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Saturday, July 14 Session A

Management

An Investigation of Variables Associated with Reduced Livability in a Broiler Complex in Mississippi

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A southern Mississippi broiler complex in an area of high poultry density has experienced persistent lower livability and growth performance compared with company averages for the state. It was hypothesized that circulating Infectious Bronchitis Virus (IBV) challenge exacerbated by underlying Infectious Bursal Disease (IBD)-induced immune suppression was the primary contributor to reduced livability and live production performance on certain farms, and that disease challenges are most prevalent on farms in areas of high bird density. A retrospective analysis of data from a three-year period (March 2014 through March 2017) was designed to investigate the role of disease, seasonal, and geographic variables in broiler livability and growth performance. A database containing flock settlement data, processing-age ELISA titers (IBD, IBV, Newcastle Disease Virus and Reovirus), broiler vaccination programs, weather trends, and geographic zones was created and statistically analyzed. Preliminary data analysis suggests that there is a correlation between reduced livability and IBV antibody titers, supporting the role of disease challenge in the complex. The highest and lowest performing farms in the complex will be selected for a subsequent sampling study to evaluate bird health and disease challenge. Information from these studies will guide future management and disease control decisions.

What are you doing to day-old turkeys?

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We often get questions on what people are doing to their poults. Sometimes the questions are around injectable vaccinations, sometimes it is around probiotics sometimes it is around bird treatments. Then the question is usually: is that a good thing or a bad thing? A consolidated dataset from the last years' worth of turkey poult placements in our tracking system will be reported that will cover vaccine usage, probiotic usage, and bird treatments that were applied to poults in our hatcheries and delivered across the US into various systems. Then where available the effect of these various treatments will be shown relative to the reported starting mortality of these birds. Data will be presented as overall data and broken down by sex. Producer identity and region/location will not be shared. If this presentation was found to be useful by the AAAP and audience, we would be willing to attempt to make this a yearly presentation at AAAP.

Necrotic enteritis and the causal pie

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The role of a production veterinarian often includes explaining complicated disease issues to non-veterinarians including farmers, technicians, and production managers. Necrotic enteritis is a complex and multifactorial disease issue. The causal pie is an epidemiological tool that is useful for understanding the contributions of multiple factors to the development of the disease.

Using Decision Analysis to Determine Quarantine Times for International Travel

Donna Carver, DVM, PhD, ACPV

Quarantine is often required to limit disease spread. Human quarantines are generally specific to one known disease. This approach allows the quarantine to be based on a known incubation period. People traveling internationally become exposed to a disease not present in the United States. Ebola virus disease (EVD) is one such disease. Potential exposure is known, the incubation period is 21 days and people likely to have been exposed to the virus are monitored for 21 days to identify any symptoms compatible with EVD. Poultry diseases are not as publicized as EVD so international travel can result in

exposure to unknown or foreign animal pathogens. This poses a threat to poultry that has contact with the traveler upon return to the workplace. Currently many travelers quarantine themselves from poultry flocks for some specified length of time (2 or 3 weeks). These times are often arbitrary but deemed necessary by people who have a vested interest in the poultry flocks. This paper explores using a combination of known pathogens in an area, survival of the pathogens, the purpose of the trip, the likely exposure to the pathogens, known reservoirs, the amount of travel time and the value of the birds being protected, to establish quarantine times. Consultants, conference participants, poultry disease laboratory visitors, and tourists do not have equal risks for returning to the workplace with viable pathogens and should not be subjected to equal quarantine times away from the workplace.

Compartmentalization: Primary Breeders Achieve First U.S. Avian Influenza Clean Compartment

Elena Behnke[b], Eric Jensen[a], Kate Hayes[c], Carolyn Miller[b], Phillip Eidson[c], Ken Powell[c], Will Dillard[c], Sara Reichelt[c], Bill Stanley[a], Gregorio Rosales[d]

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Aviagen became the first U.S. Poultry Primary Breeder Company to achieve USDA certified compartment status on October 20, 2017. Aviagen's pedigree and great-grandparent facilities were audited and certified that they met standards established by USDA APHIS. The USDA Avian Influenza compartmentalization program, which sets a high standard for disease control and prevention, was first adopted as regulation in the Code of Federal Regulations in 2006 and fully established in 2016. The program's goal is to preserve trade with key countries in the face of future Avian Influenza outbreaks when regionalization is no longer feasible. Compartmentalization offers trading partners peace of mind that if H5 or H7 Avian Influenza is detected, it will be reported promptly, and any contaminated products will be diverted from the market without risk to other countries' poultry industries. Additionally, compartmentalization can preserve interstate movement of breeding stock to domestic customers and operations in the event of future Avian Influenza outbreaks.

Reovirus

Reovirus Pathogenesis in Broiler Chickens Post *In Ovo* Infection

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Reovirus infections and associated diseases continue to be an economically-significant factor for U.S. broiler chicken producers. Currently the most important reovirus-associated disease in broiler chickens is viral arthritis/tenosynovitis. Since 2011 chicken reoviruses have continued to shift antigenically, which complicates vaccine-based control strategies. One of the ways reoviruses change is through reassortment of genome segments when different genotypes of reovirus coinfect chickens. Reovirus reassortants can be transmitted horizontally and vertically. Horizontal transmission of reovirus is well documented, but few details are known about virus transmission from hen to progeny and subsequent pathogenesis in vertically-infected chickens. Several vertical transmission models were evaluated, and one was selected for further studies. Pathogenesis, seroconversion and duration of reovirus shed in *in ovo*-infected chickens will be discussed.

Reovirus variant D as the cause of severe lameness in Ontario broilers in 2017

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Since the spring and summer of 2012, Ontario broilers had experienced increasing leg problems presenting as splay leg and difficulty walking. Since that time reovirus issues declined but, in the fall of 2016, and throughout 2017 there have been cases of Ontario broilers with severe leg problems presenting as lameness, splay leg and tenosynovitis. As previously observed, birds submitted to the Animal Health Laboratory were found to have non-suppurative tenosynovitis and epicarditis composed predominantly of lymphocytes and plasma cells with development of lymphoid nodules. These cases were reovirus positive but on viral sequencing the isolate was identified as variant D. The flocks affected with this strain of reovirus experienced increased culling rates ranging from 2% to 50%. The age of the affected flocks were variable but younger affected flocks had more severe clinical outcomes. Birds that made it to slaughter

had increased culling rates. The introduction, progression and clinical picture of reovirus variant D will be discussed.

Whole Genome Sequencing - A Key To Solve The Problem Of Re-emerging Turkey Arthritis Reovirus

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Since 2011, we at the University of Minnesota Veterinary Diagnostic Lab (MVDL), have seen an increase in the number of turkey arthritis cases. Autogenous vaccines were able to slow down the infection at first but multiple turkey flocks are now presenting with viral arthritis and the disease continues unabated. The clinical picture has also changed; signs of cartilage erosion and presence of disease in younger turkeys at the age of 5-7 weeks. We conducted this study to characterize 227 'old' and 'new' strains of turkey arthritis reoviruses (TARVs) isolated between 2011 and 2017 from 11 different U.S. states. Of the 227 isolates, 130 TARV were selected for whole genome sequencing (WGS) based on different states/geographical areas, year of isolation, turkey age group, flocks type (breeder/ commercial) and different submitters. Twenty isolates of turkey enteritis reovirus (TERV) were also included. The Illumina MiSeq 250 paired end cycle was used for WGS. The obtained MiSeq files underwent quality check assembly by using a pipeline developed in our lab. Whole genome sequence analysis will not only help us to genetically characterize the newly emerging TARVs but also to find out molecular markers to differentiate TERVs and TARVs and to select a suitable vaccine candidate that represents and confers immunity against majority of TARV strains. These results will be discussed in detail at the conference. The data should be helpful in understanding the emergence and evolution of TARVs in the United States.

High-resolution Linear Epitope Mapping of B-cell Responses Following Immunization with Avian Reovirus S1133

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Avian reoviruses are causative agents of tenosynovitis and viral arthritis. Commercially available reovirus vaccines do not protect against challenge with emerging variants associated with tenosynovitis and lameness. Autogenous reovirus vaccines are commonly utilized to help protect against disease. Neutralizing epitopes of the host protective protein, Sigma C, have not been clearly defined. In this study, linear epitope mapping of the Sigma C protein was performed on polyclonal chicken sera from SPF chickens vaccinated twice with S1133 commercial live attenuated/inactivated vaccines in the following treatments: 1) live and live 2) inactivated and inactivated 3) live and inactivated. Serum samples from individual birds were selected for epitope mapping based on virus neutralization and Western blot analysis. Epitope mapping was carried out using S1133 Sigma C sequence translated into linear 15 amino acid peptides with an overlap of 14 peptides. Multiple proposed epitopes and single peptide interactions were identified. There was a common antibody response shared among the three vaccination groups around peptides with a consensus motif located on the intracapsid region of Sigma C. The group two antibody response profile had higher detection intensities and more antigenic epitopes for peptides associated with the globular head of Sigma C than the other two groups. Identification of immunogenic epitopes for S1133 will serve as a baseline for the analysis of variants.

Genetic Characterization of Turkey Reoviruses from clinical cases of tenosynovitis in South Dakota

Luke G. Baldwin, Erich G. Linnemann, Holly S. Sellers

Avian reoviruses are non-enveloped, RNA viruses that, while ubiquitous in the poultry environment, are also the etiologic agent for viral tenosynovitis in both turkeys and chickens. Turkey viral arthritis associated with reovirus was first described in the early 1980's, but historically considered a minor clinical problem. In 2011, an increase in clinical tenosynovitis associated with variant reoviruses was observed in both turkeys and chickens across the United States. Turkeys with reovirus tenosynovitis exhibit clinical signs and gross lesions such as lameness, enlargement of the hock joints, varus and valgus angular limb deformities, uneven gait, and recumbency. Genetic characterization of chicken and turkey reoviruses is based on the sequence of the host protective Sigma C protein. In this study, genotypic characterization of 6 turkey reovirus field isolates from South Dakota, from 2016-2017, was performed. The complete coding region of the Sigma C protein was

amplified by RT-PCR then sequenced. The genotypes for each of the turkey reoviruses were determined by phylogenetic analysis of the Sigma C. The turkey reoviruses from South Dakota belong to a genetically distinct subgroup within genotype 2 along with other turkey arthritis reoviruses in the database. The amino acid similarity among the isolates ranged from 92-97% providing evidence that multiple reoviruses were circulating among the SD flocks during 2016-2017.

Molecular Characterization of Emerging Avian Reovirus Variants Isolated from Viral Arthritis cases in Western Canada

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Viral Arthritis (VA), a disease caused by pathogenic avian reovirus (ARV), has emerged as a significant cause of economic losses in Broiler flocks in Western Canada. The objective of the study was to molecular characterize the ARV strains isolated from VA cases originated from Western Canada. From 2012-2017, a total of 94 VA cases were diagnosed and 38 ARVs were isolated mainly from tendons and joints. The observed cases had 4-13% morbidity on average, followed by a spike in mortality/culling that, in extreme cases, required the culling of the entire flock. Molecular characterization of a partial segment (first 300 amino acids-aa) of the Sigma C gene showed that the 38 ARV sequences grouped in all six of the previously published ARV clusters. The most common clusters were #4 with 11 isolates (29.7% of the total), and #5 with 13 isolates (35.1% of the total). The most variable clusters were #1 with 76.7-100% aa identity between isolates, #2 with 66-99.3%, and #4 with 62-100%. To the best of our knowledge, this is the first report showing the wide genetic diversity of ARV cluster 4 in field ARV isolates and the circulation of all six reported ARV clusters in Canada. The data obtained in this project will be used to select relevant ARV isolates to be included in an efficacious ARV autogenous vaccine program.

Saturday, July 14 Session B

Bacteriology

Characterization of *E. coli* Isolates from Clinical Cases in the US Commercial Layer Industry

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The aim of this study was to identify, characterize and compare the *Escherichia coli* serotypes that are currently circulating in the US commercial layer industry. The *E. coli* isolates were recovered from clinical cases of colibacillosis in commercial layers in 2017. Early mortality (4-8 days) and cases of peritonitis and salpingitis during production were included in the study. Flocks were vaccinated against *E. coli* using an O78 live vaccine. A total of one-hundred *E. coli* strains were evaluated through O serotyping, phenotyping, PFGE, virulence profiles and genome sequencing. *E. coli* serotype O2 was the most common serovar detected (about 30%). Other serotypes included *E. coli* serotypes O15, O18, O21, O25, O45, O5, O8, O78. Multiple phenotypes and PFGE patterns were detected among and within serotypes. Virulence and genome sequencing results will be discussed. Based on these results, the control of colibacillosis in commercial layers should consider vaccination against *E. coli* serotype O2 and also against other serotypes. In addition, due to the limited cross-protection reported between serotypes, a continuing monitoring for emergent serotypes is required not only to evaluate the efficacy of the vaccination but also to determine if any modification in the vaccine formulation is needed.

Avian pathogenic *E. coli* levels across the US broiler and turkey industries.

Hutchison, E., Karunakaran, D., Anderson, S., Vang, E., Wujek, R., Rehberger, T.

Avian colibacillosis, a systemic infection caused by *E. coli* – specifically avian pathogenic *E. coli* (APEC), is an economically important disease in the commercial poultry industry due to loss of yield. APEC infection is a common problem in the US turkey, broiler, and layer industries, and has traditionally been controlled through the use of antibiotics. But as consumer demand and new regulations continue to pressure producers to phase out

antibiotics, alternative means to effectively deal with APEC are needed. One alternative is treatment with direct fed microbials (DFM), or probiotics, to combat the prevalence of APEC. In this current study, survey data from US broilers and turkeys was analyzed to determine overall effectiveness the AHAN DFM program. This analysis comprised >3,000 broiler and turkey gastrointestinal tracts (GIT) of birds that were participating in the AHAN DFM program (treated) and birds that were not participating (untreated). Ages of the birds ranged from 6 to 70 days (broilers) and 0 to 18 weeks (turkeys) and were collected from dozens of companies over a period of 3 years, using a standardized sampling method. Results show that the average level of APEC in both turkeys and broilers was significantly lower in birds treated with AHAN DFM than untreated birds. A break-down of average APEC levels by age shows that AHAN DFM-treated broilers and turkeys had lower APEC levels at every week of production tested. These results show an industry-wide trend that provides compelling evidence for the effectiveness of the AHAN DFM program at reducing APEC levels in commercial turkey and broiler systems.

Studies on Clostridial dermatitis, A disease of economic concern in turkeys

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Clostridium perfringens and *Clostridium septicum* have been isolated consistently from dermatitis lesions in turkeys. Disease pathogenesis for Clostridial Dermatits (CD) is inadequately understood. There is very little information as to how exactly these bacteria gain access and play a role in producing dermatitis lesions in turkeys in light of their (*CP / CS)* presence in most of the turkey growing environments. Our long-term goal is to understand the underlying causes and or potential risk factors that may contribute to the development of CD in turkeys. The purpose of this study was to evaluate the effect of dermal scratch to facilitate Clostridial infection and causation of dermatitis in turkeys. A total of seventy-two (9 weeks) old Nicholas turkeys were used. Birds were tagged and divided in 2 groups i.e., Scratched and unscratched. Each group was further divided into 3 subgroups. Litter was sprayed with *Clostridium perfringens* in Room 1 and 4, *Clostridium septicum* in Room 2 and 5 and Normal Saline in Room 3 and 6. Scratches were made with the help of 0.5-inch, 18-gauge needle to create two parallel lesions 2 to 3 cm in length on the anterior region of the ventral surface parallel to the keel. Blood from each bird was collected on Day 0, 14 and 28. Two birds from all subgroups were necropsied on 7 and 21 days of treatment and tissues were collected. Remaining birds were euthanized on Day 28 in CO2 chamber. Rectal swabs, Litter samples and fresh droppings were collected on Day 0, 7, 14 and 28 and analyzed for Clostridial load by Q-RT PCR. Experiment 1 has been completed and analysis of samples collected is in progress. Initial results of this experiment appear to indicate that subcutaneous injuries promote the development of dermatitis lesions from *C. septicum* and/or *C. perfringens* from bedding material. The sequential development of lesions post exposure to CP/CS with cutaneous injuries.

Evaluation of Environmental and Tissue Microbiota using 16s Metagenomics Analysis for Understanding the Etiology behind Turkey Cellulitis

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Turkey cellulitis (TC), or clostridial dermatitis, is a condition which causes mortality and plant condemnation in market age turkeys. It is a nationwide health concern and ranks as the third most important issue facing the turkey industry. The objectives of the study are to correlate the etiology of TC with various environmental factors, as well as to analyze samples with metagenomics analysis to observe potential shifts in microbiota in diseased animals. Fecal samples, water swabs, and litter were collected weekly from 6 locations in Iowa. Three control and three case farms were utilized for sample collection. Case flocks qualified if mortality from TC exceeded 0.5 per 1000 birds for two consecutive days. In case barns, fecal samples and select organs were collected aseptically at necropsy and submitted for bacterial determination and metagenomic analyses. External temperature, humidity, and litter temperatures were recorded weekly. Dry matter percentage and

particle size analysis were performed from each litter sample. Litter and water samples were cultured for determining cfu's for aerobic, anaerobic, and coliform bacteria from each sample. Preliminary results indicate an inverse relationship between the particle size of litter contents and aerobic and anaerobic bacterial growth. Trends from the case barns indicate higher aerobic and anaerobic bacterial growth in litter when compared to the control barns. Data also suggests an inverse relationship between coliform and aerobic and anaerobic bacterial growth in case barns. Further metagenomics analysis is in progress, which will help determine trends in microbiota in the environment, healthy turkeys, and diseased birds.

Ulcerative duodenitis secondary to *Clostridium perfringens*: a new disease in ABF and organic broilers?

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Clostridium perfringens has long been associated with numerous disease processes in commercial poultry production, however one of those conditions is not ulcerative duodenitis. In the past year, multiple ABF (antibiotic free) and organic flocks, typically experiencing outbreaks of necrotic enteritis, have shown multiple birds that exhibit superficial, multifocal, ulcers within the duodenum. The duodenum is red streaked with ulcers typically taking on an off white to tan appearance. Histopathology revealed the presence of true ulcerative lesions. Initially the condition was thought to be caused by *Clostridium colinum*, long associated with ulcerative enteritis in quail, however bacteriology of affected duodenums and accompanying livers and spleens yielded true cultures of *Clostridium perfringens*. This begs the question as to whether this represents a truly unique, and new manifestation of *Clostridium perfringens* that may be unique to ABF and organically produced broilers. With internal retrospective analysis, we hope to determine if there is a correlation between the severity of necrotic enteritis (in terms of lesion appearance and mortality numbers) and the presence of ulcerative duodenitis lesions. We are currently working on identifying associated risk factors (i.e. worse in organic or ABF flocks) and the effects of the condition on measurable production parameters. As more of the broiler sector transitions over to ABF and organic production, it will become increasing important to better understand all new, and old, disease challenges as the means to treat them are severely limited by the nature of ABF and organic production.

Genotyping and phylogenetic analysis of *Ornithobacterium rhinotracheale* isolates from Peru.

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Ornithobacterium rhinotracheale is a bacterium associated with respiratory disease in poultry. In Peru, *O. rhinotracheale* isolates obtained during 1998-2000 have been reported previously, all isolates presented the same Enterobacterial repetitive intergenic consensus-based PCR (ERIC-PCR) profile, representing only one genotype. In 2016, two isolates of *O. rhinotracheale* were reported in co-infection with *Avibacterium paragallinarum* from layers in Peru. However, the genetic relationship between the recent *O. rhinotracheale* isolates from Peru are unknown. In this study, 11 *O. rhinotracheale* isolates obtained during 2010 to 2017 from layers (3 isolates), broilers (7 isolates) and a backyard chicken (1 isolate) with respiratory diseases were confirmed by a specific PCR. The ERIC-PCR was used for the genotyping of the isolates, three different ERIC-profiles were identified, eight isolates presented the same ERIC-profile (profile I). However, one isolate and two isolates presented the profiles II and III, respectively. A phylogenetic analysis of the partial sequences corresponding to 16S rRNA gene of the isolates included and sequences of *O. rhinotracheale* corresponding to different serotypes reported to GenBank were clustered and formed five main well-supported clades. Four isolates formed a clade separed from the rest of the isolates. Additionally, one Peruvian formed another clade separed and was a different ERIC-PCR profile. In conclusión, genetic diversity between Peruvian *O. rhinotracheale* isolates exist. The ERIC-PCR and phylogenetic analysis of the 16S rRNA gene could be important tools for the genotyping of *O. rhinotracheale* isolates.

Overview of an outbreak of infectious coryza in California in 2017

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Infectious coryza is an acute respiratory disease of chickens caused by *Avibacterium paragallinarum*. The California Animal Health and Food Safety Laboratory system (CAHFS) typically diagnoses this bacterial infection in less than 10 necropsy case submissions per year. However, during 2017 the Turlock branch of CAHFS experienced an increase of submissions, with a total of 54 confirmed cases. The majority of cases (n=40) was received from commercial broiler operations, followed by commercial layers (n=11) and backyard chickens (n=3). Clinical history included respiratory signs, increased mortality and poor performance. Swollen head-like syndrome and sinusitis were commonly observed in broiler cases. Severe neurological signs were also described in one case. Layer submissions frequently revealed polyserositis with or without involvement of the upper respiratory tract. Diagnosis was based on bacterial cultures and PCR. *A. paragallinarum* was recovered from infraorbital sinus (n=47), air sac (n=14), heart (n=7), lung (n=4), trachea (n=4), conjunctiva (n=1), brain (n=1), cranial bone/ear (n=1) and ovary (n=1). Infections complicated by other pathogens were frequently observed. In particular, Infectious bronchitis virus (IBV) was detected from tracheal swab pools by qRT-PCR (n=24). The IBV was isolated by viral isolation technique in 12 cases and 4 different strains were identified by sequencing analysis: CA1737 (n=8), Ark DPI (n=2), Cal 99 (n=1) and Cal 3099 (n=1). A few submissions were positive for *Mycoplasma gallisepticum* (n=7) and *Mycoplasma synoviae* (n=5) by qPCR. In addition, an endemic strain of infectious bursal disease virus (IBDV) was detected by qRT-PCR on bursa pools (n=16).

A survey of enterococcal and bacterial spondylitis in broiler chickens and review of pathogenesis

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Bacterial spondylitis is a significant cause of impaired mobility, decreased production, and mortality in broiler chickens. *Enterococcus cecorum*, a Gram-positive, facultative anaerobe, is often isolated from vertebral lesions; however, other bacterial species can be cultured. *E. cecorum* infection can also result in femoral osteomyelitis, pericarditis, and hepatitis. The pathogenesis of enterococcal spondylitis has not been fully elucidated, although multiple predisposing factors have been proposed. A recent report identified a link between osteochondrosis (OCD) of the free thoracic vertebra and colonization by *E. cecorum*; mechanical stress and weight-bearing may predispose this site to OCD. The study also found earlier intestinal colonization with pathogenic strains of *E. cecorum* in birds with spondylitis compared to non-affected birds. Previously, one study found no significant difference in the occurrence of OCD lesions among four broiler strains (including a heritage strain) or other factors. Causes of vertebral OCD, sources of *E. cecorum*, and predisposing factors for bacterial colonization remain areas of research interest. In a review of cases of bacterial spondylitis presented to the Poultry Research and Diagnostic Laboratory, “leg problems”, lameness, or paralysis are reported; clinically, birds are often unable to stand. At necropsy, distortion of the free thoracic vertebra with exudate is a common finding. Histologically, lesions include mixed inflammatory infiltrates, osteonecrosis, frequent intralesional bacteria, and occasional vascular thrombi; chronic lesions include fibrosis. In some cases, vertebral growth plates are also affected, with chondrodegeneration or epiphysiolysis. *E. cecorum* is often isolated from vertebral lesions; *Enterococcus faecalis*, *E. coli*, and *Salmonella sp.* are rarely identified.

Microbiome in the upper and lower respiratory tract and standardization of sample collection methods in clinically healthy chicken layers

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Complex microbial communities (microbiome) that occupy the respiratory system have a significant influence on poultry health and productivity. To discover the role of microbiome in host susceptibility and response to respiratory pathogens, we first need to define the baseline respiratory microbiome in clinically healthy birds. We began by following a commercial chicken layer flock for one year to collect sinus and trachea washes for bacterial community profiling through the sequencing of the 16S rRNA gene. Bacterial beta-diversity was highly affected by age and habitat within the respiratory system (sinus vs trachea). The respiratory microbiome from individual birds clustered according to the brooding, grow-out, and egg laying (adult) stages of the chicken. Shannon index and species evenness and richness analysis revealed a positive correlation between age and alpha diversity, especially in the trachea. At class level taxonomic resolution, the respiratory bacterial communities were characterized by high levels of Actinobacteria and Deinococci in the sinus, Betaproteobacteria in the trachea, and Bacilli in both sinus and trachea. Microbiome in the upper respiratory tract (trachea and sinus) may not fully represent the respiratory microbiome due to constant introduction or loss of taxa through air and mucociliary clearance. In a subsequent study in specific-pathogen-free chickens, we have divided the respiratory tract into its upper and lower components. We hypothesize that the lower respiratory tract is stably colonized by a microbiome that is distinct from that observed in sinus or upper trachea. We also show that some sampling methods are not optimal for respiratory microbiome profiling.

Reducing Microbial Load On Hatching Eggs Using A Dry Hydrogen Peroxide Gas System

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It is known that eggs have an extensive microbial population on the shell which can impact hatchery cleanliness as well as chick health. Sanitation methods that reduce bacterial loads are essential, but washing hatching eggs removes the protective cuticle layer. The objective of this study was to determine if a novel dry hydrogen peroxide (DHP) gas could reduce the microbial load on hatching eggs, since it has previously been shown to reduce microbial loads on surfaces in hatcheries. In trial 1, eggs were placed in a controlled space while trial 2 took place in a hatchery egg cooler. Microbial load in both trials was evaluated by washing eggs in tryptic soy broth (TSB) after 0, 24, 72, and 120 hours of exposure to DHP. Samples were then serially diluted and inoculated onto MacConkey or tryptic soy agar, and most probable number (MPN) calculations were obtained. Trial 1 results showed that the treated group had less growth on TSA plates after 72 hours ($p=0.05$) of exposure compared to time 0, while the non-treated group had similar levels. In trial 2, the treated group showed more growth on TSA at 120 hours ($p=0.0001$) but reduced growth on MacConkey media at 72 and 120 hours ($p=0.001$). The non-treated group showed increased growth on TSA beginning at 72 hours where MacConkey agar had increased growth at 24 hours but less growth at 72 hours ($p=0.05$). This data indicates that the DHP system can prevent expansion or reduce microbial load on hatching eggs after exposure.

Bacterial composition of *in ovo* broilers.

Hutchison, E., Karunakaran, D., Smith, A., Rehberger, T.

In most animals, the early microbiome is heavily influenced by maternal microbiota. This is especially true for brooding species, such as turkeys and chickens, which endow their offspring with microbes by inoculating the egg during oviposition, and through exposure to the maternal fecal matter in the nesting environment. Many of these inherited microbes may be evolutionarily important for the fitness of the offspring, since it is known that the microbiome plays a critical role in the development and modulation of the immune system. Recent studies of commercial broiler systems have found that very few commensal bacteria, such as lactic acid bacteria (LAB), transmit from breeder hens to their day-of-hatch chicks, while opportunistic species, like *Escherichia coli* and *Enterococcus facalis*, are found in both mother and offspring. This may be due, in part, to the design of modern hatchery systems, which separate eggs from their mothers, and often sanitize egg surfaces. These processes allow persistent organisms like *E. coli* and *E. faecalis* to inhabit niches that would normally be occupied by maternally-derived commensals. In an effort to better understand when and how *E. coli* and *E. faecalis* establish themselves in day-of-hatch birds, the microbial composition of broiler breeder hens and their corresponding egg clutches within a large broiler

integrator will be analyzed. Gastrointestinal tracts (GIT) and nest boxes from breeder flocks will be sampled at lay of the targeted clutches. The eggs will be sampled at early incubation, immediately before transfer to the hatching room, 24 h post-transfer, and at hatch. All microbial analyses will include traditional plating methods as well as culture-independent community analysis. The results of this study will provide insights into the means by which pathogenic organisms establish themselves *in ovo*, helping integrators to pinpoint and reduce vulnerabilities in order to improve chick quality.

Sunday, July 15 Session A

Antimicrobial

The prevalence of methicillin resistant *Staphylococcus aureus* in Pennsylvania poultry meat

Donna Kelly, Lisa Murphy

University of Pennsylvania School of Veterinary Medicine, Pennsylvania Animal Diagnostic Laboratory System - New Bolton Center, Kennett Square, Pennsylvania, USA.

