine) at the N-terminus of the F1 protein (residue 117), indicating that these strains were velogenic. The nucleotide sequence analysis revealed that our isolates showed the greatest nucleotide identities (99.3%) with the velogenic strains from Jordan, Israel and Turkey, suggesting that the virus circulating in Egypt probably extends from the Middle Eastern region. Phylogenetic analysis showed that our isolates could be classified into three genotypes (VIId, VIIa and II), indicating that VIId is the predominant circulating genotype in Egypt, where 10 isolates were clustered. One isolate for genotype VIIa and one for genotype II were also observed. A low evolution rate, with Ka/Ks ratios ranging from 0.01-0.02, indicated negative or purifying selection. The minimum evolutionary distance detected was 0.09 to genotype VIId, whereas the maximum distance was 0.21 to

genotype II, from which most commercial live virus vaccine strains are derived. Conclusion: The control of NDV by vaccination still faces new challenges and evaluating the effectiveness of the current commercial vaccine strains against circulating NDV strains has become a necessity.

Characterizing miRNA Regulated Pathways That Modulate Avian Orthoavula Virus 1 (AOAV-1) Replication

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USDA

Newcastle disease caused by virulent strains of Avian orthoavulavirus-1 (AOAV-1) / Newcastle disease virus (NDV) is a significant concern for the poultry industry. Because current NDV vaccines only protect against homotypic challenge but do not reduce viral shedding, a key objective of our research is to identify host genes that are necessary for viral replication and shedding. We transiently increased or repressed intra-cellular function of a small subset of chicken microRNAs in chicken fibroblasts followed by AOAV-1 infection to identify key anti- and pro-viral microRNAs. Using immunofluorescence, hemagglutination and infectivity assays in embryonated SPF eggs we identified several chicken miRNAs that can alter replication of AOAV-1. Computational analysis of these miRNAs identified key interferon (IFN) and Interferon stimulated gene (ISG) pathways for validation with qRT-PCR. These data identify novel intervention targets to reduce / mitigate NDV replication and shedding.

Repeated Isolation of Avian Orthoavulavirus Serotype 13 in Ukraine

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Avian paramyxoviruses (APMVs) belong to the recently assigned subfamily Avulavirinae of the family Paramyxoviridae and have been shown to infect a wide variety of poultry and wild bird species. Currently there are 22 confirmed serotypes of APMV. The subfamily Avulavirinae is composed of three genera, namely, Metaavulavirus, Orthoavulavirus, and Paraavulavirus.In 2013, active surveillance of wild birds for APMV and avian influenza virus was conducted in the Azov-Black Sea region of Ukraine. The hemagglutinating agents that did not cross-react with avian influenza virus antisera were isolated in 9-day-old specific-pathogen-free embryonated chicken eggs. One isolate from fecal samples collected from a white-fronted goose (Anser albifrons) in the Odesa region weakly cross-reacted with antisera against APMV-1, APMV-3, and APMV-7. This agent was submitted for further characterization using next-generation sequencing at the Southeast Poultry Research Laboratory of the USDA in Athens, GA.Viral RNA was extracted from allantoic fluid using the MagMax AI/ND Viral RNA Isolation kit. Host DNA was removed using an in-house depletion protocol. Sequence-independent single-primer amplification was used to produce random amplicons that were further processed using the Nextera DNA Flex library preparation kit.. Pair-end sequencing (2x300 base pairs) of the generated libraries was

performed on an Illumina MiSeq instrument using the 600 cycle MiSeq Reagent Kit v3. Sequence data were de novo assembled using MIRA software within a customized workflow on the Galaxy platform. The final genome consensus of the isolate designated white-fronted goose/Ukraine/Prymorske/71-15-02/2013 contained six genes (3'-N-P-M-F-HN-L-5') with coding sequence lengths of 1482, 1194, 1101, 1638, 1740, and 6600 nucleotides, respectively. The genome sequence showed the highest nucleotide identity of 99.7% to Avian orthoavulavirus 13 (APMV-13) strain goose/Kazakhstan/5751/2013. Currently, this is the sixth complete genome of APMV-13 available in public databases and the second reported from Ukraine. The previously isolated Ukrainian APMV-13 strain white-fronted goose/Ukraine/Askania-Nova/48-15-02/2011 shared 96.8% nucleotide identity with the isolate presented in this study. This genomic data provides further knowledge on APMV-13 diversity and will facilitate future studies on Orthoavulavirus evolution.

Introduction of a Novel Newcastle Inactivated Vaccine (r NDV-LS-HNgVII) Developed by Phibro for the Israeli Poultry Industry. Field Trial Results and Data from Commercial Usage

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Newcastle Disease (ND) outbreaks caused by the vNDV-genotype VII occur occasionally in Israel poultry farms and can severely affect all segments, especially the layer farms. It continues to infect vaccinated birds despite strict biosecurity and intense ND vaccination, which is designed to eliminate clinical disease and decrease the amount of virus shedding. A homologous vaccine was developed to improve the protection against Newcastle disease virus genotype VII. The new recombinant inactivated vaccine, rND-LS-HNgVII (AM/HNgVII), was constructed from the Lentogenic LaSota strain by replacing the HN gene of the LaSota with the HN from genotype VII. The efficacy and safety of the vaccine was evaluated in laboratory and field trials.The field study objectives were to evaluate the safety, efficacy, and benefits of a gVII recombinant vaccine against Newcastle disease under Israeli field conditions compared to the commercial vaccines currently applied in the field.The serological response to the gVII recombinant vaccine was evaluated by the HI method using homologous (gVII) and heterologous (gII) antigens. Vaccinated layers were challenged with a Velogenic NDV field isolate of genotype VII on day 111, and after the challenge, viral shading was evaluated by qPCR. The effect on egg-laying performance and the interaction with other vaccines were then assessed.The test group vaccinated with the gVII recombinant vaccine demonstrated high HI antibody values of 15.8 (11-17) - 9.0 (8-10) at 14-56 weeks post first vaccination respectively and complete protection against challenge. In addition, the virus excretion from the test group showed better results than those vaccinated with commercial vaccines. No adverse interactions with this vaccine to other vaccine's efficacy were found as tested by AI (H9N2) HI testing. Egg-laying performance was not negatively affected by the new vaccine.Overall, we found the gVII recombinant vaccine to be safe and efficacious in preventing Newcastle disease. Following the field trial results the vaccine was introduced in August 2022 to vaccination programs of all the pullet (breeders and layers) flocks in Israel. Data demonstrating the influence of this vaccine on ND outbreaks will be presented.Abbreviation:ND = Newcastle DiseaseHN = Hemagglutinin Neuraminidaser NDV = Recombinant NDgVII = genotype 7LS = LaSota

Newcastle Disease Virus in Brazilian Pigeons

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Newcastle disease virus (NDV) can infect over 250 bird species with variable pathogenicity. NDV isolates belonging to genotype VI have birds of the family Columbidae as their reservoirs. In Brazil, NDV in feral pigeons was detected in three municipalities (Porto Alegre, São Paulo, and Recife) in the last eight years. Phylogenetic analysis based on the complete CDS F gene grouped the Brazilian strain with other viruses from subgenotype VI.2.1.2, including sequences detected in Africa. Our group reported the last detection of NDV in feral pigeons in São Paulo city in 2019. Here we describe a new bird outbreak at the exact location three years later, in May and June 2022. Birds displayed similar clinical signs and gross lesions previously reported, including neurological signs and hemorrhages in different tissues from affected birds were observed. Tissue samples were collected from two birds and tested for Newcastle disease virus by RT-qPCR test targeting the M and F gene. One virus was isolated from a pool of tissue samples and characterized by the hemagglutination inhibition (HI) and the intracerebral pathogenicity index (ICPI) tests. The complete coding sequence of the F gene was obtained by Sanger sequencing. The monoclonal antibody 617/161, specific to recognizing pigeon strains, inhibited the isolate with an HI titer of ≥ 256. The virus had a cleavage site (113-RRQKR 116*) characteristic for virulent strain, with 97.1% nucleotide identity compared to the sequence detected in 2019. Although the high nucleotide identity between the sequences, and 100% of identity in the F protein cleavage site, when comparing the ICPI, the virus had a decrease in virulence. Herein, the isolate had an ICPI of 0,06, characteristic of an avirulent NDV. The virus obtained in 2019 had an ICPI of 0,99, characteristic of a virulent strain. Amino acid substitutions were C29Y, S124G, P357S, S364N, S499O, and A500T. The phylogenetic analysis based on the complete CDS F gene grouped the detected isolate with other viruses from subgenotype VI.2.1.2, class II, including the three previously reported in 2014, 2018, and 2019. We reported the fourth independent outbreak of the subgenotype VI. 2.1.2 in Brazil, our data suggest a distinct subgenotype compared to the African NDV sequences that

were previously grouped into subgenotype VI. 2.1.2, which should be reclassified as VI. 2.1.2.1. The complete genome analysis will also help to understand the decrease of NDV pathogenicity in pigeons after three years.Funding: RedeVirus-MCTI-CNPq (Process number 403761/2020-4) and FAPESP (Process number 2019/13198-0).

Toll-Like Receptor Ligands Enhance Vaccine Efficacy Against Virulent Newcastle Disease Virus Challenge in Chickens

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To enhance the efficacy of the current Newcastle disease vaccine, we have selected potential adjuvants with well-characterized intracellular targets, the toll-like receptors (TLRs). Imiquimod is a small-molecule activator of TLR7, a sensor of dsDNA. ODN-1826 is a mimetic of CpG DNA and ligates TLR21. Activation of TLRs leads to antiviral responses including the induction of the type I interferons (IFNs). In this study, birds were vaccinated intranasally with live LaSota strain with or without Imiquimod or ODN-1826 (50 ug/bird). Two weeks after vaccination, birds were challenged with virulent Newcastle disease virus (chicken/CA/212676/2002). Both the vaccine groups adjuvanted with Imiquimod or ODN-1826 induced higher and more uniform antibody titer among birds compared with live vaccine alone group although the difference was not statistically significant. In addition, adjuvanted vaccines demonstrated greater protective efficacy in terms of reduction in virus shedding titer and number of birds shedding the challenge virus at 2 and 4 days post challenge. Tissues (Harderian gland, trachea, cecal tonsil, and spleen) were collected 1 and 3 days after vaccination and expression of antiviral and immune genes is being analyzed in relation to protective efficacy among groups. These results demonstrate the potential use of TLR-targeted adjuvants as mucosal vaccine enhancers and warrant further optimization for efficacy and practical application in chickens.

Monitoring of Serological Response to NDV Vaccination in Commercial Layers Immunized with HVT-Based Vaccines

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MSD Animal Health

Since the 1980s, Newcastle Disease (ND) has been one of the main health challenges facing the commercial layer sector in Peru, despite the use of up to 5 live and 2 inactivated vaccines during the rearing period and even boosting during the production period, the NDV field challenge (ICPI>1.92) still hits flocks under 30 weeks of age, which show sudden drops in egg production of up to 40% with severe deterioration of the quality of the shell. The use of HVT-based vaccines has been added to the current program, demonstrating improve protection. Several different HVT-based vaccines are currently being used, including single inserted HVT-ND, and dual inserted such as HVT-ND/IBD and HVT-ND/ILT. The serological response to these vaccines' were not detected by traditional ELISA kits. Herein, we present the use of Anti-F protein specific ELISA as a valid tool to identify the serological response to HVT-based vaccines. Results observed a uniform and prompt humoral response that high lives (20,000 with CV>10%) from as early as 5 weeks post vaccination up to 50 weeks-of-age. We highly recommend the use of anti-F protein specific ELISA when monitoring HVT-based NDV vaccines.

