American Association of Avian Pathologist 2021 Annual Meeting

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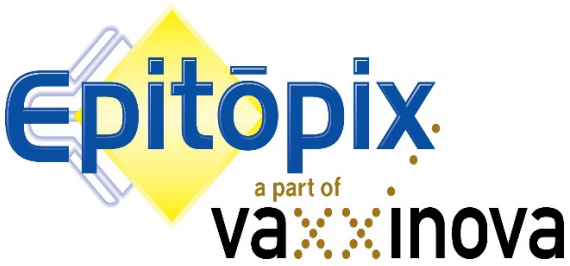
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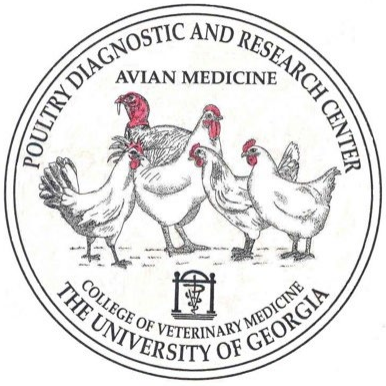
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Antimicrobial

Effect of Electrostatic Aspersion of a Novel Bound Residual Antimicrobial on Hatchability and Chick Quality

Ivan Alvarado[1]

Merck Animal Health[1]

The objective of this study was to determine the impact of electrostatic application of a novel broad spectrum, bound, silane based quaternary ammonium antimicrobial compound (Armatrex®) on hatchability and chick quality. A total of 2,700 commercial broiler fertile eggs were equally divided in 5 groups, 4 groups sprayed with a different concentration of the bound antimicrobial compound (0.1%, 0.25%, 0.5% or 1%) and one group remaining as “non-treated” group.One third of the eggs per treatment were evaluated for moisture loss, while three eggs per treatment were evaluated for water vapor conductance. Egg residues were analyzed to determine hatchability, early/middle/late dead, pips and culls. At hatch, average chick weight, yolk weigh and yolk free body mass were determined, while lung samples were submitted for mycology evaluation. At 7 days, remaining chicks were weighed to determine the effect of the different treatments on body weight. No adverse effect on water vapor conductance, moisture loss or hatchability was observed in the treated groups when compared with the control group. A decrease in the percentage of Aspergillus spp. and Penicillium spp. positive chickens was observed in all the treated groups. Finally, an increase in body weight was observed at 7 days of age in the treated groups when compared with the control group.

Avian Influenza

Adaptation of Mexican lineage H5N2 low pathogenic avian influenza virus in chickens

Mary Pantin-Jackwood[1], Sungsu Youk[2], Christina Leyson[3], David L. Suarez[4]

Southeast Poultry Research Laboratory. USDA, ARS[1,2,3,4]

Thepathobiology of avian influenza virus (AIV) changes as it circulates and adapts in different avian species. Low pathogenic (LP)AIVsubtype H5N2 hasbeen circulating and causing losses in poultry in Mexico since 1994. In thisstudy we examined the infectivityand transmissibility in chickens of an early and a more recent Mexican H5N2 LPAIV. We found that the earliervirus(A/Chicken/Hidalgo/26654-1368/1994) had a higher mean bird infectiousdose (BID50)in chickens than the2011 virus(A/Chicken/Mexico-Coahuila/IA20/11/2011) [10^3.5 andless than10^2mean embryo infective dose (EID50), respectively]. In addition, the 1994 virus did not transmit to contacts, whereas the 2011 viruseasily transmitted to contacts. This indicates that the later virus is more infectiousand transmissible in chickens, and thus better adaptedto this species. To identifypossiblemolecular markers ofadaptationin chickens, we also examined the whole genome sequencesof the two viruses studied and H5N2 viruses from 1994to date. Someof the changes identified werealso reported in other studies to be associated to increased virulence and adaptation in chickens, indicating that specific virus mutations might be markers of poultry adaptation. This information is important because viruses that are well adapted to poultry are highly infectious and transmissible, contributing to faster spread between premises.

Characterizing the Antigenic Evolution of North American H9N2 Avian Influenza Viruses

Erica Spackman[1], Jasmina M. Luczo[2]

SEPRL-USDA-ARS[1,2]

H9N2 avian influenza viruses(AIVs)were first detected in North America, and although the lineage is not the same,they are now prevalentin Asian, African, and Middle Eastern countries.H9N2 AIVoutbreaks in poultry are associated with significant morbidity, andproduction losses. Additionally, H9N2 viruses readily reassortwith andprovide internal genesto theH5 and H7 AIVs of pandemic concern. In addition to H5 and H7 AIVs, H9N2 AIVs have been associated with zoonotic transmission to the human population. Because of their importance, H9N2s are often controlled by vaccination in areas where they are endemic in poultry. Therefore, it is of interest to characterize the antigenic and biological changes that can occur by selection withantibody pressure. Here, the antigenic evolution of the North American H9N2 virus, A/turkey/WI/1/1966 (WI66), was characterized.Specifically, we assessed the antigenic evolution of the surface glycoproteins, hemagglutinin and neuraminidase.Antigenic escape mutants were selected by passaging WI66(H9N2) LPAIV in the presence of homologouschickenantiseraat 32°C, 37°C, and 40°C. Furthermore, antigenic escape mutants were selected by passaging WI66 in the presence ofheterologous antisera representing North AmericanandEurasianH9lineages. WI66(H9N2) LPAIV population genetic diversity was monitored by whole genome next generation sequencing.Effectsof antigenic escape mutationsonantigenicity wascharacterized by hemagglutinin inhibition and neuraminidase inhibition assays. This informationcontributes to our ability to predict the evolution of H9 AIVs and the threat posed to poultry.

Evaluation of the Efficacy of an Inactivated ND+AIVH9N2 Vaccine in Broilers with maternal H9N2 Antibodies against virulent LPAI H9N2 Virus Challenge

Andreas Herrmann[1], Andreas Delvecchio[2], Herve Alloin[3], Michel Bublot[4], Anges Dancer[5], Cesarino Giacomini[6], Stephane Lemiere[7], Siham Fellahi[8], Taoufik Rawi[9]

Boehringer Ingelheim Animal Health[1,2,3,4,5,6,7], Institut Agronomique et Vétérinaire Hassan II[8], MCI Santé Animale[9]

Avian Influenza (AI) H9N2 viruses remain one of the major concerns in the Middle-East, North African and Asian areas and are an emerging threat to global poultry production. Although H9N2 is a virus of low pathogenicity, it is a critical factor in the complex respiratory disease, frequently associated with other pathogens in field conditions with enhanced morbidity, mortality and egg drops. For control of the disease in enzootic countries the vaccination with inactivated Avian Influenza virus (AIV) H9N2 vaccine is widely used. The immunological cross-reaction post AIVH9N2 vaccination against antigenic variants of AIVH9N2 virus within the same lineage was recently assessed using the HI-test. Information about other immune mechanisms inducing clinical protection or from controlled studies about efficacy against virulent challenge is scarce.In a controlled study conventional broilers with maternal H9N2 antibodies received an inactivated ND+AIVH9N2 vaccine at day 7 of life followed by a virulent AIVH9N2 challenge at 21 days post vaccination. In a second study conventional broilers with maternal antibodies received or didn't receive the inactivated ND+AIVH9N2 vaccineat day 7 in addition to live Newcastle Disease (ND) and Infectious Bronchitis (IB) vaccinations followed by virulent challenge with Moroccan AIVH9N2 isolate + variant IB virus. In both studies the AIVH9N2 vaccinated groups showed an enhanced clinical protection against a virulent AIVH9N2 challenge compared to the not vaccinated control groups. The number of challenge virus shedders was reduced in all the AIVH9N2 vaccinated groups

Genotypic changes in low pathogenicity avian influenza viruses after replication in chickens and turkeys

Christina Leyson[1], Miria Ferreira Criado[2], Sungsu Youk[3], David L. Suarez[4], David E. Swayne[5], Mary Pantin-Jackwood[6]

Southeast Poultry Research Laboratory[1,2,3,4,5,6]

The pathobiology of two H7N3 low pathogenicity avian influenza (LPAI) viruses was examined in turkeys and chickens. Both viruses were isolated during the 2020 H7N3 outbreak in turkeys in the U.S. One of the viruses has a 66-nucleotide deletion in the stalk region of the neuraminidase (NA) gene. NA stalk deletions have previously been associated with adaptation of avian influenza viruses to poultry. Both of the H7N3 LPAI viruses readily infected turkeys and were efficiently transmitted to contacts when givenat medium and high virus doses, whereas the virus with the NA deletion infected turkeys even when given at a lower dose, indicating that this virus was even better adapted to turkeys. In contrast to turkeys,no difference in infectivity or transmission was observed between the two viruses in chickens, which were only infected with the higher doses of the viruses and transmitted poorly to contacts. These findings are consistent with the fact that these H7N3 LPAI viruses were obtained from turkeys and suggest that the NA deletion may have a role in adaptation of the virus to turkeys as a host. To examine changes that occur in the virus genomes as they replicate in these different hosts, we performed whole genome sequencing on oral swabs obtained from the infected chickens and turkeys. Single nucleotide variations at the consensus and sub-consensus level were enumerated and compared between the different groups. These studies provide insights into the mechanisms contributing to adaptation of wild bird-origin avian influenza viruses in poultry species and ultimately help in mitigating outbreaks poultry.

Is Artificial Insemination a Method of LPAI Spread in Breeder Turkey Flocks?

Marion Garcia[1]

Hybrid Turkeys[1]

Work from High-Path Avian Influenza (HPAI) outbreaks in the US have establishedthat “semen from a not known to be infected tom flock has a potential to transmit HPAI to inseminated hens” (Carol Cardona, personal communication). Thisis acase studyconducted during an outbreak of Low-Path AI (LPAI) to explore if the same is true for LPAI.Aturkey breeder flockbecame infected with LPAIduring production. The breeder farm is composed of two laying hen barns and one tom barn. The grower (grower A) works in collaboration with a relative (grower B) who is also in the turkey breeding business. The collaboration includes sharing of insemination crews and a tom milker. Significantly,grower A has an arrangement with grower B to supplysomesemen to his lay facility.Following a significant egg drop, the hen barns on grower A’s farm tested positive for LPAI. All areas of collaboration between grower A and grower B were immediately discontinued. The tom barn on grower A’s farm subsequently tested positivefor AI.Sequential testing of semen from grower A’s tom flock and drinker swabs from both growers’flocks are being conducted to determine ifsemen could potentially transmit LPAI to inseminated hens.

Isolation and Subtyping of Avian Influenza Strains from Wild Birds of the Coast of Peru, 2019-2020

Gina Castro-Sanguinetti[1], Ana Apaza[2], Alonzo Callupe[3], Mercy Ramirez[4], Hermelinda Ramirez[5], Juan More[6], Paulo Simas[7], Eliana Icochea[8], Vikram N. Vakharia[9]

University of San Marcos[1,2,3,4,5,6,7,8], University of Maryland Biotechnology Institute[9]

Influenza A virus (IAV) is the causal agent of an acute respiratory disease that affect birds and several mammalian species such as pigs, horses, canines, aquatic mammals andhumans. Peru is free of avian

Influenza in domestic birds, however, numerous strains have beenisolated from wild aquatic birdsin our Laboratory duringthe period of 2006-2018. The present study was carried out including420 samples from severalspecies of wild birds from wetlands onthe coast of Limaregionduringthe period ofMarch 2019 to March 2020. Viral isolation was performedin SPF embryonated eggs, identification by RT-PCR andwhole genome sequencing using Next Generation Sequencing technology. Fivestrains of avian influenza virus were isolated. The identified subtypes were H2N6, H6N2 (2 strains), H6N8 and H13N6. This project has been funded by FONDECYT-Peru andWorld Bank (Contract 02-2019-FONDECYT-BM-INC-INV).

Linking Remote Sensing to Targeted PCR based Environmental Sampling for Detection of Avian Influenza in Waterfowl

Maurice Pitesky[1]

UC Davis School of Veterinary Medicine[1]

Migratory waterfowl are the primary reservoir of avian influenza viruses (AIv) which can be spread to commercial poultry. Surveillance efforts that track the location and abundance of wild waterfowl and link those data to inform assessments of risk and sampling for AIv currently do not exist. To assist bio-surveillance and minimize poultry exposure to AIv, here we explored the utility of remotely sensed MODerate Resolution Imaging Spectroradiometer (MODIS) satellite imagery in combination with land-based climate measurements (e.g., temperature and precipitation) to predict waterfowl location and abundance in near real-time in the California Central Valley (CCV), where both wild waterfowl and domestic poultry are densely located. These data were then used to sample waterfowl and their environmental for AI. Wetland water samples were collected and processed using filtration and whole-segment PCR in order to explore improvements in sensitivity and specificity. Remotely sensed data visualized as part of the California Waterfowl Tracker (CWT) demonstrated spatio-temporal clustering of waterfowl in various locations in the California Central Valley (CCV). The ability to utilize and link remote sensing is an integral improvement toward improving AIv surveillance in waterfowl in close proximity to commercial poultry. Expansion of these types of methods linked to user-friendly web-tools such as the CWT could provide the backbone of a national surveillance system for waterfowl and AI.

Protection of chickens against North American H5N2 and H7N3 avian influenza viruses following updates in hemagglutinin inserts of licensed recombinant fowlpox vectored vaccines

David Swayne[1], Kateri Bertran[2], Miria Ferreira Criado[3], Dong-Hun Lee[4], Lindsay Killmaster[5], Mariana Sa e Silva[6], Eduardo Lucio[7], Justin Wildener[8], Nikki Pritchard[9], Emily Atkins[10], Teshome Mebatsion[11], Christopher B. Stephens[12], Hallie King[13], Erica Spackman[14]

USDA/ARS/SEPRL[1,2,3,5,14], University of Connecticut[4], Boehringer-Ingelheim Animal Health[6,7,8,9,10,11,12,13],

Vaccination to control H5N2 and H7N3 avian influenza (AI) has been practiced in the Americas since 1995 and 2012, respectively. Divergence of circulating viruses with development of vaccine-resistant field strains have occurred with virus lineages. The latest vaccination strategies used in Mexico consist of a prime-boost vaccination program using a live-vectored vaccine at 1-day-of -age and a killed oil emulsified vaccine at 2-weeks-of -age. Here we evaluated efficacy in chickens of two recombinant fowlpox virus vectors containing updated H5 (rFPV-H5/2016) and H7 (rFPV-H7/2015) inserts as sole vaccines when administered at 1 day-of -age and challenged 3 weeks later. The rFPV-H5/2016 producedpre-challenge hemagglutinin inhibition (HI) titers measured against circulating subcluster 4 H5N2 low pathogenicity AI (LPAI) virus (2010 H5/LP) and significantly decrease oral and cloacal virus shedding when compared to the sham controls following challenge with 2010 H5/LP. Similarly, the rFPV-H7/2016 vaccine produced pre-challenge HI antibodies in sera (15/20) measured using H7N3 high pathogenicity AI (HPAI) virus (2015 H7/HP virus) as test antigen and conferred 100% protection against mortality and morbidity, and

significantly reduced virus shed titers from the respiratory and gastrointestinal tracts. Bycontrast, chickens vaccinated with an older rFPV-H7/2002 vaccine mostly lacked HI antibodies measuredagainst 2015 H7/HP antigen and were not protected upon challenge with 2015 H7/HP virus. These results confirm the efficacy of the new rFPV-H5/2016 and rFPV-H7/2015 vaccines against newer divergent field H5N2 LPAI and H7N3 HPAI virus in Mexico, respectively, and confirm the importance of targeted updating of vaccines seed strains for long-term effective control AI viruses.

Bacteriology

Avibacterium paragallinarum Field Strain Persistence in Water at Two Different Temperatures

Rodrigo Gallardo[1], Simone Stoute[2], T. Santoro[3], M. Timofeyeva[4]

University of California, Davis[1,2,3,4]

Severe outbreaks of infectious coryza have been occurring in egg layer and broiler flocks since 2017in California.The disease presentationhas not been the conventionaland while we know that commercial vaccines containing C-1 and C-2 strains elicit protection in flocks vaccinated twice,this strategy cannot be used in broiler flocks.In order to protect flocks at risk, we need to better understand the persistence of this bacteria in the environment in order to avoid conditions that make thempersist for longer periods of time. Even though not proven, it is thought that outbreaks are more common in winter months,duringfoggy days.In orderto testthe persistence of AP, we spiked water at two different temperatures 5 and 20Cand we sampled them to measure livability and bacterial loaduntil 4 dayspost spike. These results will be discussed and associated with the Coryza prevalencethroughout the year.

Effects of Hops-derived Feed Additive on the Control of Gangrenous Dermatitis in Male Turkeys

Carrie Cremers[1], Michelle Kromm[2], Katherine Meyer[3]

Jennie-O Turkey Store[1,2,3]

Gangrenous dermatitis is a disease that causessignificanteconomic loss in the turkey industryannually.Along with the economic losses, there is alsoconcern on the amount of antibiotics used and antibiotic resistance while treating gangrenous dermatitis.For these reasons, it is consistently ranked in the top five disease issuesimpacting the turkey industry as ranked byturkey vets in the U.S.In this study, flocks were fedeither a control diet ora diet with ahops-derivedfeed additiveto determine if the product would help reduce gangrenous dermatitisincidencein male turkeys.Data related to flock performance and antibiotic usage willalsobe presented.

Erysipelothrix Rhusiopathiae Septicemia In Two Pastured Turkey Flocks

Jarra Jagne[1], Elizabeth L. Buckles[2], Elena Demeter[3], Timothy Wu[4]

Cornell University Animal Health Diagnostic Center[1,2,3,4]

Erysipelothrix rhusiopathiaeisa Gram-positive non-spore-forming rod-shaped bacterium that causes the disease Erysipelas in turkeys and pigs. It usually occurs as a sporadic disease but may be on the rise in pastured flocks that are exposed to the organism, which can thrive in soil for years. Domestic pigs are consideredthe main reservoir of E. rhusiopathiaeand many are asymptomatic carriers.The disease is also a zoonosis known as Erysipeloid in humans. It occurs in humansas a localized skin infection or a septicemiasometimes accompanied by the development of endocarditis. Two separate submissions of E. rhusiopathiaein Broad Breasted White turkeys were sent to the New York State Animal Health Diagnostic Laboratoryabout three

weeks apartfrom two farms owned by two brothers. Both flocks were 12 weeks old when the infection occurred. Both flocks reported increased mortality and morbidity with sudden death and lethargy as the principal signs. Post-mortem examination of affected birds in the first Flock A showed a severe, acute, diffuse necrotizing pneumonia, moderate splenomegaly, mild enlargement of the gall bladderandmild urate retention. The second Flock B was devoid of gross lesions except for an abundantthick mucoidexudate in the upper respiratory tract. Aerobic bacterial cultures from Flocks A and B spleens, and oropharyngeal area of Flock B were allpositive for E. rhusiopathiae. Flock Ain addition was also positive for E.coli. Histochemical staining with Gram stain revealed Gram-positive bacteria in lung sections.

Focal Ulcerative Dermatitis Syndrome in Midwestern Laying Hens

Kay Russo[1], Nick Evans[2], Daniel Grum[3], Diana Ayala[4], Emily Kimminau[5], Peter Karnezos[6]

Purina Animal Nutrition[1,2,3,4,5,6]

Midwestern laying flocks are experiencing an emerging dermatological disease, referred to as Focal Ulcerative Dermatitis Syndrome (FUDS). Initially only described in cage free brown egg layers, it is increasing in incidence in cage free white egg layers as well. Skin lesions generally present on the dorsum of the birds, cranial to the uropygial gland and are described on a scale from mild to severely exudative and necrotic. Outbreaks result in up to 50% cumulative mortality, presumably due to septicemia. 16S rRNA next generation sequencing was used to characterize the microbiome of environmental, cecal, ileal and skin samples taken from a flock experiencing an outbreak and compared to samples from a healthy sister flock. This presentation will review key differences noted in the microbiome analysis between the FUDS and healthy flocks.

Genomic Characterization of Avian Pathogenic E. coli Co-isolated with Enterococcus spp. in Cases of Avian Colibacillosis

Grayson Walker[1], M. Mitsu Suyemoto[2], Luke B. Borst[3]

NC State University[1,2,3]

Avian pathogenic E. coli (APEC) and Enterococcusspp. (ENT) are frequently co-isolated from poultry having died fromcolibacillosis. To discovera genetic basis for reported growth and virulence synergy between these pathogens,the whole genome sequence was determined for 28 APEC strains that were 1) co-isolated with ENTand 2) hadknownin vitrogrowth synergy with ENTunderironlimitation.Strains were characterized by phylogenetic analysis, plasmid replicon typing, and comparative genomics. There was widespread genetic heterogeneity including varied plasmid-conferred antimicrobial resistanceand virulence elements.While some APEC were closely related, 6 of 7 Clermont phylogroupswere observedwithGroup A beingmost prevalent (9/28).The number of plasmid replicons ranged from 2 to 5, with the IncFIB (25/28) and IncFII (14/28)being most frequent. A strain containing both of these replicons was cured of its plasmids, which had no effect on growth synergy with ENT or virulence to broiler embryos. The annotated genome of an APEC strain that lacked growth synergy in mixed culture with ENT was compared to that of multiple strains that exhibited a robust growth response under iron limitation. The in vitrosynergy with ENT phenotype was correlated with the presence of acolanic acid polymerase and genes in the aerobactin siderophore operon. While the molecular mechanism responsible for APEC and ENT growth and virulence synergy remains unclear, genomic comparison of APEC revealed that colanic acid metabolism and aerobactin siderophore production may play importantroles. Additional studies are neededto characterizethispolymicrobial virulence mechanism.

Case Reports

A Case Report of High Mortality in Chickens due to Infectious Bronchitis Virus

Julia Blakey[1], Ana Da Silva[2], Carmen Jerry[3], Rodrigo Gallardo[4], Art Bickford[5], Simone Stoute[6]

USDA- ARS USNPRC[1], University of California, Davis[2,3,4,5,6]

In March 2019, the California Animal Health and Food Safety Laboratory- Turlock branch received 2 submissions of broiler chickens from commercial flocks reporting increased mortality. Submissions consisted of both white and brown broilers. Submitted chickens demonstrated mild to severe depression and ruffled feathers. At necropsy, moderate to severely enlarged and pale kidneys were observed with gross lesions indicative of dehydration. Microscopically, renal tubules were degenerated and distended with necrotic debris. Interstitial edema and mononuclear inflammatory cell infiltration was observed in kidney sections. Infectious bronchitis virus (IBV) was isolated and identified by qRT-PCR from kidney tissue pools and tracheal swab pools from both cases. Sequencing of the S1 hypervariable region was most similar to a local California variant. The outbreak lasted roughly 1 week in both flocks with 2% total mortality in the brown broilers and 20% total mortality in the white broilers. IBV associated with nephritis has been sporadically reported in California broiler flocks and represents a significant pathogen due to the potential for high flock mortality.

A Wrinkle in Time: Investigation of Variant Bronchitis in Broiler Breeder

Eric Shepherd[1]

University of Georgia College of Veterinary Medicine[1]

Two broiler breeder flocks with acute onset of decreased egg production and wrinkled eggs were submitted to PDRC in late winter/early spring of 2021. These flocks also had an acute onset of lethargy with respiratory noise. Wrinkled eggs began to rise and production dropped between 2-4% at about 50 weeks and 53 weeks of age, respectively. Samples were taken for MG/MS PCR, IBV serotype specific qRT-PCR, and NDV rt-PCR. NDV and IBV ELISAs were performed on acute and convalescent sera. PCR results were negative for MG/MS in both flocks and virus isolation from trachea, cecal tonsils, and kidneys were unsuccessful for 3 passages in embryonated eggs. Trachea and kidneys were negative for IBV on a serotype specific qRT-PCR respiratory panel. Cecal tonsils were strongly positive for the universal IBV primer and probe but were negative for all others. Tissue homogenate was passed through naïve broilers without recreation of clinical signs and IBV PCR was negative on those tracheas. Cecal tonsils from the second hen flock was homogenized and passed into 50 week old hens to attempt to recreate the clinical signs seen in the field and to grow up enough virus to allow for full S1 sequencing. Multiple broiler breeder and egg layer flocks have presented with similar signs as these flocks in the last year without being able to determine which IBV serotype was to blame. Diagnostics are ongoing for this case and will be updated when the presentation is given at the AAAP meeting.

Case Report: An Atypical Mycoplasma gallisepticum Case in Commercial Layers: Serological and Molecular Diagnosis

Karen Grogan[1], Louise Dufour Zavala[2], Noala Ferguson-Noel[3], Jenny Nicholds[4], Eric Shepherd[5], David French[6]

University of Georgia[1,3,4,5,6], Georgia Poultry Laboratory Network[2]

Routine serological monitoring submitted to Georgia Poultry Laboratory Network (GPLN) from a 37-week-old Hyline W-36 commercial layer flock in Northeast Georgia identified suspect Mycoplasma gallisepticum(MG) positive hens. The flock is from a MG negative in-line complex, without of use MG live vaccines, but known to be Mycoplasma synoviaepositive. Aclinician from Poultry Diagnostic and Research Center (PDRC) serves as the veterinarian of record for the farm and duplicate serum samples were submitted to PDRC. Additional serum samples were positive on MG plate testand suspect positive (1:40) on hemagglutination inhibition (HI).Choanal swabs were collected for MG PCR at both GPLN and PDRC and sequencingconducted on both PCR products by PDRC. PCR results werepositive for MG and sequencing identified a known vaccine strain, F strain. No clinical signs were reported in the flock and production continued as expected in the flock.The flock in question was molted at 67 weeks-of-age.Routine serological monitoring at 78 weeks-of-ageidentified the flock to be serologically positive for MG on ELISA and HI. Choanal swabs were subsequently submitted for MG PCR and were strongly positive for MG (30 swabs, 6 pools, cycle threshold (CT) values=26-30). No clinical signs were reported at this time point either. Sequencing results are still pending for the post-molt samples and epidemiologic investigation is underway. The case reportwill focus on the lack of clinical signs, serological testing and results, molecular testing and identification, and epidemiological investigation.

Clinical investigation of early embryonic death in turkey hatching eggs

Marissa Studniski[1], Ben Wileman[2], Michelle Behl[3], Brianna Dierdorff[4]

Select Genetics[1,2,3,4]

A report from the hatchery indicated there was poorer hatchability and increased early dead counts on breakout analysis from a breeder flock. An investigation was started into the reason for why this was occurring and it eliminated most causes except for arecent treatment. Antibiotic administration of florfenicol was suspectedto have caused reduced hatchability and an increase in early embryonic death in turkey hatching eggs. A follow up trial was conducted on farm to confirm antibiotic administration wasthemost likelyetiology of the reduced hatch results.A subset ofbirds were injected subcutaneously with 30 mg/kg and 15 mg/kg florfenicolwhile the remainder of the flock served as untreated controls. Eggsfrom each of the three groupswere collected from the hens for 13 dayspost antibiotic administration. Hatchand break outdata was collected on the egg sets. Antibiotic administration of 30 mg/kgand 15 mg/kg caused increased early embryonic death and areduction in hatchability(34.31% and 4.88% respectively)for the first 3 days post-injection. Both field results and trial results supportthatflorfenicol induces acute toxic effects on early embryonic development in turkey hatching eggsresulting in decreased hatchability.