In an international study published in 2016 by Jesper Larsen et al, detailing an investigation of methicillin resistant *Staphylococcus aureus* (MRSA) in people, it was determined that the MRSA was of poultry origin. The patients did not have access to farms or food animals. The conclusion was the patients most likely contracted the illness from the consumption of contaminated poultry meat. Other investigations over the years in the United States have determined the prevalence of MRSA in poultry meat to be approximately 1.8%. A study was conducted in Pennsylvania to determine whether the current MRSA prevalence was similar, determine sources and to look for possible control points. Seven types of poultry meat products were collected from in southeastern Pennsylvania, from twenty-five retail facilities, in three separate sampling rounds, over a period of five months. The retailers included large commercial grocery chains, independent grocers, discount grocers and local markets. The meat samples were tested according to the United States Food and Drug Administration Bacteriological Analytic Manual for *Staphylococcus aureus* isolation. Antimicrobial susceptibility patterns were determined using the ARIS Sensititer system. Methicillin resistance was confirmed by BBL Cefinase™ and PBP2 reactions. PCR for *mec* A/*mec* C genes and sequencing were performed on the MRSA candidates. The study findings will be presented.

Effect of an organic acids based feed additive and enrofloxacin on the prevalence of antibiotic-resistant *E. coli* in cecum of broilers

Nataliya Roth[1], Sigrid Mayrhofer[1], Martin Gierus[2], Christine Weingut[1], Christiane Schwarz[2], Barbara Doupovec[3], Roger Berrios[3], Eduardo Vicuna[3], and Konrad J. Domig[1]

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Antibiotic use is monitored in some countries in order to avoid resistance. Resistance against fluoroquinolones is high in countries where this antibiotic is used. The objective of this study was to investigate the effect of feeding diets with an acids-based additive, as well as fluoroquinolone antibiotics, on the prevalence of antibiotic resistant *E. coli.* Four hundred Ross 308 broilers were randomly divided into 3 treatments: the first group, negative control, was not supplemented with any feed additive; the second group has received feed additive based on formic, acetic, and propionic acid (FA) and the third group has received enrofloxacin (AB) in water. Fecal samples of one-day old chicks were collected. On d 17 and d 38 of the trial, cecal samples from each of the 8 pens were taken, and the count of *E. coli* and antibiotic-resistant *E. coli* was determined. Antibiotic-resistant *E. coli* in one-day-old chicks was shown. Broilers supplemented with FA and treated with AB did not have a significant influence on the total number of *E. coli* in the cecal content on d 17 and d 38 of the trial. FA supplementation improved growth performance and significantly decreased ($P \leq 0.05$) *E. coli* resistance to ampicillin and tetracycline compared to the control and AB groups, as well as decreased ($P \leq 0.05$) sulfamethoxazole and ciprofloxacin-resistant *E. coli* compared to the AB group. AB treatment increased ($P \leq 0.05$) the average daily weight compared to the control group and increased ($P \leq 0.05$) the number of *E. coli* resistant to ciprofloxacin, streptomycin, sulfamethoxazole and tetracycline.

Pen Trial to Evaluate Effects of Necrotic Enteritis Disease Prevention Antibiotics on Antimicrobial Resistance in Broiler Litter

Randall Singer[1], Britta Wass[1], Charles Hofacre[2], Doerte Dopfer[3], Elise Lamont[1], Kelly Schultz[3], Bob Wills[4], Tim Johnson[1]

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Antibiotics and ionophores are used in broiler production to prevent necrotic enteritis (NE) and coccidiosis, respectively, but little is known about resistance selection following the administration of these compounds. The objective of this pen trial was to quantify the effect that these administrations have on the microbiome in the litter over time as well as on the quantity of antibiotic resistance genes in the broiler environment. The study consisted of 5 pens per antibiotic treatment group with 60 day-old chicks placed per pen. For this presentation, data from four of the treatment groups will be discussed: 1) No narasin, no antibiotic; 2) narasin (70 g/ton), no antibiotic, 3) narasin (70 g/ton), oxytetracycline (100 g/ton); 4) narasin (70 g/ton), oxytetracycline (400 g/ton). Birds were fed antibiotics and ionophore from days 1 through 28, and birds were sacrificed at day 35. Following a 7-day downtime, birds were again placed in the pens and assigned the same treatments. A total of 3 flocks were followed per pen. Tetracycline concentrations in the litter were assessed with mass spectrometry. DNA was extracted from the litter of every pen weekly. Microbiomes were assessed by sequencing the 16S rRNA gene. 47 antibiotic resistance gene quantities were assessed with a quantitative PCR microfluidic device. In the pens that used oxytetracycline, there was an accumulation of antibiotic in the litter over the flock cycle, and because the litter was reused, there was carryover of tetracycline in the litter in the high dose pens. There were no significant differences in gene quantities among the groups, although there were differences in microbial community structure. Resistance gene quantities and microbiomes did change over time as each flocked aged.

Trends in Antibiotic Use in an Integrated Poultry Company

Becky Tilley, DVM, DACPV

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FDA's Guidance for Industry #209 and #213 recommended drug sponsors voluntarily make human medically important antibiotics available for food animals by prescription only for water soluble products and Veterinary Feed Directive for antibiotics in the feed effective January 1, 2017. Prior to that date, most antibiotics approved for use in food animals were available over the counter. This presentation will look at trends in antibiotic use in an integrated poultry company over time as a result in this change. We will examine trends in the antibiotics most commonly used. Commonly diagnosed disease conditions requiring antibiotic treatment and changing disease trends will also be examined.

Quantifying Antimicrobial Use in Poultry Production

Randall Singer

Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota

With the need to better understand the ways in which antimicrobials are used in human and veterinary medicine, antibiotic use data collection efforts are now underway in different agricultural commodities. As has been described previously, simple comparison of gross use estimates of different antimicrobials is of limited value due to differences in potencies, duration of activity, relative effect on target and commensal bacteria, and mechanisms of resistance. The objective of this continuing project is to estimate the quantities of different antimicrobials used in poultry production (broiler, turkey and layer) for specific indications and applications. A survey of production companies using a top-down approach to determine the range of antimicrobial usage practices across the industry was conducted. An update of this effort will be provided along with the challenges of establishing metrics for reporting antimicrobial usage in poultry production.

NPIP

National Poultry Improvement Plan

The National Poultry Improvement Plan (NPIP) is a voluntary disease control program for the poultry industry in the United States. The objective of the NPIP is to provide a cooperative Industry-State-Federal program through which new diagnostic technology can be effectively applied to the improvement of poultry and poultry products throughout the country. Further, the NPIP establishes the regulatory standard in sample collection, diagnostic tests performed, and the laboratory protocols for conducting tests. Over the past year, modifications and changes to the NPIP federal regulations and the Program Standards document have been made. A new biosecurity program has been implemented for the poultry industry and the first compartmentalization program for primary breeders in the USA has been established.

Case Reports

The Impact of Histomoniasis on Turkey Breeder Production

Brian Wooming

Cargill Turkey Production LLC, Springdale, AR

Histominasis in turkeys has been observed with increasing frequency in our complexes since the beginning of 2016. In many cases, the disease has caused severe mortality, and in several instances, has led to the decision to terminate the flock. This case report will focus on two severe cases of the disease in breeder candidates in 2017, and will discuss treatment responses in each case. Surviving members of each flock were followed through lay, and the impact of the disease on production will be discussed.

Clinical investigation of neurological symptoms in a flock of turkey breeding toms

Marissa Garry

Select Genetics, Willmar MN, United States.

In late July, a flock of 46 week old stud toms were experiencing pronounced neurological signs. Tissue samples, serology, tracheal swabs and environmental samples were collected and sent to the University of Minnesota Veterinary Diagnostic Laboratory to rule out common causes of neurological symptoms in turkeys. Sample results were negative for viral and bacterial agents. After further investigation, toxicology of the brain tissue identified suppressed brain cholinesterase activity suggesting organophosphate toxicity.

Rickets Subsequent to Mineral Segregation in Commercial Organic Turkeys

Molly E. Parker

Butterball, LLC, 1 Butterball Lane, Garner, NC 27529

Rickets has been well described in the literature as a nutritional disease which is rarely seen in modern commercial poultry production. A flock of commercial organic turkey poults presented with the complaint of difficulty walking and weak legs. Necropsy findings, histopathology, and feed analyses confirmed the lameness was the result of rickets due to inadequate calcium ingestion. Feed sample analyses indicated that the organic mash diet was appropriately formulated; however, mineral segregation resulted in inadequate calcium intake. This case demonstrates the importance of not only proper feed formulation but also proper feed distribution, particularly for young birds which have a lower daily feed intake level.

Atypical Presentation of Fungal Pneumonia in Turkey Poults

M.E. Lighty

Jennie-O Turkey Store, Willmar, MN 56201

Fungal pneumonia, also referred to as brooder pneumonia or Aspergillosis, typically presents with white, yellow, or grey circumscribed nodules or plaques in the lungs and/or airsacs of affected turkey poults. This field report will describe a series of cases where various types of mold, including *Aspergillus fumigatus* and *Rhizopus*, were cultured from the lungs of turkey poults in the absence of grossly visible granulomas. Affected flocks presented as increased mortality with varying degrees of lung congestion. Flock history, necropsy findings, and results of diagnostic testing will be reviewed.

AN UNUSUAL CASE OF BOTULISM IN COMMERCIAL TURKEYS

Robert L. Owen, VMD, Ph.D., DACPV, Patricia Dunn, DVM, MAM, DACPV, Eva Wallner-Pendleton, DVM, MS, DACPV

A significant mortality increase was reported in thirteen-week-old male commercial turkeys. Clinical signs included lameness and inability to ambulate normally in approximately 10% of the animals. Extensive laboratory testing of multiple submissions of animals, feed samples, and water samples failed to reveal any demonstrable cause for the lameness and mortality. A sister flock from the same breeder flock sources sharing a common well and feed source were unaffected. The flock was eventually processed and the poultry house was completely cleaned and disinfected. Upon placement the subsequent flock was normal until 4 weeks of age when the birds exhibited the same symptoms. This time the sister house also became infected. Multiple submissions again revealed nothing significant until intestinal contents were submitted for PCR testing for botulism toxin and the results were positive. Managing these animals in a conventional and antibiotic free environment will be discussed.

Systemic histomoniasis associated with Marek´s disease in free-range layer hens

Fernando Ruiz, Guillermo Rocha, and Felix Sanchez

Department of Poultry Science, Faculty of Veterinary Medicine, National Autonomous University of Mexico (UNAM)

Histomoniasis is a disease of birds caused by the protozoan parasite *Histomonas meleagridis.* This parasite can infect all species of galliform birds but its pathogenicity varies markedly between species. The chicken (*Gallus gallus)* recover rapidly after an infection, and the mortality is usually low; while in the turkey (*Meleagris gallopavo)* the disease is much more severe and can reach a mortality up to 100%. Our objective is to describe an outbreak of systemic histomoniasis combined with Marek´s disease in free-range layer hens that caused a mortality of 70% of the flock. In the pathologic examination, we observed round necrotic lesions in the liver, caseous typhlitis and pneumonia. In the intestinal lumen, we found parasitic structures consistent with *Ascaridia, Raillietina, and Heterakis.* In the histopathological examination, we found necrotic typhlitis, hepatitis, and pneumonia associated to *Histomonas meleagridis.* We observed a lymphoma in the brain pons and lymphocytic neuritis in the peripheral nerves. There are very few reports of systemic histomoniasis in chickens (*Gallus gallus)* because they are able to mount a very effective immune response against the parasite. This is the reason why this outbreak could have a strong association with the free-range system productions, which, as a result of its characteristics, can facilitate the emergence of parasitic disorders, like *Heterakis gallinarum,* and immunosuppressive diseases like Marek´s disease, that affects the B and the T CD4 and CD8 lymphocytes, which are essential to the humoral and cell-mediated immunity.

This Flock 'Mite' Have A Problem!

Jenny A. Fricke[a] and Joseph E. Rubin[b]

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At 41 weeks of age, broiler breeder hens were submitted to the diagnostic laboratory for examination due to increased mortality. The results of necropsies from this flock and a second flock (31 weeks of age) on the same site were consistently septicemia characterized by peritonitis and oophoritis. *E. coli* was consistently isolated from sick birds and despite *in vitro* susceptibility to tetracycline, the flock(s) failed to respond to treatment. Treatment with enrofloxacin, while successful during therapy, was met with relapse once completed. Coinciding with colibacillosis was an apparent increase in the burden of red mites (*Dermanyssus gallinae*). Interestingly this mortality followed regulatory changes which prohibited the use of carbamates, a product class that was previously used on this farm for mite control. The observed treatment failures and the location of mortality (hens dying in next boxes where red mite populations would presumably be higher) led to suspect of hematogenous spread of *E. coli*, mechanically vectored by mites. To determine whether the mites were playing a role in the dissemination of *E. coli* and development of septicemia, isolates grown from healthy and sick birds, mites and the environment were compared using pulsed-field gel-electrophoresis. These comparisons revealed that only isolates from mites and affected birds were closely related. This finding supported the final diagnosis of *E. coli* septicemia secondary to *Dermanyssus gallinae* parasitism.

Clinical Presentation of False Layer Syndrome Caused by Infectious Bronchitis

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McKinley Hatchery (St. Marys) Ltd. 34 Revell Dr., Guelph, ON. N1G 0B8, Canada

A case report on the presentation of False Layer Syndrome (Cystic Left Oviduct) that has been linked to infectious bronchitis infections. The rate of Infectious bronchitis infections in Ontario, Canada was very high late in 2016. Numerous layer flocks that were hatched during this period presented with poor production which upon investigation was the result of damage to the left oviduct. Infection by a Delmarva strain of infectious bronchitis resulted in regional aplasia and large cystic oviducts in a large percentage of hens from seven pullet farms. The case report discusses the gross and clinical pathology findings, lab reports and sequelae to the syndrome. Discussion on the pathogenesis, epidemiology and prevention of the infection are included. Since the onset of this syndrome in Ontario, there have been several other areas in the US and Canada that have experienced the same symptoms. Recognizing the clinical signs and understanding strategies of prevention will minimize the economic and welfare impacts of this novel presentation of Infectious Bronchitis infection in North America.

Leg Weakness and Mortality in Pullets?

History of sudden onset of leg weakness in five week old pullets followed by mortality later the same day. I will discuss rule-outs, treatments options and diagnostics that were pursued in determining the ultimate cause of the weakness and mortality.

Investigation of high bacterial counts on hatching eggs at transfer

Jean E. Sander, DVM, MAM, DACPVA, Manuel DaCosta, James Vickory, Bryan Cummings

A Senior Technical Services Veterinarian, Poultry, Zoetis, 1040 Swabia Ct., Durham, NC 27703

A commercial broiler company was experiencing high bacterial counts on egg surfaces at time of transfer in two of the company's hatcheries while another two hatcheries within the same company but in a different geographic region were experiencing the low bacterial counts as would be expected on clean egg surfaces at transfer. Egg washes were conducted at egg delivery to the hatchery as well as egg washes of those same flocks at transfer in order to determine if the transfer contamination resulted from higher surface bacterial counts at the time of delivery into the hatchery or if the eggs were being continually contaminated through some practice within the hatchery. Based on these data, further investigation was conducted to determine the source of the contamination in an effort to correct the problem. The additional data collection included an egg storage facility, egg transport trucks, etc. The findings were assessed in an effort to find and correct the problems discovered.

Incubation and after hatch issues and their influence on broilers performance

Danuta Furmanek DVM, MSD AH

The XXX hatchery experienced problems with increased mortality in first week of life , at level up to 5% and sometimes even higher. Extensive analysis showed the problem was pronounced especially in spring and autumn seasons.

Mortality used to start early, on 2nd day after placement, which could suggest other than bacterial infection related origin (normally, it takes 3-4 days before bacterial infection results in increased mortality). 2nd day suggested more a sort of incubation/ developmental related problem.

Problems lasted for 2 years, before an extensive investigation was performed.

In that 2y time period, two things changed : the fertility increased significantly and the air handling unit was upgraded to an air pressure control system, to make it more efficient. We have looked at these 2 parameters as possible reasons creating problems.

Case description and necropsy results showed that problems, which seemed to be related to impaired cardiovascular system function. High numbers of d.o.c with blood in lungs were reported. Heart looked affected (heart muscle not firm enough). Residual yolk sacs relatively big. Affected flocks show also more problems with flip-overs, ascites later in rearing. All these observations suggested problem related to an imbalance between the speed of development of embryo and availability of oxygen (too limited air exchange in relation to egg shell temperature/speed of development of the embryo).This forces the embryo to use protein for energy instead of yolk fat (results in poor heart conditions and more flip overs, ascites etc.,but can already cause problems in very early period after hatching as well).

Results of additional testing revealed very surprising facts, which allowed to form a reliable scenario of problems origin, although some questions remained unanswered – why problems occurred occasionally and seasonally..?

Protection conferred by a rHVT/ILT vaccine under intense field infectious pressure (a field case report)

JF. Ríos-Cambre, EM. Trejo-Martínez

MSD Salud Animal Mexico

A broiler flock was vaccinated with a recombinant HVT/ILT vaccine at one day old at the hatchery and placed in a 17 house broiler farm located in an area with recent history of infectious laryngotracheitis (ILT) virus circulation. Around 41 days of age some of the birds in one house started to show mild to moderate respiratory signs that were consistent with ILT, and lab diagnostic tests confirmed an ILT infection. Control measures were taken for the birds in that particular house, while the rest were under strict observation, while no other measure were undertaken. At the end of the grow-out period at 56 days of age, clinical signs in the affected house had subsided, while in the rest of the farm no signs were detected, even though lab test confirmed the exposure to an ILT virus in those birds, and although the production parameters in the affected house were not satisfactory, the entire farm's ended within the expected results for the broiler company. After intense scrutiny it was concluded that some of the birds placed in the affected house had been vaccinated improperly, while the rest were adequately immunized. The overall conclusions were that it is imperative to verify that this kind of vaccine has to be properly reconstituted and applied, and that by accomplishing this, the vaccinated birds can withstand an intense field virus exposure.

Wooden Breast Syndrome as a precipitating factor in acute gastrocnemius tendon rupture and leg condemnation in roaster chickens

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With modern developments in broiler chicken genetics, nutrition, and management, Wooden Breast Syndrome (WBS) has risen to the forefront of conditions affecting breast meat quality, and negatively impacting consumer product satisfaction and poultry growers' profits. A major broiler company on the Delmarva Peninsula temporarily extended their male roaster processing age from 62 to 67 days, with respective average live weights increasing from 10.25 to 11 pounds. The processing plant subsequently condemned drumsticks for "leg problems." Investigation of gross lesions revealed acute rupture of the gastrocnemius tendon, accompanied by hemorrhage and edema in the surrounding tissues. Histological analysis of tendons and accompanying gastrocnemius muscle ruled out infectious processes, such as reovirus-associated tenosynovitis, *Staphylococcus aureus*, or *Mycoplasma synoviae*. Rather, the tendons showed microscopic lesions ranging from acute hemorrhage to chronic granulation tissue formation and fibrosis, consistent with abnormal mechanical loading on the tendon. Adjacent skeletal muscle revealed lesions of myofiber degeneration, myositis, and phlebitis consistent with WBS. The combination of WBS myopathy in the gastrocnemius muscle and increased bird weight likely contributed to abnormal mechanical loading in the tendon causing rupture. WBS should be considered as a potential precipitating factor in the acute rupture of gastrocnemius tendons, resultant clinical lameness in broilers, and increased incidence of leg condemnations at the processing plant.

Infectious Coryza in broilers in the Central Valley of California, production and economical impact

Grace Mountainspring, DVM[a], Charles Corsiglia, DVM, MAM, dACPV[a], Manuela Crispo, DMV[b]

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Infectious Coryza caused by the bacterial agent, Avibacterium paragallinarum, not typically effecting broilers, is diagnosed on a commercial broiler operation in the Central Valley of California in March 2017. The index farm had 300,000 chicks placed throughout 14 houses. First clinical signs, consisting of nasal and ocular discharge, cough, rales, and snicks were detected at 19 days of age in one house. As the disease progressed, swollen, edematous heads and and ruffled feathers developed. By 22 days of age, spread of the disease occurred, and by the time the birds reached 41 days of age, the entire index farm had contracted the disease. By

processing, the farm experienced a staggering 24% mortality and despite treatment efforts along with increased biosecurity control, this disease was not contained on the index farm. Coryza spread across the four farm complex in a period of a few weeks. Diagnostics were carried out through the CAHFS laboratory in Turlock, CA. Confirmation of the agent was done by bacterial isolation and PCR. Serology and PCR for infectious bronchitis, AI, and Mycoplasma were all negative. At processing, whole body condemn reached over 50%, and the remaining processed birds had disease involvement mainly consisting of airsacculitis at 97% of the flock. Feed conversion for this flock was 2.38. Comparing to a farm that processed within the same week with WB 1.29%, disease involvement at 7.8%, and FCR at 1.75, it is clear coryza has the potential for causing detrimental impacts on broiler production. This case demonstrates the important production and economic impact uncomplicated coryza can have on broilers.

***Streptococcus gallolyticus* subsp. *pasteurianus* in Ducklings**

Richard M. Fulton, D.V.M., Ph.D. dACPV and Rinosh Mani, B.VSc., M.S., Ph.D., dACVM

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Three contract duck growers for a commercial duck company, which produces 6 million ducklings per year, had flocks with increased daily mortality which started between day 6 and day 10 and typically lasted for 5 days. Mortality rate would return to normal levels. Necropsies of ducklings from the 3 farms were performed on separate dates. Gross lesions consisted of splenomegaly (2 to 3 X). Bacterial culture revealed the presence of *Streptococcus gallolyticus* subsp. *pasteurianus* (*S. bovis* II/2). Antibiotic susceptibility was the same on two of the farms but different on the third affected farm. *Streptococcus gallolyticus* subsp. *pasteurianus* is a commensal organism in the intestines of humans and animals and has been known to cause meningitis in newborn infants and adults as well as bacteremia in newborn and endocarditis in adult humans. It has caused disease in laying hens, broiler chickens, goslings, pigeons, and turkeys and is thought to be an opportunistic pathogen. Source or route of infection is not known.

In Depth Investigation of a Hatchery Vaccination Failure

Francisco J Rojo B., Ulises Revelo, Alejandro Rojas, Natanael Méndez, Hector Garcia

Marek´s Disease (MD) has always been a big concern for the poultry and is well known how tumors and nervous clinical presentations are responsible for mortality in commercial farms. Cutaneous presentation and condemnations due to Marek´s disease in the slaughter house have also a great impact on profitability for poultry business. Since late 1960´s and early 1970´s the use of vaccines/vaccination has been a great success to control MD, even when combined different serotypes according to the field challenge. One single dose of the vaccine given at the hatchery may be enough to immunize the birds for all their life, no matter if they are short (broilers) or long living commercial birds (layers or breeders). A clinical report was received with high mortality in a breeder complex. The objective of this report is to present the diagnostic procedures that lead to the Marek´s disease diagnosis, as well as the extent of the hatchery procedures, which went far beyond the vaccination process per se, and resulted in an improved hatchery vaccine/vaccination procedure.

Sunday, July 15 Session B

Mycoplasma

Update on MG Control in Multi-age Commercial Laying Operations: Diagnostic Approaches and Control Programs

Daniel A. Wilson

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A large number of commercial laying operations in the US are multi-age facilities which create a degree of difficulty in control of mycoplasmas including, in particular, *Mycoplasma gallisepticum* (MG). As a result, many facilities maintain a continuous MG positive status and utilize commercially available vaccines as part of their control programs. Unfortunately, approaches to monitoring and control of MG in some commercial laying operations have went relatively unchanged over recent years. For a variety of reasons these multi-age complexes don’t always have veterinary support or routine surveillance and monitoring for ongoing changes to their MG status or program. Over time these MG challenges

can pose a significant impact to overall bird health and productivity.

I intend to discuss updates to MG diagnostic and control programs that I have found helpful from field and case information. More specifically I hope to present information for monitoring flocks for failure of MG control programs through discussions of novel field sampling approaches, the benefits of frequent ELISA-based serological monitoring, and the necessity for utilization of molecular diagnostics to best determine vaccination approaches. Lastly, I hope to demonstrate the failures and successes of various commonly-used MG vaccination programs in field situations.

Incidence and epidemiology of *Mycoplasma gallisepticum* infection in turkeys over 20 years in one commercial company.

Shivaprasad H. L, M. Pitesky, J Ochoa and R. P. Chin.

California Animal Health and Food Safety Laboratory System – Tulare Branch University of California, Davis.

Mycoplasma gallisepticum (MG) can cause a highly contagious and significant economic disease in poultry especially in turkeys. The disease is often complicated with secondary bacterial resulting in respiratory signs with swollen sinuses, increased morbidity, mortality and condemnations. MG can cause drop in egg production in layers. Pathology includes conjunctivitis, sinusitis, tracheitis, airsacculitis and pneumonia with lymphoplasmacytic inflammation and lymphoid nodule formations. Disease can spread from bird to bird within a house and between houses and farms, hence biosecurity is important. There were 42 outbreaks of MG infections between 1997 and 2017 in turkeys, 5.5 weeks of age to 29 weeks of age involving 16 ranches in one commercial company. MG was often identified by PCR in the swab form the trachea. MG was isolated from the respiratory tract such as trachea, air sacs, and lungs and occasionally from the sinus. Multilocus Sequence Analysis (MLSA) of the MG isolates revealed six different patterns among 16 ranches suggesting spread from farm to farm as well as external sources through various means. It has been shown that 60 to 70 % of the backyard chickens have been positive for MG by serology in California. The backyard chickens around the farms can also be a source of MG infection in the turkeys.

Development of Multilocus sequence typing (MLST) for *Mycoplasma gallisepticum*

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Mycoplasma gallisepticum (MG) is the most pathogenic avian mycoplasma species. It affects commercial, non-commercial poultry and wild birds. Current MG sequence typing methods rely on the partial sequence of one or more surface antigen genes. Contrarily, Mulitocus Sequence Typing (MLST), a widely used typing method for many human and animal pathogens relies on conserved housekeeping genes. Recently, MLST assays have been developed for *M. synoviae* and *M. iowae*. Additionally a whole genome based core genome MLST (cgMLST) assay has been developed for MG. However, cgMLST cannot be applied to clinical samples. Here, we have developed 7-loci based MLST scheme for MG. These seven loci were selected out of 425 genes recently used for core genome MLST (cgMLST) development. A total of 101 diverse MG samples, including isolates and clinical samples, were typed using this 7-loci MLST. The phylogeny and discriminatory power of this 7-loci MLST were evaluated and compared to cgMLST and compared to surface antigens genes currently used for MG sequence typing. The 7-loci MLST provided optimum discriminatory power and congruent phylogeny to cgMLST. A public database for MG MLST was created. This assay will increase the accessibility to MG sequence typing and provide a stable and expandable nomenclature that is compatible with cgMLST. This assay represents an important tool for epidemiological investigation of MG and allow better chances for successful control and eradication of MG.

Investigation of Environmental Fomites and Their Role in the Transmission of *Mycoplasma Synoviae*

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Mycoplasma synoviae (MS) is a disease that has plagued the poultry industry for decades, costing the industry billions of dollars in losses. It often infects the upper

respiratory tract of chickens and turkeys, causing subclinical or clinical disease with airsacculitis. The aim of this research was to identify environmental fomites that could play a role in the indirect transmission of MS in Northeast Georgia. To accomplish this objective, environmental samples were collected from 6 commercial broiler breeder farms with flocks that were confirmed positive for MS infection. Samples included dust, ground insects, feathers, flies, litter, and rodents. Both quantitative real time PCR and isolation (culture) assays were used to detect MS in these materials. Results revealed that dust, feathers and litter carried the heaviest load of MS DNA with 15%, 61% and 80% of samples positive by real time PCR respectively. An estimate of as much as 2.7 x 107, 3.4 x 107, and 1.8 x 107 MS organisms were detected in the most positive dust, feather and litter samples respectively. These results indicate the materials are likely to be the most infectious (and risky) environmental fomites on an infected farm, and although a comprehensive biosecurity program is important to control the spread of several poultry diseases, special attention and resources should be focused on cleaning (dust and feathers) and disinfection of depopulated farms as well as proper litter management to avoid indirect transmission of MS to neighboring farms.

Co-infection studies with respiratory viruses and/or Mycoplasma synoviae in chickens and turkeys

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Low pathogenic avian influenza virus (LPAIV) can produce a mild to moderate upper respiratory disease in chickens that can be aggravated by other factors including other respiratory pathogens. Since commercial poultry is routinely exposed to infectious bronchitis virus (IBV) and Mycoplasma synoviae (MS), we studied the dynamics of AIVIBV-MS co-infections and their effect on disease and virus shedding. Three-week-old SPF chickens were inoculated with MS followed five days later with H5N2 LPAIV (Mexico lineage) and/or an IBV virulent field strain (Ark). Mild conjunctivitis was the only clinical sign observed in LPAIV-inoculated birds. Moderate to severe conjunctivitis was present in all birds inoculated with IBV, single or co-infected. In addition, birds coinfected with MS+IBV or MS+IBV+LPAIV had rales, swollen heads, and at necropsy, hemorrhages and exudates in the trachea. Birds inoculated only with LPAIV had mild microscopic lesions in the trachea. These lesions were more severe in birds co-infected with IBV+MS or IBV+MS+LPAIV. Co-infection with LPAIV increased IBV shedding, and an increase in MS shedding was found in co-infected birds compared to birds only infected with MS. In a second study, SPF turkeys were also co-infected with H5N2 LPAIV and MS. Co-infected turkeys were less active than single infected ones and body weights were affected. Co-infected turkeys shed significantly higher titers of LPAIV than turkeys only exposed to the LPAIV, but no effect of MS shedding was observed. These co-infection studies highlight the role of avian Mycoplasma in co-infections of poultry with respiratory viruses (LPAIV and IBV) in producing disease and lesions.