Biosurveillance of Newcastle Disease Virus in Multiple Avian Species in Pakistan during 2011-2021

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Newcastle disease (ND) is highly contagious viral disease causing serious economic losses to the poultry production facilities in several South- and East Asian, Middle-East, African and Eastern-European countries. Pakistan presents a unique opportunity to study the mechanisms of viral maintenance and spread in endemic countries. There is a wide variety of non-poultry bird species (both free-living and kept in captivity) in Pakistan; however, limited information is available concerning the potential role of these avian species in the dissemination of NDV. From the last decade, we have isolated virulent strains of Newcastle disease viruses (NDV) from more than 18 poultry and non-poultry avian species in Pakistan. The virulent strains of NDV have been isolated from vaccinated poultry. Outbreaks of virulent strains of NDV of sub-genotype VII.2, XXI.1.1, and XXI.1.2 have been frequently reported in multiple avian species and isolated from different production systems. Virulent strains of NDV have been isolated from multiple birds kept in live bird markets. To understand the relationship among the circulating viruses and to identify the avian species and the husbandry systems that might contribute to ND endemicity, we have isolated and characterized NDV of multiple genotypes from different avian species and production systems in Pakistan over a eleven-year period. These findings provide evidence for the existence of epidemiological links between Newcastle disease outbreaks in commercial poultry and infections with virulent strains in other avian species kept in proximity to poultry. Our results suggest that the endemicity of Newcastle disease in Pakistan involves multiple hosts and environments.Keywords: Newcastle disease, surveillance, VII.2, XXI.1.1, XXI.1.2, avian species, Pakistan

Parasitology/Coccidiosis

Saponin-Rich Plants Mixture Supports Coccidiosis Vaccination Program in Broiler Chickens: A Case Study of Floor Pen Challenge Model

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Vaccination against coccidiosis is on the rise. It is supported by a growing societal demand for antibiotic-free meat products. However, vaccination may be accompanied by some adverse effects, such as a negative impact on growth and/or the development of necrotic enteritis. Moreover, during the time of the establishment of immunity, the birds are only moderately protected. This study investigated different strategies for associating cocci vaccination and a mixture of saponin plants (Norponin XO2) in managing coccidiosis in. 200 day-old male Cobb 500 broiler chickens were distributed in 20 experimental units, each experimental unit of 10 chickens. Chickens were randomly divided into 5 groups: UUC: Uninfested Untreated control, IUC: infested untreated control, VACC: infested and vaccinated group at d1, VACC/NPXO: infested, vaccinated, and supplemented with Norponin XO from d1 to d42, NPXO: infested, nonvaccinated and supplemented with Norponin XO from d1 to d42. The birds were orally inoculated on day 14 of age by live strain vaccine at 15 times the dose.

Growth performances (live weight, feed intake, and FCR) were monitored at d42, 5 birds from each group were randomly selected, and intestinal samples were collected (jejunum) for morphometry. Statistical analyses were performed by analysis of variance (ANOVA) using GraphPad software. The experimental infestation model was successful. It was evidenced by the significant reduction in live weight and increase of FCR of chickens in the IUC group compared to the UUC group. all treatments compensated this loss. Villi length and area were both degraded by Eimeria spp. Infestation. Whereas all treatments, except vaccination, compensated for the loss of villi length, only VACC/NPXO and NPXO treatments were able to compensate villi area significantly. This study evidenced that NPXO2 supplementation is a valuable tool to support vaccination programs. Moreover, the obtained results demonstrate that Norponin XO2 supplementation is a "stand-alone" solution to manage coccidiosis.

The Effects of a High-Flavonoid Corn in Chickens Experimentally Infected with Eimeria Maxima as Part of a Necrotic Enteritis Model

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Flavonoids are natural compounds found in several plants that have been studied for their health-promoting properties, such as antibacterial and anti-inflammatory effects. In a previous publication, we reported that the inclusion of a high-flavonoid corn (PennHFD, Penn State University) in broiler chicken diets effectively decreased mortality, the incidence of intestinal lesions, and improved growth performance of broiler chickens undergoing a necrotic enteritis (NE) challenge. However, the mechanisms of action by which those effects occurred are unknown. Because Eimeria maxima was used as a predisposing factor to induce NE in our previous experiments, we hypothesized that the inclusion of PennHFD in the diets of broiler chickens has an impact on the E. maxima infection. The objectives of this study were to evaluate the effects of a high-flavonoid corn (PennHFD) on growth performance and oocysts shedding in broiler chickens infected with E. maxima oocysts. A total of 240 male broiler chickens (Ross 308, Aviagen) were randomly divided into 48 cages (5 chickens/cage; 12 replicates/treatment) to receive one of the four treatments: CTL A (Non-infected birds fed a commercial corn-based diet); CTL B (Non-infected birds fed a PennHFD-based diet); INF A (Birds infected with E. maxima fed a commercial corn-based diet); INF B (Birds infected with E. maxima fed a PennHFD-based diet). On day 14, birds from the infected treatments received an oral gavage of 5,000 oocysts of a laboratory strain of E. maxima. Fecal samples were collected from each cage on days 6, 7, and 8 post-infection (DPI) to estimate the fecal oocysts concentration. Chickens and feed were weighed weekly to analyze mean body weight gain (BWG), mean feed intake (FI), and mean feed conversion ratio (FCR). No significant changes were observed in performance parameters, although chickens infected with E. maxima (INF A and INF B) had on average 11% lower BWG (P=0.08), and 8.9% lower feed intake (P=0.04), and 5 points higher FCR (P= 0.39) compared to chickens in the control treatments (CTL A and CTL B). No significant differences were observed in oocyst counts between chickens fed the commercial corn line and chickens fed PennHFD (P = 0.50). On 6 DPI, the mean oocysts concentration in treatment INF B was 1.3 x 104

oocysts/g of feces compared to 8.8 x 103 oocysts/g of feces in treatment INF A (P = 0.30). On 7 DPI, a peak of oocysts shedding was observed in all treatments. In INF A, the mean oocysts concentration was 2.4 x 105 oocysts/ g of feces compared to 1.9 x 105 oocysts/ g of feces in INF B (P = 0.362). On 8 DPI, the mean oocysts concentration in INF A was 9.8 x 104 oocysts/ g of feces compared to 7.5 x 104 oocysts/ g of feces in INF B (P = 0.08). In conclusion, the inclusion of high-flavonoid corn (PennHFD) in the diets of chickens infected with E. maxima did not improve growth performance or reduced oocyst shedding. This suggests that the results observed in previous experiments are not because of the effects of PennHFD on E. maxima. Further experiments will be conducted to evaluate the antibacterial and anti-inflammatory effects of PennHFD in chickens challenged with necrotic enteritis.

Compromised Bone Homeostasis in Modern Day Broilers Challenged with Bacterial Lipopolysacchadrides and Mixed Eimeria Cocktail

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The rapid growth of modern-day broilers is associated with great economic benefits; however, they are prone to metabolic disturbances and disproportionate bone development, increasing welfare concerns. Clinical and subclinical coccidiosis is usually associated with decreased profitability and impaired gut health, increasing oxidative stress and inflammation that negatively affect bone development and bone homeostasis. Furthermore, bacterial cell wall component, lipopolysaccharides (LPS) are associated with the inhibition of bone formation which could be released into circulation followed by lysis of gram-negative bacteria during dysbiosis associated with coccidiosis. The current study aimed at bone health following the amalgamation of LPS and Coccidiosis in modern-day broilers. 360, one-day-old male Cobb500 broilers were randomly allocated into 6 treatments (T1-T6) and 5 replicates. Eimeria mixed cocktails with 12,500 E. maxima, 12,500 E. tenella, and 62,500 E. acervulina were inoculated to birds except for the non-challenged control group. Additionally, on day 14, LPS was intraperitoneally injected into treatments 3 and 4 at 1 and 2 mg/kg respectively. Moreover, treatments 5 and 6 were injected with 1 and 2 mg/kg on days 14 and 18 respectively. Statistical analyses were performed using JMP pro 16 statistical software (Cary, NC, USA). Serum sclerostin (SOST) levels, which is a molecule inhibiting bone formation and increasing bone resorption, were significantly increased in T6 injected with 2 mg/kg LPS at two-time points, indicating that coccidiosis and LPS challenge negatively affected bone homeostasis. This is further supported by Calcein labelling for dynamic histomorphometry. In contrast, gene expression of Tumor necrosis factor alpha (a potent pro-inflammatory cytokine) was downregulated along with similar downregulation of the SOST gene, which may indicate negative feedback of severe inflammation and high serum SOST levels. Besides, interleukin-1 beta and NF-kB were upregulated and downregulated, respectively. This could represent that gene expression wouldn't necessarily frame the underlying mechanisms in bone homeostasis. In summary, this suggests that bacterial lipopolysaccharides at higher levels would affect the bone health of the broilers in the concomitant presence of gut integrity concerns.Keywords: Broilers,

Lipopolysaccharide (LPS), Eimeria, Calcein, Sclerostin, Bone homeostasis

Evaluation and Comparison of Performance and Level of Coccidia Immunity of Coccidia Vaccinated Broiler Chickens Fed Various Feed Additives in a Bioshuttle Program