Clinical Investigation of Neurologic Symptoms in Turkey Breeder Hen Candidates

Jake Carlson[1], Ben Wileman[2]

Select Genetics[1,2]

In late October, one barn out of 5 barnscontaining 15 week old turkey breeder hen candidates had an acute onset ofneurologic signs and flushing. During a two week time span the barn lost approximately 200 birds total. Tissue samples, serology, drinker swabs and feed samples were collected andsent to the University of Minnesota Veterinary Diagnostic

Laboratory and to the Missouri Veterinary Diagnostic Laboratory to rule out common causes of neurologic symptoms in turkeysand to investigate the possibility of ionophore toxicity and heavy metal toxicity. Brain and Liver samples were negative for bacterial, fungal and viral etiologies and drinker swabs were negative for Avian Influenza and had a late CT for Newcastle that was most likely vaccine strain. Histopathology however indicated lymphoplasmacytic inflammation suggesting a viral etiology with the suggestion of possibly Eastern Equine Encephalitis. Virus isolation from the brain thus far has been negative. Feed analysis did not find the presence of any ionophores nor elevated levels of lead.After about 2 weeks the flock returned to normal and no other barns were affected.Investigation is still ongoing and all results will be presented.

Pickled Pullets?

Jenny Nicholds[1]

University of Georgia[1]

At 13 days of age 10 live and 10 dead Cobb pullets from one house on a two house farm were submitted to the diagnostic lab with a history of appearing drunk. Neurologic signs had been noted the previous week, were worst in the morning and lessened throughout the day. A feed issue was suspected by the submitting company. At the lab, 8 of 10 birds submitted alive were uncoordinated or unable to rise. Body weights were on target for age and blood glucose was found to be >200 mg/ml in all samples evaluated. There were no significant gross necropsy findings in any of the examined birds and only 3 were noted with empty crops. An extensive set of tissues were collected for histologic examination. Ionophore toxicity was the initial primary differential and a recommendation to remove and replace feed was made. With the exception of one brain section displaying necrotic Purkinje cells, histologic findings were non-specific or unremarkable. There were no changes to support ionophore toxicity. The flock was visited 3 days after the initial submission and it was discovered that affected birds appeared to be located at half house. At this point the focus of subsequent investigation shifted to other suspected toxins including pesticides that had been applied. The details of subsequent investigation, findings and final outcome, Imidacloprid toxicosis, will be presented.

Reoccurring Bordetella Exposure at a Commercial Turkey Brood Hub

Jolene Tourville[1]

Jennie-O Turkey Store[1]

Case report of a newly constructed brood hub with reoccurring Bordetella avium exposure in young turkey flocks. The brood hub was built in Spring of 2018. In 2019, the farm managers and service personnel noted ongoing respiratory issues in multiple flocks sourced from this facility. Necropsies and other diagnostics showed a multitude of respiratory diseases including Newcastle disease, Ornithobacterium Rhinotracheale (ORT), and colibacillosis. Bloodwork also showed high titers to Bordetella. Additional diagnostics were performed in subsequent flocks at younger ages to try to confirm Bordetella as the initial cause of the respiratory problems. Tracheal swabs, nasal swabs, and serology confirmed Bordetella avium in birds as young as 14 days old. Extensive investigation was conducted to locate the source of Bordetella and to focus on cleaning and disinfecting the brood hub. This case report will elaborate on this frustrating disease, the after-effects of Bordetella infection, as well as demonstrate the importance of communication and teamwork amongst all levels of the company.

Reoviral Hepatitis In Commercial Hen Turkeys

Laura Tensa[1], Brian Wooming[2]

Cargill Turkeys[1,2]

Reovirus is a ubiquitous virus found in turkeys, playing a role in multiple disease conditions including tenosynovitis and enteritic disease. In 2019, reovirus was described to cause turkey viral hepatitis, a condition previously described as being caused by a picoronavirus. At approximately 10 days ofage, mortality spikes in affected flocks. Necropsy of the

mortality reveal shepatomegaly, pin point whitehepaticfoci, and no other significant lesions.Mortality returns to normal levels without treatment after a few days,however as the flock ages they developmoresigns of reovirus infection: uneven flocks,stunted birds, and down birds with ruptured leg tendons.In summer 2020, a cluster of reovirus hepatitis cases were diagnosed in one production complexover the span of approximately two months. This case report describes initial findings and diagnosis withhepatitis lesionsand mortality,follow-up of affected flocks and tenosynovitis arthritis,and the final impact at the processing plantwith condemn and downgrade. Finally, steps taken to mitigate recurrence in the complex, including cleaning and disinfecting and modifying the vaccination programin the breederswill be discussed.

Sunday Morning Calls are Never Good!

Sara Throne[1], M. McConnell[2]

Simmons Foods, Inc.[1,2]

Case Report: Sunday Morning Calls are Never Good!Throne, Sara Ja., and M. McConnella aSimmons Foods, Inc.Siloam Springs, AR, USABotulism is an infectious disease caused by the botulinum toxin produced by the bacterium Clostridium botulinum. This report will describe a case of botulism in a 27-week-old broiler breeder flock of 8300 birds. This case initially presented with an extreme rise in mortality and morbidity in one house on a four-house farm. Birds were reluctant to walk, and many were moribund. In the initial necropsy, few to no clinical signs were recognized given the acute nature of the presenting symptoms. Many birds still had feed in the crop and were in production with an egg in the oviduct. Initial rule outs included an extreme heat incident, feed intoxication with a medication, or exotic infectious disease. All of these were ruled out through on-farm or feed mill investigation or diagnostic testing for Avian Influenza (AI), New Castle Disease (NDV), and Mycoplasmosis (MG/MS). Botulism was added to in the rule out list, and treatment was initiated as other tests were being performed. While botulism was initially diagnosed as a disease of exclusion and with response to antibiotic therapy, confirmation through mouse bio-assay was obtained. Recurrent antibiotic therapy was needed in the subsequent months following diagnosis. Possible avenues for the root cause have been inconclusive and the source of the botulism is still unknown. Production parameters such as livability, egg production, hatchability, and hatch of fertile will be presented for this flock, which is still in production.

Suspected Sulfonamide Toxicosis in a Broiler Breeder Flock

Richard Fulton[1]

Michigan State University[1]

A 50,000 flockof28-day-old broiler breeders was experiencing an elevated mortality event. Chicks from that flock, 5 pullets and 6 cockerels were submitted for diagnosticinvestigation.Necropsy revealed a bacterial septicemia with fibrinous polyserositis in the majority of the birds while 2 cockerels had coccidial typhlitis. Sulfaquinoxaline was prescribed at label dosage with water treatment for 2 days, followed by 3 days of nomedication,followed by 2 days of medication.The mortality jumped dramatically 8days after the last treatment. Lesions consisted of skin discoloration, subcutaneous petechiation and enlarged pale kidneys.Mortality remained elevated for 17 days. Analysis of liver and kidney revealed elevated levels of sulfaquinoxalinein relation to an experimental sulfaquinoxaline toxicosis. Dose calculation and the drug administered were analyzed and determined to be correct.

Tracking a Salmonella Gallinarum Outbreak on a Commercial Pullet Rearing Facility in South Africa

Gregory Celliers[1], Herman Bosman[2]

Hy-Line International[1], The Poultry Practice[2]

Fowl typhoid is caused by Salmonella entericasubspecies entericaserovar Gallinarum(S. Gallinarum)resulting in an acute or chronic septicemic disease that most often affects mature chickens and turkeys.S. Gallinarum is a nonmotile organism and is host specific for avian species. Europe and North America have reduced the prevalence of the disease through national prevention schemes, however in Africa, Asia, South America and the Middle East fowl typhoid remains a problem. In South Africa, sporadic outbreaks occur despite the use of live S. Gallinarum (SG9R) vaccine and biosecurity precautions. This case study discusses an outbreak that occurred on a remote commercial pullet rearing facility in South Africa where no S. Gallinarum vaccine had been used. Erraticmortalities were experienced starting at 5 weeks of age, the most affected flock ending on 4.01% at 15 weeks of age. On postmortem the pullets presented with an enlarged, liver and spleen with typical grey discoloration of the lungs. All three rearing sites on the farm eventually became affected. Potential sources of the outbreak were investigated,and changes made to the daily operations and biosecurity procedures to prevent a reoccurrence of the disease. The study tracks the depopulation and restocking of the farm under the improved practices.

Unusual cases of botulism in pullets and breeders.

Kurt Dobson[1]

George's Inc.[1]

There have been several casesof botulism that has continued for several flocks from the same pullet house. The breaks have occurredfrom onepullet house on a four house farm. The disease has been confirm by NVSL. The birds will break with botulism either in the pullet house or in the breederhouseor both. Several interventions have been tried to decrease the incidence and severity of the breaks. Depending on whether the birds have broken in the pullet house or have waited until the breeder house is a puzzle. We have not been able to determine where the clostridium came from or how it continuesto remain in the same house. This report will discuss the previous flocks and the current situation.

Coccidiosis

A practical approach to ensure proper immune response to a commercial coccidiosis vaccine in Broiler breeders

Jose Bruzual[1]

Aviagen[1]

It is well established that hatchery application is a requirement for development of strong immunity to the coccidiosis vaccine. However, field conditions necessary for the coccidiosis vaccine to cycle properly during the first 3-4 weeks of the broiler breeder life are just as important. In particular, the transition from the first to the second cycle. The field conditions that allow oocyst cycling such as oxygen, litter humidity and litter temperature are well known in the scientific community, but very rarely are these adjusted for this purpose. In this report we describe the management of a poultry integrator that had been struggling with severe reaction to Eimeria tenella and its negative impact on production parameters including uniformity and viability during the grow out phase. We will share the strategy used for adjusting density and feed space during brooding to ensure minimal conditions for proper oocyst cycling which resulted in a decrease in E. tenella reactions as well as the need for minimal or no treatment in the field.

Comparison of Three Live Coccidiosis Vaccine Protective Immunity Against Seven Field Isolates

Andres Montoya[1], Steve Fitz-Coy[2]

Merck Animal Health[1,2]

In this study we examined the efficacy of three live coccidiosis vaccine againstheterologous challenge from several field isolates in commercial broiler chickens. One hundred and fifty 1-day-old broiler chickens were equally divided in three groups and sprayed with commercial vaccines A (Coccivac-B52), B or C, following the recommendations of the manufacturers. Soon after vaccination, birds were placed in floor pens during the duration of the study. Fresh fecal samples were collected at 7, 10, 14, 17, 21, 24 and 28 days of age (DOA) to determine oocysts output. One hundred and fifty additional hatch-mates were also divided in three groups and remained as non-vaccinated and challenged controls. Protective immunity against heterologous isolates was determined at 22, 28 and 35 DOA by individual oocyst oral inoculation in birds in each group. At 22 DOA birds were selected, identified and challenged with one of the two field isolated (E. maxima or E. tenella). At 28 DOA birds were selected, identified and challenged with one of the three field isolated (E. acervulina, E. mivati or E. mitis). And at 35 DOA birds were selected, identified and challenged with one of the three undefined field isolates of chicken Eimeria. Birds were randomly selected from each of the vaccinated groups and from the non-vaccinated controls. After challenge at 22, 28 and 35 DOA evaluation was done 6, 4, and 5 days post challenge respectively. Gross lesion scoring and microscopic evaluation were performed in different regions of the gut (1=duodenum, 2=jejunum, 3=ileum, 4 = ceca and 5= rectum). Heterologous challenges at 22 DOA showed that all groups of immunized birds and challenged with either E. tenellaor E. maxima were better protected than challenged controls. However, there were differences in the levels of protection among the immunized and challenged groups. Vaccine A birds had 82% and 79% protection against the E. tenella and E. maxima,respectively. However, Vaccine B and Vaccine C immunized birds were less protected against the E. maxima challenge, 46% and 29%, respectively. Protective levels of immunity at 32 DOA post challenge showed that birds immunized with Vaccine A or Vaccine B demonstrated exceptional protection against the challenge antigens (E. mivatiand E. acervulina). Vaccine C immunized and challenged birds showed good protection against the challenge antigens. Protective levels of immunity at 35 DOA birds immunized with Vaccines A or B showed no protection against challenge with the antigen-pullet 1 (E. brunetti) isolate while Vaccine C provided exceptional protection. Birds immunized with Vaccines A or B and challenged with isolated-pullet 2 (E. brunetti and E. maxima) showed good to exceptional protection. Birds immunized with Vaccine A and C showed good protection against the back yard 2 field isolate (E. acervulina and E. mivati), while VaccineB elicited fair protection. In Summary, Vaccines A, B and C protected against eight (80%), five (50%) and six (60%) of the 10 coccidia field challenge isolates, respectively. VaccineA (Coccivac-B52) also showed protection against the E. mivati field antigens. Vaccine Cshowed some protection against the E. mivati and E. brunetti isolates.

Effectiveness of Anticoccidials and Coccidia Vaccination in Broiler Chickens Analyzed by Network Meta-Analysis

Ruediger Hauck[1], Jordan Eckert[2], Miranda Carrisosa[3]

Auburn University[1,2,3]

With the trend to antibiotic free and organic production, the control of infections with Eimeria spp. in broiler flocks has become more difficult. Vaccination against coccidia is an alternative to anticoccidial feed additives, but there are concerns that live vaccines might have negative effects on production parameters and intestinal health. Reports of experiments directly comparing anticoccidials and coccidia vaccines are rare. Network meta-analysis (NMA) is a method to compare more than two treatments in a meta-analysis of published articles. We used this method to analyze experiments testing anticoccidials and coccidia vaccines with or without Eimeria challenge in floor pen studies using commercial broilers grown

to 40 to 49 days of age. Effect sizes were mean differences in body weight/body weight gain (BW/BWG) and feed conversion rate (FCR) between the included groups. The results show that groups vaccinated against coccidia have a similar BW/BWG at processing age compared to groups given anticoccidials. The analysis of five subsets, containing (1) only groups receiving no additional antibacterialgrowth promotor (AGP), (2) receiving only ionophore anticoccidials, (3) challenged with only coccidia, (4) challenged with a high dose of coccidia, or (5) challenged earlier in life brought similar results and confirmed the robustness of the NMA. While analysis of FCR brought similar results overall, they were slightly more favorable for anticoccidials than vaccines. In the process, the analysis exposed unnecessary as well as inherent problems with data quality, which researchers working with coccidia should consider. It alsoidentified areas in which information is lacking and that should be addressed in future research.

Evaluation of the sensitivity of product freeze indicators and the effects of short-term/ mild freezing on live coccidiosis vaccine

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Huvepharma Inc.[1]

Freezingisknown to bedetrimental to the viability of live coccidiosis vaccines.Therefore,some vaccine manufacturers includefreeze indicators in thepackaging to ensure that the vaccineis not exposed to freezing temperaturesduringtransport and storage. However, the sensitivity of these indicators when exposed to sub-freezing temperatures for brief periodsandthe potentialeffects of such exposure onviabilityof the coccidiosis vaccineis unknown. The purpose of this study is to assess the sensitivity of product freeze indicators(Freeze WatchTMIndicators, 3M Company, Maplewood, MN)and the deleterious effects of mild/ short-term freezing onlive coccidiosis vaccine(AdventTM,Huvepharma, Inc., Peachtree City, GA).To accomplish this, freeze indicators and coccidiosis vaccine were exposed to two levels of freezing temperatures (-1°C and -4°C)to determine time to indicator change and time to vaccine freezing. After determining time to vaccine freezing, severalintermediate timepoints were chosen to assess in-vitro vaccine viability compared toacontrol vaccine storedat 5°C.Adye-exclusion assay(ViacystTM,Huvepharma, Inc., Peachtree City, GA)was used to asses viability of sporulated oocysts.Vaccine viability will be representedby numbers of viable oocysts as compared to the control vaccine. The results of this study are.

Examination of One of the First Coccidiosis Vaccines: anticoccidial drug sensitivity, immunity development, and broiler performance

Greg Mathis[1], Ha-Jung Roh[2], Kobus Van-Heerden[3], Brett Lumpkins[4]

Southern Poultry Research, Inc.[1,4], Ceva Animal Health[2,3]

Poultry coccidiosisis managed by either anticoccidial drugs or live coccidia vaccines. Commercial coccidia vaccine research and development began in the 1950's principally by Allan Edger, Auburn University. In the 1970's ,Eng-Hong Leealong with University of Guelphworked on producing a coccidia vaccine. In 1983 aCanadian patentwas approved: Protecting against coccidiosis in poultry by administering a vaccine containing sufficient organisms to develop an immunological response in the animal. The vaccine described in thepatent was commercially launched in 1985.and contains Eimeria acervulina, E. maxima, and E. tenella. To determine the anticoccidial drug sensitivity of the vaccinestrains a battery coccidia challenge test was conducted. The challenge was 100X the commercial recommended dose. The results showedthat all strains were highly sensitive to robendiene, decoquinate, clopidol, salinomycin, and narasin. Diclazuril, zoalene, and amprolium did not completely control all strains. This was due to efficacy or mode of action issues than to a resistanceissue. A 42D broiler floorpen study examinedthe effect ofvaccination on performance.Treatments were no vaccine, vaccine,and vaccine plus Zoalene 125 ppm D14-28. Thebest performance was vaccine plus drugfollowed by vaccine alone. Both vaccine treatmentshadsignificantly better performance than

no vaccination. A d28 mix Eimeriaspecies challenge demonstrated that Zolene did inhibit immunity development. However,by D35 both vaccine groups had strong coccidiaimmunity. These results emphasize the benefitsof a using a vaccine and the potential benefit of using a drug inconjunctionwith a vaccine.

Good Coccidia Control in Commercial Turkeys via ASTs

Steve Fitz-Coy[1]

Merck Animal Health[1]

Seven species of Eimeria are described as parasites for turkeys, Eimeria adenoeides, E. meleagrimitis, E. gallopavonis, E. dispersa, E. meleagridis, E. subrotunda and E. innocua. Four species,Eimeria adenoeides, E. meleagrimitis, E. gallopavonis, E. dispersa, are considered as having significant economic impact on commercial turkey production. For many years, pharmaceuticals were used as the primary method of coccidiosis control. However, the declined efficacy of several anticoccidials and consumers pressure to reduce the use of drugs from food animal production are challenging factors in current coccidiosis control programs.The anticoccidial sensitivity tests (AST) is the standard to measure the effectiveness of several anticoccidials and alternative products. ASTs employ traditional anticoccidials and non-traditional products. Several of these products are natural products currently used by segments of the poultry industry; however, the industry is not sure about their effect against Eimeria species.Data showed that some of the traditional products have shown a decline in efficacy against specific isolates of Eimeria. Some of the alternative products have shown fair to moderate efficacy against some of the isolates. Some of alternative ingredients used in this exercise did not exert a major impact on either the intestinal and or cecal species of coccidia.

Lessons Learned Adjusting the Application of Coccidiosis Vaccine in Grandparent Broiler Breeders

Christina Lindsey[1]

Aviagen[1]

Coccidiosis is well recognized as one of the major causes of production losses in poultry globally. Coccidiosis vaccines have become a cornerstone in the control of this parasite for much of the industry. As a follow-up to my 2020 AAAP presentation, “Lessons learned changing coccidiosis vaccines” (wherein we covered both hatchery and field topics), I’ll share the adjustments we made to our vaccine application at the hatchery for our internal flocks of Grandparent (GP) broiler breeders. Topics will include vaccine mixing methods and equipment, vaccine administration, hatchling care, and verification of homogenous administration by 7-day oocyst counts (OPGs) in the field.

Recombinant Bacillus subtilis expressing a chicken NK lysin peptide as an effective mucosal delivery strategy to prevent and control avian coccidiosis

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The chicken NK-lysin peptide 2(cNK-2) is a natural lytic peptide withdirect cytotoxicity against apicomplexan parasites such as Eimeria. Developing an effective oral delivery strategy to express cNK-2 in the intestine where Eimeriaparasites interact with the host’s gut epithelial cellswill reduce the fecundity of parasites and gut damage. Furthermore, cNK-2 modulates gut immune responsesto decrease local inflammationelicited by parasite invasion of host cells.Therefore, we developed a stable strain of Bacillus subtilisthat carries chicken NK2 peptide(B. subtilis-cNK2)to determine its effectiveness as an oral carrier of NK lysin peptide to the gut and to investigate its effect against coccidiosis infection in

commercial broiler chickens. Chickens were allocated into four treatment groups in a completely randomized design:1) negative control (NC, unchallenged), 2) positive control (PC: challenged without B. subtilis), 3) B.subtiliswith empty vector (BSEV),and 4) B. subtilis-cNK2(BSNK). All birds were challenged with 5,000 sporulated E. acervulinaoocysts through oral gavage except the NC group.Chickens givenBSEVorBSNK wereorally gavagedon day 14,15 and 16(1×1010cfu/mL)followed by oral challenge infection with E. acervulina.Infected chickens treated with BSNK showed improved ($p<0.05$) growth performance, gut integrity, and lower($p<0.05$) oocyststshedding compared to infected and untreatedcontrols.Taken together, this is the first demonstration to showdietary B.subtilisprobiotics carryingchicken NK2 peptidecan bean effectivealternative to antibiotics strategy toreduce harmful effects of avian coccidiosis.

Use of a Sporulated Oocyst Coccidiosis Vaccine to Induce Immunity through Trickle Infection Under Field Conditions

Mark Mouw[1], Daniel Wilson[2]

Wilson Veterinary Co.[1,2]

Coccidiosis vaccination of chickens is usually accomplished at the hatchery or by early feed application. The vaccine is a controlled dose of sporulated oocysts which will infect the vaccinates and proceed through the normal Eimeria life cycle for each of the species in the vaccine. Protective immunity requires exposure 3 to 5 sequential life cycles, depending upon the species. Each successive life cycle expands the numbers of Eimeria shed into the environment until immunity is achieved and oocyst production is dramatically reduced. To accomplish these life cycles, the flock must have access to the feces containing oocysts after each life cycle is completed, and the oocysts must sporulate to become infective. Sporulation requires heat and moisture that may not be at appropriate levels in many pullet management systems. Access to feces may also be difficult in cage-type rearing facilities. An alternative vaccination strategy has been explored. Instead of depending upon natural recycling of oocysts, flocks are given a small dose of sporulated oocyst vaccine via feed at weekly intervals to simulate recycling, but without the requirement of access to feces or the appropriate environmental conditions for sporulation since the vaccine oocysts are already sporulated. The dose is lower than might be expected from natural recycling, but the ability of small doses of coccidia to stimulate immunity was described as early as 1941 by Dickinson, and later called "trickle infection" by Joyner and Norton in the 1970s. Successful protection of flocks using partial doses of vaccine at each interval (to achieve economic parity with a regular coccidiosis vaccination program) or full dose of vaccine at each interval have demonstrated that protective immunity can be achieved without natural field recycling. This provides a viable alternative to conventional coccidiosis vaccination to induce immunity in replacement layer pullets or chickens in adverse environmental conditions.

Using nanopore NGS technology to differentiate Eimeria parasites

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Coccidiosis is a costly enteric disease for commercial poultry worldwide caused by a single-celled, parasitic protozoa of the Eimeria genus. The parasite reproduces rapidly causing significant damage to intestinal linings and often mortality. The most common method for the identification and differentiation of Eimeria species is classical microscopy using morphometric characteristics of size or shape. While a simple technique, microscopy can be subjective and the sensitivity is not ideal. As molecular tools advance, using PCR may afford the opportunity to further characterize Eimeria samples past simple species differentiation. The overall goal of this research is to produce high quality profiles from sequence data to promptly identify Eimeria strains. Three different genome regions have previously been used to differentiate species of coccidian on a molecular level; Internal Transcribed Spacer-1 (ITS1), Ribosomal 18s DNA (18S), and

Cytochrome Oxidase 1 (CO1). Pan-Eimeria primers have been developed for these three gene regions, and provide a method to amplify all the sequences present in a mixed sample. Our laboratory is following these PCR reactions with Oxford Nanopore's Mk1B (MinIon) next generation sequencing (NGS) technology. The MinIon platform allows our laboratory to sequence samples as they are prepared, and the sequencing and bioinformatics can be performed in house. For this project, all commercial US vaccines were sequenced. For all vaccines, all species predicted to be present per manufacturer label were identified for each gene target. Sequence coverage and depth for each gene region was sufficient, allowing for creation of detailed taxonomic tree cladograms using the bioinformatics software program Geneious. These results show the applicability of using nanopore sequencing technology for differentiating Eimeria species in a mixed sample, and for the potential in diagnostic use.

Diagnostics

Development and Evaluation of a Turkey Coronavirus Enzyme-Linked Immunosorbent Assay (ELISA) using S1 spike protein

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Iowa State University[1,2,3]

Turkey coronavirus (TCoV) is the etiologic agent of a highly contagious enteric disease of turkeys of economic significance in the United States and worldwide. There is a lack of diagnostic tests currently available for this disease in general and a lack of suitable serologic assays in particular, which may explain the lack of knowledge of the seroprevalence of TCoV in the turkey population. To date, several TCoV ELISA platforms have been described based on different antigen targets. However, the only assay currently available for the U.S. commercial turkey industry is a baculovirus-expressed N-protein competitive ELISA (cELISA) from North Carolina State (JS Guy et al, Av Dis 2002). In this study, the N-terminal portion of the TCoV spike protein (S1) was selected as antigen for the development of an indirect ELISA. The coding region of the S1 protein was synthetized, cloned, and expressed using a mammalian expression system (HEK293 cells), and purified by affinity chromatography. The diagnostic performance (sensitivity and specificity) of the TCoV S1 indirect ELISA was assessed using serum samples collected weakly from experimentally-infected control turkeys (n = 12) through 42 days post-inoculation (dpi) . Preliminary results indicated that the proposed ELISA detected anti-TCoV specific antibody 15 days post-inoculation with rate of detection increasing throughout the end of the study (100% detection after 35 dpi). This assay can be easily expanded and allow for large scale testing, allowing for surveillance and better disease prevention and control strategies. Further detailed results will be discussed at the meeting.

Non-target RNA Depletion Strategies to Improve Sensitivity of Next-Generation Sequencing (NGS) for Diagnosing Infectious Agents in Poultry

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PCR-based assays havebeen the benchmarkfor diagnosing pathogensof poultry and other livestock, however, these techniques are limited in their ability to detect multipleinfectingagents, must be frequentlyoptimized, and providelimitedgenetic informationon the pathogen. In contrast, untargeted, high-throughputsequencingcan rapidly detect allinfecting agentsin a samplewhile providinggenomic sequence information. AlthoughNGS for diagnostics offers many advantages, one ofits primarylimitations islow sensitivity to pathogens due tothe abundance of host and other non-target sequencein sequencing libraries. In the work presented here, we explore methods for improving sensitivity of NGS to detect respiratory and enteric pathogensinpoultry from RNA extracts of swab samples. We

employedcommercial and non-commercial negative enrichment strategies to selectively deplete the most abundant rRNA reads from the host andnon-target bacteria.Treatment diminishedhost signal from ~90% of total reads to ~10-20%and greatlyreduced the total number of reads mapping to bacterial 16S/23S. This resulted in up to a 200-fold increase in viral reads, detection of a greater number of viral agents, and higher average genome coverage for pathogens. Depletionassays added only 2 hours to NGS library preparation. Custom design offered significant cost savings ($7-12 per sample) compared to commercially available kits ($30-50 per sample). The custom depletion strategies can beoptimizedfor various hosts and sample typesand inclusion of theseenrichment stepscan greatly improve sensitivity of NGS for diagnostic purposes.

Precision farming: A new tool and database to monitor recombinant turkey herpesvirus vaccine applications in commercial birds.

YUN-TING WANG[1], Taylor Barbosa[2], Rik Koopman[3], Linnea Newman[4]

Merck Animal Health[1,2,3,4]

Turkey herpesvirus (HVT) is a commonly-adopted vector used in many different commercial chicken vaccines by applying it in ovo around 18-19 days of the embryonic development. Different protein gene inserts from other pathogens could be added into the HVT vector in order to provide broad protection against multiple diseases at the same time. There are many benefitsto using HVT as the vaccine vector, such as long-term disease prevention. Due to the limitation of HVT transmission between chickens, it is very critical to ensure vaccine application accuracy in order to achieve optimal protection for commercial flocks. Commercial real-time PCR (rtPCR) kitshave been used in the field to detect HVT replication cycles in chickens after in ovo vaccinations. However, there are many limitations with the rtPCR surveillance method. First, users won't be able to tell if the submitted samples contain enough genetic materials for PCR amplification, which could be heavily influenced by sampling technique. Second, as has been well documented, the biological variances between different commercial breeds and the nature of HVT replicationcould result in negative PCR results through different ages. Here we describe a unique Next Generation Sequencing (NGS) platform developed to overcome these issues. There have been more than two thousand samples from all over the world uploaded into the platform database and used to build the baselines for different commercial breeds. With this unique tool, poultry producers will be able to achieve the goal of precision farming by monitoring and advancingrecombinant HVT vaccine applications.