Tracheal Lesion Evaluation of broilers co-infected with *Mycoplasma synoviae* and Infectious Laryngotracheitis

Valerie C Marcano, Susan M Williams, Maricarmen García, Marianne Dos Santos, Naola Ferguson-Noel

Mycoplasma synoviae (MS) is a poultry pathogen of worldwide prevalence that may result in respiratory disease, airsacculitis and synovitis. The clinical presentation of MS may be greatly exacerbated by concurrent infection with respiratory pathogens such infectious Laryngotracheitis virus (ILTV). Infection with ILTV leads a decrease in egg production, acute respiratory disease and death. Vaccination with ILT CEO in broilers infected with MS leads to significant increases in airsacculitis and mortality compared to non-infected broilers. In this trial, we compared ILTV scoring techniques to scoring techniques traditionally used for MS, while investigating the impact of MS infection on the ability of the ILTV CEO vaccine to prevent tracheal lesions following virulent ILTV challenge. Broilers were vaccinated and/or challenged with ILTV in the presence or absence of MS. Preliminary results suggest that MS exacerbates tracheal lesion scores in birds infected with ILTV, highlighting the benefit of controlling MS infection as well as the risk of ILT CEO vaccines in MS-infected broiler flocks.

***Mycoplasma synoviae (MS)* Core Genome Multilocus Sequence Typing (cgMLST): The Next Level of MLST**

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Mycoplasma synoviae (MS) is a significant poultry pathogen with increased prevalence and virulence for poultry in recent. MS strain identification is essential for epidemiological outbreak investigation, prevention and control efforts. Currently, multilocus based sequence typing assays have been developed for MS, yet the resolution of these assays could be insufficient for outbreak investigation. The cost of whole genome sequencing became close to that of sequencing the traditional seven MLST targets; however, there is no standardized method for typing MS strains based on whole genome sequences. In this paper, we are proposing a core genome multilocus sequence typing (cgMLST) scheme as a potential standardized and reproducible method for typing and differentiation of MS whole genome sequences. A diverse set of 25 MS whole genome sequences were used to identify 302 core genome genes as cgMLST targets (35.5% of MS genome) and a total of 44 whole genome sequences of MS isolates from six countries in four continents were typed using this scheme. cgMLST based phylogenetic trees displayed a high degree of agreement with core genome SNP based analysis and available epidemiological information. cgMLST allowed evaluation of two conventional MLST schemes of MS. Moreover, the high discriminatory power of cgMLST allowed differentiation between samples of the same MLST type. cgMLST represents a standardized, accurate, highly discriminatory, and reproducible method for differentiation between MS isolates. Additionally, it provides stable and expandable nomenclature, allowing for comparing and sharing the typing results between different laboratories worldwide.

Using drinker swabs to monitor health status of turkey flocks for *Mycoplasma*

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Historically flocks that have clinical signs of *Mycoplasma* have been diagnosed via PCR using tracheal swabs. Since individual birds have to be handled in order to collect tracheal swabs, typically a limited number of birds are sampled. The use of drinker swabs to collect samples for PCR both expands the sample size due to the amount of birds using the drinker and is an easier sample to perform.. Four farms were included in this study. Surveillance testing using PCR was performed using samples from multiple drinkers in a turkey barn. Comparison of CT values between tracheal swabs and drinker swabs show that drinker swabs can be used to assess and monitor turkey flocks for *Mycoplasma*.

Immunology

Tracheal Immune Pathways and the Role of Viral Microbiota in Resistance to Different Infectious Bronchitis Virus Genotypes

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In previous work, we developed a “resistant-susceptible” animal model using MHC congenic chicken lines with haplotypes B2 and B19 to assess resistance and susceptibility to two different IBV genotypes, M41 and Ark. We assessed respiratory clinical signs, tracheal thickness, viral load in tears and IFN-β, IFN- γ, IgG and IgA levels in sera and tears. The major differences between the two MHC haplotype chicken lines were observed in IgG and IgA concentrations in tears. In order to investigate immune pathways that could be associated with the different levels of resistance, we performed RNA sequencing from tracheas collected at 2 and 6 DPI from B2 and B19 haplotype chickens challenged with both IBV M41 and Ark. In addition, the viral microbiota detected in the tracheas of both chicken lines were evaluated and compared. Results from these experiments will be discussed.

Effect of ammonia on the immune response to infectious bronchitis virus vaccination and protection from homologous challenge in broiler chickens

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Commercial broilers in the grow-out stage are commonly exposed to ammonia. Ammonia concentrations are higher in poorly ventilated houses and coincide with the elevated incidence of respiratory disease occurring during the winter months. Our study aims to determine the effect of ammonia on the immune response to infectious bronchitis virus (IBV) vaccination and protection against homologous challenge in commercial broiler chickens. One-day-old chicks were immunized with IBV Mass-type vaccine by oculonasal route and placed in a climate-controlled room containing 30-60 parts per million (ppm) of littersourced ammonia. At 28 days, birds were challenged oculonasally with homologous IBV M41, and protection was measured by viral detection in the choanal cleft, clinical signs, ciliostasis, and presence of airsacculitis. Throughout the study, IBV-specific antibodies were measured in serum (IgG) and lacrimal fluid (IgA), and immune cells in respiratory and systemic tissues were characterized by flow cytometry. IBV-vaccinated birds in both ammonia and no-ammonia groups were completely protected from challenge and showed significantly reduced viral load, clinical signs, no ciliostasis, and no airsacculitis. Nonvaccinated controls were not protected from challenge, and 90% of ammonia-exposed controls had airsacculitis, compared to 40-50% of controls exposed to no ammonia. Flow cytometry and antibody titers revealed trends in the timing and robustness of immune cell proliferation and activation. Our results indicate that commercial broilers exposed to moderate levels of ammonia are not more susceptible to IBV challenge if they are appropriately vaccinated, and further suggest that ammonia modulates the immune response to virus exposure at the cellular level.

Effect of dose and strain on the ability of HVT to hasten immunocompetence in chickens

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We have demonstrated that in ovo administration of HVT accelerates the maturation of cellular and humoral immune responses. This has been shown in egg-type chickens (SPAFAS) and in meat type chickens (commercial broilers), albeit the effect in broilers seem to be less remarkable than in SPAFAS. The objective of this study was to confirm previous results and to optimize the benefit of in ovo administration of HVT in both SPAFAS and broilers. In particular two factors were evaluated, vaccine dose and vaccine strain. Four different doses (2,000, 4,000, 8,000, and 16,000 PFU) and three different HVT strains (one conventional and two recombinant HVTs) were evaluated. Phenotype of immune cells in the spleen at day of age, ability of the lymphocytes to proliferate when in contact with Concanavalin A, increase in wing web thickness when inoculated with phytohemagglutinine-L, and humoral immune responses to keyhole limpet hemocyanine were used to assess immunocompetence. Results will be discussed.

Expression of immune-related genes in the jejunum and cecal tonsils of broiler chickens challenged with coccidia and *Clostridium perfringens* and supplemented with sodium butyrate and essential oils

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The objective of this study was to determine the effects of sodium butyrate (SB), and sodium butyrate plus essential oils (carvacrol and ginger; SBEO) on the expression of immune-related genes in the jejunum and cecal tonsils of coccidia and *Clostridium perfringens* challenged broiler chickens. The birds were assigned to 4 treatments (8 replicates of 58 birds each: basal diet and no challenge; basal diet and challenge; diet supplemented with SB and challenge; diet supplemented with SBEO and challenge). On d 13, the challenged birds were inoculated with ~5,000 oocysts of *Eimeria maxima* by oral gavage. On d 18-19, the same birds were inoculated with *C. perfringens* via drinking water. Jejunal and cecal tonsils samples were collected at d 12, 18, 21, and 28 to analyze the expression of immune-related genes. On d 12, SBEO upregulated the expression of IL-6 (P=0.01) in the jejunum, and IgA (P=0.02), IL-6 (P=0.02),

and TLR-2 (P=0.02) in the cecal tonsils, when compared to the unsupplemented groups. On d 18, SB supplementation upregulated IL-1β (P=0.01), and the *E. maxima* challenge downregulated the expression of IL-6 (P=0.03) in the jejunum. At 21 d, SB decreased IL-10 expression (P=0.001), and SB+EO increased the expression of IgA (P=0.001) in the cecal tonsils. On d 28, no effects were observed on the gene expression of any intestinal sections studied. In conclusion, SBEO modulated the expression of genes prior to the challenge, and SB or SBEO supplementation showed immunomodulatory effects mainly on the jejunum after the *E. maxima* challenge.

Strengthening the cell-mediated immunity post-hatch using *in ovo* delivery of nucleic acids with increased resistance to respiratory viruses

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Respiratory virus infections such as avian influenza, infectious laryngotracheitis and infectious bronchitis lead to high morbidity and mortality in poultry globally. Given the limitations of current control measures against these virus infections, it is a necessity that novel measures are developed leading to enhanced host immune responses against respiratory viruses. The objective of the study was to boost the cell-mediated immunity in chickens post-hatch *via* pre-hatch delivery of nucleic acids increasing the resistance to respiratory viruses. We delivered CpG DNA or non-CpG DNA (control DNA) or PBS *in ovo* and collected tissues from the respiratory and immune organs for the purpose of immunostaining of macrophages, T cluster of differentiation (CD)4+, T CD8+ and B IgM+ cells. In the next experiment, we delivered CpG DNA and non-CpG DNA or PBS as controls *in ovo* and subsequently infected with infectious laryngotracheitis virus (ILTV), infectious bronchitis virus (IBV) and H4N6 avian influenza virus (AIV) at day 1 post-hatch. We found *in ovo* CpG DNA increases recruitment of macrophages, T CD4+, T CD8+, B IgM+ cells in lungs and immune organs post-hatch. In correlating with the cellular responses, we also found that *in ovo* delivered CpG DNA induces protective responses against ILTV and IBV infections in terms of reduced mortality and clinical signs and inhibits AIV replication in lungs and trachea. This study suggests that the CpG DNA is a potential candidate for further investigation in order to boost cell-mediated immune response against respiratory viruses.

Hatchery Egg Shell Membrane (HESM) Nutritional Supplements Modulate Immunity of Chickens

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The need for antibiotics free poultry production has necessitated the quest for alternative strategies to improve poultry immunity. Previously, we showed that post hatch poultry given 'hatchery egg shell membrane (HESM)' supplement improves their immunity and stress resistance which was attributed to their bioactive protein constituents and microbial contaminants. Thus, we hypothesized that an early exposure to these factors improve chicken immunity. To test it, we used 3 groups of day-old chickens raised for first 2 weeks with feed containing no supplement, tryptose phosphate broth (TPB) incubated 0.5% HESM supplement, and TPB-*E. coli* enriched 0.5% HESM supplement, followed by control feed thereafter. At 5 week, each group of birds were divided into 2 subgroups with one receiving saline and the other 2X107CFU *E. coli* instilled intra-tracheally and 3 days later an intramuscular injection of *E. coli* LPS (1 mg/kg BW). The birds were weighed before and 24h post injection, bled, and killed. The serum immunoglobulin (Ig A, G, and M) levels against *E. coli* proteins were evaluated by ELISA. The results showed that chickens receiving HESM supplement alone showed significantly high levels of anti-*E. coli* antibodies compared with control fed birds and the levels of Igs did not change much on challenge with *E. coli* and LPS. Whereas the LPS challenge caused a significant loss of BW in control fed birds, the chickens that had either received HESM or bacteria enriched HESM showed significantly lower BW loss. Thus, our results indicate that post hatch supplement of HESM product can improve immunity and impart tolerance of chickens to microbial factors.

Lipopolysaccharide (LPS)-Toll-like receptor (TLR) 4 Signaling Leads To Type I Interferon Production And Antiviral Response Against Infectious Laryngotracheitis Virus (ILTV) Infection

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Engagement of toll-like receptor (TLR)4 ligand, lipopolysaccharides (LPS) with TLR4 in mammals activates two downstream intracellular signaling routes; the myeloid differentiation primary response gene 88 (MyD88)/ toll-interleukin-1 receptor (TIR) domain-containing adaptor protein (TIRAP) and the TIR domain-containing adaptor inducing interferon (IFN)-β (TRIF)-related adaptor molecule (TRAM) /TRIF. However, the existence of the later pathway leading in to the production of type one IFNs has been debated due to the conflicting observations. The objective of our study was to investigate whether LPS induces type I IFNs in avian macrophages leading to antiviral responses attributable to LPS-mediated type I IFN production. First, we found that LPS elicits type I IFN activity and nitric oxide (NO) production in avian macrophages. Second, we determined that the LPS-mediated antiviral response is dependent mainly on type I IFN activity and not on NO production. Third, we discovered that LPS-TLR signaling mediated type I IFN activity is due to IFN-β and not due to IFN-α production. Fourth, we discovered that the blocking the TRAM/TRIF cascade using TRAF associated nuclear factor (NF)κB activator (TANK)-binding kinase 1 (TBK1) inhibitor, which is important for the dimerization and translocation of IRF3 leads to inhibition type I IFN activity. Finally, we found that reduction in ILTV replication by LPS-mediated antiviral response is attributable to type I IFNs in addition to NO production. Our findings imply that LPS elicits both MyD88/TIRAP dependent and TRAM/TRIF dependent pathways in avian macrophages consequently eliciting anti-ILTV response attributable to type I IFN activity and NO production.

Wealth of Knowledge

Impacts of Mycotoxins on Poultry Health and Their Prevalence In The 2017 Corn Crop

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Mycotoxin contamination of feed materials is a global concern, as exposure to mycotoxins significantly impacts animal health and productivity. Mycotoxins primarily predispose livestock and poultry for pathogenic bacterial (necrotci eneteritis and salmonellosis) and viral diseases (Reo virus) as well as shown to compromise vaccine efficiency, leading to loss of productivity. BIOMIN has been conducting global mycotoxin surveys on commodity crops and complete feeds annually since 2004, including annual corn surveys in the United States since 2012. Corn and corn product samples were analyzed for aflatoxins, type A trichothecenes such as T-2 toxin, type B trichothecenes such as deoxynivalenol (DON; vomitoxin), ochratoxin A, fumonisins (FUM), and zearalenone (ZEN) and their derivatives, utilizing the LC-MS/MS method. Preliminary results suggest almost all surveyed samples contained at least one mycotoxin type (96%), similar to 2016 but increase from 2015 (85%). The co-occurrence of more than one mycotoxin in the same sample (43%) was brought back to similar levels seen in 2015. Deoxynivalenol continues to be the most prevalent mycotoxin at 87%, continuing the overall increasing trend since 2013. Mean contamination levels of DON were similar to 2016, a 2X increase from 2015, while the maximum contamination showed a nearly 2X increase from 2016. Mean contamination level of FUM was also similar to 2016, over 3X increase from 2015. Because of the high frequency of multi-mycotoxin contamination in samples thus far, multiple strategies of mitigating risk are needed beyond adsorption, including biotransformation and providing support to immune and liver function.

Histopathology and Histomorphometric Study of Gizzard Erosions in Broiler Chickens Associated with Gross Necropsy Findings

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Gizzard erosions in broiler chickens have been studied extensively by the poultry industry; however an evaluation comparing traditionally raised broiler chicks and antibiotic free broiler chicks has yet to be explored. A field visit revealed gizzard erosions and severe gizzard sloughing associated with multiple flocks of antibiotic

free broilers. Which prompted this research in order to compare gizzard erosions found in broiler flocks raised without antibiotics (NAE) and conventionally raised flocks. Ten birds from each flock (NAE and conventional) were evaluated on 1, 14, 28, 42, 49, and 56 days of age. Gizzards were scored grossly on a range of 0-3 (no lesion to severe lesion), and a section of each gizzard was collected in 10% neutral buffered formalin. In addition, full necropsies and allometrics were also performed on each bird. Thirty-six of the gizzards, representing each collection time and scoring category, were routinely processed for histopathologic evaluation. Individual gizzards were scored on multiple features related to the koilin layer including: adhered surface bacteria, inflammatory cell entrapment, and hemorrhage. Additionally, glandular hyperplasia, glandular inflammation, submucosal inflammation, and muscular involvement were also recorded. Auxiliary evaluations included histomorphometrics, measuring the thickness of the koilin and the glandular regions of each gizzard. This methodology allowed the quantification of subtle histopathologic differences in each gizzard compared to the previously established gross scoring system for gizzard lesions; full analysis is still in progress.

Hepatic sinusoidal vascular smooth muscle hypertrophy proceeds septox hepatitis in broilers

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Septicemia-Toxemia ("septox") hepatitis is the most economically significant FSIS category causing broiler carcass condemnations in the United States. Ten broilers were obtained from four different houses on one farm at 20, 27, 34 and 36 days of production (160 total). Five healthy and five clinically ill chickens were selected at each sampling. Gross liver lesions, hepatic sinusoidal vascular smooth muscle hypertrophy (HSVSMH), splenic histiocytosis and bone marrow density were graded (0-4) for each bird. HSVSMH was evaluated by counting enlarged nuclei of hypertrophic capillaries. HSVSMH was confirmed by immunohistochemistry for smooth muscle actin. Aerobic cultures of all livers and gall bladders from septox birds were negative. Additionally, no bacteria were observed microscopically. Ten/160 birds had severe, 7/160 had moderate, 58/160 had mild to minimal HSVSH as graded on 0-4 scale. The birds with moderate to severe HSVSMH had significant bodyweight loss on day 20, 27, 34, and 36 with the mean body weight loss of 218, 255, 415, and 565 grams, respectively. Linear regression analysis revealed positive correlation between HSVSMH, extramedullary hematopoiesis, splenic histiocytosis, bone marrow proliferation and gross septox hepatitis. Minimal to severe HSVSMH was observed in all age groups representing 53% of examined birds. Grossly visible septox hepatitis resulting in carcass condemnation is proceeded by mild (grade 0.3 to 1.9) chronic HSVSMH that has minimal effect on growth. We hypothesize that moderate to severe (grade2.0 to 4.0) HSVSMH eventually limits oxygen diffusion from capillaries causing an acute hypoxic event resulting in grossly visible centrilobular necrosis and reactive hepatitis.

Use of Intracranial Alcohol Injection in Ducks and Ratities as a Humane Method of Euthanasia

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Commercial White Pekin ducks were euthanized by 3 different methods; cervical dislocation, CO2 gas, and intra-cranial injection of alcohol. Due to the dive reflex, gas has been considered to be an ineffective method of euthanizing waterfowl. Due to the physical characteristics of ducks, cervical dislocation is technically difficult. Currently, many commercial ducks that are euthanized prior to slaughter are euthanized by intra-cranial injection of alcohol. However, there are no approved methods of euthanasia of ducks (AVMA). We evaluated parameters such as time to death (corneal reflex, cessation of heartbeat) and ease of method. Commercial Pekin ducks were used and CO2 was metered and delivered into a small individual bird chamber. The study was replicated with each replicate using a different person trained in proper cervical dislocation to perform that method. Time to death was measured in seconds and each method was compared

using all parameters. Intra-cranial injection of alcohol was the superior method in all parameters measured.

Pathological And Molecular Characterization Of Wooden Breast Disease In Commercial Broiler Chickens During The Normal Growth Period

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The emergence of Wooden Breast Disease (WBD) in commercial broiler chickens has been associated with significant economic loss in the poultry industry resulting from severe reduction in meat quality traits. To unravel the morphological changes and molecular events characterizing the pathogenesis of WBD in chickens, a time-series evaluation of 350 male broiler chickens were conducted under commercial conditions for 7 weeks. From the Pectoralis major muscles, necropsy samples were taken weekly for histologic and ultrastructural analysis, and biopsy samples were taken from weeks 2 to 4 for gene expression analysis using RNA-sequencing. Histologic evaluation of the necropsy samples revealed perivenous lipid deposition, aggregates of lipid-laden macrophages and phlebitis beginning from week 1, and increasing scope of lipidosis within the interstitium and between myofibers, myodegeneration, myonecrosis, myositis and interstitial edema from weeks 2 to 7. Ultrastructural evaluation of samples from week 4 to 6 showed presence of dense collagen fibers and multifocal nodular lipogranulomas within the interstitium. Further, gene expression analysis using biopsy samples from affected and unaffected birds revealed top canonical pathways such as ECMreceptor interaction, complement system, axonal guidance signaling, integrin signaling and fibrosis in WBD. Additionally, gene expression analysis showed enhanced response to inflammation, skeletal and muscular disorders, connective tissue disorders and dysregulation of major metabolic pathways such as lipid metabolism and oxidative phosphorylation in affected birds. This study therefore demonstrates that structural and molecular perturbations involving the vasculature, extracellar matrix and metabolism are pertinent to the onset and development of WBD in commercial meat chickens.

Using Social Network Analysis (SNA) to Better Understand Disease Transmission in Backyard Poultry Flocks in California

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According to a 2010 USDA survey, backyard poultry (BYP) ownership is increasing nationally. While BYP ownership continues to increase, the location and number of BYP flocks remains unclear. Furthermore, live BYP movement such as the buying, selling and trading of backyard poultry movement is unclear. Additionally, multiple studies indicate that BYP owners have poor biosecurity practices. This combination of poor biosecurity practices and unregulated BYP movement can facilitate the spread of disease among flocks. In this study, an in-depth survey focused on identifying key sellers, buyers and traders of BYP along with husbandry-related questions was sent to BYP owners in order to better understand how disease can spread among these operations. Network results were analyzed using social network analysis (SNA), a scientific and quantitative way of studying relationships, to identify well-connected actors (ie. hatcheries, feed stores) and counties in the spread of disease. In total, there were 356 survey participants and 40 out of 58 counties were represented. SNA results in combination with geographical data can help optimize outreach and disease mitigation efforts in California.

Digital security in managing veterinary records and compliance data

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Attention to information security and integrity is the responsibility of all parties involved in the animal health industry. Ensuring regulatory compliance is also a key component of professional practice. Currently, the move to electronic record management for veterinary feed directives and veterinary prescriptions has created apprehension due to privacy concerns and elevated the need for deeper understanding of secure data management practices. In this presentation, we provide an overview of the best practices for secure veterinary record management and how organizations can decrease their risk of security breaches. We place focus on state-of-the-art security paradigms, including the use of blockchain and distributed security mechanisms, to

combat the financial burden of information security for all parties in the animal health industry.

Development of a Web-App for Monitoring Waterfowl Habitat in Real-Time in California

Migratory waterfowl remain the focus of Avian Influenza (AI) surveillance efforts, but traditional approaches for tracking movements, such as banding and telemetry, are costly and labor intensive. An alternative approach employs the use of remote sensing via publically available satellite imagery, next generation radar analysis and the resulting model generation in near real-time in order to provide spatio-temporal localization of waterfowl habitats and movements. Here we present a publically available web-app that integrates several habitat based models that are updated daily in order to provide near real-time updates of waterfowl habitat in California. Producers and other stakeholder can input address information and get current and historic (i.e. 7-day rolling historical data) risk assessments of waterfowl habitat (roosting and feeding) at the selected location.The current version of the web-app also has historical Next Generation Radar (NEXRAD) data with the same address functionality. Future iterations of the web-app will allow NEXRAD analysis in near real-time.

Monday, July 16 Session A

Virology

A Retrospective Study of vvIBDV and vvIBDV Reassortants in California Backyard Poultry; 2009-2017

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Very virulent infectious bursal disease virus (vvIBDV) was first identified in the United States in commercial chickens in California in December 2008. Approximately one year later, vvIBDV was detected in backyard chickens submitted to the California Animal Health and Food Safety Laboratory System (CAHFS). This report describes the pathogenicity and epidemiology of all vvIBDV and vvIBDV reassortant viruses detected in backyard poultry submitted to CAHFS from 2009 to 2017. Fourteen cases of vvIBDV were detected from necropsy submissions. These cases originated from seven Counties in California and affected chickens ranged from 4 weeks to 4.5 months of age. In most cases acute morbidity was reported with mortality often occurring within 24 hours after the onset of clinical signs. Microscopically, severe lymphocyte depletion was described in bursas from all 14 cases. In some cases the occurrence of concurrent diseases was observed. Sequence analysis of both segment A and B of these vvIBDV and reassortant vvIBDV strains was used to confirm their identity. This retrospective study indicates that vvIBDV genogroups continue to circulate within backyard poultry and may pose a potential threat to commercial poultry.

Molecular Characterization of Infectious Bursal Disease Viruses from commercial poultry in Peru

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The detection and classification of infectious Bursa disease virus (IBDV) or "Gumboro" disease is of crucial importance for its surveillance and control. In this study 60 isolates of IBDV from poultry farms in the Peruvian center obtained in 2016 were characterized, which together with 3 vaccine strains, were subjected to RT-PCR (Reverse-Transcription-Polymerase-Chain-Reaction) in a single step to to amplify a 248 bp fragment of the hypervariable Vp2 region, the identity of the PCR product was confirmed by sequencing, and typed by RFLP (Restriction- Fragment-Length-Polymorphism). Likewise, the phylogenetic relationships were evaluated using the Neighbor-Joining method, and were translated *in silico* to analyze the variations of aminoacids from residues 225-307. This study was carried out in the FARVET research and development laboratories. Of the 60 isolates characterized, 2 Classic patterns were identified: Lukert 5 (8.3%) and Edgard 3 (5%) grouped into two different branches, 4 US variant patterns: Delaware-A 20 (33.3%), Variant USA-625 21 (35%), Variant USA-2512 2 (3.3%), Delaware-E 1 (1.7%) grouped into a single branch, and 3 new patterns 8 (13.3%) not previously reported phylogenetically related to USA variants. No patterns were detected for virulent strains, however, 11 amino

acid mutations were detected in V242I, 1 V256I and 1 L294I related to highly virulent strains. Vaccine strains showed patterns Lukert, Edgard and Delaware-E. This is the first study that covers the genetic variability of IBDV in Peruvian field isolates using molecular techniques, finding non-virulent patterns.

Very virulent Gumboro or not? A perennial retrospective on sequencing, phylogenetic analysis and conceptual translation of VP1 and VP2.

Martin Liman, Jennifer Haneke and Theresa Oldopp

AniCon Labor GmbH, 49685 Hoeltinghausen, Germany

Clinical relevant Gumboro infections are constantly challenging worldwide chicken production. Continuous monitoring and deeper characterization of IBDV strain present in flocks has become an essential tool for veterinary service. Besides direct pathotyping via RT-qPCR more sophisticated methods such as sequencing and subsequent phylogenetic analysis and conceptual translation are used as basis for
reassessment of choice of live vaccines applied. AniCon lab (Germany) is provider of such services for samples received from Europe, Middle-East, Africa and further countries abroad. The presentation will provide analysis of hundreds of data sets from such services and discuss the development observed. Also, feedback from customers taking such service on daily routine basis will be presented and discussed, including problems of understanding the methodology, the informative value and usability of results provided and consequences of actions taken regarding choice of vaccine.

Protective effects of a fermentation metabolite product against avian influenza virus in commercial layers

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A major component to preventing and managing avian influenza virus (**AIV**) in poultry is increasing host resistance by boosting the innate immune response, particularly a type I interferon (**IFN**) response. Recent studies have demonstrated the immunological effects of a fermentation metabolite product (Original XPC™, Diamond V) in poultry, suggesting XPC is able to modulate the innate immune response. This study aimed to determine if XPC supplementation in layers is associated with decreased AIV shedding following challenge with LPAI virus. At one day of age, layers were split into two groups; one group was fed a standard commercial diet, the other group was continuously fed the standard diet containing Original XPC. At four weeks post-feed treatment, chickens were challenged with H6N2. Oropharyngeal swabs were collected to assess viral shedding titers using RT-PCR. In both groups, spleen and thymus were collected for gene expression analysis pre- and post H6N2 challenge. Gene copy numbers of IFNalpha, IFN-gamma, IL-1B, IL-6, and IFN-B were evaluated using digital droplet PCR. No significant difference in the proportion of chickens shedding virus between groups was observed. However, a significant difference in median titer between groups was observed (P <0.0001). Across all collection days, XPC supplementation resulted in a 1.3 median Log10 reduction in shedding titer compared to the control chickens. In XPC spleen tissues post- compared to pre-H6N2 challenge, IL-1B and IL-6 were significantly downregulated and IFN-alpha was significantly upregulated. These observations could provide a possible biological/immunological explanation for reduced viral shedding in XPC supplemented chickens.