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Evaluation and Comparison of Performance and Level of Coccidia Immunity of Coccidia Vaccinated Broiler Chickens Fed Various Feed Additives in a Bioshuttle Program.Brandon F. Doss (1), Brett Lumpkins (2), Greg F. Mathis (2)(1) Huvepharma, Inc., Peachtree City, GA 30269(2) Southern Poultry Feed and Research, Inc., Athens, GA 30607AbstractCoccidiosis 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 effects of saponin-based products and anticoccidials commonly used as a “bioshuttle” with coccidia vaccine in a coccidia challenge model for impacts on performance, and coccidiosis immunity. A total of 2,450 male chicks were randomly assigned to seven treatments (7 pens/treatment; 50 birds/pen) as follows: 1) Unvaccinated, no additive (negative control); 2) Coccidia vaccinated (Advent) + no additive (Positive Control); 3) Coccidia vaccinated (Advent) + Clarity-Q D14- 35; 4) Coccidia vaccinated (Advent) + Saponin 2 D14 – 35; 5) Coccidia vaccinated (Advent) + Saponin 3 D14 – 35; 6) Coccidia vaccinated (Advent) + Anticoccidial 1 D14-35; 7) Coccidia vaccinated (Advent) + Anticoccidial 2 D14 - 35. On day 14, 28, 35, and 42, performance parameters were measured. Fecal oocysts counts were determined on day 7, 14, 21, 28, and 35. On day 21, one representative bird per pen was selected and sacrificed for ileum sample collection for villi height and crypt depth analysis. Additionally on day 21, 2 representative birds per pen were selected for FITC assessment and sacrificed for ileum sample collection for tight junction protein analysis. To evaluate coccidiosis immunity, on day 21 and 28, four representative birds were removed from each pen and challenged by oral gavage with a combination of E. acervulina, E. maxima, and E. tenella oocysts. Six days post challenge (day 27 and 34, respectively), these birds were euthanized and macro coccidia lesion scored for degree of E. acervulina, E. maxima and E. tenella infection. Microscopic E. maxima scoring from intestinal mucosal scrapings was also performed. Two representative birds per pen were also selected for FITC assessment and sacrificed for ileum sample collection for tight junction protein analysis. The saponin and anticoccidial treatments showed statistically significant reductions in adjusted feed conversion and increase in average weight gain compared to coccidiosis vaccine alone at day 42. However, the anticoccidial treatments produced a statistically significant reduction in adjusted feed conversion compared to the saponin treatments. For the birds challenged on day 21, the coccidiosis vaccine only and saponin treatments produced similar macroscopic lesion scores for E. acervulina and E. maxima respectively, which were statistically significant reductions compared to the unvaccinated group and anticoccidial treatments. All treatments, except for one of the anticoccidial treatments, showed statistically significant reductions in macroscopic E. tenella lesions scores compared to the unvaccinated birds. Microscopic E. maxima scores were similar for all groups. For the birds challenged on day 28, all treatments produced statistically significant reductions in E. acervulina, E. maxima, and E. tenella macroscopic lesion scores compared to the

unvaccinated group. However, statistically significant variations for the respective coccidia were present among the treatments. The coccidia vaccine only group and two of the saponin treatments showed statistically significant reductions in E. maxima macroscopic lesion scores when compared to the anticoccidial treatments. The coccidia vaccine only group and the saponin treatments showed statistically significant reductions in E. tenella macroscopic lesion scores when compared to the anticoccidial treatments. The coccidia vaccine only group and two of the saponin treatments also resulted in statistically significant reductions in microscopic E. maxima scores in comparison to the unvaccinated group and anticoccidial treatments.Keywords: Coccidiosis, Saponins, Broiler, Performance, Coccidiosis Immunity

Evaluation of the Development of Resistance Following Continuous Use of Synthetic Anticoccidials

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Modern poultry production utilizes chemotherapeutic agents as a tool for the control and prevention of coccidiosis, a major disease of chickens caused by protozoan parasites in the genus Eimeria. However, the extensive use of these compounds has led to the development of resistance in coccidia strains. The purpose of this study was to compare the timeline of drug resistance development to multiple synthetic anticoccidials based on bird performance and coccidiosis lesion scoring. In these experiments, broiler chicks were evenly distributed among 5 treatment groups and reared for 6 weeks: Trt 1= no treatment (negative control), Trt 2= day-of-age coccidiosis vaccine (positive control), Trt 3= clopidol 25%, Trt 4= amprolium 25% and Trt 5= zoalene 25%. Treatment groups 3-5 received an anticoccidial in all feeds at an inclusion rate of 1lb/ton. Pen weights and feed consumption were analyzed to determine body weight gain (BWG) and feed conversion ratios (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 results of this study are pending.

Calprotectin (Avian MRP126) as a Fecal Biomarker for Coccidiosis in Broiler Chickens

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Coccidiosis is a parasitic disease caused by protozoan from the genus Eimeria. This disease causes significant economic losses for the poultry industry as it produces severe intestinal inflammation reducing performance and increasing mortality in broiler chickens. Our group has previously reported that fecal calprotectin increases in chickens undergoing necrotic enteritis induced by coinfection with E. maxima and Clostridium perfringens. The present study aimed to detect the level of calprotectin in the fecal samples of chickens challenged only with Eimeria maxima. A total of 560-day-old Ross 308 broilers were randomly allocated into floor pens and divided into control and infected treatments (14 repetitions per treatment). On day 14, the birds in the infected treatment were orally challenged with 7,000 oocysts of Eimeria maxima. On the fifth day post-infection, fresh fecal samples were collected, pooled, homogenized, and kept in ice to minimize protein degradation. The NP-40 lysis

buffer was used to extract protein from the samples. A total of 27 samples (n =14 and 13 for the challenged and control treatments, respectively) were analyzed using a sandwich ELISA according to the manufacturer's instructions. Fecal calprotectin was detected in all fecal samples with an average concentration of 532±321 ng/ml for the challenged and 765±519 ng/ml for the control treatments, respectively. The difference between the two treatments was not statistically significant (P=0.181). These results indicate that avian fecal calprotectin does not increase during a single challenge with E. maxima.Keywords: Calprotectin, Broiler, Biomarker, Eimeria maxima, Coccidiosis

Evaluation of Different Coccidial Control Programs in Broiler Breeder Pullets in a Commercial Production

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A commercial broiler integrator compared four different coccidial control programs from September 2016- April 2020 looking at 1,849,200 broiler breeder pullets. The two main measurables were flock uniformity defined by bird weight coefficient of variation (CV) and mortality. The treatments were: coccidial vaccine A, Zoalene 113 grams per ton, coccidial vaccine B, coccidial vaccine B plus a phytogenic feed additive. The comparison showed some numerical as well as statistical differences between cocci programs.

Coccidia Lesion Score and Performance in Broilers Fed a Nicarbazin-Potentiated Ionophore Up to 21 Days Followed by Different Ionophore Classes in Growing and Final Phases in the Presence of a Brazilian Field Coccidia challenge

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The purpose of this study was to compare the efficacy and zootechnical parameters of a nicarbazin-potentiated ionophore (nicarbazin+semduramicin) in starter phase followed by two different ionophores belonging to different classes (semduramicin or SEM – a glycosidic ionophore, and monensin or MON – a monovalent ionophore) in grower/finisher phase with a contemporary challenge of Brazilian coccidia field strains. In Brazil, in-feed anticoccidials are the main tool used for coccidiosis control in broiler production and nicarbazin-potentiated ionophore products are often used in the first 3 or 4 weeks of the broiler's life (starter phase) followed by ionophores in the grower/finisher phase. It is a common practice to rotate between monensin and salinomycin in the grower/finisher phase, both monovalent ionophores that share similar profiles of anticoccidial resistance and efficacy as reported previously by researchers around the world. Anticoccidial Sensitivity Tests (ASTs) performed with Brazilian coccidia strains in recent years have shown that monovalent ionophores (alone or potentiated with nicarbazin) are becoming less effective when compared with ionophores of other classes. Nicarbazin associated with a glycosidic ionophore – semduramicin: Nic+Sem at 600 g/ton (nicarbazin 48 ppm + semduramicin 18 ppm) was evaluated up to 21 days in both

treatments. Treatment 1, from 22 days up to processing (42 days) was treated with semduramicin (SEM) at 22.5 ppm, while Treatment 2, had monensin (MON) at 120 ppm, in the same period. The Infected Untreated Control (IUC) had no anticoccidial present in the feed. All treatments received virginiamycin at 10 ppm in all feed phases. The experimental model used consisted of a challenge with Brazilian field strains of Eimeria acervulina, Eimeria maxima and Eimeria tenella (214,000, 63,000, 7,700 sporulated oocyst/bird, respectively). Cobb 500 male broilers were challenged via gavage at 18 d. Broilers were kept in floor pens, in a randomized complete block design for 3 treatments of 10 replicates per treatment. Thirty birds per pen were distributed for a total of 900 birds. Coccidiosis lesion score for all treatments was performed according to the Johnson and Reid (1970) methodology at day 24 (3 birds per pen). Weight gain (WG) and feed conversion rate (FCR) at 21 days of age (3 days post-infection) were not affected by treatments due to the use of the same in-feed anticoccidial (Nic+Sem) in both treatments and probably due to the short period after coccidiosis challenge. At 28 days, WG was statistically improved ($P< 0.05$) in treatments with SEM in the grower phase compared to IUC, while the treatment with MON in the grower phase was not different from IUC. Average WG at 28 days in the IUC was 1.523 kg, 1.678 kg in the SEM treatment group, and 1.624 kg in the MON treatment group. FCR at 28 days was statistically improved ($P< 0.05$) in treatments with SEM and MON in grower phase in comparison to IUC. Average FCR at 28 days was 1.345, 1.234, and 1.270 for IUC, SEM, and MON, respectively. While evaluating the 22-42-day period, where only ionophores were used in both treatments, we observed that for WG and FCR the treatment with SEM had the best zootechnical results in comparison with IUC and MON, with statistical difference ($P< 0.05$). Average FCR in the 22-42-day period was 1.624 for the IUC, 1.539 for the SEM treatment group, and 1.593 for the MON treatment group. Average WG from the 22-42-day period was 2.177 kg for the IUC, 2.320 kg for the SEM treatment group and 2.183 kg for MON treatment group. On feed intake (FI), only numerical differences between treatments in all periods evaluated was observed. Average FI from 22 to 42 days was 3.533 kg, 3.567 kg, 3.471 kg, for IUC, SEM and MON, respectively. In regards to coccidiosis lesion score, at 24 days (6 days after challenge), there was a statistical difference ($P< 0.05$) between the IUC and the two treatments on E. acervulina and E. tenella. For E. maxima no statistical difference was observed. While evaluating the TMLS index – total mean lesion score, the in-feed treatments reduced coccidiosis lesion scores by more than 50% in comparison to IUC. The results obtained in the coccidiosis lesion score can be attributed not only to the ionophore used in the grower and final phase (22 to 42 days) on each treatment (SEM or MON) but also to the anticoccidial used in the first 21 days (Nic+Sem) (because the birds were inoculated at 18 days and scored on day 24). Considering the coccidia challenge used in this study (contemporary Brazilian field strains) and that monovalent ionophores are used frequently in broiler production in Brazil in the grower and final phase (without any rest period), the differences in WG and FCR between the two different ionophores tested can be due to a lack of anticoccidial efficacy of monovalent ionophores due to overuse.

Pathology

Chronic Respiratory Syndromes in Captive Houbara Bustards: Clinicopathological Overview and Diagnostic Investigation

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Houbara bustards are terrestrial birds, highly adapted to semi desert regions and steppes habitats. As vulnerable species, they are part of many conservation projects, including captive breeding operations. Monitoring and preserving the health of captive birds is essential to minimize the risk of diseases dispersal to wild populations. Since 2012, hundreds of cases of chronic respiratory syndromes have been reported annually in captive bred Asian Houbara bustards (Chlamydotis macqueenii) in the United Arab Emirates, where several thousands of birds are raised each year (International Fund for Houbara Conservation). To better understand the etiology of this problem, diagnostic investigations were conducted on several cases registered in 2022. Affected birds were as young as 30 days of age and were kept in groups of 12 to 24 individuals, housed in outdoor aviaries. Non-specific signs, such as reduced activity, progressive weight loss and dehydration predominated. Respiratory symptoms varied, in terms of frequency and severity, and included oculo-nasal discharge, sneezing, gurgling and coughing. Variable amounts of mucoid exudate, diffuse thickening of the bronchial walls and, to a lesser extent, accumulation of caseous exudate within abdominal air sacs, were the most significant necropsy findings observed. Histopathology confirmed the presence of tracheitis, airsacculitis and bronchopneumonia, mostly characterized by marked epithelial hyperplasia, interstitial fibrosis, lymphoplasmacytic inflammation and/or lymphofollicular response. A polymicrobial infection, involving one or more viral agents and Mycoplasmas, complicated by environmental conditions, was suspected, guiding molecular testing. An overview of the diagnostic process and the non-infectious and infectious factors identified, including a novel Mycoplasma iowae-like, is provided and discussed.