Enteric Health

Analysis of intestinal microbiome of commercial broiler chickens affected with clinical and subclinical necrotic enteritis

Hemlata Gautam[1], Lisanework Ayalew[2], Alexa Dison[3], Betty Chow-Lockerbie[4], Shelly Popowich[5], Arzhang Shayeganmeher[6], Iresha Subhasinghe[7], Khawaja Ashfaque Ahmed[8], Susantha Gomis[9]

University of Saskatchewan[1,2,3,4,5,6,7,8]

Enteric pathogens exploit intestinal mucosal surfaces for, colonization invasion and infection of host. Clostridium perfringens(C. perfringens) affects gut mucosa of broiler chickens and causes necrotic enteritis (NE) in conjunction with predisposing factors such as high protein diet, immunosuppression and co-infectionwith Eimeria. Protective host immune responses and the pathogenesis of this disease have to be fully explored and no preventive strategy or vaccines are currently available.The objective ofthis study was to understand the pathogenesis of NE to develop effective control strategies. To meet this objective, we evaluated changes associated with the intestinal microbiome of broiler chickens who developed clinical and subclinical NE following C. perfringenschallenge.Challenged birds showeddistinct gross and histopathological lesions of NE. Using this model, intestinal contents were collected from healthy birds, and birds with clinical

and subclinical disease. Next generation 16S rRNA sequencing was performed to assess the degree of dysbiosis and identify differences in the composition of the microflora between healthy and diseased birds. Significantchanges in the microbiome were observed to be associated with the development of NE. The normal flora composition consisted primarily of the lactobacillaceae, cyanobacteriaand peptostreptococcaceaegroups, with a small amount of clostridiaceae. Infected birds had considerably decreased lactobacillaceae, and increased clostridiaceaeand enterobacteriaceae. The dysbiosis was more severe in birds with clinical infection compared to subclinical. The results of this study provide the opportunity for future work on the interaction of C. perfringensand commensal enterobacteriaceaon the pathogenesis of NE.

Effects of dietary maltol as postbiotics on innate immunity, gut health, and growth performance of broiler chickens infected with Eimeria maxima

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Maltol isone of 40 intestinal metaboliteswhich ishighly upregulated when chickens are given a growth promoting direct-fed microbials, Bacillus subtilis1781/747(Park, I., et al., 2020. Front Vet Sci 7:123). To better understand the role of dietary maltol in mediating beneficial effects on host immunity, gut health and growth promotion in chickens, in vitroand in vivostudieswere conducted.In in vitrostudy, itwas used to evaluate the effect of maltol on innate immune response usingchicken macrophage cells (HD11), gut integrity onchicken intestinal epithelial cells (IEC), and differentiation effectson a quail muscle cellline(QMC) and primary chicken embryonicmuscle cells (PMC).In in vivostudy, dietary effect of maltol was tested on disease parameters including gut lesion scores, fecal oocyst shedding, growth performance, gut integrity and host immune response after infecting chickens orally with Eimeria maxima. In vitro, maltol increasedthe occludin, ZO1on IEC, and MUC2 levels in IEC compared to control group, and also increased IL1β and IL8 levels in HD11stimulated by LPS compared to non-LPS groups. In vivo, maltolincreased body weight during entire infection period compared to negative control group. Additionally, high dose of maltol decreased lesion score, TNFSF15, and IL1β of jejunum and fecal oocyst shedding inchickensinfected with E. maxima. In conclusion, maltol improved immune responses in IEC and HD11in vitroand improved the growth performance, intestinal immune response, and barrier integrity of young broiler chicken infected with E. maximain vivo.

Efficacy of intestinal conditioner pronutrients (botanical molecules) on intestinal integrity and serum cytokine levels in broilers fed raw soybean

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Intestinal conditioner pronutrients are botanical active molecules that improve intestinal integrity and productive performance. They are a novel solution to replace antibiotic growth promoters (AGPs). To evaluate if intestinal conditioners can replace AGPs, 480 broilers were raised for 35 days and distributed into 3 treatments: control "CON" with a basal diet without growth promoters; a group supplemented with bacitracin and halquinol "AGP"; and a group supplemented with intestinal conditioners "IC". All diets included 3% raw soybean to challenge intestinal integrity with antinutritional factors. Performance, sections of the intestine and serum cytokine concentration were evaluated. Significant differences were considered when $P<0.05$. Productive parameters (weight and feed conversion) were significantly improved in IC compared to all other groups. CON and AGP showed a detached gut mucosa and hemorrhagic foci caused by the challenge while IC maintained the normal structure of the mucosa. Morphometric analyses of jejunum and duodenum evidenced IC had better ratio villus height:crypt depth indicating better villi structure. There was a tendency towards a decrease

of serum cytokines, particularly IL-1b (P<0.05), in IC and AGP showing a better control of gut inflammation. In conclusion, raw soybean in the diet caused alterations in gut integrity and performance. Intestinal conditioner pronutrients effectively prevented them and avoided the signs of inflammation, which was correlated with a significant improvement in performance. The positive effects of intestinal conditioners were significantly greater than AGPs, showing that they can replace AGPs in broilers with additional benefits in gut heath and performance.

Impact of Salmonella Typhimurium vaccination and coccidiosis vaccination on the intestinal health of broiler chickens

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Auburn University[1,2,3,4,5]

Salmonellosis and coccidiosis are well-known intestinal diseases in poultry production. Salmonella are gram-negative bacteria with a high zoonotic potential, residing in the chicken intestines. Coccidiosis is caused by Eimeria spp., a parasitic protozoan, and responsible for large economic losses in the poultry industry. A common strategy to fight these diseases is the vaccination of day-old chicks. The aim of this study was to investigate the interaction of both vaccinations on the intestinal health of broiler chickens in a 2 x 2 experimental design with the factors vaccination against Salmonella Typhimurium (ST) and vaccination against coccidiosis. On day 21 pi all groups were challenged with a ST field strain. Samples were taken on day 21 pi and day 42 pi. Re-isolation of ST from liver and ceca showed a higher susceptibility for systemic infection with ST in birds vaccinated with both vaccines. A gene expression analysis by qPCR was performed to investigate expression of tight junction genes in the jejunum. Changes compared to the unvaccinated control group were observed on day 21 in the group vaccinated against ST and on day 42 in the group vaccinated against coccidiosis. Microbiome profiling of the jejunum was performed by Illumina sequencing of 16S rRNA. The results revealed a significant difference in the beta-diversity on day 21 pi after vaccination with ST. Number and composition of phyla differed on day 21 pi compared to day 42 pi. These findings indicate a significant influence of coccidiosis and ST vaccine on the gut health of chickens.

Increased Incidence of Viral Enteritis in Broiler Chickens in 2020: Isolation and Characterization of Viruses from the Intestinal Samples

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Viral enteritis/enteropathy, also known as runting stunting syndrome in chickens, was prevalent during early 2000s in the United States, but has dissipated since then. However, in 2020 there was an increase in incidence of viral enteritis in commercial broiler chickens and an increase in submission of intestinal samples to the laboratory. Viral enteritis affects young broiler chickensduring the first two weeks of life.The disease is characterized by thinning of intestinal walls, wateryintestinal content, and passage of watery undigested feed, which results in growth depression and economic losses. Several types of viruses have been associated with viral enteritis in chickens including orthoreovirus, astrovirus, and adenovirus. Rotavirus and parvovirus may also play a role in disease development. Reovirus and astrovirus were detected in most intestinal homogenates submitted from affected commercial chickens in 2020, and in many cases infections were concomitant. Isolated reoviruses and astroviruses were serotyped using a panel of polyclonal antisera, pathotyped in low-maternal-antibody day-old broiler chickens, and genotyped. Findings will be presented orally.

Isolation and Characterization of Enteric Viruses in Runting and Stunting Syndrome Outbreak for the Production of Future Autogenous Vaccine

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Runting and Stunting Syndrome (RSS) causes significant economic losses in the broiler industry. Thissyndrome is characterized by enteric disease known to increase feed conversion, suppress body weight, and increase culling and mortality. The etiology of this syndrome is not precisely understood, but there is evidence for a combination of enteric viruses including Reovirus, Astrovirus, Rotavirus, and Adenovirus. In this study, duodenal loop samples were collected ~14 days of age from broiler farms with classic RSS signs. From these samples, the two most severely affected farms were selected via histopathological diagnosis of RSS. Samples from these two farms were then processed for virus isolation, PCR, sequencing, and recreation of RSS in SPF chicks. The combination of virus identification, sequencing, and disease re-creation will be used in further studies and potential autogenous vaccine production.

Managing the gut microbial population in antibiotic-free broiler production

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Arm & Hammer[1]

Antibiotic-free poultry production has overwhelmingly taken over traditional production, increasing from 4% of US broiler feed in 2013 to 58% in 2019. This significant change is due to both consumer pressure and the Veterinaty Feed Directive and is aimed at reducing the spread of bacterial antibiotic resistance genes. Managing gut microbial challenges with subtherapeutic antibiotics had been replaced by alternative methods to prevent and treat diseases, such as probiotics and prebiotics. Hatchery studies were conducted to evaluate the role of early colonization of gut microbiota by analyzing culturable early colonizers at the day of hatch and succession of mature microbiota through amplicon sequence analysis. Horizontal transmission from the environment was greater than vertical transmission of the maternal microbiota. In addition, there were significant populations of avian pathogenic Escherichia coli (APEC) in broiler chicks at day of hatch. A probiotic developed to promote colonization by beneficial bacteria and reduce the levels of APEC, without the use of antibiotics, was applied in a single dose at the hatchery. The targeted probiotic application was as effective as using gentamycin in reducing APEC levels without disrupting the healthy microbiome.

Non-Specific Enteropathy Syndrome in Commercial Turkey Poults

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Poult Enteritis Mortality Syndrome (PEMS) and/or Poult Enteritis Complex (PEC) is an economically devastating phenomenon that has plagued the Turkey Industry for a couple of decades. These are general terms that have been utilized to characterize the infectious intestinal diseases of young turkeys to which are commonly clinically described by severe runting, malabsorption, and immune dysfunction. There has been many etiologies linked to this syndrome, but also many unanswered questions in terms of exact etiology as well as additional contributing factors. It has also been in the last couple of decades that the Turkey Industry has lost many tools, including several in the antimicrobial and antiprotozoal drug classes, that may of in the past aided in the reduction of the severe effects of this condition. Thus making PEMS, PEC, or poult enteritis of unknownetiology a lingering disease topic of interest for commercial turkey producers and of top focus for preventative strategies to further modulate and reduce the overall impact on performance. Poult enteropathies, in there complex nature, highlight the overall importance of a thorough, open-minded field investigation and

therefore I plan to utilize this case report todefine one clinical perspective on an attempt totear down the walls of a regional non-specific enteropathy condition. The previously defined infectious nature of PEMS should not cloud our judgement or limit how we look into these syndromes. The research efforts are limited in conditions such as poult enteropathies, as the parameters involved in daily production are very complex, and not easily reproduced experimentally. Therefore, it is my clinical perspective that revealing the solution(s) to any poult enteritis condition be likened to peeling an onion. With diligence and critical evaluation of multiple components, both non-infectious and infectious, there is often a multitude of potential contributing factors. Open-mindedness, critical, and often curious thinking are therefore a field clinician's best tools or qualities that allow us to overtime begin peeling back these more complex conditionslayer by layer, and further illuminating the areas of potential focus for future or current best management practices.

The Sporadic Nature of Intestinal Dilatation Syndrome in Layer Breeders

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Intestinal dilatation syndrome (IDS)is a disease with acurrently unknown etiology. Flocksimpacted with IDS are usuallyWhite Plymouth Rock (WPR)female parent stock(PS), and sometimes commercial brown layinghens. This condition has been identified in all majorlayer genetic company flocks. IDSis most closely associated with pullets reared on deep litter, although presentationof the diseasedoes not typically occur until after 30 weeks. Affected henswill stop layingand appear emaciated. Necropsy revealssegmented dilatation of the small intestine around Meckel's diverticulumthat extends for 10 –20 cm. Histopathology shows a chronic granulocytic enteritis with villous atrophy. IDS has a major economic impact on breeder flocks with thedecrease in overall productivity and highmortality. For this study, we identified a PS house in Europeimpacted with IDS that hatched in November 2017 (13.56% mortality at 66weeksof age).Thenext flockhatched in March 2019with the same rearing and laying house, and every bird was wing-bandedwiththe goal of necropsyingall mortality to understand the true incidenceof the disease. All mortality was necropsied between15and 40 weeks, about half the mortality was necropsied between 40 and 66 weeks.At 66 weeksof age, 548 out of 8576 (6.40%) hens housed had died. Of the 312 birds necropsied,8 had confirmed lesions of IDS. Thefailure torecreateIDSin previously impacted houses underscores the challenge faced in findingthe etiologic agent and understanding more about pathogenesis and prevention.

Immunology

Assessment of Activated and Regulatory T cells in the Trachea of Vaccinated Chickens as a Recall to an Infectious Laryngotracheitis Virus (ILTV) Challenge

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Early studies pointed out the relevant need ofT cell mediated responses withresistance against infectious laryngotracheitis. However, little is known about the components of theT cellimmune responsesthat are associated with disease resistance. The aim of this study was to assess theactivated and regulatory T cells atprimary sites of viral replication. To this end the activation of cytotoxic T lymphocytes (CTLs)and natural killer (NK)cells and quantification of regulatory T cellsin the larynx-trachea of vaccinated chickens after challenge was evaluated. Briefly, specific pathogen free (SPF) chickens were vaccinated with either a chicken embryo origin (CEO) vaccine, atissue culture origin (TCO) vaccine or a recombinant (r) HVT-LT vaccine. Chickens were challenge at 28 days of age with a virulent ILTVstrain. Titers of systemic neutralizing antibodies post-vaccinationwere determined. Results indicated that CEO vaccine conferred complete protection based on the abilityof

CEO vaccinated chickensto block challenge virusreplication, prevent trachea lesions andclinical signs. The T cell response in thetrachea of CEO vaccinated chickenswas characterized by anearlyincrease and activation of CTLs. A significant correlation was found between increase of activated CTLs and decrease of clinical signs. The TCO and rHVT-LT vaccines were categorized to induce partial protection by their capacity to reduce but not block the replication of the challenge virus, trachealesions and clinical signs, while a moderate increase of resting and activated CTLs and NK cellsappeared in the larynx-trachea. Regulatory T and NK cells were significantly increased in the non-protected group (Non-vaccinated/Challenge). Among vaccinated groups higher titers of neutralizing antibodies induced post-vaccination coincided with superior protection after challenge.

Characterization of immune responses after vaccination and challenge with IBV in MHC lines

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In situ analysis of biomarkers is highly desirable in molecular pathology because it allows the examination of biomarker status within the histopathological context of the clinical specimens. In addition, identifying RNA expressionat the single cell level withinthe morphological context by RNA in situ hybridization provides a great deal of information on gene expressionchanges over conventional techniques that analyze bulk tissue.The goal of this experiment is to use RNA scope to detect gene expression primarily in tracheas and Harderian glands of MHC B haplotype chickens(IBV resistant-susceptible model)vaccinated and challenged with homologousMassIBV strains.In addition, we will use flow cytometryto characterize antigen presenting and T cell populations.Viral load will be measured from tears to associate IBV load and responsesin tissues.Results will be discussed.

Extracellular vesicles released from chicken tracheal cells present distinct proteomic signatures upon avian influenza virus infection and Toll-like receptor ligand stimulation

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Avian influenza viruses (AIVs) cause respiratory infection, mortality, and decline in production.During viral respiratory infections, communication and interaction between the host cells at the early stages of infection determine the quality and magnitude of the resulting immune responses. The release of extracellular vesicles (EVs) from host cells isasystem of mediated intercellular communication. These EVs contain an array of biomolecules, including proteins and RNAs, which can be regulated by viral infections and may play a role in antiviral responses.In this study, we aimed to identify theimmunoregulatory roles of EVs released from tracheal cells. We hypothesized that EVsreleased from chicken tracheal cells regulate the functions of other immune cells, such as macrophages. To this end, we characterized the protein profile of EVs secreted by chicken tracheal cells during AIV infection. Subsequently, wehaveidentifiedthe impact of EVs released from chicken tracheal cells on chicken macrophages.In this study, atotal of 140 differentially expressed proteins (i.e. > 2-fold change) were identified. Analysis of gene functions and protein-protein interactions using thePANTHERclassification systemand STRING database, respectively, revealedthat EVs secreted by chicken tracheal cells in response to various stimuli are enriched in protein markers involved in immune responses and cell signaling pathways. Currently, we are evaluating the mRNA expression of some candidate genesin macrophages following EVtreatment. In conclusion, characterizing EV protein profiles revealed a potential role of respiratory EVs in the induction and modulation of antiviral responses against AIV infectionin chickens.

Georgia Titers 102

Louise Dufour-Zavala[1], Luis Gomez[2], Roy Berghaus[3]

GA Poultry Laboratory Network[1], Phibro Animal Health[2], University of Georgia[3]

The Georgia Poultry Laboratory Network has been producing and distributing serology baselines for Georgia flocks yearly for the past 15 years. The "GA Titers" document is one that collates very large amounts of ELISA data from the commercial poultry industry, reporting it by age ranges and test type. It serves as an aid for titer interpretation for companies using ELISA monitoring programs. GA Titers got a major overhaul in 2021, as we modified the report to make it more statistically solid and more useable. We also added several titer comparisons that enhance our understanding of how vaccinated and exposed poultry respond and how that response is measured using ELISA systems. Novel uses of the data will also be discussed.

Responses of Chicken Macrophages to Clostridium perfringens isolates varying in their Pathogenicity

Ravi Kulkarni[1], Carissa Gaghan[2], John Prescott[3]

NC state University[1]

Clostridium perfringens, an anaerobic toxin-producing and spore-forming Gram-positive bacteriumis the causative agent of Necrotic enteritis (NE) in poultry. Recent withdrawal of in-feed antibiotic supplements in poultry has caused a significant surgein NE incidence and theglobal annual losses due to NE are estimated around $6 billion. The pathogenesis of NE involves intestinal damage and necrosis caused by C. perfringens toxins and enzymes. There is evidence that unique NE-causing strains which possess signature NE-associated virulence genesincluding netBand that the immunity against NE is associated with virulent (but not avirulent) strains of C. perfringens. Although NE pathogenesis is moderately well studied, avian cellular immune responsesto C. perfringensand their secretory proteins is poorly understood. In the present study, we usedC. perfringensisolates that varied in their NE virulence capacity to investigateboth cell-associatedand cell-freesecretory proteins-induced macrophage responsesin-vitro. The findings showed that macrophages treated withtwo virulent isolates and their secretory proteins had significantlyupregulated transcription of interferon (IFN)-γ cytokine and toll-like receptor (TLR)-2 pathogen-sensing immune genes, when compared to avirulent C. perfringensisolate. Furthermore, macrophage production of nitric oxide and surface expression of MHC-II antigen presenting molecule was also found significantly increasedwhen treated with virulent C. perfringenscompared to avirulent isolate. In summary, our study suggested that C. perfringensand their secretory proteins can activate chicken macrophages and that their activation is associated with the virulence potential of these bacteria.

The Influence of Ascaridia galli Development on Cytokine Gene Expression and Intestinal Microbiota of Infected Chickens

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The aims of this study were to establish a timeline of the influence of Ascaridia galli on the expression of cytokines and the composition and relative abundance of theintestinal microbiota.Fiftysixmale layer type birds were used and randomly placed into onecontrol and one infected group. Theinfected group waschallengedwith 500 embryonated A. gallieggs forthree consecutive days starting at 24 days of age. Three birds of each group were euthanizedweekly until the birds were13 weeksoldand the remaining birds were euthanized. Jejunal content was frozen for extractionofbacterial DNA and investigation of the microbiota by 16S rRNA gene amplicon next generation sequencing. Total RNA was extracted from one-gramjejunal wall for determination of expression of TGF-β4,INF-γ,IL-4,IL-8,IL10andIL13byqPCR. The rest of the jejunal wall was digested using pepsin and HCl to detect and

count A. gallilarvae.We were able to visualize larvae from week 1 to week 6 post infectionin decreasing numbers.However,no eggs wereshedinthefeces and adultswere not observed at the end of the experiment. The birds were fed with feed formulated forbroilers which is more nutrient dense. This might have contributed to the birds' ability to clear the infection before the worm were able to reach maturity. The gene expressionand microbiota analysis will provide more data tosupport or reject this hypothesis.

Mycoplasma

Application of Comparative Genomics to Mycoplasma gallisepticum Vaccine Studies

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Mycoplasma gallisepticum (MG)causes respiratory disease and significant economic losses in poultryworldwide. While the currently available MG live vaccines all have their benefits, some MG vaccine strains can revert to virulence and strain differentiation among vaccine strains and field isolates may be difficult. Also, live vaccine development and evaluation require trials withtwo or more MG strains in the same animal study,and it is valuableto develop differentiating PCR protocolsfordifferent vaccinesas well as challenge strains. Our objective in this research was to use comparative genomics to identify targets and develop strain-specific quantitative PCR protocolsfor 6/85 vaccine, as well as our standard MG challenge strain fortrials-R-strain. To achieve this, whole genome sequencing of several MG isolates, including vaccine and reference strains, was performed using Illumina technologies. Whole genome assembly, annotation and analysis were performed using tools available in PATRIC.Next,potential targetsfor strain differentiation were identified by proteome analysis, protein family comparison, and variation analysis tools in PATRIC. The specificity of the potential targets was verified by NCBI BLAST analysis; strain-differentiating primers and probes forTaqman® real-time PCR were developed using Primer3Plus and Primer-BLAST and tested on mixtures of DNA as well astracheal swabs from infected chickens and clinical samples. Utilization of these protocols for R-strain and 6/85 allows for the rapid and quantitative differentiation of the R-strain in research trials aimed at understanding the dynamics of the vaccine and challenge strain in vivo;these protocolsmayalsobe applied to clinical diagnostics to specifically identify6/85 vaccine with less time and cost than the current techniques.

Sero-conversion of a Commercial Layer Flock Vaccinated with MG ts-11 and MS MS-H is Not Associated with Wild-type Colonisation

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Bioproperties Pty Ltd[1,2,3,4,5,6]

The mycoplasma status of a commercial layer flock reared on a single-age remote site was followed from 1 week post-vaccination into production. The vaccines (ts-11 and MS-H) were mixed on farm and administered at full dose in a single eyedrop at 7 weeks of age. The pullets were moved to a multi-age production site at 16 weeks. Sero-response was detected using Bio-Chek MG and MS ELISA kits and was correlated to in-house MG and MS strain specific DIVA PCRs. Weekly choanal swabs were collected from 30 randomly selected birds for the first 4 weeks post vaccination (PV) following which both serum and swab samples were collected from 20 birds every 3 weeks until 35 weeks of age, after-which, the sampling time point was changed to every 5 weeks for the remaining laying period. PCR results show MG ts-11 colonisation peaking at 5 weeks PV, declining till end of rearing and a subsequent resurgence at point of lay, co-incident with an increase in MG seropositivity. In contrast, MS-H colonisation peaked at 7 weeks PV and remained high and was associated with a steady increase in the sero-response to MS-H post transfer. No field strain was detected in any of the samples tested by DIVA PCR and no production

disturbances were recorded in this flock during the study period demonstrating that serological conversion post point of lay is not due to wild type colonisation. This study identifies the kinetics of long-term colonization and seroconversion following a single eye-drop application of MG ts-11 and MS MS-H at 7 weeks of age. While MS-H can efficiently colonise the trachea and remain persistent, ts-11 may not be easily detected during rearing but undergoes a colonization expansion to a second peak post point of lay. In this study this was associated with seroconversion to MG and MS in lay. Identification of the mechanism surrounding this surge in vaccine colonisation and subsequent seroconversion is currently under investigation.

Salmonella

Effectiveness and compatibility of live Salmonella vaccine and live yeast probiotic in a Salmonella Enteritidis challenge model in broilers

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As USDA-FSIS has reduced the number of Salmonella positive samples allowed to meet poultry performance standards, the use of interventions on farms has become more important to reduce Salmonella coming to the processing plant. Live vaccines and yeast products have individually been shown effective in reducing Salmonella, but there has been little research to demonstrate their compatibility and combined efficacy. This study had 1350 broilers in 50 floor pens with 10 pens per treatment. The five treatments were 1) no treatment, 2) Levucell SB Titan Advantage (1 pound/ton), 3) Megan Vac1 day 1 dose only, 4) Megan Vac1 on day 1 and day 14, and 5) Levucell and the two doses of Megan Vac1. The challenge was 7.5 x 107 CFU/bird S. Enteritidis (S.E.) by oral gavage to 15 birds/pen (seeders) on day 28. On days 40 and 41, the ceca, liver/spleen and carcass rinse with feathers was collected from 8 birds/pen, half with direct challenge and half with indirect S.E. exposure. Salmonella cultures were performed with tetrathionate, then XLT-4 by micro most probable number (MPN) per Berghaus et al., 2013. At 4 days post day 1 vaccination, there was no statistical difference in vaccine recovery in the ceca, whether with or without Levucell, and 100% vaccine recovery in liver/spleen from all vaccinated birds. Overall, the live yeast product did not interfere with vaccine colonization. Also, Megan Vac1 administered as either 1 or 2 doses had significant overall impact on reducing S.E. colonization. Levucell had its greatest impact on S.E. reduction in birds horizontally exposed. All treatments had greater effect on reducing liver/spleen and ceca Salmonella in horizontally exposed birds. Megan Vac1 plus Levucell had a significant impact on reducing prevalence of S.E. in liver/spleens of direct and horizontally exposed birds.

Evaluation of the toxigenicity of lipopolysaccharide associated with chicken hepatopathy.

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Elanco[1], University of Arkansas[2,3]

Lipopolysaccharide (LPS), endotoxin, is an antigen of gram-negative bacteria that results in specific antibody production in the host. However, certain portions of the LPS may elicit an immune mediated reaction, similar to disseminated intravascular coagulopathy, evidenced by severe hepatopathy and mortality in some poultry. The focused emphasis on controlling salmonella in poultry has led to a substantial increase in the use of vaccines, both live and killed, in broiler breeders. An increase in mortalityapproximately two weeks post-vaccination has been noted in some flocks receiving inactivated emulsified vaccine. Post-mortem examination has evidenced uncoagulated blood in the abdomen and a friable, inflamed liver leading to a diagnosis of post-vaccinal hepatopathy. Two batches of autogenous salmonella vaccine containing the same isolates were prepared and used to vaccinated Ross 708 broiler breeder pullets. Mortality began to increase 17 days post inoculation following use of one but not the other. The batch associated with hepatopathy had high levels of LPS as determined using the Limulus Amoebocyte Lysate test. These two batches

and two batches containing purified LPS from Salmonella enteritidis were evaluated using a growing feather dermal-test that indicates reactivity. The results of this evaluation and potential for further use will be discussed.