Improved real-time RT-PCR tests for Class II Newcastle Disease Viruses

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Virulent Newcastle disease virus (NDV) is an exotic animal disease in the United States, but it is endemic in a large part of the world and creates a risk for introduction into the U.S. The rapid diagnosis of the virus through the National Animal Health Laboratory Network by a real-time RT-PCR test allows for a timely control effort to mitigate a future introduction. The current NDV is based on primers targeted to the matrix gene for Class II viruses, and it remains a sensitive and specific test for many lineages of the virus. Unfortunately because of the genetic diversity of NDV, some genotypes of virus may be missed or have greatly reduced sensitivity. A fresh approach to develop new real-time RT-PCR tests, capitalizing on the large NDV sequence database for class

II NDV isolates, was performed that analyzed the entire genome of hundreds of isolates to identify highly conserved regions. Using a single nucleotide polymorphism (SNP) analysis, multiple different primer sets were identified and empirically tested. Three different primers sets, 1 in the nucleoprotein and 2 in the polymerase gene were selected that had high sensitivity and specificity. Bioinformatically all three tests were more conserved than the matrix test and were able to identify more isolates than the matrix test with similar sensitivity. The availability of alternative bench validated primer sets for Newcastle disease virus provides viable alternatives if the current test does not perform as expected.

Assessment of infectious laryngotracheitis virus (ILTV) recombination in nonvaccinated and vaccinated chickens after co-infection with genotype V and VI virulent strains of the virus

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Infectious laryngotracheitis virus (ILTV) is an alphaherpesvirus that causes upper respiratory disease in chickens, resulting in significant production losses in poultry industries worldwide. Recent findings had shown that emerging virulent ILTV isolates resulted from recombination events between attenuated ILTV vaccine strains used in Australia and circulating field viruses. The development of a single nucleotide polymorphism (SNP) genotyping assay has helped to understand the recombination events that gave raise to these viruses. Following co-inoculation of specific pathogen free chickens the resultant progeny was examined for evidence of viral recombination and characterised the diversity of the recombinants over time. Results clearly identified the location of recombination breakpoints in a selection of the recombinant progeny. Furthermore, full genome sequences of field isolates from different geographical regions, including US field isolates, as compared to in vivo obtained recombinant progeny identified conserved recombination hot-spots in the ILTV genome. Preliminary analysis of co-infection with two virulent ITLV strains from the US (Genotype V and Genotype VI) in non-vaccinated, chicken embryo origin (CEO) vaccinated, or recombinant HVT-LT vaccinated chickens was performed. Preliminary results indicated that CEO vaccinated chickens significant reduced the replication of either virulent viruses in the trachea, while chickens vaccinated with the recombinant HVT-LT vaccine showed some indication of viral replication in trachea at early times post-infection. SNP genotyping of viral progeny from co-infected non-vaccinated and rHVT-LT vaccinated chickens.

Immunological characterization of monoclonal antibodies against the recombinant gpG of infectious laryngotracheitis virus

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Infectious laryngotracheitis (ILT) is produced by the Infectious Laryngotracheitis Virus (ILTV) affecting upper respiratory tract of chickens. Control programs use indirect ELISA to detect serum antibodies against recombinant viral glycoproteins, such as gpC, gpJ and gpG. However, antibodies produced several days after the infection cannot differentiate a current infection from one pass or vaccination. Infected epithelial cells secrete large quantities of viral gpG during acute infection, suggesting its potential application in the direct and early diagnosis of ILT. In the present study five monoclonal antibodies (mAbs) directed against recombinant gpG were characterized to evaluate its application in detection of native gpG from infected birds through ELISA assays. Five hybridomas were generated, four of them secreted mAbs IgG2b and other IgG2a. The mAbs were characterized by Immunofluorescence (IFI), Western Blot (WB) and ELISA assays. IFI on infected LMH cells with ILTV showed cytosolic reactivity with the 5 mAbs demonstrating the recognition of native viral proteins. The specificity of the mAbs against the recombinant and native gpG was proved by WB. Reactivity and specificity of the mAbs were estimated trough OD ratios using indirect ELISA. Reactivity against its homologous proteins showed OD ratios up to 60, while no reactivity was showed against other related virus. These mAbs can be applied to the development of

immunological methods that allow the detection of active viral infection in birds of economic importance, as well as to use in screening, evaluation of treatment efficiency and study of the role of gpG in the immunopathology of this disease.

Defining the impacts of West Nile virus on Ruffed Grouse (*Bonasa umbellus*) in Pennsylvania

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West Nile virus (WNV) was detected throughout Pennsylvania by 2002, which temporally was associated with precipitous population declines in ruffed grouse (*Bonasa umbellus*). Statewide, grouse populations have not since recovered. The present study assessed the potential impacts of WNV on Pennsylvania ruffed grouse populations through integrating laboratory- and field-derived data. Methods included both experimental infections of juvenile grouse and serology on field-collected blood samples. Grouse were susceptible to experimentally-induced WNV morbidity and mortality. Forty percent (4/10) of naïve inoculated grouse were euthanized on 7-8 days post-inoculation (DPI) due to severe clinical disease; lesions in these birds included severe myocarditis and mild encephalitis. Subclinically-infected grouse that survived to the end of the trial at 14 DPI (6/10) had moderate myocardial and more widespread neurologic lesions. These results suggest encephalitis is more likely a chronic manifestation of WNV in grouse, and that longer-term survival may have been compromised in some of these surviving birds. No in-contact sham-inoculated controls had evidence of infection. Nobuto filter strips collected from hunter-harvested Pennsylvania ruffed grouse during the 2015-2016 and 2016-2017 seasons revealed statewide WNV seroprevalence of 14% (28/202) and 22% (48/217), respectively. Seropositive birds were detected in every region of the state and in the majority of sampled counties. Collectively, experimental challenge data and field-derived serologic data provide insight into the distribution and extent of WNV prevalence and the potential impacts of WNV on Pennsylvania ruffed grouse.

Whole-genome sequencing of avipoxviruses directly from lesions using Nanopore MinION

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Avian pox is an infectious disease caused by avipoxviruses (APV), resulting in cutaneous and/or tracheal lesions. This condition may have a major economic impact in gallinaceous poultry and is an emerging concern for wildlife. Poxviruses share large genome sizes (from 130 to 360 kb), featuring repetitions, deletions or insertions as a result of a long-term recombination history. The increasing performances of next-generation sequencing (NGS) opened new opportunities for surveillance of poxviruses, based on timely and affordable workflows. We investigated the application of the 3rd generation Oxford Nanopore Minion technology to achieve real-time whole-genome sequencing directly from lesions, without any enrichment or isolation step. Two independent cases of fowlpox were diagnosed in commercial layer farms in western France. Diseased birds showed severe dyspnea and suffocation before death. All tracheal swabs and tissues sampled in both farms tested PCR positive for fowlpoxvirus. All samples tested negative for other relevant pathogens. We readily generated whole APV genomes from cutaneous or tracheal lesions, without any isolation or PCR-based enrichment. fowlpox virus reads loads ranged from 0.75% to 2.62 %. The long read size eases the assembly step and lowers the bioinformatics capacity requirements and processing time compared to huge sets of short reads (e.g. illumina data). The complete genome analysis confirmed that this Fowlpox virus clusters within clade A1 and hosts a full length REV insert. The pathobiological relevance of this REV insert, although a classical feature of FPVs, should be further investigated.

Enteric Health

Secondary enteritis and crop mycosis in commercial turkey flocks

Elise Gerken

Jennie-O Turkey Store

Multiple flocks isolated geographically to one production area within a larger production system with crop mycosis and varying degrees of secondary enteritis were identified. Many of these flocks had normal gross and histopathological findings with the exception of enteric, mainly cecal, abnormalities. Cecal wet mounts and pathology was consistent between these flocks, with enlarged ceca with watery contents ranging from dark green to brown/orange in color and a variety of secondary microbial pathogens with unknown primary causes.

Synbiotic supplementation augments protection against Eimeria challenge in turkey poults

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As consumer and regulatory opinions change, the industry is seeking alternatives to reduce usage of antibiotics and chemicals. As research develops, probiotics have been receiving increased attention for their ability to improve enteric health. The objective of this study was to evaluate the effects of synbiotic (combination of probiotics and prebiotics) supplementation on performance and intestinal health of turkeys during an *Eimeria* challenge. Day-old poults were provided either a standard diet, a diet supplemented with coccidiostat (Clinacox) or a diet supplemented with synbiotic (PoultryStar® me). On day 16, half of the pens were administered a mixed *Eimeria* challenge resulting in a total of 6 groups. The trial was conducted for 45 days with performance measurements taken on days 0, 16, 21, 35, and 45. On days 21 and 28, 5 birds/pen were euthanized for lesion scoring. Fecal oocyst shedding was also determined. Challenged birds supplemented with synbiotic showed improved body weight throughout the trial when compared to the challenged control and was similar to the coccidiostat group. Body weight gain and FCR was also improved in challenged birds administered synbiotic or coccidiostat from day 21-28. On day 21, the percentage of birds displaying lesions in the jejunum and ileum was reduced in birds provided synbiotic or coccidiostat. Similar to non-challenged controls, fecal samples from challenged birds supplemented with either synbiotic or coccidiostat displayed no evidence of oocyst shedding. Overall, these results suggest supplementation of poultry-specific synbiotic may be a viable solution to alleviate negative consequences observed during a coccidiosis challenge.

Attaching and Effacing *E. coli* as a cause of enteritis in turkeys and chickens

H. L. Shivaprasad and J. Ochoa

California Animal Health and Food Safety Laboratory System – Tulare Branch University of California, Davis.

Escherichia coli can cause various syndromes in poultry including colisepticemia, coligranuloma, salpingitis, omphalitis, osteomyelitis, synovitis and cellulitis. However, the literature on *E. coli* as a cause of enteritis in poultry is limited. Attaching and Effacing *E. coli* (AEEC) also known as enteropathogenic *E. coli* are well known to cause enteritis and diarrhea in various species of animals including poultry. AEEC are also known to increase the pathogenicity of certain enteric viruses. Between 1990 and 2017 numerous cases of enteritis associated with AEEC were diagnosed most often in turkeys but also in chickens. Most of the affected turkeys and chickens were young, less than five weeks of age and had a history of diarrhea, decreased body weight and increased mortality in the flock. Grossly, the intestines, including the ceca, were distended with watery frothy contents in most birds. Histopathology of the intestine revealed the attachment of Gram-negative bacilli to the tips of the villi, and transmission electron microscopy revealed intimate attachment of the bacteria to the enterocytes with effacement of the microvilli and formation of cup-like pedestals. *E. coli* was most commonly isolated from the ceca and virulence gene, *eae* was demonstrated in 10 % of the isolates by PCR.

Studies on the Interaction of Necrotic Enteritis Severity and Salmonella Prevalence in Broiler Chickens

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Three broiler pen studies were conducted to evaluate the effect of necrotic enteritis (NE) and respective

intervention strategies on salmonella prevalence. The NE challenge methodology was common across studies and consisted of an oral gavage of Eimeria spp. at 14 days followed by a Clostridium perfringens challenge at 18 days. The first study evaluated the effects of different times of exposure (4, 18, and 21 days) to Salmonella Heidelberg (SH) on salmonella prevalence in the presence of NE. Additionally, effects of BMD as NE intervention control were evaluated. Overall, birds that were co-infected with NE and SH at 18 days resulted in higher recovery of salmonella fro, spleens, bootie swabs and carcass rinses. Furthermore, BMD resulted in reduction of salmonella prevalence on bootie swabs (Control = 100% vs. BMD = 75%) and carcass rinses (Control = 76% vs. BMD = 33%) of 18 days SH challenged birds. It was concluded that NE severity had an effect on salmonella prevalence and that strategies that can counteract NE can have an indirect effect on salmonella recovery. Trials 2 and 3 had the objective of comparing BMD, Zoalene and a probiotic as NE interventions on the prevalence of salmonella when birds had a co-infection of NE and SH at 18 days of age. Study 2 preliminary results revealed that BMD and Zoalene decreased salmonella recovery from bootie swabs and cecae when compared to probiotic and no intervention birds. The results of study 3 are currently pending but will be presented at the meeting.

Parasitology

Gel diluents vs. water spray: practical comparisons between different gels and water

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Gel diluents have been popular for use with coccidiosis vaccine and have been proposed for other uses, such as infectious bronchitis application. Studies with different gels vs. traditional water spray have revealed some surprising differences between gels, and some interesting findings that can confound studies comparing diluents. When administered by gavage, gels stay in the crop, while water can be expectorated. In our studies, using the same vaccine, gavage with gel resulted in a significantly faster onset of immunity compared to gavage with water, likely an artifact of the expectoration. A gel containing an oral adjuvant did not result in faster onset of immunity compared to one without the adjuvant, indicating that subsequent recycling (where adjuvant has no role) is critical to the development of coccidiosis immunity. Other surprising findings included the fact that in the lower viscosity gels allow settling of oocysts over time. This must be considered when applying large volumes of coccidiosis vaccine, and may require a mixing methodology to keep oocysts suspended. Some researchers are recommending chilling diluent for use with infectious bronchitis (IB) vaccine to preserve IB vaccine titers, but if a gel is chilled, it chills the chicks much longer than water chilled to the same temperature. This is contrary to the claims of manufacturers that gel preserves chick body temperature better than water: it depends upon the temperature of the gel and the water. Precocious vaccines (with lower oocyst output post vaccination) may benefit most from gel application, based upon comparative studies.

Using Polymer Beads to Optimize Hatchery Application Of Coccidiosis Vaccines

Donald Ritter

Hatchery application of coccidial vaccine is a difficult thing to objectively measure in broilers. This talk shares information about a novel method of using polymer beads as a marker for coccidial oocysts to allow direct measurement of relative oocyst vaccine consumption. Pros and cons of actual oocyst output vs polymer beads enumeration will be discussed.

Coccidiosis Vaccines and Immunity

John E McCarty, DVM, MAM

Boehringer Ingelheim

Having been involved with coccidiosis vaccines for the past 18 years we still find users of coccidiosis vaccines have a misunderstanding of how these products help develop immunity and the management practices need to achieve this protective immunity. Multiple studies will be presented that show how these vaccines establish protection and how best to accomplish this goal.

Using a live non-attenuated coccidiosis vaccine to reduce anticoccidial resistance in a commercial facility in Ontario.

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Coccidiosis is an intestinal disease that the global poultry industry current requires $700 million annually to control. Anticoccidial medications have lost their effectiveness due to the *Eimeria* having developed resistance. Several studies demonstrate that 'seeding' a barn with oocysts from a live-coccidiosis vaccine that are sensitive to anticoccidials may reduce the level of resistance in the isolates in that particular facility. Observing fecal material for oocysts (i.e. OPG) is a reliable method for determining infection level. During a field study that involved over 40 commercial broiler farms in southern Ontario, the OPG shedding pattern documented on one farm suggested strongly that the Eimeria parasites present were likely resistant to a multitude of anticoccidial medications. OPG peaked on day 21 with several consecutive flocks on different anticoccidial programs in 2016 and 2017. OPG counts from fresh feces reached over 500,000 in each flock which was highly unusual for flocks using in-feed anticoccidials. For two consecutive flocks in the summer of 2017, a live, non-attenuated coccidiosis vaccine was administered at the hatchery (plus in-feed antibiotic) to seed the facility with drug-sensitive parasites. The first flock in the fall of 2017 consisted of the same anticoccidial program that resulted in the high OPG peak the year before. Oocysts were isolated weekly from flocks before, during, and after the vaccine administration, and an Anticoccidial Sensitivity Test was performed on each isolate. Oocyst DNA was isolated in order to identify and enumerate the Eimeria species present.

Evaluation Of Different Coccidia Vaccines, With Or Without A Bioshuttle, For Control Of A Pathogenic *E. Tenella*

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Coccidiosis is an economically significant enteric disease. *E. tenella* infects the ceca of chickens causing bloody feces, thickening and sloughing of the cecal wall, and mortality. For the past several years, a commercial poultry integrator has had pathogenic *E. tenella* challenges on multiple broiler complexes. These challenges have been unresponsive to multiple interventions including coccidiosis vaccines and anti-coccidial treatments. The purpose of this trial was to evaluate the protection properties of two coccidiosis vaccines used alone or in combination with an ionophore against the pathogenic isolate. Two challenge times were used, an early challenge to coincide with when lesions and mortality were seen, and a late challenge to determine if further cycling would improve immunity. Litter samples were collected to determine vaccinal oocyst cycling. Body weights, gross lesion scores, and oocyst count scores were collected to determine if birds were protected compared to non-vaccinated challenged and non-challenged controls. Oocysts per gram of litter counts showed that both vaccines were infective and all species of *Eimeria* were shed in the first cycle, though at different levels from each vaccine. In the early challenge, the vaccinated birds were not protected whereas in the late challenge, all vaccinated groups had significantly lower lesion scores than the non-vaccinated challenged control. Vaccinated birds had significantly lower body weights than the nonvaccinated birds. Results from this trial show that neither vaccine protected against an early challenge from this pathogenic isolate of *E. tenella*. Once the vaccine completed cycling, however, both commercial vaccines were protective.

Comparison of a commercial coccidiosis control program in broiler breeder replacement stock with and without the addition of a chemical anticoccidial.

Dr. Erin Riley[1], Wilson Benton[2], Dr. Tak Nino[3], Phil Stayer[1]

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[3]Zoetis

Two replacement broiler breeder coddidiosis control measures were assessed in a Southern US broiler integrator. One measure was commercial coccidiosis vaccination by itself and the other was using the same vaccine with chemical anticoccidial in the feed. Fecal samples and oocyst counts were performed at weekly intervals in order to determine coccidial shedding patterns. Analysis of these findings and overall outcome of livability and uniformity will be presented.

Microscopic *Eimeria maxima*: Comparing Serial Intestinal Scrapings to Composite Sample with Statistical Modeling.

Presentation will compare accuracy and variation rates of serial intestinal scraping verses composite scrapings for *Eimeria maxima* in broiler intestinal tracts. Predicative modeling will be presented as well as correlations to age of the bird and anticoccidial program. This is a follow-up study to the information presented at the 2015 AAAP meeting in Boston.

Utilizing OPG counts to monitor coccidiosis vaccination programs.

Kelli Jones[1], Nora Johnson[2], Kobus Van-Heerden[3], Hannah Menges[4], Robert Owen[4]

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There has been a major shift in the industry from ionophore and chemical use as common means of coccidiosis control to coccidiosis vaccination. As this change has occurred it is important that our means to evaluate coccidiosis control programs evolve, as well. Traditional intestinal surveys/posting sessions were initially designed to monitor the presence or absence of oocysts under programs designed to prevent cycling of oocysts. These surveys helped producers determine when to switch products based on the presence or absence of oocysts, as well as to identify when resistance may be developing. The entire concept of vaccination relies on controlled exposure of oocysts over a defined period of time, so simply identifying the presence or absence of oocysts on a given day is not enough to properly monitor a vaccine program. OPG (oocysts per gram of feces) monitoring has shown to be a very helpful tool for monitoring and troubleshooting vaccine programs in the field. Some benefits of OPGs include the fact that they are minimally invasive (only require feces), more representative (can collect as many samples as you want), and represent changes over a given time period rather than a snapshot on a given day. Opportunities and advantages of OPG counts will be discussed in detail, as well as potential lessons that can be learned through monitoring these counts.

Immunization of newly-hatched broiler chicks with a recombinant Eimeria maxima protein EmaxIMP1 conjugated to nanoparticles confers protection against coccidiosis.

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Although vaccines composed of low doses of *Eimeria* oocysts are useful for eliciting protection against coccidiosis in young chicks, there are inherent problems with this approach, including inefficient and non-uniform vaccine delivery. Incomplete vaccine coverage can lead to coccidiosis and necrotic enteritis in chicks that did not ingest sufficient vaccine because chicks are placed in litter that generally contains high numbers of *Eimeria* oocysts and *Clostridium perfringens* spores. The purpose of this study was to determine if a recombinant *E. maxima* protein, namely rEmaxIMP1 that was found by others to elicit immunity against oocyst output, could protect broiler chickens against a high dose *E. maxima* challenge. Preliminary studies using aqueous forms of rEmaxIMP1 delivered by various routes revealed negligible protection against *E. maxima* infection. However, immunization of newly-hatched broiler chicks with rEmaxIMP1 conjugated to nanoparticles (NP) conferred immunity to *E. maxima* as demonstrated in both battery cages and floor pen studies. In battery cages, weight gain (WG) in NP-rEmaxIMP1-immunized chicks was greater ($P < 0.05$) than NP-non-recombinant controls (NP-NR). In floor pen trials, protection as measured by WG was increased ($P < 0.10$) over NP-NR controls. Of interest is that NP-rEmaxIMP1 was observed within 1 hr and 6 hr in the spleen, bursa, and small intestine of NP-rEmaxIMP1 chicks. These findings indicate that NP can direct recombinant proteins, such as rEmaxIMP1, to relevant sites and elicit immunity against subsequent *E. maxima* infection. Studies are underway to figure out a practical method of delivering NP-rEmaxIMP1 to chicks in a commercial setting.

An Invention of a new method for control of coccidiosis in Chicken

Elie Barbour

The objective of this lecture is to present a compilation of data from 10 different trials performed by our research team in nine countries, aiming at invention of a

new method for the control of coccidiosis in poultry. The justification for this work is the trend in the developed and some developing countries to avoid the use of drugs in poultry husbandry, and the problems we are facing in the present control programs around the world. Accordingly, a Wide Spectrum Disinfectant (WSD) was invented that has the ability to inactivate Oocysts of 8 Eimeria spp. and a wide spectrum of bacteria and viruses. In addition, an invention of an emulsion of Essential Oil Blend In Water Extract (EOBWE) of plants was completed. A novel method, with dual approach, of application of WSD to disinfect surfaces on the contaminated farm, followed by intermittent administration of standardized dose of EOBWE in drinking water, was evaluated in many trials, against controlled and field challenges by sporulated oocysts of Eimeria spp.. Control and reference- commercial coccidiostat treatments were included in the trials for comparison. There was a consistent control of coccidiosis by the novel method, manifested by significant reduction of the oocysts output and its associated intestinal gross and microscopic lesions, with significant improvement of broilers production compared to birds in other treatments.

Is Important to Choose the Right Anticoccidial During the Winter Season?

Andres Montoya

Coccidiosis continue to be an important diseases in the poultry industry. The increase of resistance of anticoccidial medication and the increased the Antibiotic Free Production raise the important of the selection of the right anticoccidial to be part of the a rotational program for the success and sustainability of this type of production.

Detection of coccidia and other parasites in fecal samples from chicken backyard flocks

Ruediger Hauck

Keeping backyard chickens is a hobby that is becoming increasingly popular. However, backyard flocks have a lower standard of biosecurity compared to commercial poultry. For this reason, backyard flocks can be a reservoir for many pathogens, including pathogens that are less prevalent in commercial poultry, like some coccidia species, and zoonotic agents like Cryptosporidium spp. or Blastocystis spp.. As there is only limited data on the prevalence of parasites in backyard flocks, the aim of this investigation was to investigate fecal samples from backyard flocks for coccidia species, flagellate parasites, Cryptosporidium spp. and Blastocystis spp.. Forty-seven fecal samples were obtained from 41 backyard chicken flocks. Coccidia ocysts and nematode eggs were counted using a McMaster chamber. Coccidia were detected in 27 samples; oocyst counts were between 100 oocysts per gram (opg) and more than 100,000 opg with a median of 1,000 opg. The species present in the samples were determined by next generation sequencing of a fragment of the cytochrome oxidase I gene and of the 18S rRNA gene. Eight samples contained eggs of Ascaridia sp. or Heterakis gallinarum, while eggs of Capillaria sp. were detected in nine samples. Maximum worm egg counts were 1700/g for Ascaridia sp./H. gallinarum and 3700/g for Capillaria sp. Median counts were 450 and 200. Investigation for Histomonas meleagridis and other flagellates, Cryptosporidum spp. and Blastocystis spp. was done by PCR.

Evaluation of Performance and Woody Breast Myopathy in Broilers under Coccidiosis Vaccination or Anticoccidial Programs

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Overall performance and severity of woody breast (WB) lesions were evaluated in commercial high yield male broilers vaccinated with Coccivac B52 at day of age by coarse spray or under feed anticoccidial (Ionophore, Chemical or Ionophore/Chemical) programs. Randomly selected broilers were equally divided in 6 treatment groups with 8 replicates per treatment and placed in floor pen units with new wood shavings litter. Diets were formulated following the recommendations of the genetic company to fulfill the requirements for the different grow-out phases. Treatment groups were identified as follows; Vaccinated, Vaccinated & Salinomycin in grower feed, straight Salinomycin, straight Nicarbazin, Nicarbazin/Salinomycin shuttle and Control “nonvaccinated/no anticoccidial”. Body weight, feed intake and adjusted feed conversion were evaluated before changing diets with a final evaluation performed at 57 days of age. At 49 and 58 days of age, five birds from each treatment group were randomly selected for the evaluation of carcass composition and incidence/severity of WB. WB lesions were scored from 0 (normal) to 3 (severe). At 57 days of age, higher body weight, lower feed intake and lower adjusted feed conversion was observed in the Vaccinated and straight

Salinomycin treatment groups. No major differences in carcass composition among the treatment groups were observed during the 49 and 58 days evaluation. Increased severity of WB lesions was observed from 49 to 58 days of age. At 49 days of age, most of the evaluated breasts showed lesion scores between 0 and 2, while at 58 days, most of the evaluated breasts showed scores between 2 and 3. No major differences in severity of WB lesions were observed during the 49 days evaluation. At 58 days of age, breasts from the Nicarbazin/Salinomycin shuttle and control groups showed increased severity of WB lesions when compared with the Vaccinated and Vaccinated & Salinomycin in the grower feed treatment groups.

Control of Capillaria infestation with fenbendazole in chickens via drinking water treatment in combination with zero egg withdrawal

Rik Koopman

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Worm infestation is often an underestimated cause of performance loss in commercial chickens. One special challenge is *Capillaria obsignata* infestation. *C. obsignata* is common in older layers or breeders, showing infestation levels of 2000 worms per bird that can persist through production. *Capillaria obsignata* has a direct life cycle that easily persists under intensive farming conditions where constant temperature and humidity are ideal for larval development of the worms in worm eggs shed with the feces. It is often resistant to treatment regimens. Recent safety trials using the standard treatment with fenbendazole at 2mg/kg bodyweight via drinking water during 5 consecutive days demonstrated that fenbendazole was safe for all types of chickens with no negative impact on reproductive parameters. Efficacy studies demonstrated very good *Capillaria* control, with 98.1% reduction of pre-adult and adult worms of *Capillaria obsignata*. A soluble fenbendazole treatment for drinking water has been developed with user-friendly preparation and good solubility without the risk of clogging the water system for rapid response to control infections. For commercial layers, it is important to have a treatment that does not require destruction of eggs produced during treatment. Residue studies have resulted in a zero days' egg withdrawal claim for this water-soluble fenbendazole product in the EU.

Top dressing and blackhead: Fact or Fiction?

S.Genger

Several flocks experiencing various severities of blackhead were evaluated to determine the value of top dressing with new litter on mortality. Individual cases will be presented by focusing on mortality patterns of flocks breaking with histomoniasis before and after top dressing. Flocks with and without intervention were included to evaluate the effectiveness of the intervention.

Monday, July 16 Session B

Salmonella

Current Situation Of Fowl Typhoid In Latin America: Is This Disease Reaching Alarming Proportions?

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Since the end of 2016 and during 2017, there has been a significant increase in the number of cases of fowl typhoid (FT) in different Latin America countries. This disease caused by Salmonella Gallinarum (SG) is raising a singular importance because, contrary to the observations in previous years, when brown commercial layers were mostly affected, there has been an important increase in the presentation of FT cases in broiler breeders, commercial layer breeders, and broilers. Furthermore, in some cases, samples collected in hatchery plants have been positive to SG. In the majority of these cases, the clinical signs and lesions have been observed mainly in the progeny exhibiting low mortality. The environmental samples collected during routine monitoring programs in broiler breeders have been negative for SG. However, samples of organs of affected flocks, especially liver with gall bladder and bone marrow, have been positive to SG. Samples collected in the hatchery (piped egg, exploding contaminated eggs, and drag swabs), tested positive to SG. The increase in FT cases in different Latin American countries is raising concerns about the possibility that this disease may become epizootic for this region. In order to implement an effective control against FT, at

least four aspects must be identified: SG sources, risk and persistence factors, and positive reactors. It is important to highlight that in order to be successful in the control of FT, positive breeders must be eliminated.