Necropsy of Back Yard Chickens: A "Wet Lab" on Poultry Oncology

James Davis

Georgia Poultry Laboratory Network

Hundreds of back yard chickens have been submitted to the Georgia Poultry Laboratory over the years for necropsies. A wide variety of tumors have been diagnosed in some of these chickens. Gross photos of some of the tumors, with the corresponding histologic diagnosis as to tumor type, will be shown.

A Retrospective Study on Transmissible Viral Proventriculitis Causing High Condemnation Rates in Argentina

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Transmissible viral proventriculitis (TVP), caused by a Birnavirus, chicken proventricular necrosis virus is closely related to infectious bursal disease virus and produces high economic losses in the poultry industry worldwide. TVP is characterized by enlarged and pale proventriculus with thickened walls and a widened and friable isthmus. The result is poor growth performance parameters and proventricular rupture during processing, potentially contaminating the carcass. One hundred and nineteen proventricular samples were obtained from 42-to-50-day-old, broiler chickens with gross lesions of TVP over 12 episodes of condemnation at the processing plant in the Entre Ríos Province (Argentina) over a period of 5 months. Histopathology revealed TVP-compatible findings including necrosis of glandular epithelium, interstitial lymphocytic inflammatory cell infiltration and tubular metaplasia in 109 proventricular samples. The remaining 10 samples only showed interstitial lymphocytic inflammatory cell infiltration. Molecular studies were performed on representative samples of proventriculi to correlate with pathologic findings to elucidate the presence of this viral agent. This preliminary retrospective study describes massive proventricular condemnation due to TVP-compatible gross and microscopic lesions in broiler chickens in Argentina.

Validating a Model of Hypocalcemia in Laying Hens

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A major welfare and economic concern for the egg industry and the second most common cause of death of commercial egg-laying chickens is hypocalcemia. This occurs due to the high daily requirement for calcium to produce the eggshell. Postmortem diagnosis is a diagnosis 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. Surprisingly, there is no validated quantitative postmortem test to confirm this diagnosis. The goal of the first phase of this study was to validate a model of hypocalcemia. Forty

white leghorn hens aged 35 weeks were randomly assigned to three groups that were fed diets containing either low, normal, or high amounts of dietary calcium for ten weeks. Hens were humanely euthanized at 47 weeks and samples were collected for histopathology, mRNA, and mineral analyses. Statistically significant differences in ionized calcium blood levels were found in hens fed a low calcium diet relative to normal or high calcium diets after 10 weeks; blood phosphorus and calcitriol were similar among all groups. The low calcium fed group laid significantly fewer eggs and these eggs had thinner eggshells compared to the normal and high calcium fed groups. Histologic examination revealed a significantly thinner tibial bone cortex and enlarged parathyroid glands in hens fed low calcium diet relative to normal or high calcium diets. These preliminary results justify the use of this model to develop a quantitative postmortem test to confirm the diagnosis of hypocalcemia in hens submitted for necropsy.

Salmonella

Temporal Salmonella Prevalence and Quantity in Broiler Production

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Despite reduced Salmonella incidence in post-harvest broilers, an estimated 17.3% of human salmonellosis cases are linked to contaminated chicken. Reducing pre-harvest Salmonella contamination is critical to lowering the Salmonella burden in broilers arriving for processing and relies on effective pre-harvest monitoring. This study was designed to evaluate changes in Salmonella prevalence and quantity in 21 broiler flocks during grow out. We utilized bootswabs to assess environmental Salmonella at days 0, 6, 12, 18, 30, 42, 54, and 66 via selective enrichment and plating, and qPCR quantification of Salmonella-positive samples. Bootswabs were collected from four farms across two integrators. Across all flocks, 66.7% (14/21) houses were positive pre-placement, showing that residual Salmonella from previous flocks was present. Salmonella prevalence peaked between 18-30 days of production. At harvest, 52.4% (11/21) of houses remained Salmonella positive. A generalized linear model showed sampling day had significant influence on Salmonella incidence ($p=0.0016$). Salmonella quantity was below the level of detection for most Salmonella positive samples, and peaked day 0 and during the final two weeks of production. Collectively, this data shows Salmonella shedding during broiler grow out is dynamic. Importantly, Salmonella status mid-production does not compare to late-production, and this may impact the timing of pre-harvest Salmonella monitoring and subsequent interventions. Further, only 20% (4/20) of paired bootswabs with quantifiable Salmonella were within 1 log CFU/sample of each other. This demonstrates that for quantification at least, bootswabs may not be an adequate sample.

Salmonella spp. Isolated from Commercial Game Bird Clinical Cases in Missouri and Kansas: Retrospective Study

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Current literature regarding Salmonella isolation in commercial gamebirds is limited. University of Missouri – Veterinary Medical Diagnostic Laboratory (MU-VMDL) records from September 15, 2012, to September 14, 2022, were analyzed to determine the prevalence of Salmonella serovars isolated from pheasant, quail, and partridge diagnostic cases. During the 10-year period, 357 gamebird cases were submitted to the MU-VMDL. Salmonella was isolated directly or by secondary enrichment in 11% of these cases. Recorded bird age ranged from 1 day to 19 weeks, and the most common presenting complaint was increased mortality. 48 Salmonella isolates and 20 different Salmonella serovars were detected. The three most common serovars included Salmonella Typhimurium in 15 (31%), Agona in 4 (8%), and Worthington in 4 isolates (8%). Analysis of antibiotic resistance patterns for each isolate revealed 44% of Salmonella isolates were resistant to 3 or more antibiotic classes, 50% were resistant to 1-2 antibiotic classes, and 6% were susceptible to all antibiotic classes tested. This retrospective study demonstrated that Salmonella Typhimurium was the most common Salmonella serovar isolated in MU-VMDL commercial gamebird diagnostic cases from 2012-2022 and that multi-drug resistance was common.

Using Antibiotic Selection to Improve Salmonella Isolation in Infection Studies

Vinicius Lia, Roy Curtiss, Douglas Natoce

University of Florida

One of the main challenges in studies involving experimental Salmonella infection is the isolation and quantification of inoculated strains in highly contaminated samples, such as caeca content and Bursa of Fabricius. Highly selective media cannot fully inhibit the growth of background colonies and will also decrease Salmonella recovery. We have evaluated the inhibitory ability and efficiency of plating of Hektoen enteric agar, XLD, Salmonella-Shigella (SS) agar, XLT4, Brilliant Green agar, and Selenite Cystine agar when inoculated with intestinal content samples collected from chickens infected with Salmonella Typhimurium, Enteritidis, Heidelberg and Infantis. We have observed that none of the tested media can fully inhibit growth of background colonies and make it difficult to determine Salmonella titers in samples with high bacterial load.Later, we have isolated Salmonella strains resistant to nalidixic acid and rifampicin and demonstrated that a combination of both antibiotics at low concentrations in different selective media can fully inhibit growth of other bacteria while maintaining a very high efficiency of plating when compared with LB agar.

Efficacy Evaluation of Sx, A Novel GRAS Chemical Mixture, on Reducing the Salmonella enterica Serovar Typhimurium Contamination of Broiler Carcasses in a Wing Wash Model

Manjunatha Mahabalarao

Labrotur Research & Consultancy Services, LLC

USDA-FSIS performance standards for salmonella reduction in broiler meat production have been strictly implemented by poultry processing establishments. Furthermore, USDA-FSIS has approved at least fifty products in different combinations for on-line and off-line re-processing in poultry establishments. Sx is a novel mixture of GRAS (Generally Regarded as Safe) ingredients that is a patented technology discovered by biomimicry research in plants of the rainforest. Due to its broad spectrum antimicrobial antibacterial effect, preliminary studies were conducted to determine Its efficacy on the reduction of nalidixic acid-resistant Salmonella spp. on experimentally contaminated broiler carcasses (using a wing wash model). Sx was tested at 100ppm and 200ppm, with or without lecithin and was compared with an untreated negative control group and Peracetic Acid (PAA) at 200ppm as a commercial comparator. There were six treatment groups (Negative control, Sx at 100ppm, Sx at 200ppm, Sx at 100ppm with lecithin, Sx at 200ppm with lecithin and PAA at 200ppm) There were 9 replicates (one pair of wings) for each treatment group. Three replicates of each treatment group were used in three different sets. Each pair of wings were sprayed with 10mL of Salmonella enterica serovar Typhimurium inoculum – (108 cfu/mL). After 15 minutes of incubation at room temperature (25 0C), each pair of wings were immersed in the respective treatment solutions for 15 minutes. Afterwards, each pair of wings was removed aseptically and rinsed with 100mL Buffered Peptone Water supplemented with 0.1% of Sodium thiosulphate. Two mL of rinsate was collected, serially diluted and plated on XLT4 agar using the Drip method in duplicates. After incubation at 37 0C overnight, typical colonies of salmonella were counted to determine the final concentration. The final data was statistically analyzed by One-way ANOVA in the GLM of SAS (JMP 16.2, Cary, NC) for each treatment group. All the treatment groups have shown statistically significant ($P<0.05$) improvement when compared to negative control. The most significant observation was made with Sx at 200ppm (3.28 x 105 cfu/mL) with Lecithin which showed the reduction of salmonella counts by 1.09 Log when compared to the untreated control (4.21x 106 cfu.mL)and by almost 50% reduction when compared to PAA 200ppm (6.51 x 105 cfu/mL). It was concluded that Sx as a GRAS ingredient product could be a user-friendly, environmentally safe, and economical alternative for the reduction of Salmonella carcass contamination in poultry processing facilities. *Labrotur Research and Consultancy Services, LLC., Indiana£Antimicrobial Consultants, LLC, IndianaµEcoPlanet One Health, LLC, Montana†Blue River Research Services, Indiana