Evaluation of Vaccination Techniques on Development of Hemorrhagic Hepatopathy in Broiler Breeders, a Pilot Study

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Hemorrhagic hepatopathy (HH) is a syndrome observedin layer pullets and infrequently in broiler breeder pullets that results in elevated mortality following vaccination. Reported postmortem findings include hepatomegaly, splenomegaly, amyloidosis, andhepatic and splenicnecrosis, as well as significant amounts of noncoagulated hemorrhagic fluid in the coelom.Thissyndrome is frequently associated withthe administration of Salmonella bacterins and is thought to be associated with an inflammatory response to the bacterial lipopolysaccharide (LPS). This pilot study was designed to create a model to induce HH in broiler breeder pullets using differentvaccination techniques. Fifteen-week-old pullets were inoculated witheither0.5 mL ofan autogenous Salmonella bacterincontaining4 isolates or 0.9% saline. The vaccinewaswarmed to either 85°For 100°Fand administeredeither intramuscularlyin theleftbreast or subcutaneouslyin the right inguinal fold. Control groups wereinoculated with 0.9% saline using the same warming and application techniques as the bacterin vaccinated groups. Birds were necropsied at two and four weekspost vaccinationto screen for lesions associated with HH. Muscle, spleen, and liver tissues were collectedin neutral buffered formalinfor histopathologic evaluation for amyloidosis, vaccine reaction, and inflammation. Our hypothesis isthat birds inoculated with the Salmonella bacterin via the intramuscular route will have the highest prevalence of HH as well as the most severevaccinereactions. Results from this research will be used to minimizevaccination associated morbidityand mortalityin broiler breedersin the field.

Genetic characterization of Salmonella Enteritidis recovered from chicken samples collected by the USDA Food Safety and Inspection Service from Mississippi between 2016 and 2020

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Salmonella enterica subsp enterica serovar Enteritidis(SE), is the leading cause of salmonellosis in humans in the United States. Since 2013, an increasing trend of foodborne illnesslinked to chickenand caused by this serovarhasbeen reported by the National Outbreak Reporting System (NORS)from the Center for Disease Control and Prevention (CDC) in the U.S. In addition, SEisone of the most common serovarsdetected in chickens (NVSL -USDA APHIS, 2018)and the control of thismicroorganismin poultry represents a challenge nowadays when birdsare raised with none or reduced use of antibiotics.Thepurpose of this study was to improve theunderstanding of SEinfectingpoultry and ultimately guideSalmonella control practices in Mississippi. Thegenetic characteristics of 81 SE isolatescollected by the USDA Food Safety Inspection Service from Mississippi poultry establishments between 2016 and 2020were evaluated in comparison withthe SEPhage Type 4 (PT4)strain P125109 and the U.S. mouse origin SE Phage Type 13a (PT13a)NZ_CP022003 reference sequences. The phylogenetic analysis showed the SE strains from Mississippi clustered into two distinct phylogenetic clades. Clade 1was the most closely related to the SE PT13a reference strainwhile Clade 2clustered together with the SE PT4reference strain. Based on sample source, most of the SEisolatesrecovered from comminuted chicken samples were located in Clade 1, while most of the samplesrecovered from carcass rinses were located in Clade 2.A detailed description of relevantfeatures

in the SE Mississippi strains,as well as the predicted antibiotic resistance profiles and replicon or plasmid content isdiscussed.Thestudyhighlights specific genetic characteristicsin SEcurrently prevalent in poultry products in Mississippi which can potentially be used to target prevention strategies.

Histopathologic alterations with Salmonella Typhimurium and dietary tungsten in chickens

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It has been hypothesized that tungsten reduces Enterobacteriaceae expansion by inhibiting molybdenum cofactor-dependent microbial respiratory pathways that are active during inflammation. The aim of this study was to evaluate the effect of SalmonellaTyphimurium and dietary tungsten on the intestinal permeability, cecal S. Typhimurium colonization, and histopathological alterationsin different organs of chickens. The birds were separated into four treatments: control, dietary tungsten supplementation (250 ppm), control + challenge with SalmonellaTyphimurium (106 CFU) and challenged + dietary tungsten supplementation. The birds received tungsten in the diet from day (d) 1 and the challenge was performed on d 7. On d 9, the intestinal permeability of 10 birds/group was measured by the passage of FITC-d from the intestinal lumen to the blood, and S. Typhimurium colonization of the ceca was determined from 30 birds/group. Additionally, samples of liver, spleen, kidney, bursa, pancreas, duodenum, jejunum, and ileum from 10 birds/group were collected and routinely processed for histologic examination. Results showed that the treatments did not change the intestinal permeability, but, surprisingly, dietary tungsten increased the prevalence of S. Typhimurium by 50%. Histopathology results suggest that treatments had little to no observable effect on the small or large intestine. In the bursa, mild lymphoid depletion was observed across treatment groups. Further evaluation is being conducted to determine the significance of histologic changes.

Impact of AviPro® Megan® Vac 1 to Reduce Salmonella Contamination in Broilers

Jaime Ruiz[1], Sandra Aehle[2], Will Gretsch[3]

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Contamination of retail poultry products with Salmonellahas important public health implications. With increasing pressure from regulators and consumers to guarantee safe poultry products, the poultry industry must continue to develop control strategies aimed at reducing Salmonella infections in pre-harvest production. Risk management must include a plan of comprehensive standard practices, including pre-harvest interventions, such as administration of vaccines, to reduce and control Salmonella infections in poultry and environmental contamination. Vaccination of poultry against Salmonella infections is a complementary intervention in an overall Salmonellacontrol program. Vaccination against Salmonella infections aims to mimic the development of naturally acquired immunity in poultry. Live and killed vaccines, when used together, have been shown to reduce vertical and horizontal transmission of Salmonella in meat birds (Young et al 2007, Dorea et al 2010). By raising the resistance to Salmonellainfections through vaccination of breeders, Dorea et al (2010) showed that the Salmonellaburden on broilers at slaughter was significantlyreduced. A trial was conducted to evaluate the impact of a live Salmonella vaccine in commercial broilers. Significant reductions were observed between vaccinated and non-vaccinated bird rinse samples after one production cycle. Fewer pathogenic Salmonellawere recovered from rinsates of vaccinated birds compared to non-vaccinated birds at processing. These data provide evidence that vaccination with a live Salmonella vaccinereduced infections in broilers, which, in turn, reduced contamination in carcass rinses. Additionally, a shift in the serotype profile was observed away from serogroups B and D salmonellae, which are predominantly associated with food-borne illness.

On Farm Salmonella Prevention: Feed Mills Focus

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Considering the major role eggs and poultry meat have as vehicles of human cases of salmonellosis, controlling Salmonella colonization of live poultry is essential. Feed as a potential source is a reality all poultry productions must face. Many studies have observed the survival of Salmonella serovars in the feed for durations extending many months. Using raw materials free of Salmonella would be an effective way to prevent the colonization of birds, however, given the ubiquitous nature of the bacteria, this is impracticable in commercial live production. Nonetheless, mitigation of this risk can be achieved through the implementation of different procedures. This presentation is aimed to anyone within the poultry industry having an interest in controlling Salmonella risk related to feed. It will review, in detail, feel mill audit by combining current research with the experience of Hybrid Turkeys' success in providing Salmonella free feed for the last 19 years to their turkey breeders in Ontario, Canada. Topics will cover ingredient selection, reception of ingredients, storage of ingredients and finish feed, feed manufacturing, feed treatments and delivery on the farms. Proper biosecurity practices will be stated for each stages of feed production, as well as processes needing emergency plans. Special attention will be given to monitoring programs in order to aid a company in tracking risk and base decisions on data.

The distribution of salmonella spp. in a commercial turkey operation in North Carolina

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North Carolina State University[1,2,3]

Salmonellosis in poultry flocks is a costly and challenging to control that also posessignificant human health threat. Our aim is to 1) investigate the spatio-temporaldistribution of Salmonella spp. in various farms from a commercial turkey company, 2)estimate the main risk factors associated with this infection, and 3) determine the extentto which movement versus spatial proximity networks determine the distribution ofSalmonella spp. We utilized samples routinely collected in both the breeder and meatbird operations for the past 3 years. Breeder hens and toms are vaccinated withPoulvac ST modified-live Salmonella typhimurium vaccine multiple times prior to thestart of production. Farm location, production type, bird placement, and transportationwere collected. Data on transfer of 5 week old turkeys from the brooder farm to the growout farm along with feed truck movements between farms was considered as well.Samples from grow out flocks were collected approximately 14 days prior to harvest viabootie swabs. Samples from the breeder farms were collected every two weeks, alsovia bootie swabs. Salmonella serotyping was performed from positive samples. On thegrow-out side of the company the serotype infantis (11.72%) was the most represented,followed by albany (4.47%), senftenberg (4.19%) and schwarzengrund (3.91%).E valuation of risk factors and spread dynamics of Salmonellosis will be discussed.

The Role of Vertical Transmission and Egg Adaptation of Salmonella Infantis in Broiler Vertical Integrations

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Mississippi State University[1], AgroAvilab S.A[2], Federal University of Rio Grande do Sul.[3]

In recent years, Salmonella Infantis (SI) has become one of the most frequently Salmonella serotypesisolated from poultry and their products. Outbreaks of foodborne illnessinvolving S. infantis are being common in the United States, Canada, and Europe. Furthermore, this serotype has shown a marked multi-resistance to antibiotics.Analysis of SIisolation data in two regions of South America showed that in addition to the findings in the processing plants,the presence of this serotype in samples collected from grandparentand

breederfarms, hatcheries (PIPS), and day-old chicksis frequent. These findings suggest this serotype may be transmitted vertically or by the contamination of the eggshell. It is also possible that this serotype is adaptedto the egg. Considering that the vertical transmission of S. Infantis remains obscure, two objectives were set for this study:Goal1 was to evaluateS. Infantis vertical transmission. Goal 2 was to analyze the possible transmission through the egg (eggshell contamination).To simulate vertical transmission, S. infantis isolated from broilerchickens was inoculatedin two SPF embryo locations: yolk sac and chorioallantoic sac. To simulate SPF egg transmission, inoculation was carried out by contact (patch method). The results of this study will be discussed during the presentation.

Vaccinology

Deletion of Thymidine Kinase Reduces Protection of Marek's Disease Vaccine Candidate

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USDA-ARS[1,2,3,4,5]

Recent attempts to develop improved vaccines for the prevention of Marek's disease (MD) have focused upon recombinant Marek' s disease viruses (MDVs) in which various genes have been deleted with the intent of reducing MDV virulence while retaining its potency as an immunogen. One such construct, a MDV with deleted Meq gene, has been shown to be an effective vaccine but with the undesirable characteristic of lymphoid atrophy. Previous attempts have been unsuccessful in eliminating the lymphoid atrophy while retaining superior protection of this vaccine candidate. Disruption/ablation/deletion of the viral thymidine kinase (TK) has long been a reliable method of viral attenuation, often producing herpesvirus vaccine strains which are stable, of greatly reduced pathogenicity, and efficacious. We hypothesized that a combined Meq and TK-deleted virus may have sufficient attenuation to allow for viral replication in vivowith absence of lymphoid atrophy. While we were successful in eliminatinglymphoid atrophy, unfortunately this came at the cost of reduced virus protectionin chickens challenged with vv+MDV.

Field Experiences with Recombinant Vaccines and Interactions with Modified Live Vaccines in the Same Flocks

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Sanderson Farms[1,2,3,4]

Single and double insert recombinant vaccines for poultry are commercially available and are proven efficacious for the viral genetic inserts for which they are licensed. Field experiences using modified live vaccines of the same viruses as inserted into various single and double inserted recombinant vaccines demonstrated the interactions of using both technologies in the same flocks. Three different experiences from three different geographic regions will be shared. Recombinant and modified New Castle Disease virus provided superior protection to either product administered alone. Recombinant Infectious Laryngotracheitis (LT) virus buffered Chicken Embryo Origin LT vaccines. Broiler flock performance was enhanced when modified live Infectious Bursal Disease Virus (IBD) vaccine was added to recombinant IBD vaccine program

Onset of Immunity of a Recombinant HVT-IBD-ND Vaccine Against a Velogenic NDV and a Very Virulent IBDV Challenge

Sing Rong[1], Tura Bru[2], Kelly Turner-Alston[3], Candyce Pacione[4], Lauren Taylor[5], Rut Villa[6], John Dickson[7]

Zoetis[1]

A recombinantHVT-IBD-NDtri-valent vaccine was developedfor protection againstinfectious bursal disease (IBD), an acute and highly contagious viral infection of young chickens that causes immunosuppression andincreased susceptibility to other infectious agents;Newcastle disease (ND), a highly contagious and fatal disease affectingall species of birds; andMarek's disease (MD),a

common cause of condemnations and immune suppression in broilersand tumors in older birds.The objective of the following two studies was to evaluate the early onset of immunity to ND and IBD provided by the vaccination with the novel HVT-IBD-ND recombinant vaccine. In the firststudy,SPF leghornchickenswere injected with recombinantHVT-IBD-NDin ovoonE18, or subcutaneously onday of hatchat the minimum protective dose. On Day 21, birdswere challenged with avelogenic NDVHerts Weybridge 33/56. Protection of 96% (25/26) was observed for both in ovo and subcutaneously vaccinated groups with0% (0/15) protection for control group.In the second study, SPF leghornchickensthat were vaccinated subcutaneously at hatch, as well as control birds, were challenged on Day14with a very virulent IBDV. Ninty-eight percent protection(39/40) wasobservedfor the vaccinated groupwith0% (0/40) for the control group

Optimizing Infectious Bronchitis Virus Vaccination of Commercial Broiler Breeder Pullets the Day of Placement

Erin Riley[1], Randi Clark[2], Robin Gilbert[3], Phil Stayer[4], Matilde Alfonso[5], Ha Jung Roh[6], Jose Linares[7], Travis Cigainero[8], Marshall Putnam[9]

Sanderson Farms Inc.[1,2,3,4], Ceva Animal Helath[5,6,7,8,9]

The DMV1639 strain of Infectious Bronchitis Virus (IBV) was first identified in commercial broiler chickens in Delmarva in 2011. Since then, it has been detectedin other areas of the United States (US) and Canada in long lived chickens causing drops in egg production and early damage to the reproductive tract associated to False Layer Syndrome (FLS). To prevent this damage and develop protection early in the life of these birds, IBV vaccination at day of age with a live GA08 IBV vaccine with label claim against DMV1639 has been implemented recently by commercial poultry companies in the US. Contrarily to broilers, commercial broiler breeder pullets are not vaccinated with live IBV vaccines at the hatchery. Thus,IBV vaccination is being performed on the farm at placement. Multiple factors can impact the success of vaccination at this stage. The purpose of this study was to validate and optimize current IBV vaccination practices of commercial broiler breeder pullets at day of placement performed within the same company. Individual choanal swabs were taken at 5 days post-vaccination. Fifteen pullets were swabbed per house. A total of 450 swabs from 30 houses representing 10 complexes were collected. IBV virus detection and quantification by qRT-PCR were performed to identify the vaccine strain. Universal 5UTR IBV primers/probe, and variant GA08 were used. The percentage of pullets from which IBV vaccine was detected variedfrom 27 to 100%, the average variant GA08 Ct value ranged between 27.8 and 38.6, and its coefficient of variation from 6.5 to 18.4%. Factors related to better and more uniform IBV vaccine takes were analyzed (i.e. dosing, vaccination equipment, water temperature, disinfectant).

Turkey Haemorrhagic Enteritis Live Vaccination Controls Field Strains Circulation and Challenge

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Live attenuated Turkey Hemorrhagic Enteritis (THE) vaccination has been introduced in Italy in order toimprove virus (THEV)control in turkeyproduction. To monitor vaccine take and field virus circulation,a molecular method was developed and applied in field longitudinal studies.THEV field strains were sequenced in ORF1, fiber knob domain, E3 and hexon genes,thencharacterized by comparisonwith homologous sequences of field and vaccine strains available in GenBank. Vaccine molecular markers were found in the 3'region of the ORF1 gene while peculiar nucleotide mutations of fieldTHEVs were found in the hexon gene. Based on these findings,acombinedPCR and sequencing protocol was developed and applied in elevenmeat turkey flocks. Birds wereTHEV-vaccinated at four weeks of age with live (7flocks) orinactivated (2 flocks) vaccineor not vaccinated(2 flocks). Five birds per flockwereweeklysampled up to slaughterageby cloacal swabsthat were therefore subjected to PCRs.

Infour out eleven flocks, birds were also euthanized for spleencollectionfor THEV detection by PCRs. Samples of spleen and small intestine were submitted to routine histopathology. The vaccine strain was persistently detected inlive-vaccinated flocks,from 5 to 11weeks of age. THEV field strainsweredetected from 7 weeks of age to the end of the production cyclesin birds receiving the inactivated vaccineand in birds not THEV vaccinated. Histologically, the most severe enteritis, with intestinal necrosis and the most persistent reduction in spleen follicles number (consistent with THEV infection) were observed in not vaccinated flocks. Results evidencedthat live vaccination protects birds from THEV infection, at least until slaughter age, therefore controlling field strains circulation and challenge.

Virology: IBD

A Comparison of Immunogenicity between MB-1, an IBD Live Attenuated Gumboro Vaccine and an IBD Immune-Complex Vaccine Applied in-ovo in Maternally Derived Antibody positive broilers

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Royal GD, Deventer, the Netherlands[1], Hibro Animal HealthCorporation[2,3,4]

Infectious Bursal Disease is a contagious disease in chickens caused by an Avibirna virus. Vaccination of chickens in the hatchery is performed either with Immune-complex-vaccines or recombinant rHVT/VP2 vaccines. MB-1, alive attenuated vaccine represents anew approach for vaccination against IBDV and can be injected in ovoor subcutaneously at day of hatch.Recent studies demonstratedthat MB-1 replication in the bursa is temporarilydelayedand dependson maternallyderivedantibodylevels in each chicken,thus allowing individual immunizationand protection.In this study, the efficacy and protection of MB-1 and an Immune-complex vaccine containing the W2512 strain were tested against a challenge with the vvIBDV D6948 strain at 22 and 36days of agein commercial broilers. In the MB-1vaccinated groups replication of the vaccine strain was observed in the bursa at D27 and D41, thus enabling seroconversion and complete protection.Challenge virus was not identified in any of the MB-1 birds at 5 days post challenge.In the Immune complexvaccinated groupsreplication of the W2512 strainin the bursaoccurred at D27 while at D41, all birds were positive for the vvIBDV 6948 strainand were not protected against challenge. These results demonstratethat MB-1 applied by in ovoinjection provides complete protection in commercial broilers against a vvIBDV challenge.

Ability of a Novel Immune-Complex Vaccine to Successfully Immunize Long-living Chickens Against Gumboro Disease in Various Field Conditions

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Ceva Animal Health[1,2,3,4,5,6,7,8,9]

Infectious bursal disease (IBD, also called Gumboro disease) is a widespread, highly contagious, and economically important disease affecting poultry flocks. The control of Gumboro disease must include sound biosecurity and vaccination programs. Several IBD vaccines are available on the market. They must ensure safety, efficacy on the long term (including field strain displacement) and convenience of use. An immune-complex vaccine (Novamune®) has been specifically developed for egg-type genetic lines and slow growth chickens. One injection at the hatchery is enough to elicit an immune response, hence simplifying the vaccination protocol. This new vaccine has been tested in field conditions in various areas, including France, Greece, Lebanon, and the Philippines. Post-vaccination monitoring criteria included serology (IBD ELISA) and IBDV detection by RT-PCR in the bursa of Fabricius. After the physiological disappearance of maternal immunity, a clear and steady antibody response was evidenced. Molecular biology test (RT-PCR) results turned positive for the vaccine strain, which showed full protection. In some cases, late samplings provided evidence of the clearance of the vaccine strain from the bursa. No other IBDV strain was detected during

the study. Altogether, these results showed the suitability of this vaccine technology to efficiently and conveniently prevent Gumboro disease in commercial layers, and in slow-growth chickens.

Evaluation of rHVT-ND-IBD Vaccination and Challenge with Contemporary IBDV Variant Field Isolates

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The University of Georgia[1,2,3,4,5], Merck Animal Health[6,7,8]

Infectious bursal disease virus is an economically important immunosuppressive disease of poultry. Vaccination with recombinant HVT vectored vaccines (rHVT) is widely used to control IBDV. Recently, a new generation of rHVT vaccines containing dual inserts for IBD and NDV are now commercially available. The goal of this project was to determine the level of protection provided by commercial rHVT-ND-IBD vaccines following challenge with the standard E-Del and variant IBDV field isolates AL-2 and 9109. Specific pathogen free (SPF) chicks were vaccinated at day-of-hatch with a full dose of rHVT-ND-IBD followed by challenge at 14 or 18 days of age (doa). At 7 days post challenge (dpc), birds were humanely euthanized and bursa and body weights were obtained. Bursae werecollected for pathological evaluation and bursal scoring. Overall, both rHVT-ND-IBD vaccines provided protection against challenge with E-Del based on Bu/BW ratiosregardless of age of challenge had significantly less bursal atrophy compared to unvaccinated/E-Del challenged birds. Both vaccines also provided adequate protection based on Bu/BW when birds were challenged with AL-2 at 14 doa; however, only 1 of the 2 vaccines provided protection when birds were challenged at 18 doa. Neither vaccine provided protection based on Bu/BW when birds were challenged at 14 doa, but when birds were challenged at 18 doa, both vaccines provided adequate protection. Bursal scoring is in progress. In summary, rHVT-ND-IBD vaccines showed adequate protection against the standard E-Del, AL-2 and 9109, but age of challenge was an important factor for variant viruses AL-2 and 9109.

Virology: IBV

ArkDPI Vaccine-Like Viruses in Alabama Chickens

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Auburn University[1,2], Thompson Bishop Sparks State Diagnostic Laboratory[3,4]

Tissue samples of commercial and backyard chickens showing respiratory disease submitted to the Alabama State Diagnostic Laboratory were tested for the presence of infectious bronchitis virus (IBV) RNA. IBV S1 genes from 20 different submissions were amplified and sequenced. Analysis of the S1 gene sequences revealed several regionally commonly found S1 genotypes. Most sequences were classified as ArkDPI-type. These sequences were compared to those of ArkDPI vaccine virus subpopulations commonly emerging in ArkDPI-experimentally-vaccinated chickens.

Evaluating the Effects of IBV Vaccination on the Development of False Layer Syndrome

Adrea Mueller Slay[1], Mark Jackwood[2], Monique Franca[3], Brian Jordan[4]

University of Georgia[1,2,3,4]

Infectious Bronchitis virus (IBV) is an avian coronavirus thatprimarilycauses respiratory disease but can also affect the reproductive tract of layingtype chickens. If infection occurs in young pullets,False Layer Syndrome can develop. False Layer Syndrome is characterized bychanges in oviduct development andthe formationof large, fluid-filled cysts.Vaccination isused to control disease caused by IBV, but False Layer Syndrome is still seen in vaccinated hens. We hypothesize that timing of vaccination and infection with challenge virus influences the development of cystic oviducts

and False Layer Syndromeregardless of vaccine serotype used. To test this, fivegroups of 150 SPF pullets each were placed into separate colony houses at day of hatch. Groups1 and 5 were not vaccinated at hatch, while Groups 2and 4 were given Ma5 vaccine at hatch and Group 3 was given GA08 vaccine at hatch. IBV variant DMV/1639 challenge was administered oculonasally to groups 1-3 and 5 at 7 days of age. Groups 1-4 were given a Mass/Conn live attenuated IBV vaccine at 2-weeks of age. All vaccinated groups received subsequent Mass/Conn live-attenuated IBV vaccines at 6 and 12-weeks. Necropsies will be conducted every four weeks post-challenge, and at 20 weeks of age,to evaluate reproductive effectsand development of cysts. Trachea and oviduct tissue will be submitted for histologyto asses microscopic changes.The data collected in this experiment will provide an understanding of how IBV vaccination affects the development of cystic oviduct and False Layer Syndrome.

Evaluation of Serologic Responses to Various GA08 Infectious Bronchitis Virus Vaccination protocols in Commercial Breeder Pullets (Part A)

Rachel Thiemann[1], Kellie Jones[2], Natalie Armour[3], Jose Linares[4], Phil Stayer[5], Erin Riley[6], Randi Clark[7], Buddy Clark[8], Marshall Putnam[9]

Mississippi State University Poultry Research and Diagnostic Laboratory[1,3], Ceva Animal Health[2,4,8,9], Sanderson Farms[5,6,7]

DMV/1639 is a strain of Infectious Bronchitis Virus (IBV) with a tropism for the respiratory, urinary and reproductive tracts. Earlyoviductinfection with DMV/1639 IBV has been associated withFalse Layer Syndrome (FLS),characterized by cystic oviducts andpoor peak production. Immunization against FLS-causingstrains must be achieved as early as possibleto ensure protectionagainst reproductive lesions. The objective of this study was to evaluate and compare under field conditions the serologic responses ofbroiler breeder pullets on various vaccination programsincorporatinga live GA08 infectious bronchitis vaccinewithlabel claims against DMV/1639, GA08and GA13IBV. Three pullet flockswere evaluatedin this study, with each study group comprising two pullet housesperfarm. The first study groupreceived the GA08 IBV vaccine at placement(1 day of age, DOA), followed by the GA08 vaccine and a Massachusetts(Mass) serotype vaccine at 14 DOA. The secondstudy group received the GA08 and Mass vaccines at both placement and 14 DOA. The third study group (which served as the control group)received the GA08 vaccine at placementand a Mass + Arkansas (Ark) vaccine at 14 DOA.Fifteen sera per house (30sera per study group)were collected from all flocks at fourtime points: 1 DOA, 14 DOA, 28 DOA, andat15 weeks of age. Optimum immunization will be evaluated byevaluation of serologic response andvaccine detection via RT-PCR. This presentation focuses onserologic response.RT-PCR results will be covered in another presentation(Part B).

Field Observations on Bivalent Infectious Bronchitis Vaccination programs: How do combinations of monovalent IBV vaccines interact?

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Ceva Animal Health[1,2,3,4,5]

In recent years, many new infectious bronchitis virus (IBV) strains emerged globally, and in the US, GA08, GA13, DMV1639 had significant economic impacts in the broiler industry. As any existing commercial IBV vaccines could not provide effective protection against these new IBV challenges in the field, multiple efforts were made for vaccine licensure including GA08 type vaccines, and autogenous DMV1639 vaccine. In an effort to improve bird health, a combination of a monovalent Massachusetts (Mass) vaccine and a monovalent GA08 type vaccine has been widely adopted across the US broiler industry to broaden the protection against the most recent IBV challenges such as GA08, GA13, and DMV1639. However, there are multiple variations within these bivalent vaccination programs when executed across commercial broiler hatcheries: IBV vaccine sources and dosing, presence of live NDV vaccines, diluents types, spray

vaccination equipment, spray volume, etc.As previously presented, the IBV qRT-PCR test has been used as a tool for vaccine detection in order to validate and optimize day of age spray vaccination at broiler hatcheries. This work summarizes the lessons learned from validating diverse IBV vaccination practices using various Mass vaccines and a GA08 type vaccine. Distinct vaccine detection patterns have been observed for both GA08 type and Mass depending on the different combinations of the available vaccines, which could impact protection. Similarly, vaccine dosing and application methods can have a dramatic impact on the success of IBV vaccination. Addressing these issues when designing and executing an IBV vaccination program is critical for a successful control of the disease in broilers.

IBV Strains Associated with False Layer Syndrome: Surveillance in Layer Farms with a History of FLS and IBV Pangenome Studies

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University of California, Davis[1,2,3,4,5]

Infectious bronchitis virus (IBV) associated false layer syndrome (FLS), is a condition that occurs in laying hens after being infected with distinct IBV genotypes during their first week of life. The oviductal tropism of certain IBV genotypes allows earlyinfection, that ends in oviduct atrophy and false layers. The use of IBV vaccines at day of age alleviates the clinical outcomes of the infection. The consequences of this intervention strategy in the IBV field challenge are not clearly understood. We have performed surveillance in a commercial table egg farm with a history of FLS focusing onchick and adult stages in order to understand the IBV environmental challenge after the vaccine intervention. In addition, we have gathered QX strains from the UK, Israel, Hungary, China and France plus FLS associated strains from the US.These strains were full genome sequenced and their genomes compared with full genomes of other IBV genotypes using machine learning strategies looking for changes at the genomic level.Our goal is to look into genomic changes inside and outside the spike protein genes, that might explain the tropism of these FLS associated IBV strains.