Using salmonella prevalence in bird rinsates and parts to evaluate live ST vaccination performance in a southern U.S. broiler complex

Kalen Cookson, Manuel Da Costa and Jon Schaeffer

Zoetis US Poultry, Durham, NC

Live *salmonella typhimurium* (ST) vaccines are a production-side intervention sometimes used to help reduce colonization and shedding of salmonella. Carcass rinsate sampling at rehang is considered a good index of the salmonella levels that are coming into the processing plant and parts testing is an important new requirement of FSIS. The purpose of this study was to measure the impact of live ST vaccination on the frequency, load and serotypes recovered from carcass rinsates and parts samples over several weeks. A broiler complex in the Southeastern United States ran a week on/off trial for a total of 13 weeks including 6 weeks of Poulvac® ST vaccinated flocks. Each week, 3 carcass rinsates per lot were taken from several lots for salmonella enumeration and serotyping. Percent positive birds and lots were calculated as well as mean salmonella counts and serotype distribution. One parts sample per day was also tested for salmonella. Flock performance was also captured. Vaccinated birds showed a 31.7% reduction in positive rinsates and a 40.9% reduction in positive parts samples. Mean salmonella counts at rehang (with individual scores capped at 40) were 4.31 in controls compared to 3.53 in vaccinated lots. Salmonella O group and serotype distribution will be presented. Finally, vaccinated flocks also performed over 2 points better on adjusted feed conversion—a finding which has been seen enough times to suggest that the opportunity cost of salmonella infections may not be entirely in the processing plant.

Comparative evaluation of live attenuated *Salmonella* vaccines to prevent *Salmonella* infections in chickens

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We have recently improved our attenuated *Salmonella* vaccine platform to eliminate means of immune subversion and induce in vivo regulated delayed attenuation. Thus at the time of spray or oral immunization of chicks, the vaccine strain exhibits wild-type abilities to contend with host defense stresses. This enables efficient colonization, invasion, and dissemination to internal effector lymphoid tissues before undergoing regulated attenuation to prevent any disease symptoms and maximize immune induction. One of the means to stimulate regulated delayed attenuation is to induce controlled lysis in vivo which enhances recruitment of innate immunity, ensures vaccine clearance after several weeks post-immunization, and negates survival in nature if excreted. Thus the vaccine strains exhibit effective biological containment attributes that enhance safety. Novogen white light layer eggs are hatched in our facility and mixed sex chicks are orally immunized by gavage with vaccine suspensions into the crop at day-of-hatch with a second oral dose administered at ten days-of-age. Birds are subsequently challenged with 106 or 108 CFU of wild-type *S*. Typhimurium UK-1 (3761), *S.* Enteritidis (3550) or *S*. Heidelberg (3749). Five birds are necropsied at 4 and 11 days after challenge to quantify titers of *Salmonella* in various tissues. In these studies, comparative data is being obtained by immunization of chicks with *Salmonella* vaccines having classical means for attenuation (*aroA* and *cya crp*) and with the aforementioned newer means of attenuation with and without the regulated delayed lysis phenotype. All of these vaccines have been derived from the highly virulent *S*. Typhimurium UK-1 strain.

The role of genes encoding for tetrathionate respiration, SPI-1, and SPI-2 on the cecal colonization and systemic spread of *Salmonella* Typhimurium in chickens with or without coccidia coinfection

Claire-Sophie Rimet[a], John J. Maurer[a,b], Roy D. Berghaus[a], Brian J. Jordan[a,c], Luciana Antoniassi da Silva[a], Lisa J. Stabler[a], Kasey K. Johnson[a], Laura Tensa[a], Karen M. Segovia[a] and Monique S. França[a]

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Intestinal infiltration of inflammatory cells associated with coccidiosis may provide a growth advantage for *Salmonella* and may enhance its mucosal invasion and systemic spread in chickens. Our objectives were to evaluate intestinal inflammation induced by *Eimeria* spp. and *S.* Typhimurium challenges and to determine the fitness of *S.* Typhimurium strains deficient in tetrathionate reductase genes, SPI-1, and SPI- 2 for cecal colonization and dissemination in tissues, with or without *Eimeria* coinfection.One-day-old chickens were orally inoculated with a sham inoculum or with 400 oocysts of *Eimeria* spp. Five days later, birds were orally administrated with a combination of *S.* Typhimurium wild type and mutant strains (3.5 to 4.0 x 108 cfu/bird). Ceca, liver and drumsticks were collected 3, 7, 14, and 42 days post *Salmonella* inoculation for bacteriology. Intestinal inflammation was scored by histology. With or without coccidia coinfection, *Salmonella* cecal counts were not significantly different between *S.* Typhimurium tetrathionate reductase mutant and wild type strains; compared to the wild type strain, *Salmonella* counts in ceca were lower for the SPI-2 mutant and *Salmonella* prevalence in liver was lower for the SPI-1 mutant. *Eimeria* coinfection did not increase *S.* Typhimurium prevalence in liver and drumstick. Tetrathionate reductase genes of *S.* Typhimurium may not be required for cecal colonization in chickens. Deficiency in SPI-1 and in SPI-2, respectively, had a detrimental effect on *S.* Typhimurium systemic spread in liver and in *S.* Typhimurium cecal colonization. Low dose of *Eimeria* may not increase *S.* Typhimurium dissemination in tissues of infected chickens.

Refined Functional Carbohydrates Reduce *Salmonella* spp. Prevalence Among Broiler Breeders and Their Progeny

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Appropriate enzymatic hydrolysis of yeasts has been used to produce Refined Functional Carbohydrates (RFC) that have activities against gram negative bacteria. Specifically, these products possess sugars that interfere with *Salmonella* attachment to the intestinal lumen. In this study, three experiments were conducted to investigate the effects of a yeast-derived additive on *Salmonella* proliferation among and between broiler breeders and their progeny. In Experiment I, broiler breeders were fed either 50g/MT of RFC or a control diet continuously throughout an entire rearing period. A sample of ceca were analyzed for *Salmonella* at 23 and 64 wk. *Salmonella* were not detected in hens fed a RFC diet, while control-fed hens were confirmed 71.4% and 40% positive for cecal *Salmonella* at 23 and 64 wk, respectively. Experiment II investigated chick progeny intestines, and *Salmonella* were not detected in progeny chick intestines upon hatching. However, there was a greater incidence of Enterobacteracia “no growth” on selective media among cultures of RFC progeny intestines. Experiment III further investigated RFC effects on *Salmonella* proliferation when fed to broilers. *Salmonella* Senftenberg was isolated in 14.6% of the litter of control-fed broiler pens and not isolated in the litter of RFC fed pens using novel litter sampling methods. Broilers within pens had their cecas sampled for *Salmonella* after a lairage simulation to confirm these results. These data demonstrated that RFC was able to reduce natural proliferation of *Salmonella* in a model broiler integration.

Vaccinology

Does Early Exposure to Oral Gavage of *Clostridium Perfringens* Impact the Pathogenicity of a Severe *Clostridium Perfringens* Oral Challenge at 17 Days in a Necrotic Enteritis Challenge Model?

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Results from a floor pen study showed a significant decrease in the severity of Necrotic Enteritis in broilers raised on used litter with and without Clostridium perfringens challenge compared to broilers raised on new clean pine shavings that received the same severe Necrotic Enteritis challenge. This raises the question if the differences were due to the early Clostridium perfringens exposure in the used litter or was it due to the inherent differences in used litter vs new litter. Floor pen studies were conducted to compare the severity of necrotic enteritis (NE) lesion scores and mortality when day old broiler chicks were placed on used litter from a prior Clostridium perfringens challenge study including the used litter with the Clostridium perfringens challenge, used litter from the same flock that received

no challenge, clean pine wood shavings, compared to chicks receiving oral gavage of Clostridium perfringens culture prior to placement on clean pine shavings. The intent of the studies was to evaluate the potential of providing local immunity with this live autogenous vaccine type approach prior to typical age range on necrotic enteritis outbreaks and to evaluate the impact of such Clostridium perfringens exposure on the early health and performance of the broiler chicks. All treatments received the same controlled severe oral Clostridium perfringens challenge at 17 days. Results, discussion and conclusions from these studies will be presented.

Vaccination of Turkey Breeders with Poulvac® *E. coli* to Improve Progeny Health

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NCSU College of Veterinary Medicine, Zoetis, Prestage Farms

Systemic *E. coli* infection (colibacillosis) is an important cause of poult mortality in the early life period. This study evaluates the impact of breeder flock Poulvac® *E. coli* vaccination on the health and performance of progeny. Our hypothesis is that perturbation of hen microbiota by repeated Poulvac® vaccination will decrease colonization and transmission of *E.coli* with virulence or antimicrobial resistance determinants to poults. To test this hypothesis, breeder hens received Poulvac® vaccine monthly throughout the laying period. Non-vaccinated (control) hens from the same breeder complex served as age-, environment-, and management-matched controls. Breeder cloacal swabs and meconium samples were cultured for *E.coli*. Poults from vaccinated hens (PV poults) and control hens (C poults) were placed together and followed throughout the 11 week growing period. The cause of mortality was determined by necropsy evaluation. Birds with collibacillosis or yolksacculitis were cultured for *E.coli. E. coli* strains isolated from breeder cloacal swabs, meconium, and poults with colibacillosis were genotyped using ERIC PCR and selected strains were sequenced to identify virulence and antibiotic resistance genes. No differences in mortality from colibacillosis and *E. coli* population were found between the PV and C poults. Genotyping and genomic sequence provided insight into transmission dynamics and intestinal colonization in the early life period by *E.coli* strains. Detailed findings will be discussed.

Stability, safety and efficacy of a multivalent HVT-vectored vaccine against Marek's disease, Newcastle disease and infectious bursal disease

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Vectored vaccines using the turkey herpesvirus (HVT) as a backbone to express antigens of Newcastle disease virus (rHVT/NDV) or infectious bursal disease virus (rHVT/IBDV) are efficacious and widely used. These vaccines are administered in the hatchery to chicken embryos (*in ovo*) or to day-of-age chickens (subcutaneous). Due to concerns related to replication interference of the vaccine vectors, rHVT/ND and rHVT/IBD vaccines are not administered concurrently, leaving to the producer the decision of which vectored vaccine to use. In order to overcome vector replication interference and allow for concomitant vaccination against Newcastle disease (ND) and infectious bursal disease (IBD) with a single vectored vaccine, a novel dual-insert, HVT-vectored vaccine expressing antigens of NDV and IBDV has been developed. This dual-insert vaccine has been shown to be stable, safe and efficacious. After backpassage of the vaccine in chickens and passages in chicken embryo fibroblasts, there were no differences in the sequences of the inserted genes and flanking regions relative to the original vaccine, and both inserted genes were stably expressed. Furthermore, the dual-insert vaccine had similar host range, tissue tropism, and shedding and spreading properties as the HVT parent. Finally, efficacy of this HVT-vectored vaccine in combination with the Marek's disease virus Rispens strain was demonstrated against very virulent Marek's disease (MD) in SPF chickens, and against ND and IBD in SPF and maternal antibody positive chickens. These characteristics of the novel dual-insert vaccine position it as a pivotal tool toward controlling MD, ND and IBD in commercial poultry operations.

Effect of Dose and Genotype on Overcoming Maternal Immunity After *In Ovo* Vaccination with Recombinant Newcastle Disease Vaccines

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In ovo vaccines have multiple advantages and have been used for more than 20 years to protect against poultry diseases like infectious laryngothracheitis, infectious bursal disease, and Newcastle disease (ND). Current *in ovo* vaccines against Newcastle disease (ND), one of the most economically important poultry diseases, are recombinant vaccines using herpesvirus of turkeys (HVT) as a vector expressing Newcastle disease virus antigens. Although proven efficient, these vaccines have some limitations such as delayed immunogenicity, the inability to use a second HVT vaccine after hatch, and the use of only one expressed antigen at a time. The co-expression of immunostimulatory cytokines by live and inactivated Newcastle disease viruses has been suggested as a tool to improve vaccine efficacy. Additionally, the use of vaccines homologous to the challenge strain has been shown to decrease viral shedding. We have previously demonstrated that a ND vaccine expressing antisense interleukin 4 (IL-4R) administered *in ovo* in SPF eggs elicits high levels of antibodies, provides 100% protection after challenge with a homologous virulent strain, and has no adverse effect on hatchability and survival. We will further evaluate this vaccine in commercial eggs to study its efficacy in the presence of maternal antibodies. Commercial eggs from hens vaccinated against ND will be inoculated with increasing doses at 19 days of embryonation. Hatchability, survival post-hatch, and development of post-vaccination humoral immunity will be determined. The effect of vaccine dose on the ability to overcome maternal antibodies and on the humoral response after *in ovo* vaccination will be reported.

Transmission assessment of Infectious laryngotracheitis virus (ILTV) in recombinant (r)HVT-ILT *in ovo* vaccinated broilers after experimental challenge

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In recent years, the use of recombinant ILTV vaccines has significantly expanded as it offers a safer vaccination alternative for the control of the disease. Previous work in our laboratory showed that *in ovo* vaccination of broilers with standardize doses (1000, 3000 and 6000 PFUs) of a rHVT-LT vaccine decreased clinical sings, maintained weight gain, and reduced virus replication in the trachea after challenge with a virulent viral strain. However, the potential of *in ovo* rHVT-LT vaccinated broilers to shed virus has not been directly addressed. The objective of this study was to evaluate the level virus shedding after challenge of *in ovo* vaccinated chickens with standardize doses (1000, 3000 and 6000 PFUs) of a rHVT-LT vaccine. Unvaccinated age-paired broilers were introduced to groups of rHVT-LT *in ovo* vaccinated and challenged broilers at 0, 4, 8, and 12 days post challenge. Clinical signs assessment and tracheal swabs collection, to determine challenge virus genome load, was performed every 4 days from 4 to 16 days post-contact. Results from this study demonstrate that rHVT-ILT vaccination mitigated clinical signs of the disease. However, vaccination with rHVT-ILT did not reduce the challenge virus replication to levels sufficient to halt virus spread to unvaccinated chickens. However, we observed that independently of the vaccine dose reduced air circulation was capable to halt virus transmission. Further studies are warranted to better understand the roll of the air movement and dust in the viral transmission.

Comparison of IBV Vaccinations using Sprayable Gel, Droplet Gel, or Water as Diluent

Abigail Reith, Deborah Hilt, Brian Jordan

Infectious Bronchitis Virus (IBV) is an economically important respiratory disease seen throughout the poultry industry. Multiple methods of control have been employed with live vaccination being primary. Novel administration methods are also being evaluated to optimize vaccine coverage and minimize stress. In this study, two types of gel products were compared to conventional aqueous spray. Massachusetts-type vaccine was mixed with water, a spray-type gel, and a drop-type gel and applied to groups of 100 chicks. Chick activity was recorded for 5 minutes, and external and rectal body temperatures were recorded every 5 minutes for 1 hour after vaccination. The 3 groups of vaccinates and an unvaccinated control group were then reared in separate colony houses. Choanal cleft swabs of the vaccinates were taken on days 3, 5, 7, 10, and 14 to assess vaccine coverage based on real time-PCR. A random sample of birds from each house were taken 28 days post vaccination, challenged with a pathogenic Mass-type IBV and placed in isolator units. Five days post challenge, the birds were observed for respiratory signs,

swabbed for real-time PCR, and tracheas were taken for ciliostasis scoring. Chicks vaccinated with a spray-type gel experienced more severe temperature fluctuations than chicks vaccinated via drop-type gel or aqueous spray. Aqueous spray vaccination produced higher uptake and replication after vaccination and better post challenge protection than either gel types. This data suggests that application of live IB vaccine via gel has no benefit over the traditional method of aqueous spray.

Infectious Bronchitis Virus

Observation, Assessment and Utilization of Currently Available Infectious Bronchitis Diagnostic and Characterization Methods

John El-Attrache, Ha-Jung Roh, Melissa Madsen, Ellie Brimer, Jose Linares, Istvan Kiss, Tamas Mato and Vilmos Palya

Ceva Animal Health

Infectious bronchitis virus (IBV) is prevalent in virtually all countries with commercial poultry production. Vaccination against IB is not always successful due to improper vaccine takes and a continuous emergence of IBV variants. Therefore it is important to confirm infectious bronchitis (IB) clinical observations in a laboratory. This is often quite difficult due to the fact that more than one live IB vaccine is applied due to the simultaneous presence of multiple antigenic types of IBV. Virus isolation in embryonated eggs and observation of typical IB embryo lesions has long been the gold standard. This is traditionally followed by serotyping via virus neutralization assays in embryonated eggs as well. Since the mid 1990s, this gold standard has been replaced with the more rapid and efficient molecular detecting and genotyping methodologies. qRT-PCR has been more widely used for IBV detection, while sequencing (both partial and complete) of the S1 gene has replaced traditional serotyping, even though genotyping has not always been shown to correlate with serotype. Even with this advancement, a better understanding of the molecular methods being utilized by the diagnostic laboratory is needed to correctly interpret results and facilitate adequate vaccination decisions. This need is most important when variant IBV antigenic types are identified; multiple IBV antigenic types are present or when new IB vaccines are introduced. Multiple examples of the above scenarios will be presented that include interpretation of qRT-PCR results for vaccine takes, detection and characterization of IBV, as well as proper analysis of S1 sequencing.

Detection and Typing of Infectious Bronchitis Virus (IBV) Isolates in Pennsylvania

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Infectious bronchitis virus (IBV) infections cause a variety of significant clinical problems in chickens including respiratory disease (tracheitis, air sacculitis, increased incidence of secondary bacterial infections), reproductive issues (decreased egg production and increased egg shell abnormalities) and renal disease (interstitial nephritis, wet droppings, dehydration). Morbidity can be high and mortality is often increased, especially if secondary infections are present. Current IBV identifications are somewhat random and inadequate to detect trends. There is a need for routine semi-annual or annual IBV detection and characterization of strains circulating in the Pennsylvania (PA) chicken industry. Sampling is done from a list of potential flocks that are defined up on certain criteria to achieve a broad cross section of both layer and broiler flocks (and breeders if applicable) in widely diverse geographic region as possible. Choanal swabs from 125 flocks are taken and examined during a 3 month period. Samples will be subjected for virus isolation and rRT-PCR for IBV detection, and, if positive, for genotyping by rRT-PCR for several specific genotypes. The location of the IBV positive flock and the genotype is plotted using the GIS mapping system. Determination of circulating strains in PA is key to making sound, coordinated decisions about appropriate IBV vaccination programs. Additionally, the need for quick, affordable and accurate testing is likely to be ongoing as poultry densities increase and new strains of IBV emerge. The developed molecular assay generates knowledge of IBV prevalence in the clinical submissions. Additionally, the assay identifies the IBV variants and produces partial sequence data of S1 gene. Designing a whole IBV genome sequencing protocol using Ion Torrent technology (Next Generation Sequencing (NGS)) for further typing of new variants and detection of mixed infection will help further with typing of IBV specifically in case of identification of a new IBV variant.

Sequence Analysis and Challenge Study on Infectious Bronchitis DMV Strain from Canada

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Since 2016, an increased number of infectious bronchitis virus (IBV) cases have been reported in broilers, broiler breeders and layers in Ontario, Canada, and 49% of reported IBV cases were identified as DMV strain. While DMV/1639/11 cases reported in the U.S. were mainly associated with severe nephropathogenic symptoms, including severe flushing in broilers, the DMV strain from Canada has been affecting broiler breeders and layers by causing egg production drop with severe respiratory lesions. Currently, there is no licensed IBV vaccine in Canada to protect commercial chickens against the circulating DMV strain. To aid in the resolution of the current DMV issues in Canada, we conducted a challenge study to evaluate protection provided by a Georgia type vaccine. In the U.S., this Georgia type vaccine is licensed to provide protection against GA08, GA13, and DMV/1639/11; therefore, the Georgia type vaccine and in combination with Mass vaccine was evaluated for protection against Canada DMV strain. DMV virus was isolated from samples from the Animal Health Laboratory at the University of Guelph, and confirmed by qRT-PCR and full S1 sequencing. Eye drop vaccination at 1 day of age was followed by challenge at 28 days of age in SPF leghorns. At 5 day post challenge, choanal swabs were collected and processed for virus isolation and qRT-PCR to determine the protection by vaccines. Additionally, whole genome sequencing was performed for both Canada DMV isolate and DMV/1639/11 isolates to further analyze variation at the genomic level.

Understanding Innate Responses and Resistance to Infectious Bronchitis Virus in Resistant and Susceptible Chicken Lines Using Primary Cells

A. P. da Silva, K. Schat, R. A. Gallardo

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We have performed in vivo challenge experiments in different MHC haplotype congenic chicken lines to assess resistance and susceptibility to IBV. In previous experiments, we have demonstrated that MHC haplotypes B2 and B19 were relatively the most resistant and susceptible respectively to our IBV challenge. The main differences between B2 and B19 haplotype chicken lines were observed in humoral responses in tears at 14 DPI. However, it was not possible to draw conclusions from innate immunity assessments such as cytokine levels in tears and sera at 2 and 6 DPI. In order to supplement the knowledge acquired from in vivo experiments, we performed an in vitro investigation using tracheal epithelial and macrophage primary cell cultures. Primary cell lines derived from B2 and B19 haplotype chickens were challenged with IBV M41 in vitro and cytokine levels were assessed at 2, 4, 6, 12 and 18 hours post-infection. Besides minimizing the number of animals used, the in vitro setting allowed us to better control variables present in in vivo experiments and facilitated the assessment of innate immune responses in the two MHC chicken lines. Preliminary results will be presented.

Age of Vaccination Influences Infectious Bronchitis Virus Cross-protection

Zegpi, R., K. Joiner, A. Mishra, S. Gulley and H. Toro

Auburn University

Antigenically novel infectious bronchitis viruses (IBV) readily circumvent extensive vaccination with type-specific vaccines. Previous studies have suggested that vaccination at an early age, i.e. 1-day-old, generates suboptimal protective immunity. We hypothesized that postponing vaccination elicits increased cross-protective immunity. Chickens were vaccinated with a Massachusetts vaccine at hatch or on days 10 or 14 after hatch and challenged with an Arkansas virulent strain. The results of both trials indicate that vaccination at a later age is associated with increased reduction of viral load and increased production of anti-IBV antibodies. Challenged chickens which were vaccinated on day 1 showed increased (P<0.05) mucosal thickness, lymphocytic infiltration, as well as tracheal deciliation compared to chickens vaccinated on day 10. B cells in the Harderian gland were statistically increased in chickens

vaccinated on day 14 compared to vaccination on day 1 of age while the opposite occurred regarding CD8+ cells.

Outbreaks of Infectious Bronchitis virus Arkansas DPI serotype in broiler chickens in California.

Shivaprasad H. L., M. Crispo, B. Crossley, J. Ochoa, R. Gallardo and S. Stoute

California Animal Health and Food Safety Laboratory System – Tulare, Turlock and Davis Branches, University of California, Davis.

Infectious Bronchitis (IB) is a highly contagious common respiratory viral disease of chickens caused by coronavirus. There are many serotypes of the virus including Mass, Conn, Ark 99, Ark DPI, Cal 99, GA 98, *etc*. There are many vaccines including Ark DPI which are used to control IB. Between January 2014 and October 2017, isolation of Ark DPI was made from 28 different broiler cases involving 26 different ranches. The age of the broiler chickens ranged from 18 days to 53 days with a median age of 33 days. Clinical signs included respiratory signs, watery eyes, nasal discharge and increased mortality and pathology included sinusitis, tracheitis and bronchitis. Secondary colibacillosis was common, as well as, a few other concurrent infections. IBV was identified by PCR and was isolated in chicken egg embryos most commonly from the trachea and lung and occasionally from the cecal tonsils of broiler chickens. The IBV serotype Ark DPI was confirmed by sequencing up to 600 base pairs of the S1 glycoprotein. Ark DPI vaccine is not used in broiler chickens but used in layer chickens in California. Therefore, the occurrence of ARK DPI in broiler chickens is most likely a spill-over from the layer chickens.

Field experience with GA08 + Mass vaccinated broilers in a complex using Ark vaccine and experiencing Ark rolling reactions

Jose A. Linares[a], Ha Jung Roh[a], Kelli Jones[a], Leslie Johnson[b], Jessica Hockaday[b], Natalie Armour[b]

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Currently only a few Infectious Bronchitis Virus (IBV) types are available as live attenuated vaccines, whereas many different types of IBV are circulating and infecting commercial poultry globally, hence it is critical to identify and evaluate available vaccines with cross-protective potential. Among widely used IBV vaccine types in U.S, the Ark type vaccine has been often associated with outbreaks of respiratory disease and emergence of variant strains in vaccinated flocks. Various investigations and evidence suggest that Ark type vaccine may not only fail to replicate uniformly in birds after spray vaccination, but also tend to persist in the birds longer. Therefore, searching for an alternative to Ark type vaccine is imperative. Our GA08 vaccine has demonstrated efficacy against GA13 and DMV1639 as well as homologous GA08, therefore shows cross-protective potential. In controlled experiments using SPF layer type birds, our GA08 vaccine in combination with Mass vaccine repeatedly showed significant reduction of Ark challenge virus measured by qRT-PCR, virus isolation, ciliostasis, and histopathological analysis. To expand our knowledge on the cross-protective potential of our GA08 + Mass vaccine combination, we conducted a field trial with broilers in a complex with documented Ark rolling reactions. Results from this field trial will be presented.

Infectious Bronchitis Virus Vaccine Types Ma5 And 4/91 Protect Against A Wide Variety Of Challenge Serotypes

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Infectious bronchitis virus (IBV) is a highly infectious respiratory pathogen of commercial poultry. Infection with IBV causes the disease infectious bronchitis (IB), which predisposes chickens to secondary bacterial infections and airsacculitis. Airsacculitis then leads to condemnation at processing, which is a great cost to the industry. Due to the infectivity of IBV and losses associated with the disease, nearly all commercial poultry are vaccinated with live-attenuated IBV vaccines. IBV vaccines are serotype specific and single serotype vaccines do not provide adequate cross-protection against heterologous challenges. It has been shown, however, that combinations of IBV vaccine serotypes can provide adequate cross-protection against heterologous challenges, though not all combinations are effective against all challenge types. The Ma5 and 4/91 genotype (Massachusetts and 793/B serotype) IBV vaccines have been tested against many heterologous challenges, and this vaccine combination is commonly used throughout Europe to control IB. Our laboratories have tested this vaccine combination against multiple U.S. type IBVs (GA08, GA13, Ark, and DMV/1639) as well as several Canadian variants (QuMV, Canadian 4/91), and

protection from ciliostasis and a significant reduction in viral load compared to non-vaccinated chickens was seen in every experiment. Furthermore, this protectotype vaccination strategy was able to prevent challenge virus re-isolation in embryonated eggs in the DMV/1639 experiment. Taken together, the Ma5 and 4/91 IBV vaccine combination has proven to protect against challenge with different serotypes of IBV and under certain circumstances could be used as a simple but effective control program for IBV.

Effectiveness of a vaccine program based on the Protectotype® concept against an infectious bronchitis variant virus strain challenge (GA13) in Costa Rica

Tania Román[1], Martín Silva[2], Julio Barbosa[1], Luis Gómez[3], Danilo Hernandez[1], Rebeca Zamora[1] and Rebeca Elizondo[4]. Fernando Vargas[5], Linnea Newman[5]

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In 2016, a broiler producer in Costa Rica reported some flocks with slightly higher mortality occurring after 35 days of age, with mild or no respiratory signs. The same flocks where the increased late mortality was observed had also higher condemnation index at the processing plant, mainly due to airsacculitis. RNA samples were collected from the farms and sent for RT-PCR and sequencing in three different laboratories in US and Europe. Almost all samples were identified as being the same IB variant virus strain KM087780, also known as Georgia 13 (GA13), with more than 97% homology. Losses due to carcasses condemnation reached more than US$500,000 per month. The vaccination program-- a single application of H120 vaccine at day of age via coarse spray-- failed to protect the birds. Two applications of Mass vaccine Ma5 at days 1 and 14, reduced but did not eliminate the problem. In May 2017,a new program using Ma5 at day one and 4/91 (793B) strain at day 14 was introduced. The combination of two genotypically different strains against a third virus is based on the Protectoype concept (Cook, 1999) and its efficacy can be assessed by the ciliostasis test. The Ma5 + 4/91 program produced a significant improvement with lower mortality rates and lower airsacculitis condemnation. New RNA samples taken from vaccinated farms were negative for the variant KM087780 after two production cycles.

Subclinical losses caused by Infectious Bronchitis virus in broiler chicken flocks

Jorge Chacón, Leandro Bianchet, Fabiano Fabri

Ceva Animal Health, Paulínia, Brazil

Infectious Bronchitis (IB) is a highly contiguous disease that causes respiratory, reproductive and renal disturbs in commercial birds. In case of broiler flocks, the infection is detected by serology and molecular methods after appearance of clinical disturbs such us respiratory signs, increase of mortality and increase of airsacculitis condemnation rate in the slaughterhouse. These signs are normally detected in some months of the year and the appearance of the disease is associated to climatic variations. In order to determine the effect of IB field virus during the absence of evident clinical disease, 35 broiler flocks of three Brazilian broiler companies (1,3 million of evaluated chickens) were monitored for the presence of IBV by serology and PCR. The results showed high presence of IBV BR strain in the three Brazilian companies located in Paraná and São Paulo States in months without the presence of clinical disease. The no-infected flocks had better productive results (feed conversion, mortality rate and daily body weight gain) and lower condemnation rates associated to respiratory problems in the slaughterhouse. This work shows for the first time that Infectious Bronchitis virus circulates and causes economical losses in the absence of evident clinical signs.