Biofilm Formation Capacity by Salmonella spp. from Poultry Farms in Lima-Peru

Magali Salas, Nilda Castro Amaro, Nicole Lazo Campos, Antoinette Reyes Rossi

ALFA BIOL

Biofilm formation capacity by Salmonella spp. from poultry farms in Lima-PeruNilda A. Castro-Amaro. 1, Magali Salas-Martínez.2, Antoinette Reyes-Rossi.3, Nicole Lazo-Campos. 41, 2, 3, 4 Microbiology Laboratory ALFA BIOL S.A.C, Lima, PerúBiofilms are single or multispecies microbial communities form of viable and non-viable microorganisms, aggregates in an extracellular polymeric substance (EPS) that themselves produce as a survival and protection mechanism. The aim of this study was to investigate biofilm producing ability of Salmonella spp. isolates. For this, 1064 samples of boot swabs, drag swabs and reuse litter, were analyzed from poultry farms in the north and center of Lima- Peru, during the year 2022. The methodology used for the detection of Salmonella spp., was National Poultry Improvement Plan Program Standards APHIS- USDA 2019, obtaining 45 positive samples (4.2%), of which 29 strains were chosen to evaluate the biofilm formation capacity. The classification of the strains of Salmonella spp. biofilm-forming, was carried out according to the cut-off point optical density (ODC) established by Stepanovic (2004).The results indicate that all Salmonella spp. strains have the ability to produce a biofilm when they are at environmental temperature. Of the samples tested, 86% were weak biofilm-forming strains, and the remaining 14% have moderate capacity to form biofilms; strains with strong capacity for biofilm production were not obtained.The presence of strains of Salmonella spp. with moderate capacity to produce biofilms raises concern for the poultry industry and public health since the production of biofilm is an efficient mechanism of bacterial resistance to antimicrobials and disinfectants that are constantly used in the processing poultry, storage of poultry products.Keywords: Biofilm, Salmonella spp., optical density

Vaccinology

CpG-Oligonucleotide Can Enhance Recombinant Herpes Virus of Turkey-Laryngotracheitis Vaccine-Induced Immune Responses

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Infectious laryngotracheitis (ILT), caused by alphaherpes virus, is an economically important disease of broiler chickens. While the recombinant vaccines can be advantageous in reducing the the clinical disease, they are also associated with poor immunogenicity and delayed onset of immunity. The present study used CpG-oligonucleotides (ODN) as an adjuvant in boosting the responses induced by the commercial recombinant ILT vaccine (rHVT-LT) in one-day-old broiler chickens, when administered in-ovo. The results showed that while the CpG-ODN adjuvanted vaccine could induce significantly increased transcription of IFNï§ and IL-1ï¢ in the spleen and IFNï§ in lung tissues, the expression of IL-1ï¢ gene in the lung was significantly downregulated in the lung compared to unvaccinated control. Additionally, the splenic transcription of inducible nitric oxide synthase (iNOS) in the groups receiving rHVT-LT with or without CpG-ODN was significantly reduced, whereas the toll-like receptor (TLR)21 gene expression in the spleen and lung was also significantly downregulated compared to control. Furthermore, the splenic cellular immunophenotyping showed that the adjuvanted vaccination led a significantly higher

number of macrophages, CD4+ T cells, including the frequency of activated T cells (CD4+CD44+) when compared to control. Collectively, the findings suggested that CpG-ODN can boost rHVT-LT vaccine-induced T-helper-1 responses in broiler chickens.

Identification of Vaccine Candidates Against Novel Campylobacter Using Phage Display Technology

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Spotty liver disease (SLD) caused by C. hepaticus is an emerging cause of morbidity, mortality, and loss of production in commercial laying hens. Currently, the commercial layer industry relies on C. hepaticus autogenous killed vaccines. However, the slow growth of C. hepaticus, the detrimental effects of the high-volume use of killed vaccines on pullet performance, and the cost of production prohibit the use of killed vaccines. Therefore, a live-vectored vaccine expressing protective C. hepaticus antigen/s is a logical approach to control SLD.Material and methods: The phage display reverse vaccinology was used to select candidate vaccine antigens. First, the antibodies against killed whole-cell C. hepaticus were raised in specific pathogen-free female Leghorn chickens. Then, the protective antigen epitopes were identified using Ph.D.-12 phage display peptide library kit from the New England Biolabs and hyperimmune serum raised against C. hepaticus. Finally, DNA sequencing, Basic Local Alignment Search Tool, and subcellular localization prediction identification were used to prioritize the identified epitopes (mimotopes).Results and conclusion: We discovered 16 mimotopes matched with 13 different proteins of C. hepaticus as potential vaccine candidates. Some of these proteins have already been tested as potential vaccine candidates against other Campylobacter species, such as Campylobacter jejuni and Campylobacter coli. These epitopes' immunological and protective abilities have to be confirmed through in vivo and in vitro research.

Statistical Report on Zootechnical Results on the Use of a Recombinant HTV-ND ILT in a Broilers Company in Colombia

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The inclusion of recombinant vaccines has become a very useful tool for the global poultry industry since it provides benefits in logistics, management and health, and Colombia is no stranger to this premise. Newcastle disease (NDV) and avian Laryngotracheitis (ILT) continue to be a concern in the Colombian countryside, controlling them can facilitate the management of other entities such as Avian Infectious Bronchitis.A statistical analysis was carried out comparing batches before and after the inclusion of a recombinant HVT-ND-ILT vaccine (includes NDV F protein, gD and gI of ILT) in a Colombian company located in the department of Cundinamarca. The history of the farm and the area presents a very strong challenge in ILT, with conditions of high poultry population

including broiler breeders, commercial layers and a predominant broiler population.Forty-eight batches with zootechnical data were included in the analysis. Regarding feed conversion, a statistically significant difference was observed in favor of the batches that received the biological. This may indicate better performance and expression of genetic potential when using vectored vaccine, in addition to seeing a serological trend with less reaction to the disease. This is supported by the multivariate model, where it is adjusted for confounding variables.In addition, a better consumption behavior was observed, which may indicate that by having sanitary control, the productive indices can improve. This analysis supports the literature, where it is evident that the use of this biological in farms that maintain an adequate biosecurity status, zootechnical and health (serology) results are obtained that express the potential of high-performance birds.

An Avian Pathogenic E. coli Vector System for Delivering Heterologous Vaccine Antigens in Poultry

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Poultry constitutes the most important protein source for human consumption worldwide. One of the biggest threats to the poultry industry are infectious diseases, which are primarily controlled by antimicrobial and antiparasitic drugs, vaccines, and biosecurity practices. Due to the increasing consumer demand for “organically-raised” or “antibiotic-free” poultry, vaccination has been one of the most common approaches to control economically significant diseases. However, the current volume of vaccines has negatively affected the industry due to many reasons, including rising vaccine production and administration costs and poor growth performances associated with some vaccines. Avian pathogenic Escherichia coli (APEC), a major pathogen causing severe illness in poultry, affects all bird types and ages. In this study, we constructed two attenuated strains of avian pathogenic E. coli lacking the asd gene (Δasd single mutant and ΔasdΔaroA double mutant), which is involved in synthesizing diaminopimelic acid (DAP), to deliver heterologous vaccine antigens via an asd+ expression plasmid. Because the Δasd mutants will have an obligate requirement for DAP, they will not survive in the host without the asd+ plasmid. This system provides a balanced lethal combination to deliver antigens obviating the need for an antibiotic-selective marker in the live vaccine. This vaccine vector strain is expected to deliver vaccine antigens of other pathogens while providing protection against colibacillosis. In vivo experiments are currently underway to demonstrate the safety, immunogenicity, and efficacy of the APEC mutants expressing a novel Salmonella vaccine antigen to protect commercial egg-type chickens against colibacillosis and Salmonella colonization.

Field Trials of FAdV Inactivated Trivalent Vaccine (4, 8b, 11) in Korean Poultry Farms

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Fowl adenovirus(FAdV) is a pathogen causing huge economic losses in poultry farms. World-wide occurrence of FAdV infection underscores the importance of proper vaccination. In order to prevent outbreak of prevalent FAdV strains in Korea (4, 8b, 11), inactivated trivalent FAdV

vaccine (PoulShot IBH 4/8b/11) was manufactured and applied to 3 poultry broiler breeder farms. Here we evaluated efficacy of novel inactivated trivalent FAdV vaccine by analyzing neutralizing antibody of FAdV-4, 8b and 11. Testing and control groups were vaccinated with inactivated trivalent vaccine and commercialized FAdV-4 inactivated vaccine (PoulShot® Adeno) respectively. All breeders were vaccinated at 14~15 weeks of age. Blood samples were collected at 0, 4, 8, 12, 16, 20 weeks-post vaccination in order to conduct virus neutralization tests. According to the data all farms were infected with FAdV-8b and 11 by showing positive titers of neutralizing antibody from 0 wpv sera or 4 wpv sera. Additionally, breeders vaccinated with FAdV trivalent vaccine showed relatively high titers of neutralizing antibody of 8b and 11 from all sera(~20 wpv) compared to control group indicating possibility of higher duration of immunity. Results once again prove that FAdV-8b and 11 are prevalent strain in Korean breeder farms implicating the need of trivalent FAdV vaccine.

Serological Evaluation of (Siderophore Receptor and Porin Proteins) SRP® Response Under Natural Conditions

Chris Parmer

Vaxxinova

Broiler breeder pullets were evaluated for their serological response to vaccination. The vaccine was an SRP bacterial extract containing the siderophore receptor proteins of Escherichia coli and Salmonella enterica. ELISA was used to measure antibody response. Pullet flocks from multiple locations were evaluated. The data set includes pullets naturally exposed to E. coli and Salmonella. All pullets were vaccinated, and blood was collected at strategic time points. The serological response to SRP vaccination will be discussed.

Efficacy Evaluation of Vaccine Programs Containing Live and Inactivated Salmonella Vaccines Against Challenge by Salmonella Gallinarum in Brown Laying Hens

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Poultry farming in Brazil and other countries in Latin America and Asia are constantly affected by outbreaks of avian typhus, a disease caused by the serovar Salmonella Gallinarum, a host-specific bacterium of birds. Avian typhus brings losses to the sector due to the mortality of affected birds, including adult birds with a long cycle and, therefore, with greater individual value, such as commercial laying birds and breeding birds. The control of these bacteria by vaccination is currently a high priority demand, both within the scope of the poultry production industry, as well as by the vaccine industry, international animal protein trade, retailers and consumers. The aim of this study was to evaluate the effectiveness of vaccine programs using combinations of live vaccine and inactivated vaccine against Salmonella Gallinarum (SG), in the face of an experimental challenge in brown laying hens. Three groups of birds were evaluated using different vaccine schedules: GT1 – Vaxxon SG 9R (live) at 7 and 10 weeks; GT 2 – Vaxxon SG 9R (live) at 7 and 10 weeks + Vaxxon SE SG (inactivated) at 14 weeks; GT3 – Vaxxon SE SG (inactivated) at 9 and 14 weeks, as well as a Positive Control group (GCP) was performed. All groups were challenged with Salmonella Gallinarum at 18 weeks of age, and birds were evaluated up to 22 weeks. ELISA results showed good seroconversion at 18 weeks of age for the 3 vaccinated groups: 5496, 5680 and 6307 of GMean for GT1, GT2 and GT3 groups

respectively. The mortality that occurred in the groups after challenge with SG was 7% in GT1, 3% in GT2 and 10% in GT3, varying significantly between groups GT3/GT1 and GT2, as well as with the vaccinated groups and positive control group that did not receive vaccine, that had a mortality rate of 47%. The use of combined live and inactivated vaccines proved to be more effective in controlling avian typhus than the exclusive use of live or inactivated vaccine.