Infectious bronchitis vaccine program comparison against DMV/1639 challenge

Kalen Cookson[1], Manuel Da Costa[2], John Dickson[3], Andrew barker[4], Jennifer Strickland[5]

Zoetis[1,2,3,4,5]

Last year we presented acomparison study between the two commercially available GA08 vaccines which showed both vaccines afforded solid protection against a DMV/1639 challenge in SPF leghorns. Follow-up studies in commercial broilers showed that a GA08 vaccine by itself did notattain that same level of efficacy but combining GA08 with Mass vaccine dramatically improved DMV/1639 cross protection. The broiler study presented here is a directcomparison ofthe two GA08 vaccines in combination with a Mass vaccine for their cross protection against DMV/1639. Commercial broilers were spray vaccinated with full doses on day of hatch. Tracheas were sampled weekly for vaccine take using direct PCR. At 25 days birds were challenged with 10^4.0 EID50 of aDMV/1639 isolate and 5 days later tracheas were tested for both PCR and histopathology. Vaccine takes and persistence will be compared by serotype specific PCR probes. Tracheal protection based on histopathology and PCR Ct values will also be presented. The results will show thatboth vaccine treatments gave significant protection against all major histological indices including lymphocytic infiltration, gland and cilia loss, cumulative tracheal pathology and tracheal mucosa thickness. PCR results will show that both vaccine treatments also showed significant tracheal protection (80-100%) at a CT-35 level as well as significant reductions in viral loads of more than 1,000-2,000-fold.These results show that the combination of Mass and GA08 vaccines can be an effective vaccination program against DMV/1639 challenge in broilers.

Understanding Infectious Bronchitis Virus Vaccine Takes Using Real-Time Polymerase Chain Reaction

Brian Jordan[1]

The University of Georgia[1]

Infectious Bronchitis Virus (IBV) is a significant respiratory pathogen of commercial poultry. To prevent infection from IBV, nearly all commercial poultry are vaccinated with live-attenuated vaccines. It is expected that IBV vaccines will infect and replicate in respiratory tract tissues, inducing an immune response in the birds. Despite mass vaccination, we still have disease breaks from IBV infection. But are these breaks due to vaccine failure or failure to give vaccine? It has been shown that the vaccination process, from reconstitution to diluting to application, can be inefficient and negatively impact IBV vaccines. One way to analyze vaccination efficacy is to detect replicating vaccine virus 5-7 days after vaccination using real-time polymerase chain reaction (qRT-PCR). For this procedure, choanal cleft swabs, tracheal swabs, or tracheas aretaken from 15 birds per house or farm, for up to 6 sample sets (15x6=90 samples) per 96 well plate. Those samples are then tested with primer and probe combinations corresponding to the vaccine program. The results of this analysis fall into one of three categories; either all vaccine types are present in the same amount, one vaccine will be present in more birds and in greater quantity than other vaccines, or no vaccines will be present in a majority of birds or in sufficient quantity. Each of these scenarios can mean different things based on the vaccines used and typical results will be explained. Routinely monitoring vaccine takes provides valuabledata on IBV vaccine programs, and helps troubleshoot vaccine issues.

Virology: ILT

Dynamics of ILT CEO Vaccination in Commercial Broilers

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Boehringer Ingelheim[1,5,6], University of Georgia[2,3,4]

The chicken embryo origin (CEO) Infectious laryngotracheitis (ILT) vaccinesarecommonly used to prevent and control ILToutbreaks in commercial poultry flocks. Mass vaccination via spray or in the drinking wateris the most common application method. Inorder to understand the prevalence of the CEO vaccine following its removal froma broiler vaccination program, the following studywasconducted.Four houses at four different broiler farmsthat wereusingtheCEO vaccine in the drinking water either switchedto HVT-ILTvaccination (houses in high ILT challenge area) or eliminatedvaccination for ILTfrom their program(houses inlow ILT challengearea).After CEO vaccination was stopped the houseswere heatedat 100oF for 100 hours, windrowingwas utilized forin house litter composting,and cleaning and disinfection of the water lineswas performed.Individual tracheal swabs were collected fromthe first flocksplacedafter endingCEO vaccination. Ninety tracheal swabs were collected throughout each house and bird mortality and flock clinical status was observeddaily.Swabsampleswere processedbyILT real time PCRto determine persistenceof ILT vaccine virus in each house.In broilers farms changing from CEO to vectored ILT vaccines, the prevalence of vaccine virus was extremely low and virtually became undetectable after a couple of grow-outs using recombinant ILTvaccine. Similar results were seen in low challenge areas after discontinuing all ILT vaccinations.

Efficacy of recombinant herpesvirus of turkey laryngotracheitis vaccine against Canadian wild-type infectious laryngotracheitis virus infection

Catalina Barboza Solis[1]

University of Calgary[1]

Infectious laryngotracheitis (ILT) is endemic in backyard flocks in Canada. Wild-type ILTV are the second most common cause of ILT outbreaks in backyard flocks raised in Alberta. It is not known if Canadian origin wild-type ILTV can be control by recombinant ILT vaccines. The main objective of this study was to assess the efficacy of a recombinant herpesvirus of turkey LT vaccine against a Canadian wild-type ILTV infection. One-day old specific pathogen free chickens (n=44) were separated into four groups. Two groups were vaccinated while other two groups were mock vaccinated. At 3 weeks of age, one of the vaccinated groups and one of the mock vaccinated groups were infected while other two groups were mock infected. The chickens were observed twice a day for clinical signs for two weeks and body weight was recorded every other day. At 3, 7, 10 and 14 days post-infection, feather tips, cloacal and oropharyngeal swabs were collected for genome quantification. At day 4 and 11 days post-infection, blood was collected to isolate peripheral blood mononuclear cells and quantify CD4+ and CD8+ cells using flow cytometry technique. Serum was also collected to perform ELISA tests at both dates. At 14 days post-infection the chickens were euthanized, and tissue samples of trachea and lung were collected for histology and viral load quantification. Results showed that the vaccine prevent weight loss in the vaccinated and infected group. Clinical signs were significantly higher at 4-days post infection in the infected group. Other samples are still being processed.

Transition from CEO LT to HVT vectored ILT vaccines in a multi-age layer complex: Lessons learned

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Merck Animal Health[1,2]

Infectious laryngotracheitis (ILT) is a highly contagious acute respiratory disease of chickens that causes severe production losses, increased mortality, decreased egg production, weight loss, and predisposition to infection with other respiratory diseases. Control of ILT is mainly achieved by vaccination with conventionally attenuated live virus vaccines that are characterized by their suboptimal attenuation and frequent reversion to virulence (1). ILT continues being an economically significant disease in commercial egg producing flocks especially in multi-age complexes, causing multimillion-dollar losses as a result of high mortality and drops in egg production. A fourteen million commercial layer complex located in the United States used a live attenuated chicken embryo origin vaccine (CEO) program for approximately 15 years. Although CEO vaccines provided a protection, overtime this multi-age complex faced complicated respiratory reactions during the grow-out period due to the difficulty of placing the ILT CEO vaccine properly spaced between other respiratory live attenuated products, causing negative effect on body weight gain, body weight uniformity and bird health.The objective of this study is to present information related to the transition from CEO ILT vaccine program to a recombinant vaccine program (rHVT-ILT and rHVT-ND-ILT)performed in the 14 million commercial layer complex. Production parameters such as mortality and rate of lay will be shown, as main indicators of improvement, as other immunizations with respiratory live attenuated vaccines were relocated in a more proper fashion to improve protection and post-vaccination reaction. Experiences and lessons learned throughout the process of transition will be presented (3-4).

Virology: Miscellaneous

A ten-year retrospective study of inclusion body hepatitis in meat-type chickens in Spain (2011-2021)

Kateri Bertran Dols[1]

IRTA-CReSA[1]

Inclusion body hepatitis (IBH) caused by different fowl aviadenovirus (FAdV) serotypes has been described in several countries in recent years. In Spain, a sharp increase in number of outbreaks in broiler and broiler breeder flocks has occurred since 2011, causing considerable economic losses for the industry. Such dramatic increase prompted the use of vaccination in broiler breeders in some regions of Spain from the end of 2012 until 2018. Our epidemiological study (2011-2020) revealed that the majority of cases were reported in broilers (92.8% of the 293 PCR positive cases), which were mainly acquired via vertical transmission. Most IBH cases in broilers and breeders were caused by serotypes 8b and 11, based on the ICTV. However, serotype 2 was detected in 1 of the 3 IBH cases in broiler breeders in 2020. No seasonality was observed in the incidence of IBH cases. Based on geographic distribution, IBH has virtually affected every region in Spain with broiler and broiler breeder holdings, suggesting a widespread incidence within poultry and revealing the importance of sourcing broilers from few broiler breeder producers. Based on our epidemiological study, the number of diagnosed IBH cases peaked in 2015 and subsequently decreased starting in 2016, suggesting that the control measures taken were effective against the circulating serotypes. However, IBH cases still occur and a new serotype was reported this year, reiterating the importance of surveillance, serological monitoring of breeders, and vaccination against circulating serotypes for maternal antibody production in breeders in order to protect progenies.

Host range barriers for replication of SARS-CoV-2 (COVID-19) in avian species

Darrell Kapczynski[1], Erica Spackman[2], Ryan Sweeney[3], David L. Suarez[4]

USDA-USNPRC[1,2,3,4]

The zoonotic SARS CoV-2 virus has caused a world-wide human pandemic which has resulted in the infection of more than 60 million people with over 1.5 million deaths. The beta coronavirus, which causes COVID-19, is thought to have its ecological reservoir in bats, and transmission of the virus to humans has likely occurred through an intermediate animal host that has not yet been identified. While birds have yet to be shown susceptible to SARS-CoV-2 infection, the barriers to this limitation are under investigation. The human receptor utilized by SARS-CoV-2 to infect cells is the Angiotensin-converting enzyme 2 (ACE2). Following receptor attachment, SARS-CoV-2 is processed by a plasma membrane–associated type II transmembrane serine protease, TMPRSS2, which is critical to release the viral contents into the host cell cytosol. To determine if SARS-CoV-2 host restriction is strictly due to differences in the receptor or protease specificity, we utilized gene-editing techniques to develop an avian cell lines expressing the human ACE-2 and TMPRSS2 genes. Expression of the human genes in avian cells was confirmed by RT-PCR and western blot techniques. In vitro studies indicate that DF-1 cells expressing the human ACE2 and TMPRSS2 genessupport replication of the SARS-CoV-2 virus. The implications of using avian species as a model for virus transmission will be discussed.

Hyperimmunized Chickens Produce Neutralizing Antibodies against SARS-CoV-2

Emily Aston[1]

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The novel severe acute respiratory syndrome (SARS)-like coronavirus, SARS-CoV-2, is responsible for the global COVID-19 pandemic. Effective interventions are urgently needed to mitigate the effects of COVID-

19 and likely require multiple strategies. Egg-extracted antibody therapies are a low-cost and scalable strategy to protect at-risk individuals from SARS-CoV-2 infection. Laying hens were hyperimmunized against the SARS-CoV-2 S1 protein using three different S1 proteins and three different doses. Sera and egg yolk were collected at three and five weeks after the second immunization for enzyme-linked immunosorbent assay and plaque reduction neutralization assay to determine antigen-specific antibody titer and neutralizing antibody titer, respectively. In this study we demonstrate that hens hyperimmunized against the SARS-CoV-2 S1 protein produced neutralizing antibodies against SARS-CoV-2. We further demonstrate that antibody production was dependent on the dose and type of antigen administered. Our data suggest that antibodies purified from the egg yolk of hyperimmunized chickens can be used as immunoprophylaxis in humans at risk of exposure to SARS-CoV-2.

Pathology and Immunohistochemical Findings of Avastrovirus Infection in Chicks with White Chick Syndrome

Holly Sellers[1], Monique Franca[2], Andrew Bishop[3], Tyler Gamble[4], Jenny Nicholds[5]

University of Georgia[1,2,3,5], Pilgrim's Pride Corporation[4]

White chick syndrome(WCS)was diagnosed in day-old chicks and pipped chicken embryos frombroiler complexes in North Carolina and Minnesota. A thorough evaluation of gross and microscopic lesions was performed to better understand the pathology and pathogenesis of this disease. Necropsy revealed green or dark brown liver and enlarged yolk sacin most affected chicks. Several affected chicks had pale intestines containing watery and frothy contents, white pinpoint foci in the kidneys,and mild urolithiasis. Histopathology revealed marked heterophilic cholangitis with prominent bile duct hyperplasia as well asextramedullary granulopoiesis (EMG) in portal areas in many chicks with this disease. Other microscopic lesions seen in affected chicks included mild heterophilic enteritis, mild renal tubular nephrosis, and atrophy of exocrine pancreas associated with prominent EMG. Immunohistochemistry (IHC) revealed moderate to abundant Chicken Astrovirus (CAstV) antigen in the cytoplasm of crypt epithelial cells and villus enterocytes in the duodenum, with milder staining in jejunum and ceca. Mild to moderate amounts of CAstV antigen werealso detected in pancreatic acinar cells and renal tubular epithelial cells, with minimal amounts of CAstV antigen found in hepatocytes in a few liver samples.Astrovirus of the genus Avastroviruswas isolated from samples of liver, kidney, intestine and bile from affected cull chicks.Viral replicationand tissue damage caused by Avastrovirusin liver, small intestine and pancreas most likely affect the absorption, metabolism and transport of carotenoids derived from yolk, which may explain the abnormally white plumage in chicks with WCS.

Risk of Egg Drop Syndrome 76 transmission in broiler chickens

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Southeast Poultry Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture[1]

Egg Drop Syndrome 76 (EDS) virus is an aviadenovirus that typically infects ducks, but it can spread and cause disease in chickens where it causes problems with egg qualityand a drop in egg production. The virus was recently detected in layer chickens in Pennsylvania and has become a trade issue. An experimental study was performed in SPF broiler chickens to evaluate if virus could be detected in several clinical samples including in muscle tissue and an evaluation was made to determine if transmission through oral ingestion was possible. The virus was inoculated into birds at different ages, 1 day 11 day, and 21 days of age, and cloacal and blood samples were taken at 5 day intervals. Virus could be detected from most cloacal swabs and blood samples at 5 days post-infection and only sporadically after. All the directly inoculated birds

seroconverted by commercial ELISA by the end of the experiment. A study on transmission of day old chicks, where 2 inoculated chicks were placed with 20 uninoculated chicks showed evidence of horizontal spread by the end of the experiment. Testing of contact control birds showed only sporadic detection of virus and cloacal swabs and suggests that the viremia and cloacal shedding period is short, but most birds seroconverted by the end of the experiment. Testing of tissue samples is ongoing and will help characterize if there is a risk of infection though feeding of poultry products.

Virology: NDV

Characterization of the Thermal Inactivation Profile of Newcastle Disease Virus in Poultry Litter

JONGSEO MO[1]

USDA Southeast Poultry Research Laboratory[1]

Newcastle disease (ND), caused by the avian paramyxovirus type-1, can deliver severe socioeconomic impacts throughout the poultry industry. According to the United States Department of Agriculture (USDA) response guidelines for ND outbreaks, once the disease is introduced to the premises, infected poultry must be depopulated, in addition to a 120-day mandated period where the premises must be empty of birds. The restocking of birds cannot occur until the elimination of the residual virus is confirmed. Although sanitary disposal of organic material such as poultry litter is highly recommended, it is costly, laborious, and has the risk of spreading the virus during transport. Thermal inactivation by heating poultry houses could work as a viable and cost-effective alternative method for eliminating the residual virus in some climates. It is also crucial to confirm that ambient temperatures are sufficient to inactivate the virusduring the fallow period when heating is not a viable option. The primary objective of this study was to determine the thermal inactivation profile for APMV-1 in organic mixtures like litter across a wide temperature range (50-110°F/10-43.3°C) that could be manageable in terms of 'heating-up' different types of poultry houses and that are consistent with ambient temperatures above freezing. To simulate different litter conditions on surfaces within poultry houses, low (LM) and high moisture (HM) litter were used. For a rigorous approach, a thermostable APMV-1 strain was utilized.

Efficacy of VHVT-ND Vaccines Administered to Day-Old Turkey Chicks Against Velogenic Newcastle Challenge at 28 Days of Age

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Boehringer Ingelheim Animal Health[1,2,3,4,5,6,9], Lycée Agricole de Vendôme, Areines, France[7,8]

In the recent years, recombinant vaccines using Herpesvirus of Turkeys as vector expressing the F protein of the NDV (vHVT-ND) were developed and they arecommonly used to protect poultry flocks against velogenic Newcastle Disease (ND) worldwide. The efficacy of vHVT-ND vaccines has been proved in experimental and field trials in chickens. Nevertheless, few information has been published about their efficacy in turkeys. The objective of the present study was to evaluate the efficacy of 2 commercial vHVT-ND vaccines administered by subcutaneous (SQ) route to day-old turkey chicks. The study also examined the vaccine virus replication profile in spleen and feather pulps and compared the serological profiles for Newcastle Diseaseantibodies. 90 conventional day-old turkey chicks were divided into 3 groups; Group 1 received one SQ dose of the vHVT-ND A, group 2 received vHVT-ND Bsubcutaneously and group 3 vHVT-ND A subcutaneously in combination with one dose of live attenuated ND vaccine by eye drop. Spleens and feather pulps were collected on FTA card and analysed by qPCR for HVT virus at 7, 21 and 28 days post vaccination in order to monitor the replication profiles of the vaccines. Blood samples were collected and antibodies were titrated at different ages for vaccine monitoring by different serological techniques. Finally, in order to evaluate the efficacy,

a velogenic ND challenge (Herts 33 strain) was performed by oculo-nasal route at 28 days of age. The results clearly showed that both vHVT-ND vaccines were effective in protecting the turkeys against velogenic ND challenge and reducing the viral shedding. The best results in respect to protection and reduction of viral shedding were obtained by the association of the vHVT-ND with a live attenuated ND vaccine.

Newcastle Seroconversion to an HVT-IBD-ND Vaccine vs. a Modified Live ND Vaccine in Broilers

Jose Linares[1]

Ceva Animal Health[1]

Newcastle seroconversion to an HVT-IBD-ND vaccine vs. a modified live ND vaccine in broilers Jose A. Linares, Ha Jung Roh, James Mills, Marshall Putnam Immunity against Newcastle disease is derived from neutralizing antibodies against viral glycoproteins. The purpose of this presentation is to share results from a field study looking at Newcastle seroconversion in a broiler complex comparing flocks on a dual insert recombinant HVT-IBD-ND vaccine applied inovo vs. flocks vaccinated with a modified live Newcastle vaccine applied via spray at the hatchery. Serum samples were collected at 35 days of age and prior to processing from flocks on both vaccination programs. The serum was tested with an ELISA kit validated to quantify NDV antibody levels in birds vaccinated with HVT-NDV vector vaccines.

Protection Against Various Velogenic Newcastle Disease Challenges of a Trivalent HVT-Vectored Vaccine Against Marek's Disease, Infectious Bursal Disease and Newcastle Disease

Stephane Lemiere[1], A. Delvecchio[2], A. Herrmann[3], F. Prandini[4], H. Alloin[5], M. Jeunet[6], S. Crussard[7]

Boehringer Ingelheim[1,2,3,4,5,6,7]

Infectious bursal disease (IBD), Newcastle disease (ND) and Marek's disease (MD) are key diseases of commercial poultry that are currently controlled with vaccination. This control routinely includes the use of HVT-vectored vaccines, expressing either the ND virus or the IBD virus antigens. The single vHVT310-IBDV-NDV construct based on a vHVT013 (VAXXITEK HVT+IBD) backbone with one additional insert expressing a modified fusion (F) gene from velogenic genotype VII.2 NDV was the tested vaccine. This vHVT310-IBDV-NDV (VAXXITEK HVT+IBD+ND) vaccine was more particularly checked in the context of control and prevention of velogenic ND, a worldwide concern. Velogenic ND is highly prevalent and deserves special attention in terms of vaccination, with the concomitant use of a live vaccine, such as the VG/GA Avinew. It can be sprayed at day-old, and then possibly administered around 10 days of age. Main selected parameter of monitoring of vaccine take was serology using HI, anti-fusion and anti-nucleoprotein ELISAs. Challenges models of velogenic Newcastle disease were respectively based on the use of four different strains of velogenic ND virus, Herts 33 (related to genotype IV, possibly new genotype), Malaysia (related to genotypes VII), Chimulhuacan & Honduras (related to genotypes V). Mortality, clinical signs associated with Newcastle disease, shedding of challenge virus by oral and cloacal routes were monitored at D28 of age. The HVT vectored vaccine, alone or alongside, with one or two VG/GA Avinew sprays, displayed significant differences with unvaccinated challenged positive controls in terms of mortality, clinical signs and ND challenge virus shedding, oral and cloacal

Virology: Reovirus

Antigenic Cartography as a Tool to Determine Antigenic Relatedness of Avian Reovirus Variants

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Antigenic cartography is a computational method used to understand the antigenic andgenetic evolution of pathogens.Avianreovirusgenomes are in constant evolution, reason whyit is important to

associate genetic changeswiththe antigenicity of variant strains. This information is crucial tostreamline theselection of candidate viruses to include in autogenous vaccines.Eighteen avian reoviruses, including conventional and variant strains, were selected, plaque purified, whole-genome sequenced, and used to produce hyperimmune sera in SPF chickens. Cross viral neutralization was performed, and the obtained datawastransformed to calculate antigenic distances (AD). The ADbetweenviruses wasplotted into anantigenic map. In addition, genetic distances (GD) between viruses were calculated based on amino acid substitutions in the virus most variable proteins λC, μB, μBN, σC, and σB. Correlations betweenAG and GD will bedone by using regression analysis.

Infectivity Studies of Avian Reovirus Variants in Egg-Laying Hens

Huaguang Lu[1]

The Pennsylvania State University[1]

Infectivity studies of avian reovirus (ARV) were conducted recently for 3 ARV layer variants of genotype 2, 3 and 5 in egg-laying hens. These layer chickens were provided by Hy-Line North America, LLC, PA at time they were hatched and raised at Penn State Poultry Education and Research Center. These layer chickens remained unvaccinated, grew healthy and reached normalegg production. We started the ARV infection studies on egg-laying hens when they reached normal egg production.The three ARV variants were propagated in LMH cell cultures and measured in TCID50/ml. Each experiment included 10 egg-laying hens per group, each henreceived 1.0 ml of 105.0 ~ 107.0 TCID50dose/mL of one ARV variant via a combination of oral, nasal and ocular inoculation. These experimentally ARV-infected hens remained normal egg productions, no observable clinical signs were seen except some watery droppings during the 1st week post inoculation (pi). During the bird trial period, cloacal swab and oral pharyngeal swab samples were collected daily or every other day to monitor the virus shedding and infectious status of the ARV inoculated hens. The virus isolation results on the swab samples indicated that the ARV-infected hens started virus shedding via intestine/feces as early as 24 h pi, heavy virus shedding occurred at 2-3 d pi, then were light shedding at 5-7 d pi, and were rarely shedding after 12-14 d pi. Only a few birds' oral pharyngeal swabs tested weak positive during the first 1-3 d pi, after that they were all negative.

Investigation on factors associated with rupture tendon at processing - a review of 12 years

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College of Veterinary Medicine North Carolina State University[1]

Gastrocnemius tendon injury or rupture is a common consequence from various etiologies and conditions in poultry production. The occurrence of tendon injury can cause significant morbidity and lameness in chickens as well as quality downgrades and increased trimming on carcasses at processing. In this study, 12 years of data from a poultry processing plant were compiled and analyzed. Association between grower, season, shift, and time were investigated in relation to the prevalence of ruptured tendons and carcass condemnations.

Response of Turkey Breeding Hens to Vaccination with a Commercial Modified Live Chicken Reovirus

Ben Wileman[1], Marissa Studniski[2]

Select Genetics[1,2]

Reovirus continues to plague the poultry industry through increased mortality rates, poor performance and increased condemnation. One of the main concerns for both broiler and turkey producers is the threat of vertical transmission of Reovirus to offspring. There have been a number of clinical cases in which vertical transmission of turkey Reovirus has been implicatedin Reovirus breaks of varying severity. It has also been observed that Reoviruses tend to evolve and are not stable based on sequencing data. The most practical approach for preventingor at least limiting the risk of vertical transmission in many species isvaccination of the

female to enhancethe amount and type of passive antibody available to the offspring. This trial was conducted to determine the response of turkey breeding hens to vaccination with a commercial modified live chicken Reovirus vaccine (Reoguard™;Merial) containing strain 1133. Birds were individually gavaged either once or twice with the live vaccine to ensure maximum intake of vaccine and accurate dosing. Birds were observed for gross reactions, morbidity, mortality and shedding of Reovirus in responseto the vaccination.Birds were then vaccinated using the standard killed Reovirus product(Autogenous; Hygieia)and at the normal vaccination agesfor this complex. Immune response via antibody response was also measured throughout the study and compared to birds from the same source flock not vaccinated with the modified live vaccine.

Sequence based pathotyping of Turkey Arthritis Reovirus

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Turkey arthritis reovirushas beenassociated with lameness in turkey flocks mainly older than 10 weeks of age resulting in significant economic losses since 2011. The aim of this study was to optimize sequence based pathotyping of turkey arthritis reovirus. In this preliminary study, a set of 30 well characterized isolates with known pathotypes and serotypes were included. The whole genome sequencing of aliquots of virus isolates inoculated in birds for pathotyping was performed. These isolates were divided intwo main groups (high and low pathogenic). Whole genome sequence analysisuncovers the key differences between the two groups. In this analysis,nucleotide sequencesof all 10 segments were translated into amino acids and then key differences were screened in the primary structure of proteins. Of the 10 segments, L1, L2, L3, M1, M2 and S1 shows the differences between high and low pathotypes. Of the 10 genome segments, two segment variants (protein variant) each of L1, L2 and L3 and one of M1, M2 and M3 were present exclusively in the highly pathogenic group.These were tentatively named as high-path-variant of respective segment. The pathogenicity is not all or none property but have a gradient degree. In our study,we found that some isolates have high-path-variant of one segmentand other have high-path-variant of more than one segment in their genome. Further analysis of whole genome sequences of known pathotypes will be helpful toknow which segment contributes how much for the pathogenicity of respective isolate. This studywill determine pathogenicity of viral isolates without doing costly in vivostudies.

Wealth of Knowledge

A Practical Platform For Evaluating Multi Variable Factors In A Production Setting

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FSTAATS Agri-Business Solutions[1]

There are several possible risk factors that influence the occurrence and severity of disease in a production setting. Furthermore, interactions among these risk factors may further complicate the clinical picture. Using looseness (enteritis) in young turkeys as a disease model, a cohort of farms was designated for the study. The cohort consisted of farms that were matched for feed mill source, placement dates, barn facilities such as feeding and drinking systems and other possible confounders. The cohort was first evaluated using conventional statistical methods and then followed up using practical analytical platforms.

Classical Interspecies Transmission of Histomonas meleagridis Causing a Blackhead Outbreak in Turkeys from a Private Farm in Eastern Tennessee.