Farm and slaughterhouse parameters affected by BR strain of Infectious Bronchitis Virus

Leandro Bianchet, Fabiano Fabri, Haiala Coelho, Jorge Chacón

Ceva Animal Health, Paulínia, Brazil

Several epidemiological studies demonstrated very high detection rate and wide geographical distribution of Infectious Bronchitis Virus (IBV) belonging to the BR genotype in Brazil. Genetic analysis of S1 gene showed up to 70% of molecular relationship between this predominant genotype and Massachusetts (Mass) viruses. Experimental studies showed the failure of Mass vaccines to confer full protection against BR challenge virus while full protection was observed using homologous virus in the vaccination. This work presents for the first time the economic benefits of the effective control of IBV based on productive results of more than 26,4 million of broiler chickens of nine Brazilian poultry

companies. The flocks vaccinated with vaccine strain homologous to the most prevalent field strain (BR virus) had better financial results in the farm (feed conversion, daily body weight gain, mortality rate and use of antibiotics) compared to the Mass-vaccinated flocks. In the slaughterhouse, the flocks vaccinated with BR vaccine strain had lower mortality rate during the chicken transportation to the slaughterhouse, lower total and partial condemnations by airsacculitis and colibacillosis and reduction of carcass processing time, compared to the Mass-vaccinated flocks. The results obtained in the field trials are according to the scientific literature information that indicates that the genetic similarity between vaccine and field strains is a crucial point for effective control of IBV.

Marek's Disease

Comparison of different Marek's injection protocols on the livability of commercial laying hens

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Globally, Marek's disease remains a concern for egg producers. The primary method for mitigating the effects of Marek's infection in laying hens is day of hatch subcutaneous vaccination with Rispens vaccine (CVI988). In areas with a high Marek's burden, some hatcheries have used a double dose of the Rispens vaccine, or given chicks two separate injections at least one hour apart. Providing two separate injections allows any birds that may have been missed on the first injection to be more likely injected the second time. The trial herein evaluated 3 different day of hatch layer pullet Marek's vaccine injection protocols with a sham-vaccinated control group. Within each vaccine treatment group half the birds received an antibiotic with the Marek's vaccine and half received no antibiotic. Group one received a single subcutaneous injection of CVI988 using the Nova Tech PSP single injection module. Group 2 received two separate CVI988 injections using the Nova Tech PSP single injection module, performed one hour apart. Group 3 received two CVI988 injections using the new Nova Tech PSP dual injection module. Group 4 was injected with diluent. Birds were reared in a facility with a known Marek's virus challenge, actively shedding virus particles. Birds were evaluated for tumor lesions at 16 weeks of age. The goal of this study was to observe the difference between clinical Marek's disease in birds with differing vaccine application protocols. Final results are not available yet; full data and analysis will be available by the AAAP Symposium.

Comparison of Marek's disease virus challenge strains for vaccine evaluation

John R. Dunn

USDA – ARS Avian Disease and Oncology Laboratory, East Lansing, MI

The Code of Federal Regulations requires an immunogencity test for Marek's disease vaccines be conducted in a group of SPF chickens (9 CFR 113.330(c)). For testing a serotype-3 vaccine, a "virulent" label claim requires at least 80% of the unvaccinated chickens to develop lesions, however, multiple companies have recently reported inconsistency in development of Marek's disease in unvaccinated commercial SPF chickens inoculated with standard challenge strains. We evaluated three alternative challenge strains using commercial SPF chickens from two sources in an effort to provide more consistent results. Marek's disease virus strain 617A produced the highest percentage of positive birds in both trials, but had less tumors and more nerve enlargement compared to other strains. GA/22 and the RB-1B BAC clone produced the highest percentage of birds with tumors. Results of a protection study using 617A and GA/22 strains will also be discussed.

Study of MHC class I downregulation by highly virulent MDV strains and its role on Marek's disease virus-induced immunosuppression

I.M. Gimeno, A.L. Cortes, S.M. Reddy, B. Lopez de Juan Abad, A. Limsatanun

North Carolina State University, College of Veterinary Medicine, Raleigh, NC. a Texas

A&M College of Veterinary Medicine, College Station, TX. In previous studies, we have developed a model that allows us to reproduce under laboratory conditions MDV-induced immunosuppression (MDV-IS). Using that model, we have demonstrated that infection at hatch with the most virulent vv+MDV strains jeopardized the protection conferred by infectious laryngotracheitis (LT) vaccines in commercial meat type chickens. Furthermore, none of the currently used MD vaccination protocols protected against MDV-IS. Only one experimental vaccine lacking two copies of the oncogene meq (Md5-BACΔMEQ) was able to protect against both

tumors and MDV-IS. The specific objectives of this work were: 1) to evaluate if vv+MDV strains that induce MDV-IS (648A and 686) down-regulate MHC-I to a larger extent than MDV strains of lower virulence that do not induce MDV-IS (Md5, 617A); 2) to demonstrate that attenuation of vv+MDV strains results in reduced ability to downregulate MHC-I; 3) to demonstrate that vaccination with Md5-BACΔMEQ, but no other vaccine protocols, protects against the ability of vv+MDV strain 686 to down-regulate MHC-I expression. Our results show that splenocytes of chickens inoculated with vv+MDV were severely impaired to proliferate when exposed to Concavalin A. Furthermore, vv+MDV induces severe changes in the splenocyte population by 28 days after infection that do not occur after infection with v or vvMDV strains. Particularly, there is a cell subpopulation (CD45+MHC-I+ within the large activated lymphocytes) that greatly decreases in chickens inoculated with vv+MDV strains. MHC-IA transcripts were increased but B2-microglobunin transcripts were decreased in chickens inoculated with vv+MDV. Vaccination with Md5-ΔMEQ protected against all these negative effects.

Generation of transgenic chickens expressing Cas9 and gRNAs targeted to Marek's disease virus.

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We previously reported the construction of CRISPR/Cas9 plasmids directed against ICP4 of Marek's disease virus (MDV) which interfered with MDV replication in vitro and were tolerated in chicken primordial germ cells. We have initiated the production of germline transgenic chickens that constitutively express Cas9 and MDV-specific gRNAs. Sixty-four of 178 stage 14 HH chicken embryos injected with Tol2 constructs encoding Cas9-GFP or MDV gRNAs-DsRed and transposase plasmid hatched. Twelve males containing sgRNA-DsRed and 13 males containing Cas9-GFP were raised to sexual maturity. Semen from these males was collected and analysed by qPCR using Tol2 specific primers. We identified 3 chimeric roosters for both the sgRNA-DsRed and Cas9-GFP constructs which are now in a breeding program to generate germline transgenic chickens that stably express MDV specific sgRNAs and Cas9.

Tuesday, July 17 Session A

Diagnostics

The use of a NEW NDV-F ELISA to detect antibodies following vaccination with recombinant HVT vectored vaccines for NDV

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This presentation will highlight the results obtained on a new NDV-F ELISA after vaccination with the recombinant (r)-HVT-ND vaccines. Results will also provide guidelines to assess successful vaccination and aid in the interpretation of field results. Furthermore, results from samples collected at various times after vaccination with different r-HVT-ND vaccines alone and in combination with conventional live vaccines will be presented. Finally, a comparison of results at different time points following vaccination with r-HVT-ND vaccines using the NDV-F ELISA, NDV ELISA and HI tests will be presented.

Development of infectious bronchitis (IBV) enzyme-linked immunosorbent assay (ELISA) using pseudotyped vesicular stomatitis virus (VSV) particles expressing IBV spike proteins

Infectious bronchitis is a highly contagious upper respiratory tract disease of chickens that is caused by infectious bronchitis virus (IBV) and constitutes a major cause of economic loss throughout the industry. Vaccination against multiple IBV serotypes in commercial poultry operations is routinely practiced, therefore it is imperative to detect and differentiate these serotypes in an accurate and rapid fashion. The major determinant of IBV serotype specificity is the spike protein as it contains epitopes for serotype-specific antibodies. Enzyme-linked immunosorbent assay (ELISA) is the standard test that is widely used to detect and quantify IBV antibody titers in flocks due to its high sensitivity. The diagnostic antigen used for most commercial kits is inactivated whole virus, so commercially available ELISA tests for IBV do not distinguish between different serotypes. Vesicular stomatitis virus (VSV) has been widely used to produce pseudotyped virus particles, which contain the envelope glycoproteins of heterologous viruses. By using VSV as a

backbone, it is possible to generate a pseudotype that has the spike protein of IBV. Thus, the preservation of the native form of the spike protein can be achieved, and it only requires knowledge of the nucleotide sequence of the spike gene, which can be cloned for expression. These newly prepared antigens will only contain the serotype determinant spike, thus making the development of serotype-specific ELISAs possible. The goal of this study is to access the feasibility of developing a diagnostic test that can distinguish serotype-specific antibodies against various IBV types using pseudotyped IBV particles.

Recent observations in the use of ProFLOK® IBV Ab ELISA kits in detecting variant Infectious Bronchitis Virus (IBV) infections

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Infectious bronchitis (IB) continues to be an important disease in commercial poultry. New variants of infectious bronchitis virus (IBV) are often detected and isolated worldwide. The rapid detection of these new variant viruses is critical in controlling the disease. The ProFLOK® IBV Ab ELISA test was designed to detect field infections in highly immunized flocks. A question of interest is whether or not this established ELISA is able to detect new circulating IBV variants. A series of studies were conducted to demonstrate the ability of ProFLOK IBV to detect antibodies to various IBV variants commonly observed in the US and other poultry relevant countries. Serum samples from commercial broilers and SPF chickens exposed to various field and/or vaccine IBV serotypes were tested. ProFLOK IBV was able to detect antibodies to common and all variant IB viruses included in this study as early as 5 days post-challenge even in vaccinated flocks. These studies demonstrate that ProFLOK IBV Ab ELISA is more sensitive than other antibody ELISA kits used.

Analysis and Application of Average ELISA Titers for Mississippi Poultry

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Enzyme-linked immunosorbent assay (ELISA) is a valuable tool for poultry disease diagnostics and vaccination program monitoring. Mississippi State University's Poultry Research and Diagnostic Laboratory (PRDL) performs the bulk of serologic testing of commercial poultry in Mississippi, generating data which is broadly representative of the immunization and disease challenge status of commercial poultry in the state. The first objective of this study was to perform a retrospective analysis of average Newcastle disease virus (NDV), infectious bronchitis virus (IBV), infectious bursal disease virus (IBD) and reovirus (REO) ELISA geometric mean titers (GMTs) and coefficients of variation (CVs) for Mississippi broiler breeder and broiler chicken flocks at different age intervals over a two year period from January 2016 to December 2017. The second objective was to develop specific serologic baselines for a Mississippi poultry company based on an analysis of NDV, IBV, IBD and REO ELISA titers from healthy flocks on a particular vaccination program. Finally, the application of the 2016/2017 Mississippi average ELISA titers and the company-specific baseline titers will be demonstrated using ELISA results from different farms.

Serological surveillance of wild and pen-reared pheasants in the Central Valley of California

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The release of pen-reared ring-necked pheasants (*Phasianus colchicus*) on wildlands is a common management practice for the purpose of augmenting local pheasant populations and providing recreational opportunities to hunters. However, this management practice may be a risk factor in disease transmission to

wildlife and domestic poultry. Studies focused on disease exposure measured via the presence of antibodies are a key first step toward understanding risk in both pen-reared and wild pheasant populations. We investigated exposure to diseases in wild and pen-reared pheasants in the Central Valley of California during 2014 and 2015. We found positive serology for antibodies against hemorrhagic enteritis (HE), Infectious Bursal Disease (IBD), and Newcastle Disease (ND) in pen-reared pheasants. Wild pheasants also showed positive serology for antibodies against HE, IBD, and ND, as well as, Infectious Bronchitis virus, Infectious Laryngotracheitis, and *Pasteurella multocida*. While the nature of this study (i.e. cross-sectional serology study) cannot be used to understand the temporal transmission of disease between wild and pen-reared pheasant, the results do show that wild and pen-reared pheasants appear to be have historical exposure to the above mentioned organisms. Additional research including isolation and molecular characterization would benefit our understanding of disease interaction between wild and pen-reared pheasants.

Use of Ultrasound to Diagnose Cystic Oviducts in Infectious Bronchitis Virus (DMV Strain) Positive Immature Leghorns

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Infectious Bronchitis Virus (DMV strain) has been diagnosed in a few layer flocks in the summer of 2017 in Quebec, Canada. Affected flocks showed variable percentage of egg production at peak of lay ranging from 40 to 77%. Birds from these flocks and identified as false layers, exhibited at necropsy variable size cystic left oviducts, oviducts presenting partial to total aplasia. Similar cystic left oviducts were also observed as early as 8 weeks of age in IBV-DMV positive replacement pullet flocks with variable prevalence. Because an owner wanted to salvage as many pullets as possible for a flock destined to produce eggs for a pharmaceutical company, and because it is becoming increasingly ethically challenging to eliminate a whole flock when a majority of birds can still lay, solutions to predict the number of affected pullets were searched. Serial necropsies were conducted at different ages to determine the prevalence of cystic oviducts and it was decided to try ultrasonography to determine the presence or absence of a cystic oviduct in immature leghorns. Ultrasounds have been used in animals for numerous years to evaluate their reproductive status. However, birds possess air sacs which usually hinder the use of such technique in avian species. An intravaginal probe introduced in the cloaca was used to visualize the reproductive tract of immature leghorns and followed by a necropsy to verify the oviduct. Ultrasonography proved to be a quick and effective method to diagnose for the presence of a cystic oviduct in immature leghorns.

Development of Reliable Techniques for the Differential Diagnosis of Avian Tumour Viruses by Immunohistochemistry and Polymerase Chain Reaction from Formalin-Fixed Paraffin-Embedded Tissue Sections

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A variety of techniques have been developed as diagnostic tools for the differential diagnosis of tumors produced by Marek's disease virus (MDV) from those induced by avian leukosis virus (ALV) and reticuloendotheliosis virus (REV). However, most current techniques are unreliable when used in formalin-fixed paraffin embedded (FFPE) tissues, which many times is the only sample type available for definitive diagnosis. A collection of tumors was generated by the inoculation of different strains of MDV, REV or ALV singularly or in combination. FFPE tissue sections from tumor and non-tumor tissues were analyzed by optimized immunohistochemistry (IHC) techniques and polymerase chain reaction (PCR) with newly designed primers ideal for DNA fragmented by fixation. IHC and PCR results were highly sensitive and specific in tissues from single-infected birds. IHC furthermore allowed detection of the

specific cells that were infected with different viruses in tumors from birds that had been inoculated simultaneously with multiple viruses. These new protocols can be immediately applied for both diagnostic and research purposes to help accurately identify avian tumor viruses in routine FFPE tissue sections.

Assessment of Practical Infectious Laryngotracheitis Vector Vaccine and Field Virus Differentiation Methodologies

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Next Generation Sequencing (NGS) is a DNA-sequencing test, available at commercial scale. The NGS test facilitates high throughput multiplex reactions that can detect and quantify multiple gene targets. NGS allows the detection of specific vector vaccine and whole virus sequences that can be analysed to differentiate vaccine (whole virus or recombinant vaccines) from true field origin virus of Infectious Laryngotracheitis (ILT). An ILT Indirect ELISA test has been developed for the specific detection of antibodies against glycoprotein I (gI), allowing the monitoring of flocks vaccinated with HVT-ILT recombinant vector vaccines. This test may also be used for monitoring conventional vaccines (chicken embryo or tissue culture origin), with equivalent performance to standard commercial ELISAs. The objective of this study was to correlate results of samples from birds vaccinated with a vector LT vaccine at one day of age using NGS and ILT gl Indirect ELISA testing at 22 days of age and 9 weeks of age, respectively. For this purpose, spleen and feather follicle impressions on FTA cards were sampled from a commercial layer pullet flock located in the west cost of the U.S. Serum samples were processed to detect antibodies against gI. Complete background and history of the sampled flock involved in the study, in addition to the testing results and correlations will be presented.

Troubleshooting an infectious laryngotracheitis outbreak on a multi-age commercial layer pullet operation using next generation sequencing

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A multi-age commercial layer pullet operation with a history of chick embryo origin (CEO) vaccine use began to suffer severe infectious laryngotracheitis (ILT) breaks in the spring of 2017 through the fall of 2017. This farm was geographically isolated from most of the poultry production in this province, and the initial sequencing of the virus revealed that it appeared to be of vaccine-origin. Modifications to the vaccination program and changes to dosages of CEO vaccine failed to control the outbreak. Over time, ILT-related mortality in pullets began to occur at progressively younger ages. The decision was made to discontinue the use of CEO vaccine and implement an alternate vaccination strategy. The objective was to transition the site using an rHVT-ILT vaccine and a tissue culture origin (TCO) vaccine. In order to measure success on this high-challenge site, it would be necessary to quantify how many birds were missed at the hatchery in order to exclude vaccine failure as a cause of ILT-related mortality. The efficacy of in-hatchery vaccine application was evaluated using next generation sequencing to determine the percentage of birds that did not receive a protective dose of rHVT-ILT at the hatchery. Next generation sequencing was further employed to monitor the transition of this site in subsequent flocks. This case report is a clear example of how new molecular technologies can be employed in complex field challenge situations.

Dissecting respiratory integrity and co-infections in poultry using RPN-PCR (Respiratory Panel Nanofluidic real-time PCR): field investigations in Asian countries

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Proper diagnostic of poultry respiratory diseases using clinical clues is challenging due to the co-infection of several pathogens, the impact of environmental factors and management practices. In most Asian countries nowadays, the access to a quality diagnostic laboratory is an issue for many reasons, including laboratory capabilities, prices of analyses and lack of education of the poultry workers. The RPN-PCR, an innovative quantitative PCR method based on a microfluidic technology, targets more than 20 respiratory pathogens

(virus, bacteria and aspergillus). This study presents PCR results from Asian commercial broiler breeder and layer chickens showing acute respiratory field cases (general respiratory symptoms, mortality, egg-drop production, egg quality issues). Tracheal swabs samples, spread on FTA cards, were sent to the famous virus detection laboratory of the Toulouse Veterinary School, France (VIRAL lab). AIV, IBV, ILTV, MG, MS, ORT, *Pasteurella multocida*, *Bordetella avium*, *Ecoli* and *Avibacterium paragallinarum* were identified in several samples. 75% of the cases showed co-infections from two to seven pathogens. The relevance of the co-infections depends on the vaccination program, the local context, the positivity level of each PCR and the number of positive analyses per case. These analyses resulted in a comprehensive picture of respiratory diseases in Asian commercial chickens, suggesting widespread and diverse co-infections. This screening approach has proven to be useful for commercial poultry farmers to understand the dynamics of respiratory diseases in their flocks and therefore to take the appropriate action to improve the flock protection against these diseases using biosecurity, vaccinations and prevention antibiotics.

Rapid and sensitive characterization of Newcastle disease virus genotypes by MinIONTM nanopore sequencing

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Newcastle disease outbreaks are global challenges to the poultry industry. Rapid identification and characterization of the circulating Newcastle disease viruses (NDV) during disease outbreaks is crucial for disease management. Current real-time PCR diagnostic assays can rapidly detect avirulent and virulent NDV, but cannot differentiate different genotypes of NDV or detect a mixed NDV infection. Genotyping of NDV is important to assist with proper vaccination procedures and track effectiveness of control measures. Reverse transcription PCR coupled with Sanger sequencing provides genetic information of the agent, but it requires expensive equipment, maintenance of specific laboratory facilities, and several days for results. Here, we demonstrate that a fourth-generation DNA sequencing technology based on the MinION Oxford Nanopore device provides highly sensitive, specific, and cost effective genetic characterization of fifteen clinical samples with a short turnaround time (~6 hours). Total RNA from 33 egg grown viruses and 15 clinical swab samples containing different NDV viruses was reverse transcribed and amplified with NDV specific primers. DNA libraries were prepared with 550 to 700 ng of purified, barcoded, pooled amplicons and were sequenced on the MinION device. Data analysis was performed in a customized bioinformatics workflow within the Galaxy platform. All NDV genotypes were accurately detected with a sensitivity of EID50 101/0.1ml and a 99.8% sequence identity to the expected consensus obtained in sequencing with alternative methods. Furthermore, cost efficiency was achieved by multiplexing using a barcode system. Reproducibility of results was demonstrated by 100% sequence identity among two repetitions.

Differentiating vaccine-related Fowl Cholera from naturally occurring disease.

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Poultry Diagnostic and Research Center, The University of Georgia

Vaccine-related fowl cholera must be considered when flock mortality increases after use of a live vaccine product. Three live *Pasteurella multocida* vaccines are available but all are derived from the original Clemson University vaccine strain. All vaccines serotype as Heddleston 3,4, however this is also the most common serotype of outbreak isolates in broiler breeders and turkey. Therefore serotyping may not be useful for diagnosing vaccine-related fowl cholera. The goal of this research was to produce a vaccine-specific test to differentiate vaccine related disease from naturally occurring outbreaks. A vaccine specific PCR was developed from loci identified by genome sequencing

and validated against the type strains, previously strain typed turkey 3,4 isolates, and clinical poultry isolates archived at the Poultry Diagnostic and Research Center. Preliminary results indicate that vaccine strains were commonly isolated from the lesions of broiler breeders exhibiting signs of fowl cholera post vaccination. These isolates typed as serotype 4, but were indistinguishable from the vaccine in PFGE strain typing. These results suggest that vaccine-related disease may not be uncommon in broiler breeders and suggest that serotyping may not be reliable in distinguishing serotype 4 isolates from the vaccine.

Diagnosis of turkey enteritis: multiplex PCR versus next generation sequencing (NGS)

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Enteric viruses have been associated with enteritis and growth deficiencies in commercial turkeys and broilers. The MVDL has offered electron microscopy and virus isolation to detect viruses in feces/intestinal contents of affected birds, and more recently has applied molecular diagnostic tools. Since 2009, we have offered a multiplex RT-PCR for the three most common enteric viruses of turkeys e.g., astrovirus, rotavirus type D and avian reovirus. We are now developing a new real time enteric multiplex PCR for the simultaneous detection of astrovirus, rotavirus, picornavirus and picobirnavirus. Additionally, we now test feces/intestines with NGS using Illumina MiSeq. Both Illumina MiSeq and multiplex PCR were applied to fecal samples collected from clinical cases of poult enteritis syndrome, mid-growth turkey enteritis, malabsorption in broilers, enteritis in commercial broilers, and egg production drops. NGS has detected calicivirus, picornaviruses, rotaviruses and picobirnaviruses in some of these samples. Astroviruses were commonly detected by both methods and in all age groups but picorna-and picobirnaviruses were detected mainly in turkeys (> 5 weeks) with diarrhea or malabsorption. Multiple infections with emerging and re-emerging viruses were common. The hypothesis-free metagenomics strategy enables NGS to detect mixed infections with different microorganisms and to identify novel pathogens requiring no prior knowledge of the pathogen(s). The enteric multiplex PCR detects specific viruses with high sensitivity and is completed in few hours. NGS is costlier and more time consuming than multiplex PCR, but it is invaluable for detecting novel pathogens and comparing viral sequences.

Identification and survey of Avibacterium paragallinarum (Infectious coryza) in backyard and commercial poultry flocks of Georgia using PCR.

Douglas Anderson

Georgia Poultry Laboratory Network, Forsyth, Georgia, USA

Infectious coryza is an acute respiratory disease of chickens caused by the organism, Avibacterium paragallinarum. It is characterized by nasal discharge, sneezing, and infraorbital swelling of the face. In the USA, coryza normally affects pullets, layers, breeders, and occasionally broilers. Historically, the prevalence appeared to be low and occurred below the 34th parallel. However, more recently the disease has been identified further north. Avibacterium paragallinarum is a gram-negative, pleomorphic, nonmotile, catalase-negative, microaerophilic rod that requires nicotinamide adenine dinucleotide (V-factor) for in vitro growth. In recent years, occurrence of strains without the V-factor requirement in neighboring Mexico has made more difficult the ability to culture diagnostic cases. The need for an accurate diagnostic test has further arisen as agreement between practitioners and laboratories appears to have little consensus as to disease or organism prevalence. An Avibacterium paragallinarum PCR was developed and validated at our Laboratory. It was used to survey the prevalence among commercial poultry flocks as well as backyard flocks (historically believed to be the disease reservoir). The use of the PCR was beneficial in determining flock status in the State of Georgia.

Development of *Mycoplasma gallisepticum* F-Strain Vaccine Specific PCR Protocols

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Mycoplasma gallisepticum, the causative agent of chronic respiratory disease in chickens and the most pathogenic of the avian mycoplasmas, continues to produce significant economic losses to the worldwide

poultry industry despite extensive attempts to control the effects of infection. Live attenuated vaccines are increasingly used in the poultry industry to control avian mycoplasmosis but unfortunately, some vaccines may revert to virulence and vaccine strains are generally difficult to distinguish from natural field isolates, so techniques that allow differentiation of field strains of *M. gallisepticum* from the vaccine strains are increasingly important. In this research, whole genome sequencing and comparison of F-strain vaccines and "F-strain-like" isolates from vaccinated and non-vaccinated commercial poultry. Whole genome sequencing was performed using Illumina and compared to *M. gallisepticum* Rlow reference genomes. The collective contigs for each isolate were annotated using fully annotated *Mycoplasma* reference genomes. The analysis revealed genetic differences among several alleles including *vlhA* alleles and a gene annotated as α-amylase. PCR protocols were designed to target sequences unique to F-strain and to differentiate the vaccine from other live vaccine strains, reference strains and field strains. The PCRs can be used to detect F strains without the time, expense and specialized equipment needed for DNA sequencing.

Tuesday, July 17 Session B

Avian Influenza

Infection and transmission of LPAIV H9N2 viruses in SPF chickens.

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Low pathogenic avian influenza viruses (LPAIV), subtype H9N2 are responsible for economic losses in the poultry industry worldwide. Using multiple strains of H9N2 LPAIV isolates from different years and countries, we inoculated 3-week old SPF laying hens intranasally to evaluate infectivity and transmissibility of these viruses. Oropharyngeal swabs and blood were collected to determine virus shedding and antibody production. Birds inoculated with a 104 and/or 106 EID50 of multiple U.S. H9N2 isolates showed no clinical signs of infection. However, swab and serological analysis showed that one bird challenged with A/Turkey/TX/89 was shedding virus 2 and 4 dpi and seroconverted based on HI titers. Additionally, one bird challenged with A/Mallard/MN/304/98 was positive based on HI titer but was not shedding detectable virus. SPF chickens inoculated with a 102 or 104 EID50 of foreign H9N2 LPAIV isolates resulted in infection and transmission in chickens with differences in seroconversion, virus shedding, and transmission seen between isolates and doses. Chickens inoculated with isolates at the higher dose (104 EID50) had higher HI titers and virus shedding with increased transmission. However, only one isolate resulted in infection and transmission at the low dose (102 EID50). These results suggest that recent foreign G1 lineage or Chinese lineage H9N2 isolates are more poultry adapted than U.S. H9N2 isolates which showed low infectivity and transmission compared to the foreign isolates.

Tropism of clade 2.3.4.4. H5N8 HPAI viruses in feather pulp

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Highly Pathogenicity Avian Influenza viruses (HPAIVs) display a tissular pantropism, which implies that they could spread in feather pulp (FP). The detection of HPAIVs from feather samples has been positively evaluated for H5N1 or H7N2 HPAIVs. These studies suggest that viral loads detected in the pulp of immature feathers are equivalent or often higher than those detected on swabs. In this study, we investigated on field cases the suitability of feather pulp samples for detection of clade 2.3.4.4. H5N8 HPAIV in ducks or geese. Six flocks, confirmed H5N8 positive, were included from January to March 2017: on each flock, at least 10 birds were sampled: tracheal (TS) and cloacal swabs (CS) were taken, as well as immature wing feathers. RNA was extracted from swabs or FP and real-time RT-PCR was performed for M and H5 genes. An absolute quantification of M or H5 RNA copies was performed for each sample and the distribution of viral RNA loads was statistically analyzed. In all flocks included in the study, viral RNA loads detected in FP were at least equivalent and in most cases up to 103 higher than those detected in either TS or CS. We estimated the sensitivity and the specificity of each sample type for different thresholds, using a Bayesian model and confirmed a better sensitivity of FP. An experimental H5N8 infection was performed on 10-week-old mule ducks. Elective tropism of clade 2.3.4.4. HPAIVs in feather pulp was confirmed, suggesting that FP could be considered in surveillance sampling protocols.