The Development of a Novel Vector for mRNA Vaccines Against Respiratory Pathogens of Poultry

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Vaccines are the single most cost-efficient and equitable way to combat and eradicate infectious diseases of poultry. While conventional licensed vaccines consist of inactivated/attenuated versions of the entire pathogen, subunits of it or recombinant viral vectors expressing the antigens, there is a need to develop experimental vaccines that employ nucleic acids to produce the antigen of interest directly in vivo. Decades ago, DNA plasmid-based vaccines were developed, but their commercialization failed due to the large quantities of DNA (micrograms to milligrams) needed to elicit protective immunity. With recent advances in nanoparticle formulations and ways to eliminate sensing of foreign nucleic acids, mRNA-based vaccines have gained in popularity. The advantages of using nucleic acid vaccines include their ability to induce durable immune responses, high vaccine stability, and ease of large-scale manufacturing. To this end, we have used the untranslated regions of the chicken β-globin gene to construct novel mRNA vectors. These vectors produce full length capped and polyadenylated RNAs encoding antigens of infectious laryngotracheitis virus and infectious bronchitis virus. We will present an overview of their design, the method to generate the RNAs and their characterization in vitro.

Virology

Serological Profile During the Induced Molting Process in Commercial Layer Flocks

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Induced molting is a common practice in many commercial layer companies in Latin America to extend the egg production period over 100 weeks. However, there is no information indicating if this very stressful practice can affect the duration of the humoral immune response present in bird that undergo molting. A controlled trial was conducted in a commercial layer farm to verify the humoral immune profile before, during and after molting process. Seventy-five-week-of age Hisex commercial layers were submitted to feed and water restriction for a 9 day period to induce molting. Serum sampling (20 samples) were collected at 0, 4, 9 and 38 days after molting started. Elisa serology using commercial kits (Idexx, IDvet and BioChek) was carried out for measuring antibodies (Ab) against Infectious Bursal Disease virus (IBD), Chicken Infectious Anemia virus (CIA), Infectious Bronchitis virus (IB), Newcastle Disease virus (ND) and Egg Drop Syndrome virus (EDS). Results were compared in terms of titers and Ab-positivity. In case of IBD, CIA, IB and ND, 100% of Ab-positivity was found in all monitored

ages with similar Ab titers. In case of EDS, Ab positivity varied between 29 and 64%, but titers were similar among the collected ages. Titers were analyzed and statistically compared using One-way ANOVA test. No statistical difference ($p>0,05$) was found between ELISA titers measured at the beginning, during and after molting for all evaluated diseases. These results indicate that molting process does not affect the humoral antibody titers against the evaluated diseases in the blood circulation of molted layers.

An IBH Outbreak In Meat-Type Grandparent Stock in Brazil Caused by Fowl Adenovirus Serotype 11

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Fowl adenovirus (FAdV) is common etiological agent in poultry industry in several countries in Latin America genotyping/serotyping is done based on its hexon gene sequencing. Previous publications reported the presence of serotypes 4, 8 and 11 circulating in Brazilian poultry farms. However, vaccination is not usually practiced in the industry since severe clinical outbreaks have not been clearly linked to FAdV laboratory detection. An outbreak of a sudden increase of mortality with characteristics IBH liver lesions were observed in two flocks of meat-type grandparent stock of the same great-grandparent flock origin. Affected flocks showed sudden onset of mortality at 12 days of age with peak at 18 days of age and return to normal levels after 8 days. Mortality ranged between 7 to 9 %. Clinical signs included lethargy, huddling with ruffled feathers and inappetence. At necropsy, enlarged, pale and friable liver was noted. Mild hydropericardium was observed in some birds. Liver, pancreas, gut intestine samples were collected of diseased birds for histopathology and molecular analysis. Microscopic lesions were observed in all collected organs and FAdV-11 was detected by hexon gene sequencing. IBDV and CIAV were not detected in the sampled birds. FAdV was also not detected in the neighbor flocks which housed birds of different origin that never presented clinical signs. This observation and the type of disease presentation strongly suggest vertical transmission.

Pathogenicity in Chicks of Goose Tembusu Virus Isolated in Taiwan

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Since 2010, the Tembusu virus (TMUV) has caused severe outbreaks with neurological signs and egg-drop diseases in ducks and geese in neighboring countries of Taiwan. In late 2020, an infectious disease outbreak characterized by white diarrhea, depression, lameness, prostrate and increased mortality occurred in a 45-day-old white Roman geese flock in southern Taiwan. TMUV infection was diagnosed in diseased geese by RT-PCR. TMUV was successfully isolated using minimal-pathogen-free duck embryos and designated NTU/C225/20. The full-length genomic sequence of the virus was determined by next-generation sequencing. Genomic

analysis revealed that the NTU/C225/20 shares approximately 87% nucleotide identity with recently reported TMUV strains in China, Malaysia and Thailand, and 91% with the prototype strain MM1775 identified back in 1955. TMUV NTU/C225/20 was propagated in minimal-pathogen-free duck embryos, specific-pathogen-free (SPF) chicken embryos, and the DF-1 cell line. Following concentration and titration, the virus was further used to infect day-old SPF chicks for pathogenicity analysis. The results revealed that TMUV NTU/C225/20 exhibits pathogenicity in the day-old chicks via intramuscular inoculation, characterized by growth retardation, hyperthermia, and slight reduction of activity. Gross lesions in infected chicks were characterized chiefly by hepatomegaly, hyperemic thymus, and splenomegaly. Tissues including brains, hearts, livers, and spleens were detected as positive for TMUV by the NS5-specific real-time RT-PCR assay. Non-inoculated co-housed chicks also became infected, evidenced by virus-positive detection results in tissues. This is the first study to investigate the pathogenicity of Taiwan TMUV in chicks.

Deletion of the Chicken TVA Receptor Abolishes ALV-A Infection in Vitro

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The USDA Avian Disease and Oncology Laboratory (ADOL) currently uses live chickens of specific genetic backgrounds to produce the embryonic fibroblasts that are used to detect and subtype isolates of avian leukosis virus (ALV). We developed a cell line in which tva, the gene encoding the viral receptor for ALV subtype A (ALV-A) was deleted. Starting with the DF-1 cell line (derived from chicken embryonic fibroblasts) we used the CRISPR-Cas9 system to disrupt the tva gene. After cell sorting we identified several DF-1 clones that had suitable (i.e., bi-allelic and homozygous) indels at the target site in the second exon of tva. When we tested these cells for their ability to host ALV-A, five clones with frameshift mutations that disrupted the tva gene were not competent to support ALV-A replication in vitro. These results demonstrate that engineered cell lines can be used as part of a battery of tests to determine ALV subtype for isolate characterization, thus conserving resources and removing our need to use live animals in this subtyping assay.

Detection, Isolation, And Molecular Characterization of Distinct Group of Avian Reovirus from Chicken Flocks

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The recent emergence of new avian reovirus (ARV) variant strains 'malabsorption syndrome (MAS) and infectious arthritis' in chickens have caused a significant negative economic impact on the poultry industry across Egypt. In this study isolation and genetic characterization were performed for ARV. 16 ARV PCR positive of collected 53 bird tissue samples from different Egyptian poultry farms, vaccinated with ARV, showed clinical signs of arthritis/MAS. 3 samples were isolated successfully on ECE and cell

cultures (LMH, Vero, CEF, and CELi). Sequencing and phylogenetic analysis of the partial σC "genetic marker" for the 3 isolated samples was done, revealing that the 3 isolates were located in genogroup cluster IV with amino acid identity to each other, and with genogroup cluster, I different vaccinal strains "S1133, 2177" were (77-98 %), and (39-45 %) respectively. In conclusion, Egyptian isolates located in a gene cluster IV are distinctive from the commercial vaccinal strains. Updating of effective ARV vaccines and synchronized surveillance strategies are recommended to control the ARV situation in the poultry flocks in Egypt.

Development of an Indirect Enzyme-Linked Immunosorbent Assay for the Diagnostic and Vaccination Monitoring of Fowl Adenovirus Group I

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Innovative Diagnostics

Fowl-adenovirus (FadV), belonging to the gender Aviadenovirus, are responsible for many infections and economical losses in poultry flocks. Infections caused by these avian Adenovirus are widespread and endemic in chicken, turkey, and quail. As today, at least 12 serotypes were identified and classified as Fowl-adenoviruses. Some of them do not necessary lead to the development of the disease, such as serotype 1 which is low pathogenic, and responsible for Adenoviral Gizzard Erosions (AGE). However, other serotypes could induce different syndromes in the field: Inclusion Body Hepatitis (IBH), caused by the serotypes 2, 8 or 11; and Hydropericardium syndrome (HPS), caused by the serotypes 4. As to control the evolution of the disease and reduce the economic losses, vaccination with commercial or autogenous vaccines, based on serotypes 2, 4, 8 or 11, are more and more used. Given the need of serological kits for the monitoring of vaccination, Innovative Diagnostics (IDvet) has developed an indirect ELISA for the diagnostic and vaccination monitoring of fowl adenovirus group I. The performances of this indirect ELISA were evaluated with experimental and field samples vaccinated or not with fowl-adenovirus vaccines. The following poster summarizes the preliminary validation data obtained for ID Screen® Fowl Adenovirus Group 1 Indirect.

Field Monitoring of the Mareks Hybrid Vaccine RN1250 Take by Quantitative PCR

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Marek's disease (MD) has been recorded for decades as one of the major diseases of poultry. This Gallid Alphaherpesvirus 2 that causes atrophy of the Bursa of Fabricius and thymus within 3 days of exposure to chickens and targets various lymphocyte subpopulations, induces tumor formation and immunosuppression. Vaccination has been the most relevant tool together with cleaning, disinfection and biosecurity measures to reduce the incidence of the disease worldwide. A new generation of Marek's vaccine was licensed in the USA in 2016. The vaccine strain is a hybrid virus that combines CVI988 with the RM1 and Md5 strains of the Marek's disease virus (Prevexxion RN – RN1250 strain). A specific quantitative PCR (qPCR) test has been developed to the CIV988 vaccine construct for vaccine monitoring. qPCR can be performed on FTA cards containing pressed spleen and feather pulp samples. This test complements field monitoring of oncogenic MDV and RN vaccine virus which is highly

relevant in the context of layer and broiler-breeder pullets. Overall, in this study, vaccine virus detection was correlated with no detection of Marek's disease.

Genomic Surveillance of Avian Paramyxovirus in Wild Birds in South Korea during 2017-2021

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Avian paramyxoviruses (APMVs) are one of the economically important avian viruses and are mostly isolated from domestic poultry and wild migratory birds. A total of 22 serotypes have been defined by the International Committee on Taxonomy of Viruses (ICTV). We collected fresh fecal samples from wild bird habitats in Korea during 2017-2021 and identified 117 APMVs including APMV type 1, 4, 13, and 16. We conducted whole genome sequencing using Illumina next-generation sequencing and sequence analysis to investigate their genetic characteristics. Phylogenies showed high genetic diversity of APMVs recently isolated in Korea. No virulent APMV type 1 was identified. Our data suggest that wild waterfowl play a key role as a natural reservoir in the maintenance and dissemination of APMVs. Enhanced surveillance is therefore crucial for the understanding of global APMV transmission, ecology, evolution, and epidemiology.