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Sadat City University[1], University of Tennessee[1,2,3,4]

Histomonas meleagridis is the causative agent of blackhead disease, a protozoal disease affecting gallinaceous birds. In August 2020, a mortality event involving five (100%) turkey poults from a privately owned farm in eastern Tennessee that had chickens, ducks and turkeys housed in adjacent pens was investigated. The turkeys died rapidly once clinical signs were event with several having sulfur-yellow diarrhea. Grossly, the turkeys had characteristic blackhead lesions in liver and cecal tissues. Histological examination showed intralesional protozoan in the cecal and liver tissues along with necrosis and large number of inflammatory cells. Conventional PCR to amplify histomonads 18S ribosomal DNA (rDNA) and trichomnads internal transcribed spacer 1 and 2 (ITS) regions of the rDNA followed by sequencing of PCR products disclosed dual infection of H. meleagridis and Tetratrichomonas gallinarium in four turkeys. Additionally, one turkey also had Simplicimonas spp. Cloacal swabs from all birds on the farm were collected and inoculated in Dwyer's media or TF InPouches. Samples from three ducks on the farm were inoculated only on TF InPouches. In total, 61% (11/18) InPouches were positive and showing characteristic trichomnad movement while 47% (7/15) Dwyer's flasks showed growth of histomonads. Interestingly, 33% of inoculated Dwyer's media flasks showed dual growth of H. meleagridis and T. gallinarium. Furthermore, we analyzed litter samples from the pens and found Heterakis gallinarum eggs in litter samples. This is a classical outbreak of H. meleagridis acquired by turkeys possibly through ingestion of H. gallinarum eggs containing H. meleagridis. The most likely route of transmission is via defecation of H. meleagridis infected H. gallinarum eggs by chickens housed adjacent to the turkeys. Education of backyard and hobby poultry breeders on the hygienic practices to prevent the spread of H. meleagridis among bird species is warranted.

Flooring Type Affects Incidence of Bacterial Chondronecrosis with Osteomyelitis Lameness and Intestinal Integrity in Broiler Chickens

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BIOMIN America, Inc.[1,6], University of Georgia[2,9], University of Arkansas[3,4,5,8,10], BIOMIN Holding GmbH[7],

The combination of weak intestinal barriers, bacterial translocation, and bacterial colonization of micro-fractures in fast-growing bones can lead to bacterial chondronecrosis with osteomyelitis lameness (BCO) in broilers. While wireflooringhasbeen frequently used in BCO trials, the use of litterfloor models has been increasingtomore closely resemble commercial production. One objective of this trial was to determine the effect of flooring typeson BCO and intestinal integritywhen the infection is acquired naturally. A total of 360Cobb 500 chicks were allotted to either flat-wire (WF)orfreshlitter(LF)floors with 6 pens per treatment; 30 birds per penfrom d 0–14 and 25 from d 14–57.Starting on d 21, birds were walkeddaily to assess BCO based on reticence to move. On d 42 and 56, intestinal integritywas assessed via recovery of 3-5 kDa fluorescein isothiocyanate dextran (FITC-d) from serum1 h postgavagewith 8.32 mg FITC-d/kg BW. On d 57, birds were necropsied and BCO assessed. Overall, nomortalitydifferences ($P>0.05$) were observed (WF 5.8%, LF 7.9%), while BCO incidence on WF was greater (WF 68.0%, LF 37.1%; $P<0.05$). However, FITC-d recovery in sera was greater ($P<0.05$) in birds on LF compared with birds on WF on d 42 (LF 43.4 ng/ml, WF 14.0 ng/ml)and on d 56 (LF 10.9 ng/ml, WF 0.4 ng/ml).While we can infer that both flooring types contributed to BCO, their modes of action may be different as indicated by FITC-d. It can be hypothesized that BCOin WF reared birds may result frommechanical stress whereas BCO in LF reared

birds may result fromreduced intestinal integrity.Correlating intestinal integrity and bacterial translocation into the bloodstream would help further understand the relationship betweenflooring typeand BCO.

HTSi Review by Feeding Programs

Francene Van Sambeek[1]

Elanco Animal Health[1]

Elanco Animal Health developed a Health Tracking System (HTSi™) for tracking lesions at broiler posting sessions. Data was analyzed by anticoccidial feeding program looking at enteric and other health parameters. That analysis will be presented.

Relationship of bursal lesions to other comorbidities in commercial broiler chickens

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Auburn University College of Veterinary Medicine[1]

A healthy immune system is essential in mounting an effective response to both pathogen challenges and immunization. In chickens and other avian species bursal health is critical to this objective, especially as early damage to the bursa results in a lifelong negative impact on immune response. Given this, it would be expected that the presence of bursal lesions would correlate with other pathologies, particularly those of infectious nature. This study is being undertaken to explore this premise, by determining the types of comorbidities seen in broiler chickens with bursal lesions of varying severity. Data will be taken from recent archived commercial broiler case records held by the Alabama State Veterinary Diagnostic Laboratories system. The majority of these cases are from farms located across the state of Alabama, with few neighboring states represented. From these cases, those diagnosed histopathological bursal lesions, most commonly bursal atrophy and/or necrosis, will be further analyzed. All bursal scoring will be normalized to one standard grading system for further analysis. Case characteristics to be analyzed concurrently include age, complex location, anonymized submitter, and bacterial and viral comorbidities. Data will be analyzed to determine the most common comorbidities and whether there is a relationship between the severity of bursal lesions and other case characteristics. Results and conclusions will be discussed.

The Economics of Neoplastic Disease

Guillermo Zavala[1]

Avian Health International, LLC[1]

Neoplastic diseases of poultry can be of infectious and non-infectious origin. Infectious agents potentially causing tumors and, insome cases, severe immunosuppression in poultry include Marek's disease (MD) virus (MDV), avian leukosis virus (ALV), reticuloendotheliosis virus (REV) and lymphoproliferative disease virus of turkeys (LPDV). MDV is ubiquitous and thus the poultry industry is forced to vaccinate 100% of commercial chickens, incurring a significant cost. MD vaccination is mandatory in some countries. In addition, MDV is an important cause ofcondemnations in broiler chickens. Agricultural statistical data was used to estimate historical annual losses due to MD in the broiler industry. REV has been associated with large sporadic outbreaks of neoplastic and immunosuppressive disease occurring after natural infection in the field; or after vaccine contaminationwith REV. Examples of significant outbreaks and their estimated associated losses are presented. ALV has also been a historical cause for very significant losses in the commercial egg layer and broiler breeder industries. The economic impact of ALV-J is estimated for the critical years in which the poultry industry was severely affected with this congenitally and horizontally transmitted virus. MDV, ALV and REV continue to circulate in commercial populations of chickens around the world. LPDV remains confined to wild turkey populations of at least North America.

Yet another case report on Blackhead

Jewell Bremer[1], Robert Edson[2], Ben Wileman[3]

Select Genetics[1,3], Avigen Turkeys[2],

A flock of 8 week old tom breeder turkeys were assessed on a Wednesday afternoon after the grower noticed a slight increase in mortality to of one bird per thousand. This particular flock had about 4,200 birds in it and they werehoused allin one barn8Xbirds in a flock of 4,200 in a single barn on a multi-age rearing site in North Carolina.On presentation a few birds were weak, lethargic,laterallyand laterallyrecumbentand torticollisand difficulty staying upright. These birds were culled immediately and necropsied. Gross lesions revealed small pinpoint to large focal and coalescing target lesions on the liver and thickening of the cecal lining. The ceca were filled with a foul smelling hemorrhagic fluid and feces.Samples were taken of the liver and sent to Rollins State Lab in Raleigh, NC. Histomoniasis was diagnoseddiagnosis was confirmedand aggressive management strategies to mitigate losses were put into place.All strategies for managing this case and mortality outcomes will be presented.At the time of this writing the outcome has been favorable and is worth discussing.

Posters

Antimicrobial

Antimicrobial Use in Canadian Poultry Identification of High Users of Antimicrobials and Understanding Reasons for Use

Agnes Agunos[1], Anne E. Deckert[2], David F. Leger[3], Sheryl P. Gow[4], Richard J. Reid-Smith[5]

Public Health Agency of Canada[1,2,3,4,5]

The poultry industry in Canada has implemented their antimicrobial use (AMU) reduction strategy (https://www.chickenfarmers.ca/the-antimicrobial-use-reduction-strategy/;a phased approach for reduction from 2014 to 2020). Thispresentation aimsto describe flock-level AMU distributionusing milligrams per population correction unit (mg/PCU), an AMU indicatorused by CIPARS/FoodNet Canada in reporting national level results and for providing feedback to participating producers and veterinarians.Data on AMU and AMR in enteric bacteria, collected from 2013 to 2019from broiler chickens (n= 947flocks) and from turkeys(n= 427) were used.Flocks were categorized based on the percentiles of the milligrams per/population correction unit (mg/PCU)distribution: “medium” to “low” users (≤75thpercentile) and “high” users (>75thpercentile). The odds of being a high user in both broiler chickens and turkeys were significantly increased: if water medications were used, and if trimethoprim-sulfonamides, bacitracins, and tetracyclines were used. Trimethoprim-sulfonamides and tetracyclines were largely used for the treatment of colibacillosis and bacitracins were largely used for theprevention of necrotic enteritis.Flock-level analysis and providing feedback to producers and veterinarians can guide the development of AMU stewardship measures and transition from antimicrobial-dependent poultry production to reduced AMU production system in Canada.

Bacteriophage (CTC-Bio) ability to reduce Salmonella Enteritidis using an in vitro crop assay

Avery Duncan[1], Charles Hofacre[2], Virginia Baxter[3], Emily Kimminau[4], Kay Russo[5], Peter Karnezos[6]

Southern Poultry Research Group, Inc.[1,2,3], Land O’ Lakes, Inc.[4,5,6]

Foodborne Salmonellainfections have become a major worldwide public health concernwith thousands of cases in the United States alone each year. Salmonellais often associated with raw meat and animal products, mainly from poultry. As a way to circumvent this issue, bacteriophages have been studied to understand the biological mechanism in which they can be used to control Salmonellain poultry. Bacteriophages are a group of viruses that can offer highly specific and effective reduction of Salmonella.The goal of this experiment was to

simulate a bird's crop and the environment in which Salmonella enteritidisis allowed to grow to a specific concentration andthenaviable bacteriophage(CTC-Bio)is introduced. Inthis experiment, an in vitro crop assay was conducted with different bacteriophage concentrations(undiluted, 1:10, and 1:100) to determine the extent of the bacteriophage'sability to reducevaryingconcentrations of Salmonella enteritidis(8.0 x 106 cfu/ml and 8.0 x 103 cfu/ml).Following the 2-hour incubation, the undiluted bacteriophage reduced Salmonella enteritidisby 44%, whereas the undiluted bacteriophage reduced Salmonella enteritidisby over 60%during the 6-hour incubationfor samples inoculated with 8.0 x 106 cfu/ml.When comparing the 1:10 bacteriophage dilution, there was a 16% and 34%Salmonella enteritidisreduction for the 2 and 6-hour respectively. There was little difference in reduction between the two concentrations of Salmonella enteritidisat the 6-hour time point, however,at the 2-hour time point, the amount of Salmonellain samples inoculated with 8.0 x 103 cfu/ml was markedlylower than samples inoculated with 8.0 x 106 cfu/ml.This could be a direct result of the shorter incubation period and the Salmonella'sgrowth stage.Overall, thisdata demonstratesthe bacteriophage's ability atreducing varyingSalmonella enteritidisconcentrationsin an in vitro crop model for poultry.

Avian Influenza

Molecular Changes in the Hemagglutinin of North American Lineage H7 Subtype Avian Influenza Viruses in Incursions from Wild Birds into Poultry

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North American lineage H7 avian influenza viruses (AIV's) are maintained in wild aquatic bird species as shown by years of extensive wild bird surveillance. The occasional incursion of viruses from this H7 lineage into domestic poultry have caused several outbreaks of low pathogenicity (LP) and highly pathogenic (HP) avian influenza. The repeated introductions into domestic poultry indicates that these H7 viruses have some genetic advantage that facilitates this switch in hosts and further adaptation in poultry. This could include changing the virus hemagglutinin (HA) binding preference to cell receptors that are more common in gallinaceous species. To this end, we traced the molecular evolution and potential structural changes of the receptor binding pockets in the H7 HA's of recent AIV's. In addition, ten select North American H7 AIV poultry outbreaks were scanned for common (recurring) mutations. No consistent structural changes in receptor binding sites were predicted within H7 AIV's circulating in wild birds, but when the H7 viruses are introduced into poultry species, multiple mutations in the vicinity of receptor binding pockets were found, with recurring mutations possibly affecting the steric interactions with the host sialic acid receptors. These results provide insight into the evolutionary path of recent H7 HA's upon introduction to poultry species and should be useful in predicting AIV evolution in domestic poultry.

Monitoring of Antibodies against Avian Influenza Virus in commercial birds from Peru using a competitive ELISA test

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Since 2006, active epidemiological surveillance has been carried out in Peru, which has shown that the country is free of avian influenza in domestic birds. In the present study, carried out between January and July 2020, a total of 1200 serum samples were collected from commercial birds and analyzed by competitive ELISA test in order to detect the presence of antibodies againstavian influenza virus (vAI). Among this samples, 600 samples belonged to broiler breeders from of Lima and La Libertad departments; 400 samples from broiler chickensfrom Lima, Loreto and Arequipa

departments; and 200 samples from commercial layers of Lima, Ica and La Libertad departments. All samples (100%) were negative for antibodies against vAI, demonstrating that birds from the main poultry production centers in Peru have not been exposed to avian influenza virus, maintaining avian influenza disease-free status in Peruvian domestic birds. This study has been financed by FONDECYT-Peru and the World Bank (Contract 0b-b0a9-FONDECYT-BM-INC-INV)

Bacteriology

Contribution of Heavy Metal Resistance to Avian Pathogenic Escherichia coli

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Heavy metals are often usedas feed additives and supplements in animal production, but little attention is paid to theirpotential impact on pathogenic bacteria associated with poultry health. We assessedthe prevalence of heavy metal resistance genes in avian pathogenic Escherichia coli(APEC) of productionpoultry and its correlation with other antimicrobial resistance genes and phenotype analysis.Acollection of APEC (n = 169) recovered from the lesions of birds diagnosed with colibacillosiswas screened for genes associated with copper, arsenic, silver, mercury, cadmium, zinc, chromium, cobalt, nickel and leadresistance using PCR. Isolates were also screened for phenotypical resistance to metalsusing broth microdilution. The frequency of heavy metal resistance genes ranged from 0to 82% for arsenic resistance; other metal genes includedcopper (1.8%), silver(18.9%), and mercury (25%). Phenotypical analysis found MICs for copper ranged from 800-1600ug/ml while those for mercury ranged from 1.56 to 25 ug/ml, silver 12.5-50 ug/ml and MICs for nickel and manganese ranged from 800 –1600ugml. Althoughno strict breakpoints are identified for heavy metals the results are consistent with similarstudies and demonstrate that heavy metal resistance genes are widely present in APEC.The association between phenotype and genotype suggest that some of these resistances are expressed at high levels and likely linked with other antimicrobial resistance traits supporting co-resistancein APEC. Surveillance is warranted to determine the emergence of resistance traits withthe potential to select for APEC.

Evaluation of Candidate Reference Genes in Clostridium perfringens for Gene Expression Normalization

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Clostridium perfringens(C. perfringens) is the causative agent for many diseases in a wide range of hosts including necrotic enteritis in chickens (NE). Recent reports have shown a marked increase in NE associated with C. perfringensespecially in organic and antibiotic-free production systems (ABF). Several Clostridial toxins play a key role in C. perfringenspathogenesis. Studying toxin gene expression is essential for understanding C. perfringenspathogenesis. Identification of stably expressed reference genes for quantitative real-time polymerase chain reaction (qPCR) is required to avoid bias in these studies. However, most previous studies used unvalidated reference genes for estimating gene expression in C. perfringens. Therefore, the purpose of this study was to identify a set of stable reference genes that can be used to normalize C. perfringensexpression data under different growth conditions. Ten candidate reference genes (adk, ftsZ, gyrA, gdhA, recA, rpoA, rho, rrs, rpsJ, and tpiA) were assessed in 3 C. perfringensreference strains representing toxin types (A, G, and F) and expressing all Known C. perfringenstoxins. The findings of this study should facilitate studying gene expression in C. perfringensand enable researchers to better understand C. perfringenspathogenesis in different hosts.

Model Development: Evaluation of three Necrotic Enteritis challenges

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Necrotic enteritis (NE) is a severe intestinal disease in broiler chickens. Toxin-producing strains of Clostridium perfringensand intestinal epithelium disrupting Eimeriaspecies are common to commercial poultry productionand contribute to the severity of the disease. Groups studyingthisentericcondition use variousmodels in order to achieve experiment goals.In a floor pen experiment, three NEchallenge models were assessed to understand the impact on mortality and performancefrom 0-42 days. Twelve replicate pens of 25 male commercial broiler chickens (Ross x Ross) were placed in each of threetreatments. Treatments included (1) new litter plusClostridium perfringens(CP)challenge on14, 21, and 28 days, (2) new litter plusClostridium perfringenschallenge on14 and 15days, and(3) usedlitter from a previous necrotic enteritis experimentwithno additionalClostridium perfringenschallenge. All groups received a commercial coccidiosis vaccine at day of hatch. Birds placed on the reused littersourcedfrom the NEchallenge gained less weight in the starter phase than the birds placed on fresh shavings and challenged at 14,21, and 28 days or 14 and 15. This lower weight persisted relative to the other groups throughto42 days($p<0.05$). Mortality adjustedfeed conversion was not different between groups. The birds challenged with CP at 14, 21, and 28 days (21.3%) and 14and 15 days (20.0%) had higher NEmortality than the reused litter challenge (1.0%)($p<0.05$). The models with controlled exposure toClostridium perfringensdid not differ statistically. While the new litter challenges created greater separation in clinical disease metrics (mortality), it is less successful at evaluating subclinical diseasethan the reused litter model.

Case Reports

Case report: Evidence of Reemerging of Salmonella Enteritidis Infection in Poultry Layers

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In Peru, clinical infections by Salmonella Enteritidisin commercial birds have been effectively controlled with vaccination, and nocase has been diagnosed in our laboratory since 2007. In November 2020, live and dead birds were received from a flockof 57-week-old commercial brown layers from a commercial farm in Lima department, which areraised on the floor. Signs of diarrhea, increased mortality and decreased egg production were reported during the last week. The clinical examination showed depressionand diarrhea. The lesions observed were splenomegaly, severe enteritis, thickening of the abdominal air sacs, ovary regression, internal posture, egg peritonitisand severe salpingitis. Salmonella Enteritidiswas isolated from the liver,spleen, peritoneum and oviduct. The strain wasidentified by microbiological, biochemical and serological tests and it has been processed for sequencingto be genetically characterized. This report evidences the reemergence of paratyphoid due to Salmonella Enteritidispathogenic for birds.

Coccidiosis

Performance of a real-time PCR assay for Eimeria sp. of chickens

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A quantitative polymerase chain reaction (qPCR) assay as described by Vrba, et. al. 2010 for Eimeria sp. in chickens was optimized and the limit of detection determined (LOD) for five of the seven species referenced. The present study tests the sensitivity in our lab using a dilution method followed by spiking negative feces and blanks to assess matrix effects. The sensitivity of the assay was <7 to 93 for E. acervulina, E. brunetti, E. maxima, and E. tenella except E. necatrix which has a lower sensitivity with LOD of 1133 – 2266 oocysts. All the assays had the efficiency ranges between 90.5 – 100.9, and the reproducibility greater than 95.7%. Current coccidia vaccination monitoringconsists of oocyst counts collected over the cycling periods and provides a general trend of oocyst cycling without speciation. To evaluate the qPCR assays, field samples were collected at 1-4 weeks post vaccination and compared for oocyst counts per gram (OPG) and qPCR tests. The results showed a large percentage of under representation of E. necatrix in the samples. In a subsequent study, the late cycling of E.necatrixcompared to other Eimeria sp. was clearly indicated when pooled fecal samples (7, 8, and 9 days post vaccination) were tested. Also confirmed was the shedding level of each Eimeria sp. by each individual bird varied greatly on the same post vaccination time point. The results show the timing of sampling is critical for accurate oocyst speciation after vaccination. Testing of samples collected at one-time-point might cause false negative results of late cycling Eimeria sp. likeE.necatrix. Also even though the PCR assays can be very valuable tools for speciation, other variables (management, sampling and number of birds, other environmental factors) might affect the sensitivity of the results therefore caution has to be taken for interpretation of the PCR results.

Diagnostics

A Comparison of MinION and Flongle Flow Cells for the Rapid Detection and Identification of Avian Influenza A Virus in Clinical Samples

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All naturally occurring highly pathogenicavian influenza (HPAI)viruses to date have been H5 and H7 subtypes resulting frommutation of the fusioncleavage site of the haemagglutinin(HA) gene. TheaffordableFlonge flow cell adapter for Nanopore's MinION and GridION devicesallowsfor cost-efficient, real-time access to smaller, frequently performed assays. We compared the performance ofMinION and Flonge flowcells for the fast and targeted sequencing of clinical samples in conjunction with the in-house barcodedprimers for the amplification of a large part of H5 and H7 HA genes.With this aim,SPF birds were experimentally infected with a high dose (106EID50/0.1ml) of seven different HPAI H5 and H7 strains.Oral swabs were collectedat2 dayspost-infection. Viral titers in each sample were determined by qRT-PCRand ranged from 3.2 to 7.5 log10EID50/ml.Barcoded amplicons of 7HPAI were pooled together in equal volume and used for the preparation of two Nanopore libraries to be sequenced usingMinION and Flonge R9 flow cells. In just 1 hour of MinION and Flongesequencing, the total reads quantity was 234,969 and 45,060, respectively. One sample withthe lowest viralload was undetectable using both Nanopore flow cells. In the rest of the samples,viruseswere successfully detected and the correct HA type was identified. The numberof BWA-MEM mapped reads range from 8,662 to 81,570 reads for MinION and from 1,674 to 14,192 reads for Flonge flow cell.As per its lower numberof available pores, sequencing using a Flonge flow cell predictably provided fewerreads which consequently resulted inlowermeanquality

comparedto a MinIONflow cell. However, our study confirmed that the Flonge flow cell can be cost-effectivefor the rapid detection and identification of HPAI in clinical samples during anoutbreak or mass screening

An Immunochromatographic Strip for Antigen Detection of Avian Infectious Bronchitis Virus

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Avian infectious bronchitis virus (IBV) causes considerable economic losses in the poultryindustry worldwide, including Taiwan. IBV is among the most important pathogens in chickens, and it spreads rapidly among flocks. In addition to dozens of known serotypes, new viral variants have emerged due to the viral evolution and antigenic variation in IBVs. Therefore, the development of a sensitive, specific, and easily performed assay is crucial for the rapid detection and surveillance of IBV infections. A rapid and simple immunochromatographicstrip (ICS) was developed in this study by employing monoclonal antibodies against nucleocapsid proteins of IBV as the tracer and the capture antibody. The ICS showed high specificity in detecting IBV antigens, including several IBV genotypes and novel variants, as opposed to three other common avian respiratory viruses. The detection limit of the strip reached 102.1350% embryo-infective dosein the allantoic fluid. In the experimental chicken model, the strip test demonstrated consistency in detecting IBV with RT-PCR gene detection. Moreover, using RT-PCR as a standard, a sensitivity of 88% and a specificity of 93%were obtained as evaluated with fieldswab samples.Taken together, this antigen detection strip has the potential to serve as an on-farm rapid test for IBV; therefore, it may facilitate surveillance and control of the disease.

Development of antigen-capture ELISA to analyze the immunological functions of chicken interferon-kappa using specific monoclonal antibodies

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Interferon(IFN)-κis a type I IFN and plays a central role in hostantiviral defense and immune response.The functionsof type I IFN in birds havenot been clearly defined, andlimited informationof IFN-κhasbeen reportedin poultrycompared to its counterpart in mammals. In this study, we developed anantigen-capture ELISA that can determinethe productionof chickenIFN-k(chIFN-κ)using antigen-specific mouse monoclonal antibodies (mAbs).Recombinant chicken IFN-κ expressed in E. coliwasused to immunize the mice. Five mAbs that specifically recognize chIFN-κ were selected and characterized based on theirspecificity and binding activitytoward chIFN-κby indirect ELISA and western blot. For captureELISA development for chicken IFN-k, two sets of best capture anddetection mAb combinationswere identified by pairing assay. IFN-κproduction induced by polyinosinic: polycytidylic acid (poly I:C) inchicken HD11 macrophage cells was effectively detected by the newly developed capture ELISA, and the gene expression of IFN-κwas validated using qRT-PCR.Neutralizing effects of anti-chIFN-κmAbs were tested by their ability to block the induction of IFN-stimulatedgenes (ISGs) in DF-1 cells upon the stimulation by recombinant chIFN-κ protein. AllmAbs blocked the mRNAexpression of ISGs in a dose-dependent manner. The newly developed capture ELISA and anti-chicken IFN-κmAbs will serve asvaluable immune reagents for poultry scientists.

Metastatic melanoma of ocular origin in a duck

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A 12-year-old mixed breed duck from a zoowas submitted dead. The duck had a history of buphthalmiaof the right eye and poor body condition. Grossly, the duck's right eye was enlarged (buphthalmia), protruded from the orbit and the cornea was opaque. The liver was greatly enlarged(5x) with a myriad of round black massesof various sizes,visible on the capsular and cut surfaces. A black mass extended from the equator of the globe and filled the orbit. The lungs had multiple black masses of various sizes. Microscopically all masses were those of neoplastic melanocytes. The globe had marked expansion of the iris, ciliary body, and retina. Neoplastic melanocytes penetrated thesclera through the optic nerve and expanded into surrounding tissues filling the orbit caudal to the eyeball. This was a case of iridal metastatic melanoma.Metastatic iridal melanoma has been described in mammals.

Monitoring strategies of new vectored ILT vaccine

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In order to monitor the effects of vaccination in the field, Antibody monitoring strategies such as ELISA, have traditionally been employed, due to their low cost and efficiency for use in large flocks. Widespread use of vectored vaccines in poultry has led to the need for a change in strategies for effective vaccine monitoring, due to differences in the antibody response to vectored vaccines. Commercial ELISA kits, which often use whole antigen coatings can vary on their ability to detect immunity generated from vectored vaccines. We will discuss alternative ways to monitor vaccinated bird health using traditional ELISA results and simple flock performance metrics as a means of verifying vaccine performance. The methods discussed will include monitoring flock mortality patterns, performance and strategic use of antigen based testing (rtPCR).

Enteric Health

Cimenol ring, botanical molecule, to improve performance, egg quality and to reduce egg contamination in comparison to organic acids and formaldehyde

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Cimenol ring (CR) is a botanical molecule that eliminates pathogenic microorganismsboth in feed, acting as a preservative, and in the gut, to balance the digestive flora. A trial was conducted toevaluate the efficacy of CRin layerscompared toorganic acids and formaldehyde. 224 layers (15-29 weeks) were distributed into 4 groups: "CON" withoutantimicrobials, "CR" with cimenol ring; "OA" with organic acids, and "FO" with a product with33% formaldehyde. Performance, egg quality and microbial coloniesin gut, feces and eggs were evaluated. Significance was considered if $P<0.05$.Thelaying rate was significantly better in CR compared to allgroups. OA obtained better resultsthan FO and control. Egg weight significantly increased in CR during mostweeks, compared to all groups.Dirty eggs were significantly lessin CR and OA. In microbiological analyses CR obtained the lowest counts for E. coli and Campylobacter in egg content;for E. coli and Clostridium in cloacal swabs;and for E. coli, Clostridium and Campylobacterin intestinal contentwitha 90%CFU reduction compared to the control.These results are related to better gut health and food safety.In conclusion, cimenol ringhad the greatestpositive effect on performance, egg quality and pathogen elimination. Organic acids achieved the second-best results while formaldehydenegatively affected performance and egg quality, probably due to itis highly toxic and irritant. Therefore, CR is a useful and safe tool to improveegg production and quality anddecrease the risk of egg contamination.