Comparison of Manual and Mechanical Defeathering for Airborne Virus Production During the Processing of Asymptomatic H5N1 Virus-Infected Chickens

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Airborne transmission of H5N1 highly pathogenic avian influenza (HPAI) viruses has occurred among poultry and from poultry to humans during home or live-poultry market (LPM) slaughter of infected poultry, and such transmission has been experimentally reproduced. Manual defeathering is one of the most inefficient steps of the standard chicken processing and has been gradually replaced in LPM by automatic or mechanical defeathering equipment. In this study, manual and mechanical defeathering methods were tested and compared for the generation of infectious airborne HPAI virus particles. Chickens were inoculated intrachoanally with H5N1 HPAI virus and, after confirmation of asymptomatic infection based on virus shedding, birds were anesthetized and slaughtered following standard processing using either manual or automatic defeathering. During the processing, a cyclone air sampler was used to detect infectious virus in large (>4 µm), small (1-4 µm), and fine (<1 µm) airborne particles; additionally, a particle counter was used to measure particle emissions at each step of the slaughtering process. The use of a mechanical defeathering device did not significantly increase generation of infectious particles when slaughtering H5N1 virus-infected chickens, but it did significantly increase total particles in the air compared to the use of manual defeathering, in particular during the cleanup step. These results suggest that the use of automatic defeathering devices should be avoided or validated for reduced-aerosol generation in order to decrease risk of airborne generation and transmission of H5N1 HPAI viruses to other poultry or humans.

Pathology and tissue distribution of H5N2 HPAIV Clade 2.3.4.4. in vaccinated and experimentally challenged commercial Broad Breasted White turkeys.

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Three different vaccination regimens were tested for protective efficacy against challenge with the A/turkey/Minnesota/12582/2015 (H5N2) HPAIV strain clade 2.3.4.4 in commercial Broad Breasted White turkeys under experimental settings. The turkeys were primed at 2-days of age and boosted 3 weeks later with different combinations of the clade 2.3.4.4 H5N2 inactivated vaccine and a live recombinant alphavirus-vectored vaccine expressing the H5 hemagglutinin of the clade 2.3.4.4. Subsequently, birds were divided into two groups challenged at two different ages (respectively, 6 and 16 weeks of age). Among birds challenged at 6 weeks, only the unvaccinated showed lesions in vital organs associated with the presence of influenza virus nucleoprotein. At 16 weeks, mortality, lesions and viral systemic dissemination affected both vaccinated and unvaccinated groups signing a drop in protective efficacy from the vaccines. The alphavirus-inactivated vaccine combination was the most effective at both challenge timepoints. The least protective vaccine regimen was the homologous alphavirus prime-boost, as mortality and lesions occurred at both challenge timepoints. Lesions consistent with HPAIV infection ranged from none to hemorrhagic and fibrinoheterophilic pneumonia, acute multifocal necrotizing pancreatitis and splenitis, heterophilic meningoencephalitis and myocardial degeneration and necrosis. Influenza A virus nucleoprotein was detected by immunohistochemistry in multiple cell types including neurons, glial cells, ependymal cells, respiratory epithelial cells, pulmonary pneumocytes and macrophages, myocardiocytes, endothelial cells, splenic reticuloendothelial cells, pancreatic acini and ductal cells. In conclusion, the heterologous prime-boost live-inactivated vaccine approach conferred the best and longest-lasting protection from mortality, lesions and viral dissemination among the vaccine protocols tested.

A rapid-response antiviral for poultry: aerosolized ammunition against avian influenza outbreaks

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Our team is developing a patent-pending antiviral technology, SiVEC-AIV, for rapid and large-scale aerosol application to treat and prevent avian influenza virus (AIV) in poultry. SiVEC-AIV combines RNA interference with a delivery platform that utilizes non-pathogenic bacteria to generate and deliver silencing RNAs to respiratory tissues, thereby inhibiting viral replication at the site of infection to prevent viral spread. Previous studies using SiVEC-AIV (intranasal administration) demonstrated safe and effective use to protect against AIV in commercial chickens. Chickens treated with the SiVEC-AIV antiviral demonstrated up to 6,300-fold less virus shedding and a median of 400-fold less virus shedding compared to untreated chickens ($p<0.0001$). The objective of the work presented here was so to further demonstrate that this technology can be administered to chickens as an aerosol using industry standard equipment and protect against AIV (H6N2), by reducing viral shedding. Results indicate SiVEC-AIV reduces AIV viral shedding in aerosol-treated compared to untreated chickens. Testing to assess efficacy against HPAI is currently underway and preliminary results from this study will also be discussed. The overarching goal of this work is to develop this novel technology for commercial application as an economical and effective solution, ideal for rapid, large-scale protection. Overall, this antiviral has the potential to substantially improve avian influenza outbreak response and minimize reaction times to rapidly protect the poultry industry against AIV. Moreover, this is a platform technology that could be modified for effective and timely control for a wide range of avian respiratory diseases.

Genetics, receptor binding, and pathogenicity investigation of H6 avian influenza virus in Taiwan

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H6 AIVs have been identified as potential progenitors of the highly pathogenic H5N1 AIV that emerged in Hong Kong in 1997 and the H5N2 AIV in Taiwan in 2004. Epidemiological data revealed that H6 sero-prevalence rate was 25.9%, and the H6 virus prevalence rate was 7.3% in the field of Taiwan during 2013-2014. In this study, we further studied the genetic/antigenic relationship, receptor binding profiles, growth kinetics and pathogenicity of the H6 chicken isolates identified since 2000. The results showed these H6 AIVs undergo antigenic changes, presenting frequent substitutions in the globular head domain of the HA protein. Most of the H6 chicken isolates carry the molecular signature for mammalian adaptation, ie. a G228S substitution in the HA protein, and for the pathogenicity determinant, ie. a 14-a.a. deletion in the NA stalk. Of note, the HA and NA proteins of the new clade of chicken isolates share high sequence homology with the H6N1 human isolate identified in 2013 and the dog isolate found in 2014. As some studied viruses exhibited moderate binding to the 2,6-linked sialic acid (mammalian type), they can grow efficiently in MDCK cells without prior adaptation. Upon the experimental infection in SPF chickens, the new clade of H6N1 AIVs showed enhanced kidney pathogenesis, rapid shedding, and productive virus replication in major organs. In summary, H6 AIVs are prevalent in Taiwan, causing sporadic outbreaks in chickens. The “human (or dog)-infecting”-like H6N1 strains continue to circulate among domestic chickens and accumulate changes, which pose a clear threat to public health.

Toll-like receptors in different poultry species and their role in influenza virus pathogenesis

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Toll-like receptors (TLRs) are important endosomal receptors that recognize structurally conserved molecules of pathogens and mediate the induction of innate immune responses. Although several reports have shown antiviral roles of specific TLRs against influenza virus in mammals, limited studies have been conducted in poultry species. Our study initially focused on quail, an important intermediate host for influenza virus, and characterized TLR3 gene and its biological significance in pathogenesis. We newly determined the coding DNA sequence of quail TLR3 and found it to be highly identical with TLR3 sequences of other poultry species. We found an alternative splicing site that results in three different forms of TLR3 in quail and turkey but is absent in chicken. In addition, the proteolytic cleavage site reported to

activate and induce signal transduction in mammals is missing in poultry species due to the absence of nine amino acids at the cleavage site of avian TLR3 ectodomain. The presence of alternative spliced forms and absence of cleavage site indicates the different mechanisms of TLR3 mediated immune response induction in poultry species. In a separate study, we optimized an *in vitro* system to upregulate the expression of TLR3 and also developed a poultry specific CRISPR/Cas9 system to efficiently generate TLR knockout cell lines. Our study using TLR3 knockout and over-expression cells indicated involvement of TLR3 in influenza pathogenesis *in vitro.* Our efforts on generation and comparative analysis of TLR knockout cells from different poultry species will lead to better understanding of different TLRs in avian influenza pathogenesis.

Potential mediators of *in ovo* delivered double stranded (ds) RNA-mediated antiviral response against low pathogenic avian influenza virus infection

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Toll like receptor (TLR) 3 is a critically important innate pattern recognizing receptor that sense many viral infections. Although, it has been shown that double stranded (ds) RNA can be used for the stimulation of TLR3 signaling pathway in many host-viral models, it's effectiveness as an antiviral agent against low pathogenic avian influenza virus (LPAIV) needs further investigation. In this study, we delivered TLR3 ligand, dsRNA, *in ovo* at embryo day (ED)18 and infected with H4N6 LPAIV 24-hours post-treatment. Our results demonstrate that the pre-hatch treatment of eggs with dsRNA reduces H4N6 replication in lungs. Further studies revealed that the *in ovo* delivery of dsRNA increases TLR3 expression, type I interferon (IFN) production and number of macrophages in addition to mRNA expression of interleukin (IL)-1β in lung 24-hours post-treatment. Moreover, we discovered that the dsRNA elicits antiviral response against LPAIV correlating with type I IFN activity in macrophages *in vitro*. Our findings imply that the TLR3 ligand, dsRNA has antiviral activity *in ovo* and *in vitro* correlating with macrophage numbers and functions highlighting the importance of using dsRNA as an antiviral agent against LPAIV infections.

Adaptation of Wild-bird Origin H5Nx Highly Pathogenic Avian influenza Virus Clade 2.3.4.4 in Vaccinated Poultry

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The 2014-2015 incursion of H5Nx clade 2.3.4.4 high pathogenicity avian influenza (HPAI) virus caused the largest animal health emergency in U.S. history and renewed interest in developing vaccines against these newly emergent viruses. Our previous research demonstrated several H5 vaccines with varying genetic relatedness (85-100%) to the 2.3.4.4 HPAI challenge viruses (A/Gyrfalcon/Washington/2014 H5N8 or A/Northern Pintail/Washington/2014 H5N2) provided variable protection of poultry against lethal challenge. In these studies, virus adaptation to immune pressure was captured using NGS analysis of swab samples taken at various time points after challenge of vaccinated animals. Whole genome SNP analysis was performed on virus sequence obtained from two separate vaccine experiments. The variant frequency for each gene sample analyzed was determined at 1000 reads per nucleotide or above, with 5% minimum change required for inclusion. Overall, analysis of 50 samples containing complete viral genomes identified 198 amino acid (AA) mutations in either sham or vaccinated birds. The greatest percent of AA mutations were observed in the replication machinery (PA, PB1, PB2) or nucleoprotein (NP), suggesting viral adaptation into poultry. Approximately 14% of mutations were observed in the hemagglutinin gene, and were mainly found in vaccinated animals. Interestingly, identical mutations in polymerase and nucleoprotein genes were observed in groups of animals that died (PB2-T683S and NP-R100K) compared to animals that survived (NP-M105I/V), implicating a potential role in pathogenesis. Taken together, these studies demonstrate that evolution of HPAI virus populations could be influenced by immune pressure and species change.

Infectivity, pathogenicity, and transmission of a 2016 H5N8 2.3.4.4 highly pathogenic avian influenza virus in mallards and chickens

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Highly pathogenic avian influenza (HPAI) viruses subtype H5N8, clade 2.3.4.4, have caused outbreaks in wild birds and poultry across many countries in 2016-17. In this study, we examined the infectivity, pathogenicity, and transmission of a virus isolate from the current H5N8 outbreak in Europe (A/Tufted-duck/Denmark/11740/LWPL/2016/H5N8) in chickens and mallards. Birds were infected with varying doses of virus and were observed for clinical signs and mortality. All mallards were infected when given moderate to high virus doses [104-6 mean embryo infective dose (EID50)]. In contrast, chickens were infected only when given the high dose (106 EID50), indicating that they were less susceptible to infection with the virus. The mortality was high in infected chickens, as expected with HPAI viruses. Interestingly, high mortality was also observed in the mallards (88%). This result is different than what was shown with a H5N8 virus from the 2014-15 outbreaks in North America, which did not product mortality in mallards. High virus titers (107-8 EID50) were shed by infected chickens and mallards. In addition, high titers were also observed in brain, spleen, heart, lung, and muscle tissues. The virus transmitted to direct contact mallards but not to chickens. In conclusion, the high virus replication in infected mallards and easy transmission to contacts explains the fast spread and associated mortality of this H5N8 virus in wild birds worldwide in 2017. Sequencing of viruses shed from infected mallards and chickens will determine genetic changes associated with virus adaptation in these two bird species.

A comparison of the pathways for the introduction and/or spread of influenza A viruses between commercial upland gamebird and other poultry productions

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Up-to-date knowledge on disease transmission mechanisms is critical for the designing of targeted control strategies. Pertaining to influenza A viruses (IAV) in poultry, the pathways of introduction may not necessarily be the same as those for its subsequent spread. Also based on the findings from our previous study, we hypothesize that the pathways may not be similar across poultry sectors. In this study, we use literature review, expert elicitation and outbreak analysis techniques to test this hypothesis. First, we gathered data on day-to-day farm contacts that we deemed important for IAV transmission. Then, we compared the frequency of and biosecurity practices during these contacts across poultry sectors. Lastly, we used the United States 2014 - 2015 HPAI epizootic data to compare the vulnerability of the different poultry-type premises. Specifically, we calculated the total number of days that each poultry-type premises was in a designated Control Area (CA) during the entire outbreak vis-à-vis the eventual infections of each type. Preliminary results indicate that the contact structure is different across poultry sectors which implies that sector-specific pathways could influence IAV infection dynamics. CA survivability analysis results will be discussed.

Application of Diagnostic Test Results to Predict Epidemiological Variables Relevant to Choosing Disposal Options for LPAI Infected Poultry Flocks

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Options for disposition of LPAI infected poultry flocks include mass-depopulation with off-site or onsite disposal and moving recovered birds to processing. The choice of the disposal option and timing of movement is partly informed by the stage of LPAI infection (time since flock infection), and prevalence of infectious birds in the

flock, along with several other risk management considerations. This analysis aimed to estimate the prevalence of infectious birds in an LPAI infected broiler breeder flock and predict the mean number of days until the flock stops shedding, given the observation of a set of diagnostic test results. We used stochastic simulation models to evaluate various diagnostic testing options, including serological testing of 15 samples using the Agar Gel Immunodiffusion (AGID) assay, RRT-PCR testing using pooled samples of 11 swabs each, and combinations of RRT-PCR and serological tests. We discuss the relative efficiency of different protocol options for reducing the uncertainty in the outcome variables and providing confidence that the number of infectious birds at the time of movement to disposal is acceptable. Preliminary results indicate that combinations of serological and RRT-PCR testing may have a greater efficiency for estimating the days until an infectious flock stops shedding, and other relevant outcomes.

Highly pathogenic avian influenza virus survival in turkey carcasses

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Disposal of carcasses by on-site burial or landfilling after an outbreak of highly pathogenic avian influenza virus (HPAIV) has led to questions about how long the virus can survive in the carcasses at ambient temperatures above freezing. Data are very limited and are generally for processed meat at refrigeration or cooking temperatures. Therefore, risks associated with environmental contamination from burying or landfilling HPAIV infected have not been determined. In order to address how long HPAIV would survive in poultry carcasses, two-week old commercial broad-breasted white turkeys were infected with A/chicken/IA/13388/2015 H5N2 HPAIV. When clinical signs were apparent the birds were euthanized. The carcasses were individually sealed in bags and held at 72-77°F. Organ and tissue samples (breast muscle, thigh muscle, heart, spleen, lung, small intestine and feather pulp) were collected at the time of euthanasia and then daily for nine-days post euthanasia. Fluid leaking from the carcass was collected starting 2-3 days post euthanasia. A total of five of each sample type were collected at each time point. Samples were tested for virus infectivity by isolation in embryonating chickens eggs.

Controlled marketing, is it still relevant for the control of low pathogenicity avian influenza?

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Controlled marketing is a systematic approach to walk a low pathogenicity avian influenza (LPAI) virus infection off of a single- or multi-age poultry farm and to recover the economic value of the bird. Since 1978, controlled marketing has been a favored and successful strategy used by the Minnesota turkey industry as part of the Minnesota AI Control Plan. In an attempt to deal with the financial losses from recurring seasonal LPAI outbreaks, the MN AI Control Plan was structured with the goal of preventing as much economic impact during outbreaks as possible. Between the years of 1980 and 2003, other states outside of Minnesota such as Pennsylvania, Utah, and California have instituted controlled marketing during LPAI outbreaks with degrees of success. In addition, between 2009 and 2011, LPAI outbreaks in the states of Illinois, Tennessee, Missouri, and Nebraska were contained via a combination of foam depopulation as well as controlled marketing. Poultry farms and production systems have changed dramatically since controlled marketing was last described in the literature. What does the practice look like today and is it still effective given what we know now about the potential mutation of LPAI viruses to become highly pathogenic? We will explore the history and current applications of controlled marketing in turkeys and other species and its potential applications in modern poultry systems.

Using geospatial methods to measure the risk of environmental persistence of avian influenza in South Carolina

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Avian influenza (AI) is a highly contagious virus affecting wild birds and domesticated poultry. In addition to exposure from wild birds, outbreaks in domestic poultry

can be initiated and propagated by exposure to virus surviving in the environment near poultry operations. This study aimed to define areas of South Carolina at heightened risk for environmental presence of the AI virus using geospatial methods. Environmental covariates known to influence AI survival were determined based on published studies. Data on the distribution of these variables within South Carolina were downloaded from publicly available sources. All covariate layers were mapped at a 1-km resolution for ecological time periods (e.g. breeding, fall migration, winter, spring migration) and weighted based on their influence for virus survivability. Environmental suitability maps were created using these layers and ESRI ArcGIS 10.4 software with the Predictive Analysis tool. After classifying suitability map values based on World Organization for Animal Health (OIE) risk assessment guidelines, < 1% of the 1-km geographic areas showed a high risk of AI persistence in the four-time periods assessed. A higher number of geographic areas showed either moderate or low risk (1–2% and 17–19%, respectively), with a higher percentage of risk present in winter and spring migration. When farm density data were combined with AI suitability maps, there was a very low percentage of locations where moderate or high environmental risk co-located with low, moderate, or high farm density areas (0.001 – 0.120% of areas based on time period). These results improve our knowledge of environmental suitability for AI in South Carolina and may be used to support planning and preparedness efforts that aim to mitigate and/or quell agricultural outbreaks of AI within the State.

Posters

Antibiotic Resistance

Antimicrobial Resistance in *Enterococcus* species isolated from Poultry Production in Alberta

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Antimicrobial resistance remains an important problem in food animal production and foodborne bacteria despite changes and reduction in use of antimicrobials. Environmental reservoirs of resistance genes and horizontal gene transfer maintain prevalence of resistance genes even in absence of selection pressure. Enterococci inhabit the gastrointestinal tract of animals, and may cause foodborne illness when ingested. Use of avoparcin and virginiamycin in poultry has been implicated causing resistance against vancomycin and teicoplanin, glycopeptides used in human medicine. This is a multi-partner study between University of Calgary, Agriculture and Agri-food Canada, Alberta Agriculture and Forestry, and Public Health Agency Canada. Strains were isolated from 30 poultry farms within the CIPARS sampling framework. This study compares resistance to 4 drug classes in 96 *Enterococci* isolated from poultry production in Alberta during 2015-2016. The 2015 *Enterococcus* species were determined to be *faecium* (40%), *faecalis* (59%), *durans* (1%). Resistance prevalence assayed by disc diffusion was: quinolone (5.1%), macrolide (48.9%), streptogramin (46.9%), and glycopeptide (5.7%). The 2016 isolates were determined to be 39% *E. faecium*, 59% *faecalis*, and 2% *durans*. Prevalence of resistance among 2016 isolates was: quinolone (2.1%), macrolide 46.9%), streptogramin (46.9%), and glycopeptide (2.1%). Overall 50% were multi-drug resistant (3+ classes). There was no statistically significant difference in resistance prevalence between collection years for any antibiotic class. Resistance gene identification by PCR yielded: *emeA*, *efrA*, *efrB*, *ermB*, *isaE*, and *vanA*. A previously undescribed *vanA* gene variant was detected using degenerate primers and amplicon sequence analysis. Whole genome sequencing will confirm this finding.

Antimicrobial

VFD and Prescription Compliance in Poultry: Lessons Learned in 2017

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With the implementation of FDA Guidance Document #213 on January 1, 2017, many veterinarians implemented processes for managing Veterinary Feed Directives (VFDs), as well as veterinary prescriptions (scripts). Many over-the-counter (OTC) drugs changed to VFD or script status. Electronic software solutions, such as, FeedLINK® (VFD) and ScriptLINK® (prescriptions) from GlobalVetLINK, LLC (GVL®, Ames, IA), reduce time spent on VFDs and scripts, reduce or eliminate paperwork, help enable accuracy of drug label information, verify completeness of certificates and assist with regulatory compliance. In 2017, FeedLINK recorded approximately 1,000 poultry VFDs and ScriptLINK over 3,000

prescriptions. Primary concerns recorded by GVL during 2017 included: (1) educating producers to label use of medicated feeds and water solubles; (2) awareness of which drugs are now VFD and prescription status; (3) ability to attach pictures to prove Veterinarian-Client-Patient Relationship (VCPR); (4) location accuracy, i.e. premise ID, latitude, longitude; (5) identify key point person at the clinic for oversight; (6) custom templates for frequently used scripts; (6) prompt technical support and training; (7) flexibility to accommodate changes. FDA Center for Veterinary Medicine established an "Ask CVM" process to respond poultry industry questions related to the VFD ruling. A comprehensive list of these poultry-related questions and answers is referenced1 the most commonly asked questions related to water soluble prescriptions, such as, major differences in pharmacy laws/requirements between major poultry producing states? Will Rx label be required on every pack of prescribed antibiotic to a turkey flock? Implementation of the VFD and Scripts created several challenges for stakeholders in the poultry industry in 2017. The intent of this poster is to share key learnings to minimize future issues.

Shield Plus, A Natural Animal Welfare Enhancer and Disinfectant For Inactivation Studies On Avian Influenza And Other Avian Viruses

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The Shield Plus is a mineral complex with a powerful drying capacity that sanitizes animal beddings. It is composed of organic/salicylic acid, anhydrous mineral salts with an accelerated power of absorption of water and ammonia, and citronella that acts as an insect repellent and aromatic compound. To ensure its effectiveness for disease pathogen disinfection, we prepared the Shield Plus powder in sterile dH2O in different concentrations from 1% to 20% for avian virus inactivation studies. Our test results indicated that avian influenza virus (AIV) subtypes H1 through H9 were completely inactivated when mixed with 2%, 5% and 10% concentrations in 10-15min reaction time, and with 1% concentration up to 30min reaction time. The AIV inactivation results were confirmed with no growth of AIV after inoculation to embryonating chicken eggs for 72 hours of incubation. The Shield Plus product was also tested for inactivation of other avian viruses using LMH cell cultures for confirmation of test results. We prepared 5 concentrations of 5%, 8%, 10%, 15% and 20% of the Shield Plus powder in cell culture medium. Infectious Bursa Disease Virus (IBDV), Avian Reovirus (ARV) and Fowl Adenovirus (FAV) were tested in each concentration for 5min and 15min reaction times, respectively. Results showed that Shield Plus in 8% and higher concentrations inactivated IBDV, ARV and FAV in 5min or 15 min. These virus inactivation results indicate the Shield Plus powder product could be effectively used for animal bedding treatment to protect animals from infections of viral pathogens.

Susceptibility of antibiotics used in commercial farms in Peru

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Antibiotics are used in treatment of respiratory diseases mainly caused by secondary bacterial infections such as *Escherichia coli*. The widely use of antibiotics in poultry farms of Peru have led to reduce their efficacy when they are tested in antibiograms, increasing the resistant of bacteria. The local sanitary authority has banned the use of clorafenicol, nitrofurans (furazolidone and nitrofurazone), olaquindox and nitroimidazole (dimetridazole, ipronidazole, metronidazole and ronidazole, but also has stablished the maximum allowable limit of antibiotic residues in poultry meat. During 2017, 209 antibiograms were registered from clinical cases submitted for diagnostic to the Laboratory of Avian Pathology of National San Marcos University in Lima-Peru. Bacteria were isolated from air sacs, pericardium, lungs, peritoneum, liver and spleen. First, the samples were cultivated on Mc-Conkey and trypticase soy agar; afterwards the isolates were evaluated for sensitivity utilizing the agar disc diffusion test. Among isolated bacteria are *E. coli, Streptococcus aureus, Salmonella gallinarum, Pasteurella multocida, Pseudomona aureginosa, Proteus sp,* and *Klebsiella sp.* In order to show a comprehensive study, the susceptibility has been classified in type of poultry production (broiler, layer, quail, duck. and turkey), bacterium, type of antibiotic, misused of antibiotic and geographic origin. Additionally, antibiotics of more frequent used are

doxycycline, enrofloxacin, norfloxacin, florfenicol, sulfas, sodic fosfomycin and trimethoprim.

Extended spectrum beta-lactamases (ESBLs) detected since 2007 among E. coli isolates from broiler breeders, hatchery and broilers in Mexico

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Bacterial resistance is one of the most relevant issues in the world today, several organizations such as FAO, WHO and OIE have proposed different strategies to control this problem. One of the measures that is considered is the restriction in the use of antimicrobials in food animals, due to the risk that exists of the transmission of bacteria or genes of resistance to the human intestinal biota. Of particular interest, have been the Extended spectrum beta-lactamases (or ESBLs for short), so in our laboratory a work was done to determine its presence in chicken meat, in which genes such as *blatem, blactx and blacmy* were identified in various samples taken from chicken carcasses in processing plant, public market and supermarket. However, its presence in the trail was scarce, so the present work was proposed to determine the presence of these genes in strains from the collection of the Department of Medicine and Animal Husbandry of the UNAM, which had been isolated in 2007 from a batch of breeders, incubator and broilers in the central region of Mexico. In the present report we showed that these genes were already circulating in avian strains since this year among poultry industry.

Avian Influenza

Development of a Transgenic Plant Vaccine against Avian Influenza Virus

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Avian influenza virus (AIV) can cause severe disease in poultry flocks worldwide. Highly pathogenic (HP) H5N1and H7N9 AIVs are important causes of morbidity and mortality. Effective vaccines against AI in poultry need to be developed for use in emergency responses for a limited area to stop viral spread. A transgenic Arabidopsis expressing the hemagglutinin (HA) gene of the low pathogenic LP H1N1 subtype was constructed, and immune response evaluated in chickens. The study demonstrated that chickens given high or low doses of HA transgenic total soluble protein (TSP) orally had a higher HI antibody enhanced cytokine responses, and a reduction in virus shedding compared to chickens given a commercial inactivated vaccine. Chickens given the transgenic plant vaccine (TPV) had better weight gain than those given the commercial vaccine. The TPV has the potential to serve as a vaccine against AIV in poultry.

The pathogenesis of H7 HPAIV in Lesser Scaup (*Ayhthya affinis*)

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Lesser Scaup (Aythya affinis, LESC) are a North American diving duck for which there is limited data on susceptibility to avian influenza virus (AIV). Because some of the most closely related H7 AIVs to the H7N8 that caused an outbreak in the US in turkeys were from LESC, the 2016 H7N8 HPAIV was evaluated in LESC for pathogenesis including calculation of the 50% bird infectious dose. Body weights and temperatures were also monitored. An additional HPAIV, the 2017 US H7N9 from chickens was also evaluated. Although some results are still pending, there was a difference between the isolates in infectious dose. Neither isolate caused any clinical disease. The data support that LESC are similar to Mallards in that they can be affected by HPAIV, but do not develop disease.

Double stranded RNA-mediated antiviral response against low pathogenic avian influenza virus infection attributable to type 1 interferon activity and expression of toll-like receptor 3

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Toll like receptor (TLR)3 signaling pathway is known to induce type 1 interferons (IFNs) leading to antiviral response against many viral infections. Double stranded (ds) RNA has been shown to act as a ligand for TLR3 and, as such, has been a focus as a potential antiviral agent in many host-viral infection models. Yet, it's effectiveness and involved mechanisms as a mediator against low pathogenic avian influenza virus (LPAIV) have not been investigated adequately. In this study, we used avian fibroblasts to verify whether dsRNA induces antiviral response against H4N6 LPAI and to clarify the mechanism of dsRNA-mediated antiviral response. We found that dsRNA induces antiviral response in avian fibroblasts. We also confirmed that this antiviral response elicited against H4N6 LPAIV infection correlates and is attributable to type 1 IFN activity. The treatment of avian fibroblasts with dsRNA also increases IFN- and IFN- production. We also learned that dsRNA treatment of avian fibroblasts increases the expression of TLR3. Our findings imply that the TLR3 ligand, dsRNA can elicit antiviral response in avian fibroblasts against LPAIV, which is attributable to type 1 IFN response highlighting potential value of dsRNA as an antiviral agent against LPAIV infections.

Infectivity, pathogenicity, and transmission of Mexican H7N3 highly pathogenic avian influenza viruses in chickens and mallards

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The infectivity and transmissibility of 2012, 2015, and 2016 Mexican H7N3 highly pathogenic avian influenza (HPAI) viruses were evaluated in chickens to examine for virus adaptation in this species as it has circulated in poultry. No difference in infectivity was found between the three viruses and all three caused high mortality in chickens when given in moderate to high doses [104-6 mean embryo infective dose (EID50)]. High virus titers were present in tissues and oropharyngeal and cloacal swabs in birds infected with the three viruses with minor differences between strains. In order to address the role of waterfowl in the spread of H7N3 viruses, the infectious dose and transmissibility of the 2012 and 2016 H7N3 HPAI viruses were examined in mallards and compared to a mallard origin low pathogenic avian influenza (LPAI) virus. The mean bird infectious dose (BID) for the LPAI virus was <102 EID50 and the virus transmitted to all contacts, indicating that this virus was well adapted to mallards. For the 2012 H7N3 virus, the mean BID was 103 EID50, and the virus transmitted to all contacts in the groups infected with the higher doses. For the more recent 2016 Mexican HPAI virus, although the mean BID was similar, the virus replicated poorly and didn't transmit to contacts, indicating this virus was less adapted to mallards. No virus-infected mallards showed clinical signs or died. In conclusion, mallards can become infected with HPAI viruses, but infectivity and transmissibility depend on the virus and passage history in poultry.