Pathotype Determination of Very Virulent Plus Marek's Disease Virus with Multiple Nucleotide Changes in Virulence-Related Genes

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Marek's disease (MD) is a highly infectious lymphoproliferative disease with significant economic impacts. Gallid alphaherpesvirus 2, better known as Marek's disease virus (MDV) has been categorized based on virulence rank as three pathotypes, virulent, very virulent, and very virulent plus. A comparative genomics study suggested that single nucleotide polymorphisms (SNPs) in eight open reading frames (ORF), UL22, UL36, UL37, UL41, UL43, R-LORF-8, R-LORF7, and ICP4, were most significantly associated with the different pathotypes of MDV. We validated these SNPs by substituting the nucleotides in the suggested eight ORFs in the genome of vv+MDV strain 686 with unique nucleotides observed in vMDV strains. All modifications resulted in nonsynonymous amino acid substitutions of the MDV genome. Initial pathogenicity studies indicated that these substitutions of SNPs showed reduced MD incidence and increased survival of challenged birds compared to those of the parental virus. In this study, the pathotype of the modified viruses were evaluated by the best fit pathotyping method to rank the modified viruses with reference pathotype viruses. The modified genome of the vv+MDV 686 virus resulted in the reduction of its pathotype/ virulence similar to the vvMDV Md5 strain, but these changes in eight ORFs alone were not sufficient to reduce to the vMDV pathotype.

Seroprevalence of New Castle (ND) and Avian influenza viruses (H5, H7 and H9) in Commercial Poultry Flocks of Punjab, Pakistan

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Poultry industry in Pakistan is affected by different viral pathogens such as the Newcastle disease virus (NDV) and Avian influenza viruses. Despite the introduction of vaccination program against these pathogens, outbreaks are commonly observed every year in the poultry farms. Therefore, the present study was designed to estimate the seroprevalence of ND and Avian Influenza subtype H5, H7 and H9 in different commercial poultry farms around the Punjab province. A total of 828 serum samples were collected from 53 poultry flocks from January 2022 till December 2022 and examined by hemagglutination inhibition (HI) test for specific antibodies against ND and Avian influenza subtypes H5, H7 and H9. The HI test revealed a significant difference in the seroprevalence of H5 (8.9%) compared to H9 (0.9%) and ND (1.4%). However, none of the sample was positive for H7. The antibody titer of these flocks were significantly found lower at the start of winter (November, Av: 3.1 ± 0.3) and summer season (April: 4.5 ± 0.3) compared to other months (Av: 6.7 ± 0.7). It is pertinent to note that (397/828 = 48%) of these samples only showed the protective level of antibody (>6) titer. Based on these results, it can be concluded that sporadic occurrence of avian influenza and ND are visible in poultry flocks apparently linked to seasonal winter bird migration, furthermore, antibody titer of vaccinated birds warns that serious bio-security measures must be implemented on the farms.

Field report: Variant Reo Virus Strains in Broilers from Colombia

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Avian Reovirus (ARV) infection are considered a reemerging pathogen worldwide. During this decade, the poultry industry has faced the consequences of ARV variants with clinical cases of tenosynovitis and severe runting stunting syndrome (RSS) in vaccinated broiler breeders and their progeny. This is the case for Colombia broiler industry where green tendons, leg condemnations are accompanied by field lameness consistent with viral arthritis in broilers despite the vaccination programs in place, with substantial associated economic losses. Synovial fluid was collected in FTA cards form affected broilers at harvest age and processed for PCR and sequencing. The FTA samples were positive for reovirus by RT-PCR using the reovirus S1 primer set. The amino acid sequence of the reovirus Sigma C product was 81% similar to variant group 1 / genotype 5 field isolates previously characterized in the USA. The viruses are less than 50% similar to vaccine strains S1133, 2408, 1733 and 2177. This is the first report of variant reovirus strains circulating in Colombia. Further studies are in place to fully characterize the field challenge and investigate the consequences for the local industry.

Wealth of Knowledge

Genetic Characterization of Immune Related ChB6, IAP-1 and IL-15Rα Genes in Native Poonchi Chicken from International Borders of India and Pakistan

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Poonch is one of the remotest districts of the Jammu and Kashmir (UT) and situated on international borders. Backyard poultry farming with local native indigenous chicken is common practice in these hilly areas. This native poultry population is quite hardy and thrives well in adverse climatic conditions. They are considered to be more disease resistant than their commercial counterparts. However, it is difficult to ascertain whether a single or a set of gene (s) define this property to indigenous chickens. Therefore, present study was conducted for characterization of ChB6 (Chicken B-cell marker), IAP-1(inhibitor of apoptosis protein-1) and IL-15Rα (Interleukin-15) genes in indigenous Poonchi chicken population. Material and Methods: RNA was extracted from fresh blood samples of Poonchi chicken and Kadaknath chicken. After cDNA synthesis from RNA, of ChB6, IAP-1 and IL-15Rα of 1110, 554, 243 bp size respectively was amplified from cDNA using specific primers designed by Primer 3 software. Direct sequencing was carried out by Sanger sequencing and results were analyzed using BioEdit and MEGA X software's. The phylogenetic tree was constructed using Neighbor-Joining method based on the aligned sequences.Results: The results obtained from sequence analysis showed that there was no variation in the sequences of Poonchi, Kadaknath and Leghorn chicken populations for ChB6 and IAP-1 genes but there was variation with Fayoumi and other reported Gallus gallus sequences and other species. For IL-15Rα gene there was variation within Poonchi population as well as between different chicken populations like Kadaknath, Leghorn, Fayoumi. For IL-15Rα in the present study T → C SNP change was detected. It was observed that the AT content was higher in case of ChB6 and IAP-1 gene in Poonchi chicken, whereas, GC content was higher in IL-15Rα gene in Poonchi chicken. The highest genetic distance for ChB6 gene was observed between Poonchi chicken and Miniopterus fuliginosus with a value of 2.2672. The genetic distance for IAP-1 gene of Poonchi chicken with Fayoumi breed was 0.0097. The highest genetic distance for IAP-1 gene of Poonchi chicken was observed with Tympanuchus phasianellus with value of 1.4851. Within the Poonchi chicken population the genetic distance was 0.0078 for IL-15Rα gene. The highest genetic distance was observed for Poonchi chicken with Homo sapiens with value of 3.0053. The genetic distance for IL-15Rα gene with Kadaknath population were 2.5348 & 2.5712, respectively with Poonchi_1 and Poonchi_2. Phylogenetic tree of ChB6, IAP-1 and IL-15Rα gene of Poonchi chicken was analyzed with other sequences. Conclusion: The multiple alignment and genetic distance studies indicate that there is sustainable variation between the different breeds and species. Therefore, further association studies of these genes with different diseases in large population would be helpful to identify disease resistant / susceptible genotypes in the indigenous chicken population.

National Poultry Improvement Plan (NPIP) as a Model Program for Other Sectors within APHIS: Swine Health Improvement Plan (SHIP) Administration Moving Beyond the Pilot and the Growth of the Comprehensive Aquaculture Health Program Standards (CAHPS)

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The USDA APHIS Veterinary Services Strategy and Policy Aquaculture, Swine, Equine and Poultry (ASEP) Health Commodity Center has been active in recent years with assisting the development of new programs. Recently, to combat African and Classical Swine Fever in pigs, a pilot program was developed – the Swine Health Improvement Plan or SHIP – that is now moving beyond the pilot phase to become integrated as an official program into USDA APHIS. The SHIP program bases some of its principles on the National Poultry Improvement Plan (NPIP). Besides the SHIP, a substantial grant has recently been awarded to support the 2023 National Aquatic Health Plan and Standards (NAHP&S) as well as the Comprehensive Aquaculture Health Program Standards (CAHPS), again with the NPIP model in mind. CAHPS aims to improve the health of US farm-raised aquatic animals, to facilitate interstate and international trade and/or movement of live aquatic animals, and to improve marketability of its participants. If fully implemented and embraced by the States and industry, CAHPS would establish site-specific plans for biosecurity, surveillance, reporting, and response related to animal health events. Developed by the industry and APHIS, CAHPS is purposeful, premises-specific, and science-based, abiding by the five principles of Aquatic Animal Health Team; Risk Characterization and Management; Surveillance; Investigation and Reporting; and Response.The NPIP is widely recognized as the gold standard for poultry monitoring and surveillance. The 87-year-old federal-state-industry cooperative program is now the model for other commodities within APHIS. This presentation will focus on describing key features of the Comprehensive Aquaculture Health Program Standards, track progress of the Swine Health Improvement Plan as it moves into the next phase of administration, and compare and contrast the structures of each program with NPIP.

Laying Hens in Saint Kitts: Understanding Basic Demographics, Biosecurity, and Sanitary Status

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In the Federation of Saint Kitts and Nevis, there is a local market of commercial egg producing poultry farms whose demographic is unknown. This agricultural sector has not been researched, existing only one study on feral chickens in Saint Kitts, that found seroprevalence of antibodies against infectious bursal disease (86%), infectious bronchitis virus (84%), Mycoplasma (37%), and Avian avulavirus 1 (Newcastle disease virus, 31%). This exposure in the island's non-commercial chicken population raises concern for amplification and transmission to the commercial poultry farms and their potential biosecurity risk. This pilot study focuses on describing the demographics of commercial poultry farms in Saint Kitts and evaluating their biosecurity and sanitary status. To date, 9 poultry farmers registered with the Saint Kitts Ministry of Agriculture completed a biosecurity and sanitary status questionnaire. A current demographic was established, with small-scaled

laying farms with sizes ranging from 40 to 2600 (average 1000) hens at the time of survey, with an overall average daily production of 592 eggs per day, and one farm having 0 eggs produced daily. Most farms (78%) are non-caged systems housed inside barns, except for one free-range and one caged hen's facility. Only 2 farms surveyed record daily egg production, feed consumption and mortality, with many farms not having any records collected (67%). Biosecurity proved poor, with all farms sharing a lack of set biosecurity protocols, proper perimeter excluding wild animals from entering, and footbaths upon entry. While a few farms did not required designated clothing (33%), farmsthat used designated working clothes used them for all farm activities, rather than exclusively used for the hen enclosures. Cleaning and disinfection routines were reported as variable, ranging from every other day to semiannual cleaning, with some farms (67%) disinfecting with varying products, including vinegar, chlorine, Lysol, etc. All eggs produced were not refrigerated, with most farms washing eggs (75%), only 2 farms never washed eggs. Egg washing varied in technique, including use of bowls with sitting water, cloth wipes, and/or disinfectants, like vinegar or chlorine. Farmers using cloths declared to clean them every 2 days to "never".While the island has few known, small-scaled egg producing farms, we have found a lack of biosecurity and varying methods of egg processing to be areas of concern risking pathogen spread and disease in flocks. Regardless of egg washing status, farmers reporting the need to wash eggs due to contamination could be reduced by providing more hygienic environments and increasing nests available per hen. While the future of this project will explore seroprevalence of known poultry pathogens in commercial hens, information from this study can be used for many extension services to support better commercial poultry farming in Saint Kitts.