Intestinal Morphometrics - is it the right tool to objectively measure intestinal integrity

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Phibro Animal Health[1], University of Georgia[2,3]

Morphometrics of the intestinal tract in poultry has been around for quite some time and yet is not part of routine evaluations of the intestinal integrity of poultry. In this experience we based the ability of coccidiosis to cause morphological changes to the intestinal mucosa resulting in reduction of total absorptive surface in order to document the differences of the changes produced with Eubiotics. These substances may induce changes in the intestinal mucosa that can be evaluated by using intestinal morphometry. To investigate the effects of different Eubiotics on body weight, feed conversion ratio, crypt's depth, villus height and width, and Lesion Score and OPG to coccidiosis in male broiler chickens, five hundred Cobb 500 birds were housed in floor pens and divided into 8 groups. At 21 days of age, three birds from each group were euthanized by cervical dislocation to obtain the parameters mentioned before and contrast them with control birds in low challenge conditions(and contrasted with previous experiments with moderate to high challenge conditions). The experiment was finalized at 42 days of age.

Epidemiology

Continuous detection of GI-13 lineage Avian Coronavirus strain in poultry flocks from Peru.

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Ceva Animal Health[1], Ceva Animal Health[2], Universidad Peruana Cayetano Heredia[3], Ceva Animal Health[4]

Infectious Bronchitisvirus(IBV)is an important pathogen ofindustrialpoultry, capable of producing significant economic loss to the industry. Vaccination plans toprevent/control thedisease must be addressed in accordance to the epidemiological situation of thetargeted region. In Peru, the first official report ofan avian coronavirus infection was made in the late 60s from cases related to non-vaccinated flocks with severe respiratory clinical signs. In 2013 and 2014 epidemiological reports showed the detection of GI-16 genotype of avian coronavirus strains (Q1-like variant). Virus wasdetected molecularly andisolated from flocksvaccinated with Massachusetts-type live strains only,which were presentinga diversity of clinical signs. In an attempt to control the disease, control/prevention measures that includedstricter biosecurity procedures like adjusted vaccination programs and more intense cleaning and disinfectionwere implemented. Toevaluate the efficacy of those procedures, a field IBV monitoring was implementedin2018 and continued untilthe present year (2020).Molecular analyses confirmed the presence of the previously detected Q1-like variant. Analyses alsoshowed thepresence of793/B-typevariant strainsof IBV (GI-13genotype). Haplotype and network analysisindicated the detected strains to be of likelyvaccine origin. Theseresults confirm the circulation ofa793/B-typevariantof Avian Coronavirus in Peruduring the years 2018, 2019 and 2020.

Digital Platform for Monitoring and Analyzing the Molecular Epidemiology of Worldwide Infectious Bursal Disease Virus Isolates.

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In 2019, there were 18 scientific papers published in the National Center for Biotechnology Information (NCBI) site discussing phylogenetic information for infectious bursal disease virus (IBDV) strains isolated from different countries or regions. Many IBDVs have been identified throughout the globe, some with unique characteristics such as early bursal atrophy (variant IBDV strains found in the United States). Due to the different impacts to the production system from different IBDV strains, specific prevention and vaccination strategies will

need to be designed to prevent disease outbreaks. Most of the published papers focused on detailed investigation of the relationships between country-wide or regional virus isolates. However, we all learned from COVID-19 how quickly disease can be spread throughout the globe. Therefore, it is critical to closely monitor IBDV prevalence globally. As of today, there still is not a global database available for poultry veterinarians or producers to instantly and closely monitor the ongoing field IBDV situation and compare it to nearby countries or the rest of the world. The information remains in local or regional "silos". A first of its kind digital platform based upon next generation sequencing (NGS) technique was built beginning in 2019, with accumulation of seventeen hundred IBDV isolates from all over the world and still growing. With this user-friendly analysisplatform, veterinarians will be able to capture the worldwide IBDV overview easily and to also perform deeper phylogenetic analysis. Hence, they could utilize the field strain evolutionary information to better design and monitor IBDV vaccination programs.

GIS Tools for Poultry Health Reporting: Development of an Interactive Web Map for BREWS

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Georgia Poultry Laboratory Network[1,2]

Bronchitis Early Warning System (BREWS) report to keep Georgia's commercial poultry industry informed about cases of infectious bronchitis virus (IBV) within the state. Provided via email to key individuals, a map of Georgia showing county-level IBV isolations serves as an important component of the monthly BREWS report. Today's web-mapping technologies make it possible not only to deliver this map product in an online environment, but to encode additional information using interactive elements. Version 1.0 of the BREWS web map is set to launch in late 2020. This project explores its progress and the features being developed in 2021 for version 2.0.

Immunology

Evaluation Of Infectious Bronchitis (IB) Vaccine Induced Immune Response In Egg Layers: Comparison Of Two Vaccination Strategies

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Liveattenuated and killed vaccinesare used to control infectious bronchitis virus (IBV) infection in layersin Alberta, Canada. Depending on the geographical area, different vaccination protocols are used.Theaimof the study was to compare the immune response induced by two vaccination strategies that are commonly used by Alberta egg layer industry.The chickens that were assigned vaccination strategy1was vaccinated with IB live attenuated vaccine, Mass serotype at 3,8,2 and16 weeks of age via eye drop route andMass and Conn combination vaccine given at 5 weeks of age. The chickens that were assigned vaccination strategy 2 wasgiven the same vaccines as strategy1 except at16 weeks age,when the chickens were vaccinated withinactivated Mass vaccinerather than live attenuated vaccine. Thegroup 3 was themock vaccinated controls.Atpeak of lay, chickens in all 3 groups were euthanized and blood, reproductive tract washes and tissues (lung, trachea, kidney and spleen) were collected. Serum was used to quantifyanti-IBV antibody response. Tissues collected were used to extract nucleicacidto quantify immune genes and immunecell recruitment.Vaccination strategy 2induced significantly higher anti-IBVantibody response systemically andhigher percentage ofimmune cell recruitment in the reproductive tract and kidney tissues.This is a work in progress,and we are in the process of determining the cytokinegene expressionlevels in tissues.

Immunomodulant effect of in ovo vaccination with herpesvirus of turkey supplemented with toll-like receptor 3 agonist (poly I:C) in meat-type chickens

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We have demonstrated that in ovovaccination of herpesvirus of turkey (HVT) hastensthe immunocompetence of 1-day-old meat-type chickens by accelerating the maturation ofinnate and cell-mediated immunity. Thispositiveeffect wasoptimizedby modifying the vaccine dose. The recommended dose (RD, 6080 plaque forming units, PFU) had thebest immunopotentiating effectscomparedto half-dose(3040 PFU), quarter-dose (1520 PFU) and double-dose (12160 PFU). The objective of the present studywas to evaluate if we could enhance the positive effect of in ovo vaccination with HVT by adding atoll-like receptor 3 (TLR-3)adjuvant, polyinosinic-polycytidylic acid (poly(I:C)). The effect of a conventional HVT vaccine given at half-dosesupplemented with 50µgpoly(I:C)[HVT-1/2+poly(I:C)] was compared with the effect of HVTat RD[HVT-RD],HVT-1/2, 50µgpoly(I:C)alone[poly(I:C)], and vaccine diluent [sham-inoculated]. Frequencies of various immunophenotypes in the spleens of 1-day-old meat-type chickens were evaluated by flow cytometry. Results were compared to sham-inoculated chickens. HVT-RD and poly(I:C) exhibited the strongest immunomodulatory effects. There was a significantly increased frequency of T-cell receptor gammadelta (TCRγδ) cells, macrophages with MHC-II+expression (KUL01+MHC-II+),and T-cell subsets expressing activation molecules (CD3+MHC-II+, CD8+MHC-II+, CD4+CD28+, CD8+CD28+, CD8+CD44+) in all treatment groups. Intra-group analysis showed HVT-RD and poly(I:C) also had a significantly increased frequency of CD4+CD44+and CD4+MHC-II+T-cell subsets.Results show that addition of poly(I:C) to HVT-1/2 did not enhance the immune stimulant effect of HVT. In fact, it seems like HVT and poly(I:C) compete for similar mechanisms.

Synthetic CpG-ODN rapidly enhances antimicrobial functions of immune cells in broiler chickens

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Immune stimulatory activities of oligonucleotides containing Cytosine phosphodiester Guanine (CpG-ODN) have been recognized against different bacterial pathogens in chickens. CpG-ODN binds with Toll-like receptor 21 (TLR -21) which is the functional homologous to mammalian TLR-9 in immune cells,subsequently stimulate to express genes responsible to produce several inflammatory cytokines and chemokines includinginterleukin (IL)-1, IL-6, interferon (IFN)-γ and IFN-α.As the first line of defense,heterophils play an important role in the chickens' immune system and macrophages also imperativephagocytic cells. Mechanisms of these cells connect both innate and adaptive immune system and protect birds from infectious pathogens. Information islackingon the effect of CpG-ODN on antimicrobial functions of innate immune cells in chickens. In this study, our objectives were to optimize flow cytometry techniques to detect antimicrobial functions of chicken heterophils and macrophage/monocytes and utilize these optimized methods to observe phagocytosis, oxidative burst, and degranulation in macrophage/monocytes and heterophils in chickensfollowing CpG-ODN induction. CpG-ODN was injected intramuscularly and peripheralblood was collected at 24, 48,and 72 hours after injections. We have observed higher phagocytosis, oxidative burst and degranulation functions in macrophage/monocytes and heterophilsin CpG-ODN given group compared to the control group. This response was superior at 48 hours than 24 and 72 hours. Resultsof our study provide evidencethat CpG-ODN has the ability to stimulateinnate immune activities in broiler breeder chickens. Conclusively, CpG-ODN can be recognized as a potentialcandidate to be used as an alternative to antibiotics in chickens

Unveiling the immunological attributes of chicken tumor necrosis factor-a via the development of specific monoclonal antibodies

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Tumor necrosis factor-a (TNF-a) is a type II transmembrane protein with either membrane-bound or soluble forms and is a prototypical member of TNF superfamily. TNF-a is a pleiotropic cytokine associated with the regulation of systemic inflammation and host defense. Chicken TNF-a (chTNF-a) is a long missed avian ortholog and the immunological properties of chTNF-a remains largely unknown compared to the mammalian counterpart. Here, we report on the functional characterization and immunomodulatory roles of chTNF-a using the new sets of anti-chTNF-a mouse monoclonal antibodies (mAbs). A chTNF-a-specific antigen-capture ELISA was developed using compatible mAb partners via the screening and validation of ten different mAbs (3G11, 4C4, 4F3, 6A52, 6E6, 10E8, 12G6, 12H7, 14H2 and 15G7). Employing 3G11 and 12G6 as capture and detection antibodies, respectively, the levels of native chTNF-a in the circulation of Clostridium perfringens-, Eimeria- or dual C. perfringens/Eimeria-infected chickens were determined. Meanwhile, intracellular expressions of chTNF-a in primary cells or cell lines derived from chickens were validated in immunocytochemistry and flow cytometry assays using both 3G11 and 12G6 mAbs. Additionally, both 3G11 and 12G6 neutralized chTNF-a-induced nitric oxide production in chicken HD11 cells in vitro. Collectively, all these data provide a better understanding of the functional characteristics of chTNF-a and these anti-chTNF-a mAbs will serve as valuable immunological tools for the fundamental and applied studies in avian species.

Mycoplasma

Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) Multilocus Sequence Typing (MLST) Public Databases: Update and Invitation to Use

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Mycoplasma gallisepticum (MG) and Mycoplasma synoviae(MS) are pathogenic avian mycoplasma species. Both of them results in significant economic losses for the commercial poultry industry. Identifying the source of the infection and understanding the disease epidemiology are required to facilitate the control and eradication efforts of both pathogens. Recently, our group has developed MLST schemes for both MG and MS. These schemes are using the sequence information of multiple amplified gene segments for differentiation between strains, filed isolates and DNApositive clinical samples. We have established an online database for sequence and isolates information for both pathogens at pubMLST.org. Currently, for MG there are morethan361 allele sequences,185sequence typesand 269 isolates has been submittedand typed using two MLST schemes. For MS, there aremore than 249 allele sequences, 148 sequence types and 208isolates submitted. In this presentation, we will providean update and epidemiological analysis of the genotypes of MG and MS populationcirculatingglobally based on the submitted samples to the databasefrom 21 different countries. These online databases becamea platform for MLST typing and recording of isolates information of both Mycoplasma species worldwide. These databases represent a valuable aid for field clinicians that will help identification of the source of infection and outbreak investigations. This in turn will facilitate the control and eradication effortslocally and globally.

Salmonella

Case report: Severe Post-vaccinal Reaction induced by the Inactivated Oil Emulsion Vaccine against Salmonella Enteritidis applied Intramuscularly to Broiler Breeders

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In recent years, several flocks of birds, mainly broilers breeders with a clinical history of mortality and lesions in breast and liver, have been received in our laboratory. The birds were recently vaccinated against Salmonella Enteritidis using an oil-inactivated bacterin. Serious lesions were observed including hepatomegaly, hepatic necrosis, hepatic rupture, hemoperitoneum and infiltration of caseous material in breast. The case was reproduced experimentally with inoculation of the vaccine to three groups of birds, applying 0.25 mL at different temperatures: 4°C, 36°C and 39°C,intramuscularly. All groupspresentedvery severe inflammatory reaction in the area of inoculation of the vaccine, extending to a large part of the pectoral muscles of the birds. The bacterialcultureswere all negative, the liver and muscle samples are being processed for histopathological examinations. The severe vascular and necrotic lesions observed mainly inliver and musclesevidence a vascular disorder withformation of thrombi, similar to that reported in mammals and humans as a consequence of the activation of the innate response to high doses of mobile Salmonella sppflagellin.

Evaluation of Compatibility of Drinking Water Acidification With Live Salmonella Typhimurium Vaccination in Broilers

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Zoetis[1,2,3,4]

A strong food safety program encompasses the usage of multiple interventions for Salmonella spp.control from the live side to the processing plant. Live Salmonella Typhimuriumvaccination(LVST) in broilers is a strategy that has gained ample traction andsuccess reducing Salmonella spp.levels in broilers at the processing plant in the past few years.In addition, some poultry producers provide acidified water (WA) for the first days of life in order to reduce the loads and early colonization of Salmonella spp.Considering that the usage of WA at bird placing could potentially reduce the efficacy of LVST applied at the hatchery, the objective of this trial was to evaluate if the combination of both interventions would impair LVST efficacy against a Salmonella Heidelberg challenge.A total of 360 broilers were vaccinated with LVST at hatch by coarse spray and divided among 4 pens (2 isolation rooms each with 2 pens). One pen per room had the WA(Sulfuric acidplus Sodium sulfate anhydrous) to a target pH of 3.3 for the first 12 days whereas the other pen provided water at a pH of 7. All birds were boosted with LVST at 13 days. At day 28, half the birds per pen were orally gavaged with 108CFU SalmonellaHeidelberg. All birds were terminated at 39 days with cecae and liver/spleens sampled for Salmonella spp.enumeration and prevalence.LVST vaccine takes were 100% positives at 3 and 6 days of age. Overall, the salmonella loads were negligible (MPN<4) and there were no statistical differences on salmonella recovery from cecae or liver/spleens between WA birds versus the controls for both direct and indirect challenged birds. In conclusion, it was shown that WA did not affect LVST efficacyunder a SalmonellaHeidelbergchallenge.

Feed challenge model for Salmonella Enteritidis colonization

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Salmonella contaminated feeds have often been associated with the contamination of food producing animals and ultimately with human food-borne Salmonellosis. Feed can be a major source of introduction of Salmonella into commercial poultry production situations when contamination occurs at feed production facilities and in feed ingredients. Contamination is in part due to the wide variety of environmental origins for Salmonella and its ability to easily cross-contaminate during feed processing and storage. Since Salmonella spp. have been shown to survive for several months in feed products, it can remain a continuous problem for poultry production without intervention. In this pilot study we analyzed Salmonella enteritidis (S.E.) colonization in broilers with S.E. contaminated feed with a feed intervention product. Nalidixic acid-resistant S.E. was cultured in Meat and Bone Meal (MBM) to a final concentration of 105 CFU/g of feed. The S.E. contaminated MBM was mixed into the feed with the addition of a chromium marker to ensure the MBM was distributed evenly throughout the feed. The S.E. inoculated feed was fed for seven days, upon removal the S.E. level was 104 CFU/g of feed. On days 14 (20% positive) and 19 (9.4% positive) of the study, cloacal swabs were taken to evaluate the S.E. colonization in the broilers. On day 21 liver and spleen pool and ceca samples were collected from 10 birds per pen for S.E. prevalence and enumeration. There were 26% S.E. positives in the liver and spleen pools, and 55% positive in the ceca. This study demonstrated the transfer of Salmonella from feed to poultry to humans is a potential route of contamination and infection; practical and effective methods must be utilized to avoid Salmonella from entering the feed supply.

Vaccinology

Assessment of safety and efficacy of a live attenuated freeze-dried vaccine in effervescent tablets against Newcastle disease in SPF turkeys

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Boehringer Ingelheim Animal Health[1,2,3,4,5,6]

Newcastle disease (ND) is an infection of birds caused by a virus of avian paramyxovirus serotype 1 (APMV-1) with an intracerebral pathogenicity index (ICPI) in day-old chicks (Gallus gallus) of 0.7 or greater, or with multiple basic amino acids at the cleavage site of the virus fusion protein. Due to the severity of the infection and to the cost of control measures, the disease has an important economic impact worldwide, either in rural areas where backyard poultry is reared and in contexts with industrialized poultry production.Infection with Newcastle disease virus (NDV) has been virtually reported in all avian species. Clinical signs can be different depending on poultry species, breed, etc. Generally, the order of birds showing the most severe clinical signs, to the least susceptible, are chickens, turkeys, pigeons and ducks. Besides control measures such as international and national control policies and biosecurity, an important role is played by vaccination with live, inactivated and vector vaccines. However, a very limited number of ND vaccines is currently registered for use in turkeys.Studies were performed to assess safety and efficacy of a live attenuated freeze-dried vaccine, based on VG/GA Avinew strain, against ND in SPF turkey poult groups, where the vaccine was applied at day-old by nebulization. In the safety study, the birds were regularly monitored up to day 21 of age for safety parameters. In the second study, at day 21 poults were blood sampled to measure the antibody response to ND and challenged intramuscularly with a velogenic NDV. The results of the studies will be presented and discussed.

Compatibility Between a Vector rHVT Vaccine Against Highly Pathogenic Avian Influenza H7N3 Virus (rHVT-H7) and a Vector HVT Vaccine Against Velogenic Newcastle Disease Virus (rHVT-F) in Commercial Layers

Luiz Sesti[1], Francisco Rojo[2], Ricardo Franco[3], David Duenas[4], Vilmos Palya[5]

Ceva Animal Health[1,2,3,4,5]

Two challenge experiments were carried out in commercial layers positive for maternal antibodies against Avian Influenza (AI) H7N3 and Newcastle Disease (ND). In experiment 1, layers were vaccinated (SQ) at day 1 of age with a vector rHVT-H7 vaccine that contains a herpesvirus of turkeys which carries the H7 hemagglutinin gene of a highly pathogenic avian influenza (HPAI). Birds were then oculo-nasally challenged at 70 days of age with either the Mexican HPAI H7N3 virus – 2015 government's official isolate (10 birds; group 1) or with the Mexican HPAI H7N3 virus – 2018 government's official isolate (10 birds; group 2). Two non-vaccinated and challenged control groups were also used (10 birds each; groups 3 & 4). In experiment 2, day old layers were vaccinated (SQ) with the vector vaccine rHVT-H7 in association (diluted in the same diluent bag) with a vector rHVT-F vaccine that contains a herpesvirus of turkeys which carries the F protein gene of the Newcastle Disease virus. At 70 days of age birds where challenged with either the Mexican HPAI H7N3 virus – 2015 government's official isolate (10 birds; group 1) or with a velogenic Newcastle Disease virus (NDV; Chimalhuacán strain; 10 birds; group 2). Two non-vaccinated and challenged (either HPAI H7N3 2015 or Chimalhuacán strain) control groups were also used (10 birds each; groups 3 & 4). All groups were clinically observed for 2 weeks and samples (choanal and cloacal swabs) were taken at 2, 6- and 9-days post-challenge for viral excretion measurements. % livability in experiment 1 was 100% for both vaccinated groups and 0 (H7N3 2015 challenge strain) and 50% (H7N3 2018 challenge strain) for the non-vaccinated control groups. In experiment 2, all birds in the control groups were dead by 9 days post-challenge. In the vaccinated groups livability after the AI challenge was 100% and was 90% for birds challenged with the Newcastle virus. At both experiments, the amount of challenge virus excreted at 2 days post-challenge was higher ($p \leq 0.05$) for the control groups although no differences were found in excretions rates at 6- and 9-days post-challenge in experiment 2. In conclusion, the vector vaccine rHVT-H7 when applied alone induced 100% clinical protection against both HPAI H7N3 virus isolates. % livability after challenges at 70 days of age, with a HPAI H7N3 or a velogenic NDV demonstrate the compatibility of the two vector rHVT vaccines when applied in association at day one in commercial layers.

Construction, Stability and Efficacy of a Recombinant HVT-IBD-ND Vaccine against IBDV, NDV and MDV Challenges

Sing Rong[1], Yugang Luo[2], Kelly Turner-Alston[3], Candyce Pacione[4], Lauren Taylor[5], Tyler Brown[6], John Dickson[7], Jennifer Embrey[8]

Zoetis[1,2,3,4,5,6,7,8]

Infectious bursal disease (IBD) is caused by thebirnavirus IBDV. IBD is a worldwide problem,and all types of chickens in all regions are routinely vaccinated for the disease.Epitopes responsible for stimulating virus neutralizing antibody (VN) responses by the host are located on IBDV viral protein 2 (VP2). Newcastle disease virus(NDV) causes a highly contagious and fatal disease affecting all species of birds. NDV fusion protein (F) is one of the major viral glycoproteins present in the viral envelope and is the main immuno-protective NDV antigens.Marek's disease (MD) is a common cause of tumors, condemnationsand immune suppressionin chickens. The etiologic agent, serotype 1 Marek's disease virus (MDV),is a member of the family Herpesviridae. Herpesvirus of turkeys (HVT), is an avirulent turkey virusthat is capable of replication in chickens. HVT has been demonstrated as a useful vector for delivering major avian antigens, as well as an effective vaccine for MDV. Weconstructedforty-threeHVT-IBD-ND recombinants using various promoters, antigen sequencesand poly A signals,and the target gene expression cassettes were inserted

at various sites onthe HVT genome. AnHVT-IBD-NDrecombinant vaccinewasidentified and selected forits excellentin vivoefficacy(100% protection, 30/30) atDay 28 in SPF (specific pathogen-free) birds against avirulent classic IBDV challenge for both in ovovaccinated and subcutaneously injected at hatch, with0% (0/30) protection for control birds. In another study, challenged with a velogenic NDV, 95% (38/40) protection was observed for in ovovaccinated birds, and 100% (40/40) protection was observed for birds vaccinated bysubcutaneous injection, with0% (0/40) protection for the control group. In addition, efficacy of this vaccine againsta virulent MDVchallenge was observed.Furthermore,recombinant vaccinestability was demonstrated utilizingPCRbyin vitropassaging of aviral culture, as well as IFAand DNA sequencing.Recombinant vaccine stability was also demonstrated by PCR for viruses derived from in vivopassages.

Control of Mexican Highly Pathogenic Avian Influenza (HPAI) H7N3 Virus in Broilers Through the Application of a Vector rHVT-H7 Vaccine Alone or in Combination with a Reverse Genetics-Origin Inactivated Vaccine

Luiz Sesti[1], Francisco Rojo[2], Vilmos Palya[3]

Ceva Animal Health[1,2,3]

Commercial broilers positive to maternal antibodies against IA H7N3 were vaccinated (SQ) at day 1 with either a vector rHVT-H7 vaccine that contains a herpesvirus of turkeys which carries the H7 hemagglutinin gene of a highly pathogenic avian influenza (HPAI; experimental group 1; 15 birds) or in combination with an inactivated reverse genetics-origin vaccine (SQ at day 9 of age; experimental group 2; 15 birds). Both experimental groups plus two non-vaccinated control groups (experimental groups 3 & 4; 15 birds each) were challenged (oculo-nasally) at 28 days of age with a HPAI H7N3 virus isolated in the state of Guanajuato (Mexico) in 2015. Birds were clinically observed for 2 weeks and samples (choanal and cloacal swabs) were taken at 2, 6- and 9-days post-challenge for viral excretion measurements. All broilers in both control groups died up to day 6 post-challenge. % livability at 14 days post-challenge in groups 1 & 2 (93 and 100%, respectively) were similar ($p \geq 0.05$). Virus excretion at day 2 post-challenge was significantly higher ($p \leq 0.05$) in control groups than any of groups 1 & 2. Broilers vaccinated with the rHVT-H7 vaccine alone excreted similar amount of virus ($p \geq 0.05$) than the birds vaccinated with the vaccine combination. In conclusion, the vector vaccine rHVT-H7 was significantly efficacious in controlling mortality and diminishing virus excretion after an experimental challenge in broilers with a HPAI H7N3 virus applied at 28 days of age.

Duration of Immunity of a Recombinant HVT-IBD-ND Vaccine Against Two Different Strains of Velogenic NDV, and virulent and very virulent IBDV Challenge

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Zoetis[1,2,3,4,5,6,7,8,9]

A recombinantHVT-IBD-NDvaccine was developedas a tri-valent vaccine for protection againstinfectious bursal disease (IBD), an acute and highly contagious viral infection of young chickens that causes immunosuppression andincreased susceptibility to other infectious agents;Newcastle disease (ND), a highly contagious and fatal disease affecting all species of birds; andMarek's disease (MD),a common cause of condemnations and immune suppression in broilersand tumors in older birds.In one study with SPF leghornchickens, recombinantHVT-IBD-NDvaccine wasinjected either inovoatE18 or subcutaneously athatch. On Day 63, birds fromeach treatment group were challenged with avelogenic NDVTexas GB. Protection of 97% (29/30) was observed for subcutaneously vaccinated treatment group,while100% (28/28) protection was observed forthein ovovaccinated treatment, with0% (0/29) protection for control group.In a separate studywith SPF leghornchickens, recombinant HVT-IBD-ND vaccine was injected either in ovoat E18 or subcutaneously at hatchandchallenged with a velogenic NDV Herts Weybridge 33/56 on Day 63.

Protection of 100% (30/30) was observed for both treatment groupswith subcutaneous orin ovo vaccination, with 0% (0/30) protection for control birds. In addition, a study was conducted to examine the duration of immunity by challenging the vaccinated birds with a virulentclassicIBDVon day 63. One hundred percent (30/30) protection was observed forboth treatment groups (subcutaneous or in ovovaccination)of HVT-IBD-ND, with7% (2/30) protection observed for control group.In a separate study, duration of immunity against challenge of a very virulent IBDV is being tested. The details of experimental design and study results will be presented.

Field Evaluation of a Novel Immune-Complex Vaccine against IBD for Layers

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Ceva Animal Health[1]

Anovel immune-complex (IC) vaccine against infectious bursal disease (IBD) indicated forcommercial layers wasevaluatedin Colombia under field conditions involving a total of 197,425 birds. Two commercial layer operations were used for this study comparing different routes of vaccines administration. Each treatment group was assigned to a separate poultry housein the farmsselected.The first field study compared the protection against IBDV field challenge by either ICor rHVT-IBD Gumboro vaccinesadministered at the hatchery. A second studycompared the protection given by the IC administered at the hatchery vs. field vaccination by individual oralbeak administrationat 12 and 18 days of age. Parameters measured in both studies wereserology, histopathology, PCR for IBDV vaccines strain detection and sequencing, body weight, feed intake, mortality,and uniformity of the vaccinated flocks.PCR results on bursal tissue forvaccine strain detection was performed at 5, 6,and 7 weeks of age showed 80% and 87% for the IC treatment group vs.90% (IBDV Variant E) and 73%vaccine strain detection for the alternative vaccination methods used for the comparison. Serologicalvaluesand bursal lesion scoresalso demonstrated IBDV bursal colonization. Mean lesion score at 7 weeks of age were 3.0 and 3.0for the IC treatment groupsvs. 3.2and 2.6for the alternative vaccination methodsrespectively.The cumulative mortality in study 1 was lower in the IC group (P=0.017) in comparison with the alternative vaccine group. Mortality in study 2 was numerically lower for the IC group vs. the comparative group.The field results of this evaluation showedthatthe IC vaccinated flocks had greater IBD sero-conversion; lower cumulative mortality, higher body weight and better vaccinestrain detection by PCRin bursal tissues compared toalternative vaccination methods.