Identification of Optimal Sample Collection Locations for the Detection of Environmental Viral Contamination in Chicken Farms

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Environmental testing of poultry farms after an outbreak of infectious viral diseases such as infectious bronchitis virus (IBV) and avian influenza virus (AIV) is essential to promptly verify the virus-free status and subsequently return to normal operations. To find optimal sampling locations within and outside of chicken farms, a laboratory study simulating a respiratory virus-infected poultry house with wire layer cages was conducted using a commercial live attenuated IBV vaccine strain as a surrogate virus. Oropharyngeal swab samples of chickens, air, and four locations within the poultry house, including, manure conveyor belt, floor, dust, and shoes were evaluated on the day before and 1, 3, 4, 5, and 8 days after spray vaccination. Tools that are used outside the poultry house, such as shoes, masks, gloves, vehicles, and egg transport pallets were also evaluated. The vaccine virus was quantified by quantitative real-time RT-PCR. The highest viral RNA loads inside and outside of the farm were observed from the manure conveyer belts and shoes, respectively. Samples taken from floors and shoes worn inside also consistently showed high amounts of viral RNA. Our data show virus accumulation sites that could be used as target sampling areas for practical environmental sampling of viruses in poultry farms.

Supplementation with a Formulated Blend of Essential Oils Elicits an Hormetic Dose Response for Feed Conversion in Commercial Broilers

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The medicinal plants used in Traditional Medicine are known to act on the host and can have beneficial effects on inflammation, metabolism, and health. Essential oils (EO) are phytochemical metabolites derived from medicinal plants that have been used for years in poultry diets to decrease disease susceptibility and improve performance. In the scientific literature, many medicinal plants elicit hormetic dose responses in both laboratory species and in humans, such that low doses are beneficial but higher doses can have negative effects. Despite this, there is very little dose response work done on EO in commercial broilers, especially under production conditions. Given the economic impact of unrefined dosage recommendations, as well as potential negative responses to high doses, information on EO effects across a range of doses is essential for any EO-based feed additive. In this study, a multiple-trial analysis was conducted to determine the dose response curve of a formulated feed additive containing EO of cinnamon, clove, and oregano (CCO). Analysis across a set of studies, rather than a single individual study, made it possible to capture the variation around the response to EO across different conditions. The analysis included five trials, in which various doses of CCO were used and compared to a negative control. Trials were conducted in two different contract research facilities in the US. Ross708 or Cobb500 broilers were assigned from d1 of life to no supplement (CON) or supplementation with CCO; doses of CCO tested were 150, 300, 600, and 1200 g/MT of feed. All birds were vaccinated against Marek's, Newcastle disease, Infectious Bronchitis, and coccidiosis. Feed and water were available ad libitum, and an inoculum of used litter was applied to the litter to mimic production conditions targeting 5-8% mortality. Performance for the entire study period (42 days for 4 trials; 35 days for 1 trial) was summarized, and responses relative to CON were calculated within each study to eliminate the random effect of trial. The resulting data were then subjected to one-way ANOVA to determine the linear dose response of CCO, and a one-sample t-test was used to determine the significance of differences from zero at each dose. Total feed intake averaged 3.9 kg and was not impacted by supplementation at any individual dose ($P > 0.20$), nor was there linear dose response ($P > 0.90$). Final body weight averaged 2.2 kg and was increased by CCO at 150 and 300 g/MT ($P< 0.03$), but not at the higher doses ($P > 0.40$). However, there was no linear effect of CCO on final body weight ($P > 0.50$). The feed conversion ratio was adjusted for mortality and body weight and averaged 1.68. CCO decreased FCR by 4 ±1 points at the 150 g/MT dose ($P< 0.001$), and by 2±1 points at the 300 g/MT dose ($P< 0.04$). Interestingly, this positive response was negatively associated with increasing dose in a linear fashion ($P< 0.02$), and even approached a negative response at the highest dose (+1.4 points; $P< 0.20$). In conclusion, supplementation with 150 g/MT of CCO consistently improved FCR of commercial broilers across multiple studies. In addition, the effect of higher CCO doses on performance was neutral at best and detrimental at worst. These results highlight the importance of extensive dose response work in developing EO-based feed additives for broiler production.

Yucca Schidigera and Seaweed Extracts Augment Antibody Response to Newcastle Vaccination and Improve Egg Production and Quality, Blood Constituents, Antioxidant Enzymes, Immunoglobulins in Commercial Laying hens

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The current study aimed to evaluate the effect of a commercial product containing Yucca schidigera and seaweed extracts (YSE) on performance, egg quality, blood profile, antioxidant activity as well as the immune response to Newcastle disease vaccination in commercial layer chickens. A total of 144 commercial Shaver laying hens aged 45 weeks old were assigned to 6 treatments and were supplemented with 0.5 or 1 ml /L of YSE until 49 weeks of age. The results obtained in this experiment showed non-significant differences in live body weight, feed consumption, feed efficiency, or egg production due to YSE supplementation while egg weight and egg mass significantly increased with YSE supplementation. Also, there was an increase (P< 0.05) in yolk percent and yolk-to-albumen ratio and a decrease in albumen per¬cent compared with the non-supplemented group. Serum constituents [total cholesterol, LDL-cholesterol, albumin, immunoglobulin (IgG) and (IgM)], Zn-superoxide dismutase (SOD1), reduced glutathione peroxidase (GSH-Px) and egg cholesterol were significantly (P< 0.05) influenced by YSE supplementation, while total protein, triglycerides, HDL-cholesterol, malondialdehyde (MDA) were non-significantly (P< 0.05) influenced. The antibody titer against Newcastle disease virus was significantly higher in the YSE-supplemented groups. In addition, 0.5 ml/L treatment mainly acted on immunity and anti-oxidation whereas 1 ml/L treatment mainly improved egg weight and egg mass. In conclusion, Yucca schidigera and seaweed extracts could be used as a feed additive due to their capability to improve performance, immune response, and antioxidative function in layers.

Welfare

Precision Farming Strategies for Reducing Cage-Free Hens' Pecking and Cannibalism

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US Egg production is transitioning from conventional cage to cage-free system due to concerns of animal welfare. While cage-free system allows birds to perform natural behaviors such as dustbathing, foraging, and perching, an inherent challenge is feather pecking. Pecking is one of the primary welfare issues in commercial cage-free hen houses as that can seriously reduce the well-being of birds and cause economic losses for egg producers. After beak trimming is highly criticized in Europe and the USA, alternative methods are needed for pecking monitoring and management. A possibility for minimizing the problem is early

detection of pecking behaviors and damages to prevent it from spreading or increasing as feather pecking is a learned behavior. The researchers at the University of Georgia developed a machine vision method for detecting the hens and their pecking behaviors in the cage-free facilities. Two deep learning models, i.e., YOLOv5s-pecking and YOLOv5x-pecking, were developed and compared in tracking hens' pecking behaviors in research cage-free facilities. According the performance based on a dataset of 2000 images, two deep learning models have both reached 85% of precision in identifying birds' pecking behaviors. YOLOv5x-pecking model had a 3.1%, 5.6%, and 5.2% higher performance in precision, recall, and Map than YOLOv5s-pecking model, respectively. After identifying birds with pecking damages, we isolated injured birds in isolated cages for preventing further pecking from other birds. An anti-peck lotion was tested effective in preventing further pecking on old scars or damages. In addition, pecking stones or blocks were introduced as enrichment for birds to perform pecking behaviors, so as to reduce pecking on birds.

Efficacy of a Stationary Cervical Dislocation Device for Humane Euthanasia of Broiler Breeders

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Manual cervical dislocation is an AVMA approved method of euthanasia in chickens up to 2.3 kg (5.1 lb). However, birds such as broiler breeder chickens can grow to a size that makes manual cervical dislocation difficult to achieve consistently and effectively. This can cause both physical and emotional strain for those who euthanize mature broiler breeders. Therefore, it is important to identify more effective and accessible tools for the stewards of birds to assist in the euthanasia process to ensure appropriate cervical dislocation in larger birds. Therefore, the objective of this study was to evaluate a stationary cervical dislocation device in ensuring cervical dislocation is performed properly in mature broiler breeders (20-21 weeks of age). A total of 60 broiler breeders enrolled in the study were blocked by sex and randomly allocated to one of two treatments: Control (manual cervical dislocation; n=30) and CD+ (stationary cervical dislocation device; n=30).Chickens were euthanized by one trained person with over 12 years of experience. Immediately post-euthanasia treatment, the following parameters were collected: latency to insensibility, cardiac and respiratory arrest. Once death was confirmed (as defined by lack of heartbeat detection by pulse oximeter and negative corneal reflex), macroscopic and microscopic scoring was performed including skin laceration, damage to trachea, and transection of spinal cord. Subcutaneous hemorrhage at the site of cervical dislocation, subdural hemorrhage on the brain, damage to the trachea, and transection of the spinal cord were also evaluated by histology.Successful euthanasia of large commercial poultry can be challenging. Hereby, we report our findings on the pairwise comparison of manual and mechanical cervical dislocation in a practical field-oriented approach.

Improvement of Animal Welfare using Enrichment Devices for Poultry in a Research Setting

Laura Rose, Lorri Jensen

Zoetis

Animal welfare is a great ethical focus within the research community. Practicing a gold standard of animal care and welfare is not only important for humane reasons but is critical for public trust and acceptance of this discipline. The Five Freedoms state that in addition to general husbandry and veterinary care, animals have the right to express normal behavior and to be free from fear and distress. One predominant and well-known method to achieving these freedoms, is enrichment. In today's market, there is a real lack of enrichment devices designed for and available for use in the poultry species. What is available, is inappropriate either in use within the research environment (caging) or is a risk for contamination (non-certified pathogen free) in pathogen free animals. In order to improve our enrichment program, we had to creatively discover ways to evoke the natural behaviors of foraging, pecking, dustbathing, exploratory, and roosting behaviors while using products marketed for other animal species. Various enrichment devices were chosen and placed with chickens housed in different environments. Over several months, the chickens were observed while interacting with these enrichment devices. Observations were recorded and findings were discussed to determine the effectiveness of these enrichment devices in eliciting the natural behaviors mentioned previously. This method was repeated several times and end of trial necropsies were performed for additional investigation into product safety. Once these enrichment devices were included within their environments, chickens readily expressed natural behaviors in an otherwise unnatural environment. The chickens also displayed levels of reduced fearfulness while in the presence of humans. With the incorporation of these enrichment devices in future studies, we will improve our animal welfare standards by more effectively implementing The Five Freedoms.

BALANCE Stress & Dehydration Aid Water Retention and Weight Gain Trial in Turkeys

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The overall goal of this project was to examine the effect on turkey production through the implementation of BALANCE Hydration Aid (Aurora Pharmaceutical, Inc.) – an all-natural water additive with immunomodulatory properties developed to improve average daily gains (ADG) and water retention in healthy and/or stressed turkeys. The study was designed to test this low-cost alternative to antibiotics for increased growth promotion and improved ADG in healthy, non- stressed birds. A primary goal of this trial was to see if utilizing BAL- ANCE Hydration Aid in non-stressed birds could provide an economic level of ADG, water retention and improved performance.