Generation of hemagglutinin (HA)-expressing infectious bursal disease virus

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The present study was carried out to explore the feasibilityof infectious bursal disease virus (IBDV)as a replication-competent viral vector by using IBDVgenomic sites to carry an influenza A virus hemagglutinin (HA) epitope. The HAepitopewas fused tothe N-terminus of VP5 (HA5-IBDV),N-terminus of VP3,or both N-and C-terminus of VP4 or VP1 without deletion of any viral gene sequences by the reverse genetics approach. The HAtagged viral proteins were confirmed by immunofluorescence antibody assay (IFA) and DNA sequencing. In additionto HA5-IBDV, HA-expressing IBDVs weregenerated when the HA epitope was fused to the N-terminus of VP4 (HA4-IBDV) or the C-terminus of VP1 (1HA-IBDV). The HA tagged viral proteins expressed by HA-IBDVs were further confirmed by Western blotting. Viral titers were 1.3x104, 3.7x103and 3.8x104pfu/ml for HA5-IBDV, HA4-IBDV,and 1HA-IBDV, respectively.The HA tag expression remained stable after 10 serial passages in DF-1 cells when the tag gene was inserted to the vp4and vp1genes, but it was deleted in HA5-IBDV. After inoculation of HA-IBDVs tospecific pathogen free (SFP)chickens, all HA-IBDVs did not cause any

pathogenicity, but only HA4-IBDV and 1HA-IBDV induced specific anti-HA antibodiesby ELISA. The results indicated that IBDV expressing the foreign gene at N-terminus of VP4 and C-terminus of VP1 is stable and can potentially serve as a bivalent viral vector.

Use of a Novel Fluodot Nanoparticle as a Delivery Vehicle for Infectious Bronchitis Virus Antigen

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FluoDot is a novel single protein, serumal bumin-based nanoparticles, with a size range 10 to 100nm. In this study, we used a core of BSA, a natural drug delivery vehicle which has the ability to bind a number of different small molecules due to ~160 reactive functional groups present on its surface (99COOH groups and 59NH2 groups). The single BSA molecule is surrounded by diacid chains via carbodiimide chemistry resulting in a particle a size of 10+1nm, which are strongly negatively charged due to the COOH groups of the diacids. We used this novel FluoDot nanoparticle as a delivery vehicle for CD8+Tcell and Bcell epitope of S1 protein of Infectious Bronchitis virus which is a highly infectious coronavirus of chickens. It causese normous economi closses in the U.S. poultry industry. The study showed that there was an increase in both the humoral and cellular immune response on prime-boost immunization in chickens. Real time RT-PCR results indicated that after injection of IBV-FluoDots challenged with IBV viral infection in chickens, virus shedding was reduced in vaccinated chickens with IBV-FlouDots. Further improvements and refinements to the potential vaccine against IBV are underway in the lab to increase the humoral and cellular immune response to a significant level.

Virology: IBD

Ability of an immune-complex IBD vaccine to successfully displace vvIBDV and variant IBDV strains in field conditions

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Ceva Animal Health[1,2,3,4,5,6,7,8]

Gumboro disease (IBD)is one of the main poultry industry threats. Its causative agent (IBD virus) isclassifiedintoclassic virulent, very virulent or variant. All forms are able tocausesevereeconomic losses. Its high resistance in the environment explains its persistence despite cleaning and disinfection. Theimmune-complex IBDvaccine (Transmune) is capable to overcomethe interference of MDA,to replicate within the bursa and to eventually be shed,hence replacingthe field virus.Such a vaccination strategy was implementedin the Middle East broiler farms. Field monitoring assessedvaccine take and field challengesat processing age (ca. 28 days of age)using over600 PCR testson bursa samples from vaccinated chickensfor a period of threeyears(early 2018 -end 2020). Molecular results showedlow to moderatevaccine take during the 1styear (0 to 80%, average 23%). Highly contaminated farms with both vvIBD and Variant IBD were selected to follow up the ability of this vaccineto displace the field virus contamination.The obtained results during 2018, 2019 & 2020 showeda steady improvement ofvaccine strain recovery all theway up from 23%to 72%withmarked regressionofthe field virus detection from 55% to 12%.Most of thefarms showed the complete displacement of the field virus and 100% vaccine strain take with consistent usagecycle after cycle.According to the obtained results, Transmune IBD immunecomplex vaccinewas able todisplace the field challenge isolates throughconsistent hatchery vaccination.

Virology: IBV

IBV Surveillance in Broiler Farms of the California Central Valley (2012-2020)

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IBVcauses severe economic losses among chicken flocks worldwide. A constant surveillance approach should be conducted to better understand the prevalent strainsas well as target preventative strategies including vaccination and biosecurity. Even though, IBVsurveillance in broiler chickens has been performed for years, few analyses have been done to determinecorrelations withsecondary infections,which usually are the primarycauses of death.The goal of this project is to understandwhich are the most common co-infections and if they are responsible for the death or condemnation of the affected birds.Diagnostic laboratory reports were compiled and analyzedbetween 2012 to 2020. Infectious bronchitisconfirmation was based on positive RT-PCR and sequencing of the IBV S1 hypervariable region. Thefinal data was analyzed usingcorrelation matrixandbivariate analyses.The correlationand distributionof IBV co infectionswill be analyzed, reported,and discussed

Increased Mutation Rate in IBV ArkDPI-Derived Vaccines due to Changes in NSP14

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USDA[1], Auburn University[2,3]

The use of ArkDPI-derived IBV vaccines has been associated to the emergence of novel vaccine-like viruses that circumvent vaccination programs and are commonly isolated from chicken respiratory disease. In previous work in our lab, genetic changes were reported after adaptation of an embryo-attenuated ArkDPI-derived IBV vaccine to chicken embryo kidney (CEK) cells. The vaccine virus population shifted toward genetic homogeneity and showed a reduced incidence of genetic changes in chickens after vaccination. At first, the increase in vaccine stability was attributed to the higher homogeneity induced by the CEK cell adaptation of the commercial vaccine. Nevertheless, analysis of the genetic changes that occurred during the CEK adaptation of the ArkDPI vaccine pointed to the non-structural protein 14 of IBV (NSP14) as another possible factor influencing ArkDPI-derived IBV vaccines replication fidelity and the subsequent emergence of IBV variants in vaccinated chickens. In the current work, we hypothesized that a change of one amino acid on a zinc finger of NSP14 hinders the proofreading activity of the protein and causes the appearance of mutations with higher frequency during IBV replication.

Nephropathogenicity of Canadian 4/91 Infectious bronchitis virus in Chickens

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Infectious bronchitis virus (IBV) initially establish the infectionin the respiratory tract and then, spread to other tissues depending on the virulence. During 2011-2018, the 4/91 IBV strain has been isolated from poultry flocksin Eastern Canadaaffected with decreased egg production and quality. We conducted an in-vivostudy inlaying hensto observe if Canadian 4/91IBV isolate affect the egg production and qualityand its nephropathogenicity. During the peak of egg lay the specific pathogen free chickens were infected with 4/91 IBV using a standard dose and routes maintaining uninfected controls. Oropharyngeal and cloacal swabs were collected in predetermined time points. Sixchickens fromeachinfected and control groupwere euthanizedat 6 and 10 days of infection.No gross lesions were observed in tissues of infected chickens.

The IBV genome was quantified in swabsand tissues. The serum antibody response against IBV was detectedin 4/91 IBV infected chickens. As the nephropathogenicity is severe in younger age we also conducted another experiment in one-weekold chickens. We observed histological changes in kidney and recruitment of immune cells.Overall, the data show that Canadian 4/91 IBV is not associated with egg production issues in laying hens with various tissue tropism including kidney,where histological lesions and immune cell recruitments were evident.

Part B: Evaluation of RT-PCR Takes to Various GA08 Infectious Bronchitis Virus Vaccination Protocols in Commercial Broiler Breeder Pullets

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Ceva Animal Health[1,3,4,5,6,12], Mississippi State University[2], Sanderson Farms, Inc.[7,8,9,10], Mississippi State University[11]

DMV1639 is a strain of Infectious Bronchitis Virus (IBV) with a tropism for the respiratory, urinary and reproductive tracts. Early oviduct infection with DMV1639 IBV has been associated withFalse Layer Syndrome (FLS), which is characterized by cystic or atretic oviducts,and a poor peak production in affected flocks. Immunization against FLS-causing strains must be achieved as early as possible,as reproductive lesions more commonly develop in birds exposed duringthe first 20 daysof life. The objective of this study was to evaluate, and compare under field conditions, immunization of broiler breeder pullets on various vaccination programsincorporatinga live GA08 infectious bronchitis vaccinewithlabel claims against DMV/1639, GA08and GA13IBV. Three pullet flocks were evaluated in this study, with each study group comprising two pullet houses located on each of the three farms. The first study groupreceived the GA08 IBV vaccine at farm placement(1 day of age, DOA), followed by the GA08 vaccine and a Massachusetts(Mass) serotype vaccine at 14 DOA. The secondstudy group received the GA08 and Mass vaccines at both farm placement and 14 DOA. The third study group, which served as the control group, was vaccinated according to the company's current program with the GA08 vaccine at placementand a Mass + Arkansas (Ark) vaccine at 14 DOA.Optimum immunization will be evaluated by vaccine detection via RT-PCR and evaluating the serologic response. This presentation focuses on the RT-PCR results.Trial design and theserologic response will be covered in another presentation(Part A). Samples for RT-PCR were collected from all flocks at twotime points: 6DOA (5 days post DOA vaccination) and again at20DOA (5d after the 14day boostvaccination).

The Effect of Water Temperature on Infectious Bronchitis Virus Vaccine Reconstitution

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Infectious bronchitis virus (IBV) is a common respiratory challenge to the commercial poultry industry worldwide, resulting in respiratory disease, loss of production, increased feed conversion ratios, and increased condemnations at the processing plant. To protect birds from infection, live attenuated vaccines are given to day-old chicks at the hatchery. Reconstitution of the live virus is paramount to maintaining viral titres and there are multiple points where water temperatures may affect the vaccine, including the thawing water bath and diluent that the vaccine is mixed with. In this study, two lyophilized vaccines were tested (Merck Ma5 and Zoetis Ark) and three frozen vaccines were tested (Zoetis Poulvac 08, BI Mass, and Merck Ga98). Effect of the vaccine diluent was measured at two points for the lyophilized reconstitution: 13°C/55°F (cool) and 24°C/75°F (room-temperature) over a 2-hour period. Effect of the water bath temperature was measured at two points (25°C/77°F and 37°C/99°F) and two incubation times (2 vs 5 minutes) for frozen vaccine reconstitution. Samples of the vaccine working solutions were taken immediately after reconstitution, and then every 30 minutes for 2 hours and titres of the virus were determined through SPF embryo inoculation. By determining the effect of temperature on live vaccine titers, better

preparation of vaccines at the commercial hatchery level can be implemented to better manage and protect against IBV outbreaks in the field.

Virology: ILT

Avian infectious laryngotracheitis: Innovative serological and molecular assays for diagnosis, vaccination monitoring and diva testing

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Avian infectious laryngotracheitis (ILT) is a respiratory disease of chickens caused by the infectious laryngotracheitis virus called Gallid herpesvirus 1. ILT leads to major losses as a result of mortality and/or decreased egg production. Vaccination is an essential tool for poultry disease control. Different types of vaccines are commercially available. Conventional vaccines (TCO and CEO) based on native virus (partially or totally inactivated) offer good protection but can produce latent infections and reactivation of the virus in the field. Vector vaccines are created by genetic modification(s) of vector microorganisms and the integration into their genomes of exogenous gene(s) encoding for immunogenic protein(s) from viruses responsible of diseases of interest. In the case of poultry vector vaccines, the Fowl Pox Virus (FPV) or the Herpes Virus of Turkey (HVT) are commonly used as vector virus. One or more exogenous genes may be inserted to ensure stronger protection or to widen the spectrum of protection to more diseases. Benefits associated with this technology include biosecurity, efficiency, ability to breakthrough passive immunity, and long-lasting immunity. Additionally, vector vaccines may be used as part of DIVA strategies (Differentiation between Infected and Vaccinated Animals). In the case of ILT, two types of vaccines exist, one based on the gI protein, and the other on the gB protein. Given that the conventional serological kits do not efficiently detect seroconversion to vector vaccines, the ID Screen ILT gB Indirect and the ID Screen ILT gI Indirect innovative ELISA's were developed to monitor respectively FP-ILTgB and HVT-ILTgI vaccines.

Humoral Immune Response from the Revaccination of a Recombinant Fowlpox Virus Infectious Laryngotracheitis Vaccine in Commercial Layers

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Ceva Animal Health[1,2,3,4,]

The control of Infectious Laryngotracheitis (ILT) is particularly difficult in multi-age farms where ILTV usually perpetuates by latent infection. Currently, recombinant ILT vaccine based on HVT and Fowlpox vector viruses have turned into a preferred method for immunization chiefly because of their total safety as compared to conventional live ILT vaccines. Because strong and prolonged protection is needed in long-living birds, revaccination of recombinant Fowlpox vector virus vaccine expressing the ILTV's gB glycoprotein (rFP LT) is usually carried out in the field. This trial measured seroconversion from the revaccination with a rFP LT vaccine. Two groups of commercial layer hens received rFP LT vaccine at day 1, 4, 11 (group 1) and 13 (group 2) weeks of age. Throughout the trial seroconversion was measured by using the IDvet ELISA kit (ID Screen® ILT gB indirect) that detect specific antibodies against gB. Both groups showed a clear increase of gB Ab titers four weeks after each rFP LT revaccination with 100% of birds testing positive. FP vaccine take inspection (swelling or formation of a nodular lesion or scab at the site of inoculation)was performed at 6 days post vaccination. rFP LT-revaccinated birds presented a vaccine take weaker than in the control group that did not have previous FP vaccination. These results show than FP revaccination causes weak tissue reaction at the vaccination site which is not correlated with the immune response because as measured by seroconversion post revaccination. In conclusion, revaccination using the same rFP LT vaccine increases the humoral response against ILT and most probably induces a stronger and longer duration of protection.

Seroconversion Efficacy and Field Safety of a Recombinant Fowpox Virus Infectious Laryngotracheitis RFP LT Vaccine Applied at Day 1 of Age in Commercial Layers

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Severe cases of Infectious laryngotracheitis (ILT) are frequently observed in multi-age layer farms with outbreaks causing up to 20% of mortality rates. In farms with high infection pressure, ILT outbreaks can be observed from 5 weeks of age in flocks vaccinated with recombinant Turkey herpesvirus - rHVT LT vaccine in the hatchery. Likewise, flocks vaccinated at 6 weeks of age with a recombinant Fowlpox virus – rFP LT can also present very early ILT breaks between 5 and 8 weeks of age. Efforts to stimulate strong and early protection against ILT have led to the use of rFP LT vaccine at the hatchery. This new vaccination approach has shown to be tremendously efficient to control early challenge under field conditions. To verify the immune stimulation of rFP LT vaccine when applied at day of age, ILT Ab titers were measured of layers vaccinated at 1 day of age (Group 1) and at 4 weeks of age (Group 2) using IDvet ELISA kit (ID Screen® ILT gB indirect) that detect antibodies specifically against the ILTV B glycoprotein (gB). Clinical post-vaccination reaction and mortality were not observed in the two vaccinated groups indicating the safety of the rFP LT vaccine applied in 1-day-old layer chicks. In both groups, 95% of birds seroconverted at 3 weeks post vaccination (pv) presenting high GMT Ab titers: 8208 (Group 1) and 6954 (Group 2). Even though GMT Ab titers measured at 3, 6-7 and 9-10 weeks pv were slightly higher in Group 1, no statistical difference was found between both groups ($p>0.05$). These results indicate that rFP LT vaccine is safe to be applied from the first day of age conferring fast and strong humoral immunity that may explain the efficacy of such a program observed in the field for the control of early and heavy ILT challenges.

Virology: Miscellaneous

Genotyping of Fowl Aviadenoviruses (FAdV) field isolates from Southern U.S. during 2015 to 2021.

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This is a retrospective study that describes the molecular characteristics of Fowl Aviadenoviruses (FAdV)isolated from poultry samples from Mississippi, Alabama, and Texas between years 2015 to 2021. Most of the samples were from commercial broiler flocks with ages ranging between seven days to 42 days, with the highest number of the presentations between two to three weeks of age. Most of the submissions were associated with high mortality and the presence of intranuclear inclusion bodies in hepatocytes. Viral isolation was conducted using either primary cultures of chicken embryo liver cells or a hepatocellular carcinoma cell line (LMH). Positive isolation was determined by the presence of cytopathogenic effect (characterized by rounded and refractile cells). Partial genotyping of the isolates was conducted by a PCR method to amplify 900 bp of the hexon gene including the L1 loop region, and the amplicons were sequenced. The most commonly isolations were identified as FAdV species E, (serotype 8b), followed by species D (serotype 11). Few isolations of species C (serotype 10), species E (serotype 8a), and species A (serotype 1) were also obtained. The phylogenetic relationships between among are also presented.

Virology: NDV

Comparative efficacy test of different ND vaccination programs in commercial broilers against a Peruvian velogenic NDV isolate

Claudia Carranza[1]

Ceva Animal Health[1]

Comparative efficacy test of different ND vaccination programs in commercial broilers against a Peruvian velogenic NDV isolate C. Carranza1,2, L. Alzamora1, V. Palya3, B. Felfldi3, E. Walk-Kovcs3, T. Tatr-Kis3, L. Sesti4 1 Ceva Animal Health, Peru, 2Avian Pathology Laboratory, Universidad Nacional Mayor de San Marcos, 3Facultad de Ciencias y Filosofa, Universidad Peruana Cayetano Heredia, 3 SSIU-Phylaxia Ceva Animal Health, Latin America, 4Ceva Animal Health, Latin America. Newcastle Disease is a major threat to the poultry industry. In certain geographic areas like Peru, the disease is endemic, causing constant exposure and leading to cases of 90% mortality or higher. The objective of this trial was to compare the efficacy of different ND vaccination regimes against a velogenic NDV strain from Peru (genotype VIIb / most recent classification genotype XII-Diel et al.). A vaccination regime based on conventional regional ND vaccines was compared to rHVT-F alone, or in combination with other conventional ND vaccines. A quite high challenge (10 8 EID 50 /bird va intranasal route) was performed at 4 weeks of age in commercial broilers positive for MDA against Newcastle. Humoral immune response was monitored weekly, starting at day one. Anti-NDV antibody levels in serum samples were measured with Haemagglutination Inhibition test against LaSota strain and BioCheck NDV-F Antibody test kit for rHVT-F vaccines. A 14 day period of observation after challenge was conducted to evaluate clinical protection. The results show that all vaccination programs prevented ND specific mortality, except one vaccination protocol. rHVT-F alone proved to be efficacious in prevention of clinical signs and mortality against NDV challenge.

Virology: Reovirus

Reovirus Growth Kinetics in Embryonated Eggs

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Avian reoviruses (ARV) are ubiquitous in domestic poultry and are widelyheterogeneous in pathogenicity. Currently, viral arthritis/tenosynovitis in broiler flocksreportedly presents the major problem attributed to reoviruses. Many studies have been conducted to detect and characterize ARVs. While virus detection may be done directlyon affected tissues, other molecular techniques typically require isolation of the virus from tissues like tendons, heart, liver, etc., and subsequent propagation in cell culture or chicken embryos. High amounts of virus are usually needed for further work such as genotyping, pathogenicity studies or vaccine production. Thus, a sensitive, less labor- intensive and cost-effective virus propagation technique is needed.Quantitative PCR (qPCR) for ARV S2 gene was used to determine growth efficiency or characteristics of ARV in chicken embryo yolk (CEY) and chicken embryo tissues (CE).Six day old embryonated specific pathogen free chicken eggs were inoculated with ARV(EID50 = 1.6 x 101). After incubating the eggs for 1 hour, as well as after 1, 2, 3, 4, and 5 days post infection, CEY and CE were harvested. The inoculation dose was chosen to not cause embryo mortality. RNA was extracted from CEY and CE samples and then used as templates for qPCR to quantify viral load. Knowing the peak growth and therelative virus content in the compartments will allow harvesting material with the highestvirus titers. Furthermore, ARV growth kinetics can be compared to that of other ARVpropagation techniques such as those grown in primary or permanent cell cultures

Wealth of Knowledge

National Poultry Improvement Plan - A Model Program for the Swine Health Improvement Plan

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National Poultry Improvement Plan[1,2,3], Iowa State University Veterinary Diagnostic Lab[4]

The National Poultry Improvement Plan (NPIP) is a cooperative federal-state-industry program for the application of new diagnostic technology. The program allows participants to earn certifications after testing and demonstrating compliance with monitoring, surveillance, and biosecurity standards according to Title 9 Code of Federal Regulations Part 145, 146, 147, 56 and the NPIP Program Standards, in order to facilitate domestic and international trade. The program, now in its 85th year, serves as the gold standard for poultry monitoring and surveillance. A pilot program to combat African and Classical Swine Fever in pigs is using the programs within the NPIP as a model. This presentation will focus on updates to the NPIP, including highlights from the 45th Biennial Conference, the new Newcastle Disease program, the new Subpart J for game bird participants, and will highlight key features of the new Swine Health Improvement Plan (SHIP)

Physiology and Pathology of Avian Liver.

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Chicken Liver is one of the most important organs in the body which has many functions in maintaining health and productivity. Physiologically, Liver helps in nutrient metabolism, toxins removal, synthesis of certain vitamins, secretion of digestive enzymes etc. Liver is also an edible part of the body along with gizzard and heart. Liver is considered as one of the key indicator of bird's health. Its shape, color, size, consistency indicate many disease conditions. Though the Liver has highest proliferative or regenerative capacity, it functions always with pressure to deliver many physiological activities. At the same, Liver is the primary target for multiple infectious and non-infectious insults. Liver exhibit very typical, pathognomonic lesions for all those insults. Careful and thorough study of Liver's physical nature due to insults will help the clinician to funnel down the diagnosis. With the assistance from laboratory in terms of isolation, identification and histopathology, the diagnosis will be further funneled down. Starting from the vitamin deficiency with the change in size and color, bacteria, virus, protozoa, mycotoxins, management or metabolic conditions will affect the Liver. When the insults go beyond threshold level, then the liver function gets affected which leads to either subnormal performance or mortality. In this presentation, we will be presenting various different types of liver lesions collected from the field cases. Some of the pictures are also taken from reliable sources with due credits to the author. Each picture will be presented with description on gross pathology, etiology, impact on bird health, diagnostic tools, control measures.

Post Cleaning and Disinfection Monitoring by using Real-Time PCR Test in Poultry Houses

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Aviagen Inc.[1]

It is important to determine Cleaning and disinfection (C&D) procedures are well done to reduce the pathogen load in the poultry house before introducing the new flock. The cleaning process is the physical and chemical removal of organic materials (i.e., manure, feed, blood, and dust or feather, and the disinfection process involves the use of disinfectants to reduce the viral and bacterial load. There are many methods to monitor the environment and farm equipment for C&D efficacy. Bacteria can be traditionally monitored by culture on agar. Meanwhile viruses can only be monitored by Viral Isolation and Polymerase Chain Reaction (PCR) test. However, non-viruses such as mycoplasma (MG/MS) and even for Salmonella Spp. nowadays

can be monitored by Real-Time PCR testing with quicker results than conventional method such as isolation.

Supplementation of Bacitracin Methylene Disalicylate or Glucose Oxidase in Broilers Feed on Productive Parameters and Pigmentation

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Excelling SA de CV[1,2], Centro Nacional de Investigación Disciplinariaen Fisiología y Mejoramiento Animal[3,4]

Without the use of antibiotics as growth promoters, chicken and pork production systems require new strategies to protect intestinal health. The challenge is to find solutions to maintain the function of the intestinal mucosa and gut-associated lymphoid tissue to maintain the animals' health and productivity. Glucose oxidase (GOX) is a flavoprotein that catalyzes the oxidation of ß-D-glucose to D-glucono-Õ-lactone (acidifier) and hydrogen peroxide (bactericidal) using molecular oxygen as an electron acceptor. The enzyme has been shown to improve weight gain and feed conversion in broilers and piglets. The experimentconsisted of three groups supplemented with 200ppm of GOX +75ppm of xanthophylls (xan), 200ppm of GOX + 60ppm of (xan), and 500ppm of Bacitracin Methylene Disalicylate (BMD)+ 75ppm of (xan), respectively. Three hundred and sixty male broilers (Ross 308)randomly distributed in three treatments, eight replicates per treatment, and 15 chickens per replica were used. General linear SAS models, ANOVA, least-squares measures, standard error of the means, and comparison of means were used as statistical analyses. As a result, at six weeks of age, we obtained the following productive parameters: weight (AVG 3.200kg), breast weight (AVG 1.974kg), food consumption (AVG 180g/d), weight gain (AVG 80.08g/d), feed conversion (AVG 2.31kg), viability (AVG 95.00%), mortality (5.0%) and productivity index (AVG 445.36), The results about Coliforms stayed in 6.12 UFC log10 for all the groups, and Salmonella sppantibodies (AbX2 IDEXX) and UFC remained negative in all groups. The (AVG) of skin color density (MinoltaCR400) yellow achieved between 42 –52, for red density between 3.5 –10.44. None of the groups presented significant statistical differences ($P<0.05$). We can conclude that the use of GOX in broiler feed proved to be an effective alternative as a non-antibiotic additive, matching the Bacitracin group's conditions.

Trends in Mycotoxin Contamination in United States Corn and Corn DDGs

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Mycotoxins are fungal metabolites that may have detrimental effects on animal health and, even at low levels, can compromise performance. Classic signs such as reduced feed intake, impaired growth, and oral and intestinal lesions are often used as indicators of exposure in the field; however, other costs of mycotoxicosis are often underestimated, including increased frequency and severity of diseases via inflammation, immunosuppression, and modulation of the gastrointestinal environment. For this survey, corn and corn DDGs samples were analyzed for aflatoxins (Afla), type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin-A utilizing LC-MS/MS. Data from the 2020 US corn harvest were compared to previous years to examine contamination trends. Data were analyzed using GLIMMIX procedure of SAS with fixed effect of harvest year. Over the past decade, the average B-Trich and FUM contamination levels in corn have been significantly ($P < 0.05$) affected by harvest year. However, over the last 5 years, B-Trich levels have remained similar, whereas FUM was decreased ($P < 0.05$) from 2019 to 2020. Contamination levels in corn for Afla, A-Trich, and ZEN have not been statistically ($P > 0.05$) different over the past decade. Over the past 5 years, B-Trich, FUM, and ZEN contamination has been significantly ($P < 0.05$) affected by harvest year in corn DDGs samples. The B-Trich contamination level was decreased ($P < 0.05$) from 2019 to 2020, whereas FUM and ZEN contamination levels remained similar ($P > 0.05$). The

risk profile for this year's crop is likely to change as the sample pool expands. Mycotoxin risk of this harvest season is still coming into focus as the combination of hot weather, storm events, and drought during the 2020 growing season resulted in crop stress and damage, ultimately leading to grain quality and mycotoxin contamination concerns.