Using Size Exclusion Chromatography As Method For Avian Influenza Virus Detection in Delta Wetlands

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Every fall wild birds travel south to warmer climates and congregate in California's wetland habitats for the winter. Anseriformes and Charadriiformes are the main reservoir for Avian Influenza virus, and most prevalent at these wetlands. The main transmission pathway of AIv is through fecal-oral routes, and the spread of AIv in these wetlands contaminates the water and allows for the virus to infect resident species and other migrants. The current method of detection for Avian Influenza includes taking small surface water samples, but is not an effective method of detection for a virus that is sensitive and shed by reservoir hosts quickly. In order to create a more efficient methodology for AIv detection in water we used size exclusion chromatography to test larger quantities of water. We compared 50 mL surface water samples to a concentrated sample using the size exclusion chromatography columns by running RT-qPCR to determine if the size exclusion chromatography sample was more sensitive and efficient at providing AIv-positive results. The use of an evolved method of AIv detection would be beneficial in further understanding

the viral ecology, persistence in water, and overall spread of virus in migrant and resident bird species.

Persistence of LPAI and HPAI in Reused Poultry Litter and LPAI in Poultry Carcasses

Alejandra Figueroa, Theodore Derksen, Robyn Lampron, Pramod Pandey, Daniel Rejmanek, Beate Crossley, Rodrigo A. Gallardo.

Avian influenza (AI) viruses have greatly affected the poultry industry worldwide. Their interactions with the host, waterfowl as carriers, and with the environment have been studied extensively. However, research on AI persistence and elimination inside commercial poultry barns is limited. We have been particularly interested in bedding material treatment and carcass composting in order to have a better understanding of potential problems and improve biosecurity practices. We have studied the effectiveness of common footbaths used in the commercial industry, and the persistence of LPAI and HPAI in bedding material of broilers, turkeys, and layer feces. The current research project examines the effect of reused and treated litter on the persistence of both LPAI and HPAI. Reused broiler litter (1 and 11 cycles) and turkey litter (1 and 4 cycles), treated with acid compounds and wind-rowing technique, was spiked with LPAI and HPAI. Presence and viability of the virus was measured before infection and every 12 hours until 96 hours. In addition, the effect of carcass composting on LPAI was evaluated in laboratory settings. Results of these experiments will be discussed.

Investigation of tissue tropism of the Hemagglutinin glycoprotein of High Pathogenicity and Low Pathogenicity Avian Influenza H5 viruses in Poultry species and Wild birds using Tissue Microarrays

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The Avian Influenza Virus (AIV) hemagglutinin (HA) is a major determinant of viral attachment and infection. The interface between wild and domestic birds poses significant threat of transmission of AIV between wild and poultry species and is of paramount economic importance. To compare the receptor binding of the H5 HA, in different tissues from wild and domestic birds, recombinant HA protein was created using gene sequences from a low pathogenicity (LPAIV) and high pathogenicity (HPAIV) H5 AIV obtained from NCBI GenBank. The HA of (A/Northernpintail/ Washington/ 40964/ 2014 (H5N2)) (high pathogenicity) and (A/ mallard/ MN/ 410/ 2000 (H5N2)) (low pathogenicity) were codon optimized and cloned into mammalian expression vectors, then transfected into HEK 293T cells to produce the HA proteins. Representative species from the orders, Galliformes (broiler chicken and turkey), Passeriformes(American crow), Passeriformes (Mallard and Pekin duck), Charadriiformes (Ring billed gull) and Accipitriformes (Red tailed hawk) were used to construct tissue microarrays (TMA). Each TMA contained sections of respiratory and digestive tracts as well as cloacal bursa when available, to reflect routes of infection. Initial investigation showed increased tropism of both HA proteins to intestinal tract epithelium of Mallards as compared to Pekin ducks. In chicken, binding of the HPAIV H5 was seen in epithelial cells of the trachea and cloacal bursa, with negligible staining in the intestine. Faint binding of the LPAIV H5 was observed in the trachea of turkeys. Evaluation of the tropism of the AIV HA using tissue microarrays may explain the infectivity and pathogenesis and of AIV in wild and domestic bird species.

Influenza Active Vaccine stimulates the humoral immunity strong and reduce virus shedding.

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Avian Influenza Virus is a global concern in humans and animals. One of the tools to control the diseases is using vaccination. Mexico has the most experience with the use of vaccines to control H5N2 and H7N3 subtype. Mexico have approved Inactivated and vectorized Influenza vaccine in chicken. Active vaccine is not approved yet. In order to control the disease is necessary to have good level of protection as well,cellular and humoral immune response. We evaluated active vaccine to H7N3 subtype using Reverse Genetic to make the vaccine and its protection. The combination of active and inactivated vaccine, got the stronger immune response and reduce the virus shedding after challenge, compared with inactivated and active alone. The use of active and

Inactivated vaccines are the suitable method in order to control and could possible the eradication of the disease.

Bacteriology

Molecular epidemiology of *Ornithobacterium rhinotracheale* isolates from chicken and turkeys

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Ornithobacterium rhinotracheale (ORT), an important bacterial pathogen of high economic concern to poultry production. This bacterium causes severe infections in chicken and turkeys worldwide. The infection caused by ORT has been responsible for huge economic losses to the poultry industry by decreasing the production parameter and increasing medication cost. Efficient diagnosis may help in reducing prevalence of the disease. The purpose of this study was to characterize *Ornithobacterium rhinotracheale* isolates on the basis of their geographic location, year of isolation and specie of origin. A total of 56 ORT collected from two states in the USA i.e., California (n=32) and Minnesota (n=24) were analyzed by multilocus sequence typing (MLST). All of the 56 isolates were confirmed as ORT by PCR targeting *16SrRNA* gene. The results of MLST for overall ORT isolates revealed 8 different sequence types (STs). ST-1 was the predominant sequence type among all isolates followed by ST-9 and ST-8. Only one isolate i.e., NCF34 was identified as ST-2. Four STs i.e., ST-32, ST-33, St-34 and ST-35 were identified as novel and submitted to PubMLST database. No significant variation was seen in sequence types isolated from different years. On the basis of origin 38 isolates were from turkeys and 18 were from chickens. From turkey origin, 76% belonged to ST-1 and 16.6% to ST-8 while from Chickens 66% belonged to ST-1 and 16.6% to ST-9. ST-1 was the most prevalent sequence type circulating in chicken and turkeys in both states. ST-9 was second mostly identified sequence type in California and ST-8 from Minnesota.

Coryza Outbreaks in Chickens and the Role of *Galibacterium* anatis on the Clinical Presentation

Rodrigo A. Gallardo, Rudiger Hauck, Ana Paula da Silva, Charles Corsiglia, Nancy Reimers

Since January 2017 an increased number of Coryza cases have been reported in California. Some of these cases have occurred in egg layer flocks receiving inactivated vaccines as part of their vaccination schedule. Molecular typing, using the hemmaglutinin gene, of the Avibacterium grouped isolates from broilers and layers closer to isolates of the serogroup C-1. After studying the persistence of the isolates we found that the bacteria did not persist in the environment where infected chickens were raised. On the contrary in the field the clinical picture was: drop in egg production, chronic Coryza and mortality associated with peritonitis. During the Avibacterium persistence study we screened broilers to the presence of Gallibacterium anatis finding it in a considerable number of chickens. We are currently screening normal and Coryza affected flocks for the presence and load of Gallibacterium anatis measured by qPCR. The presence and load of the Gallibacterium and its association with the clinical Coryza outbreaks will be discussed.

Triple-sugar regulated *Salmonella* vaccines protect against *Clostridium perfringens*induced necrotic enteritis in broiler chickens.

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Gram-positive *Clostridium perfringens* type A, the causative agent of necrotic enteritis (NE), has gained more attention in the broiler industry due to restrictions on use of growth-promoting antibiotics in poultry feed. To date, there is only one commercial NE vaccine available based on the *C. perfringens* alpha toxin. Our previous work proved that regulated delayed lysis *Salmonella* vaccines with plasmid pYA5112 encoding an operon fusion specifying synthesis of the C-terminal adhesive part of alpha toxin and a GST-NetB toxin fusion were able to elicit significant protective immunity in broilers against both mild and severe *C. perfringens* challenges. We recently improved our *S.* Typhimurium antigen delivery vaccine strain by integrating a rhamnose-regulated Oantigen synthesis gene enabling a three-sugar regulation system for virulence and antigen-synthesis traits. The new system confers on the vaccine strain a super safe profile and much improved induction of immune responses. The strain with triple-sugar regulation systems delivering pYA5112-encoded antigens protected chickens in a field setting at levels

observed for antibiotic-treated chickens. Feed conversion and growth performance were also superior. These studies made use of a severe *C. perfringens* challenge with lesion formation and mortality enhanced by pre-exposure to *Eimeria maxima* oocysts. The vaccine achieved similar effectiveness through three different immunization routes, oral, spray and in the drinking water. The vaccine can thus be applied under commercial hatcher and broiler-rearing conditions. Further improvement of this vaccine is ongoing with addition of two newly discovered protective antigens enhancing level of protective immunity in one clinical trail so far completed.

Challenge study against conventional and emergent serovars of *Avibacterium paragallinarum* in SPF vaccinated chickens.

F. Lozano1, M. Lechuga2, V. Morales2, E. Soriano2, J. Elattrache1

The spectrum of protection of a polyvalent coryza bacterin containing serovar A-1 (Strain 221); serovar B-1 (Strain 2671) and serovar C-2 (Modesto strain) was evaluated against conventional and emergent serovar C-1 (Strain ESV-135) and serovar B (Strain ESV-185) of *Avibacterium paragallinarum* (AP). SPF birds were vaccinated at 5 weeks of age (Via sub-cutaneous route) and at 9 weeks of age (Via intramuscular route) with 0.5 ml dose/bird. A total of 88 vaccinated birds vaccinated were randomly distributed into 5 groups and challenged either with the following homologous or heterologous strains. Conventional strains A-1 (2.14 x 108 ufc/ml); B-1 (3.4 x 109 ufc/ml); C-2 (1.15 x 108 ufc/ml) and emergent strains C-1 (3.25 x 108 ufc/ml) and B (2.53 x 108 ufc/ml). Serological response previous to the challenge was performed using HI test 48 hours before the challenge. A total of 28 non-vaccinated SPF birds were equally challenged with one of the 5 serovars used in this study and observed for clinical signs during 7 days. Results in the percentage of protection against each serovar was as follows: A-1 (82%); B-1 (88%); C-2 (78%); C-1 (78%) and B (78%). Corresponding non-vaccinated birds had A-1 (0%); B-1 (20%); C-2 (17%); C-1 (17%) and B (17%). The protection achieved after double vaccination and challenge demonstrated the effectiveness of this bacterin against conventional and emergent strains of *Avibacterium paragallinarum*.

Feed acidification to improve performance and reduce necrotic enteritis when fed to broiler chickens challenged with Clostridium perfringens

Greg Mathis

Clostridium perfringens-induced Necrotic Enteritis (NE) has become a great concern to the poultry industry, which has resulted in a significant decrease in growth performance, poor feed conversion, and increased mortality. One proposed method to reduce NE is feed acidification. This research examines the use of feed acidification to reduce necrotic enteritis

Characterization of Avian Pathogenic *E. coli* (APEC) Associated with Turkey Cellulitis and Litter Quality in Iowa.

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Turkey Cellulitis (TC) has been identified as the second-greatest concern of turkey producers nationwide. The disease is characterized by locally extensive inflammation of subcutaneous tissues in the inguinal, tail, and breast regions, often striking Toms at or near market age, resulting in increased flock mortality. Previous research suggested *Clostridium spp.* is responsible for TC, but Avian Pathogenic *E. coli* (APEC) has also been isolated from cellulitis lesions. APEC can cause cellulitis in broiler chickens; colibacillosis in turkey flocks, and can persist in the production environment. This study examined *E. coli* (APEC) isolated from the lesions of birds diagnosed with colibacillosis with *E. coli* found in the environment (litter), and mortality rates from facilities with and without a history of TC in flocks. Three barns in Iowa were sampled weekly, from 10 through 18 weeks of age. Two barns with no history of TC in the past 12 months were used as controls and did not show signs of TC during the study; the third barn with a previous disease history experienced significantly increased mortality due to TC. In the control barns, APEC was detected in the litter without a corresponding change in mortality rate. In the barn that experienced increased mortality due to cellulitis, data showed a corresponding increase in APEC present in environmental samples and disease rate. The role of APEC in TC is often considered secondary to other predisposing conditions; however, our data suggest APEC in the environment can be linked with outbreaks of Turkey Cellulitis.

Necrotic enteritis in quetzals (*Pharomachrus mocinno)*

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Necrotic enteritis is an infectious disease of the domestic birds that usually presents in broilers less than 4 weeks old and it´s caused by the bacterium *Clostridium perfringens* types A and C. There are few reports of necrotic enteritis wild birds and infections in birds of the family *Trogonidae* haven´t been described. Therefore, the objective of this study is to describe an outbreak of necrotic enteritis in captive quetzals and the pathological findings associated with this disease. Three dead quetzals were submitted into the Laboratory of Diagnostic of Diseases in Poultry from the Faculty of Veterinary Medicine, UNAM to perform the post-mortem examination. In the pathologic examination, we observed necrotizing hemorrhagic enteritis and necrotic hepatitis. In the intestinal lumen we found a moderate amount of acanthocephalans. The microscopical findings were severe necrotic enteritis and hepatitis with Gram-positive bacilli distributed along the organ tissue. *Clostridium sp.* and *Escherichia coli* were isolated from intestine and liver samples. The gross and microscopic findings along with the bacterial isolation show that the cause of death is strongly associated to a clostridial enteric infection, very similar to necrotic enteritis disease, widely described in poultry. Probably, this bacterium entered into the digestive system via contaminated food or water and the elements that triggered its proliferation and toxin generation were the parasitic infestation and the diet. The results of this case are described, and we discuss the probable causes that can lead to the infection and the importance and impact of this disease in wild birds.

Identification and genotyping of *Gallibacterium anatis* isolated from clinically affected layers in Peru.

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Gallibacterium anatis is a Gram-negative bacterium, a member of the family *Pasteurellaceae*. The importance of *Gallibacterium* as a poultry pathogen has been debated, however, some reports indicated important pathogenic potential. In this study, a total of 17 isolates of *Gallibacterium* recovered during 2013-2017 from infraorbital sinuses of clinically affected layers in Peru were analyzed. The isolates were confirmed by specific PCR, the genotyping was performed by enterobacterial repetitive intergenic consensus-based PCR (ERIC-PCR). The phylogenetic analysis was performed with the sequences obtained from the partial sequencing of 16S rRNA gene of the isolates obtained and reference strains reported in GenBank. All isolates were identified by specific PCR. Genotyping by ERIC-PCR resulted in fifteen different ERIC patterns for the 17 *Gallibacterium* isolates and different to *Gallibacterium anatis* reference strain (F-149) included in the study. The phylogenetic analysis with the sequences of 16S rRNA showed a monophyletic group with the *Gallibacterium anatis* reference strains reported in GenBank. In conclusion, *Gallibacterium anatis* isolates are present in Peru and the diversity of high (15 ERIC-patterns from 17 isolates). Studies focused on the identification of the pathogenic potential of *Gallibacterium* isolates from Peru are necessary.

APEC-associated Virulence Genes in Production Turkeys in North Carolina

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Avian pathogenic *Escherichia coli* (APEC) the etiologic agent of colibacillosis, is an important cause of morbidity and mortality affecting all facets of the poultry industry. The ColV plasmid-linked genes *iroN*, *ompT*, *hlyF*, *iss*, and *iutA* have been identified as defining traits of the APEC pathotype and considered predictors for virulence of APEC. In this study, we compared the presence of APEC virulence genes and necropsy of sick bird findings with APEC virulence genes isolated from samples taken from healthy birds. *E. coli* were isolated from two commercial light hen turkey flocks. Flock “TAU” was raised for teaching purposes at the North Carolina State University College of Veterinary Medicine and Flock “SIB” was a sibling flock raised on a North Carolina commercial farm. Each flock contained 50% Hybrid turkeys and 50% Aviagen-Nicholas turkeys. Samples for *E. coli* isolation were collected regularly from each flock from placement through processing. Fecal samples (*n* = 48) and environmental swabs (*n* = 28) were cultured weekly from

each flock, while tissues and organs were cultured as needed during necropsy of mortality (*n* = 158). Cloacal swab samples (n = 20) were taken at processing from both breeds of both flocks. All isolated *E. coli* were tested for the presence of 9 virulence genes: *cvaC*, *iroN*, *ompTp*, *hlyF*, *etsB*, *iss*, *iutA*, *ireA*, and *papC*. With annual multi-million dollar losses and animal health at risk, knowing the prevalence of predicted APEC in production flocks can be used to improve the quality and health of turkey production.

Case Reports

A case-control field report: Rickets in turkey poults

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A group of 13,300 male turkey poults was randomized by box and split between two similar brooder houses for a trial intended to compare performance on different rations. The poults were healthy, vigorous and uniform at placement. On day 2, flip-overs and spraddle-legs began to appear in one of the 2 brooder houses. On days 4-6 some poults were found dead with full crops and gizzards; other poults that were euthanized for lack of mobility had no feed or litter in crops and gizzards and were mildly dehydrated. By day 6 at least 60% of poults appeared reluctant or unable to stand, had shaky mobility, were wing-walking or hock sitting. Many poults were down with legs splayed or prone with heads on the litter. A clinical diagnosis of rickets was made on the basis of clinical presentation and pliable beaks and long bones. On day 8, morbidity was estimated to be 90-100% of the flock. Euthanasia was recommended. The other brooder house had no abnormal findings. This field report is unique because it has a case-control study design. Samples and data were obtained from both case and control flocks, including feed analysis, mortality records, water consumption, body weight, gross and histopathology, and bone ash. Photo and video of case and control flocks were obtained. Results will be reported and discussed.

Case Report – Neurological Signs in Pullets

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Neurological signs and paralysis affecting the legs within a commercial layer farm are usually considered to be due to an infectious origin. Marek´s Disease (MD) nervous presentation is known to cause edema and enlarged nerves, showing lameness in birds. The use of vaccines to control MD has been successful since the late 1960´s and early 1970´s, even when combined with different serotypes according to field challenge. One single dose of the vaccine given at the hatchery is enough to immunize the birds for all their life, no matter if they are short (broilers) or long living commercial birds (layers or breeders). A clinical report was received from a new layer complex, with clinical signs of lameness. The aim of this report is to share the cause of these lesions which were found in the new complex. The cause of lameness will be presented.

Diagnosis of Liposarcoma in a Backyard Chicken with Retrospective Analysis of Neoplasms Diagnosed in California Animal Health and Food Safety Laboratory Backyard Chicken Submissions from 2008 – 2017

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This case report describes the evidence supporting a diagnosis of metastatic liposarcoma in a backyard Silkie chicken. On September 28, 2017, a dead 3-year-old backyard Silkie chicken was submitted to California Animal Health and Food Safety Laboratory System (CAHFS)-Turlock branch for necropsy, with a history of unknown skin lesions involving the entire body and severe weight loss. At necropsy, raised necrotic lesions involving the majority of the skin and multiple nodules in the liver, spleen, and bone marrow were noticed. Microscopically; stellate, spindle, and myxoid cells containing large lipid droplets were observed infiltrating the dermis and underlying a necrotic epidermis; with metastasis to liver, spleen, bone marrow, and ovary being the most significant findings. PAS, Oil Red O, Ziehl-Neelsen, Congo red, Gram, and Von Kossa stains along with immunohistochemistry (IHC) for pan cytokeratin, vimentin, S100, CD3, pp38, and Meq were used to classify the lesions. Intensely positive vimentin IHC, along with large quantities of Oil Red O positive lipid droplets within the neoplastic cells were supportive of our diagnosis of liposarcoma. The incidence of neoplastic diseases diagnosed in backyard flock submissions to

CAHFS system wide from 2008-2017 will also be reviewed.

Fowl typhoid in Coturnix japonica in Perú Case Report

Eliana Icochea**,** Nelly Cribillero, Maribel Castope, Ofelia Alzamora and Rosa González

Laboratory of Avian Pathology, School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos In four flocks of Coturnix japonica with birds from 4 to 17 weeks of age, located in an area at Lima Department, white diarrhea and high mortality are reported. At the clinical examination, the birds showed alopecia, depression and feces around cloaca feathers. At necropsy were observed mild tracheitis and mucus, hepatomegaly, liver with necrotic foci and discolored liver, splenomegaly, uratosis and regression of ovarian follicles. The culture of the liver and spleen was positive to Salmonella, also the real time PCR. The identification in selective media, motility and serotype test determined Salmonella gallinarum confirming the diagnosis of Fowl typhoid in the four cases. The antibiotic sensitivity test of the isolated strains showed that they were sensitive to most of the antibiotics used.

Pseudomonas aeruginosa systemic infection in young broiler breeders, Korea

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Pseudomonas spp. is ubiquitous in soil, water and humid environments. It is generally considered to be an opportunist that produces respiratory infections or septicemia in Avian species. *Pseudomonas aeruginosa* is the most common species among *Pseudomonas spp.* causing yolk sac infections and septicemia in young chicks. The suddenly increased mortality was shown in a broiler breeder flock, September 2017 Korea. The flock was consisted of 16,000 heads. The mortality was 31% from 4 to 13 day old chicks. The affected flock showed depression and lameness. The size for the affected chickens was uneven. At necropsy, moderate fibrinous epicarditis and swollen liver were shown in 5 day old chicken. Additionally some birds showed fibrinous serositis in liver. Histopathologically moderate subacute epicarditis and myocarditis with intralesional bacterial colonies were observed. Moderate necrosis, congestion and thrombosis were seen in liver, spleen, lung, kidney and brain. At bacterial isolation, *Pseudomonas aeruginosa* was predominantly isolated from liver, heart and joint. Additionally some colonies of *E. coli* and *Enterococcus spp*. were also isolated from same specimens. Based on histopathology and bacterial isolation, we confirmed that the case was the systemic infection by *Pseudomonas aeruginosa.* We assumed that this case was caused by contamination in hatchery. The *Pseudomonas aeruginosa* infection in young broiler breeder was rare in Korea.

Diagnostics

Establishment Of Hy-Line Commercial Laying Hen Blood Gas And Chemistry Reference Intervals Utilizing Portable i-STAT®1 Clinical Analyzer

Zachary Sauer

Iowa State University CVM

Blood gas and chemistry reference values are examined in six genetically distinct commercial varieties (CV) of Hy-Line laying hens (Hy-Line W-36, Hy-Line W-80, Hy-Line W-80+, Hy-Line Brown, Hy-Line Silver Brown and Hy-Line Sonia) utilizing the iSTAT®1 analyzer. Each line had a minimum sampling population of 89 in the beginning of the study, and each respective CV of laying hen was sampled every two weeks for six total replicates. Blood samples were obtained pen-side from the brachial vein and subsequently analyzed by the iSTAT®1 portable device. iSTAT®1 reports blood gas and chemistry values for the parameters **pH**, partial pressure of carbon dioxide (**PCO2** mm Hg), partial pressure of oxygen (**PO2** mm Hg), bicarbonate (**HCO3** mmol/L), base excess (**BE** mmol/L), saturation of oxygen on hemoglobin (**sO2** %), glucose (**Glu** mg/dl), sodium (**Na** mmol/L), potassium (**K** mmol/L), total concentration of carbon dioxide (**TCO2** mmol/L), ionized calcium (**iCa** mmol/L), hematocrit (**Hct** % Packed Cell Volume [PCV]), hemoglobin (**Hb** g/dl). A total of 3,188 sample collection results were utilized in the establishment of reference interval values for the 13 parameters between the 6 distinct CVs. Statistical analysis via ANOVA and Tukey's test revealed significant differences between CVs for all 13 applicable blood gas and chemistry parameters. Several striking differences were noted between the brown and white egg laying commercial varieties such as iCa and Hct/Hb. The blood gas and chemistry parameters collected in this study will serve as reference values to set the framework for future correlations to genetics, physiologic derangements and production performance.

Increased interleukin-6 expression is associated with pre-cancerous, inflammatory lesions in the ovary of the aging laying hen

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The laying hen (*Gallus domesticus*) is a robust animal model for epithelial ovarian cancer (EOC). Interleukin-6, an inflammatory, pleiotropic promoter, has been linked to chronic inflammation in the ovary as women age. We examined a possible link between IL-6 expression and pre-cancerous, inflammatory lesions in ovaries of aging laying hens. Reproductive tissues were collected from two groups of aging laying hens with reduced egg production (18 Leghorn hens aged 18 months and 22 Leghorn hens aged 21 months). H&E-stained slides of ovarian tissue were examined by light microscopy and levels of IL-6 in serum and ovarian tissue lysates were evaluated by ELISA. The 18- month-old hens had dense heterophil infiltrates in the ovarian cortex that were accompanied by an absence of small follicles. Hens with this lesion had higher serum IL-6 (mean = 426 pg/mL) compared to age-matched hens without this type of lesion (mean = 242 pg/mL) (P = 0.01). In 21-month-old hens, three additional ovarian pathologies were identified: cysts, hypervascularity, and proliferative ovarian surface epithelium. Hens with abundant proliferative ovarian epithelium had elevated serum IL-6 (mean = 2936 pg/mL) compared to age-matched hens with other lesions (mean = 1865 pg/mL) (P = 0.01). Expression of IL-6 in ovarian tissue lysates from hens with abundant proliferative epithelium exceeded 3000 pg/mL compared to hens with other lesions. We hypothesize that, as hens age, dysregulated inflammatory and tissue repair processes along with increasing levels of IL-6 may be associated with the development of pre-cancerous lesions that can ultimately lead to EOC.

Comparison of two commercial ELISA kits adapted to monitor immune response induced by recombinant HVT-ND vaccine

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Recombinant turkey herpesvirus vaccines (rHVT) are being widely used for the prevention and control of Newcastle disease (ND) in endemic countries and also to replace the use of conventional live ND vaccines in countries where velogenic ND viruses (NDV) are absent. Since rHVT-ND vaccines express only the F gene of NDV, they induce exclusively an anti-F NDV antibody response. Therefore, the majority of ELISA kits used for monitoring ND antibody status of flocks show low antibody level and low sero-positivity in rHVT-ND vaccinated flocks compared to the ones seen in birds vaccinated with conventional live and killed ND vaccines. This is most obvious in young age (4-6 weeks of age). In recent years new ELISA kits, claiming better detection of immune response to rHVT-ND vaccines, have been developed and introduced to the market. The aim of our study was to compare the performance of two of these commercial ELISA kits, with serum samples collected from controlled laboratory trials. SPF layers or commercial broilers were vaccinated either in ovo or subcutaneously at hatch. Immune response to vaccination was monitored during the period between 2 to 6 weeks of age. Performance of the two methods was comparable based on onset of detectable immune response to vaccination and rate of sero-positivity. Differences will be discussed. Based on the results obtained in laboratory studies both ELISA kits proved to be equally suitable to detect the immune response elicited by rHVT-ND vaccines with NDV F insert from a reasonably young age.

Establishing reference values for monitoring vaccination with a recombinant HVT by real time PCR: experimental data and seven year survey of field cases

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Monitoring Marek's disease vaccination by real time PCR has become a widely used technique in the recent years. However, our laboratory has demonstrated that expected results are affected by vaccine strain, origin, dose, vaccination protocols, age at sampling, and evaluated tissue. In this study, we have used experimental data to establish reference values for a recombinant HVT when used in different vaccine protocols and evaluated in feather pulp at 7-15 days. We have also conduct a worldwide 7 year survey of field cases to monitor vaccination with this vaccine in commercial flocks. Our results show that having reference values for each vaccine in a given vaccination program is critical to make this technique meaningful.

Salmonella pullorum Microtiter Assay Compared to Tube Agglutination

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Georgia Poultry Laboratory Network

Serum samples submitted to GPLN for the Salmonella pullorum tube agglutination assay were also tested by the S. pullorum microtiter assay. A comparison of the two assays was made to determine which assay should be used at GPLN according to specificity and sensitivity. Com-parison of cost was also a factor in evaluating the two methodologies as approximately 65,000 assays are conducted annually. When testing this volume of samples the antigen for the tube agglutination assay becomes costly. Many flocks are vaccinated for Salmonella, often before samples are collected for serology. The effect of vaccination on these two tests was also compared.

Synopsis of broiler and broiler breeder diseases diagnosed at Alabama State Diagnostic Facilities, 2016-2017

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In April 2016 to March 2017, a total of 480 poultry cases were submitted to the Alabama Veterinary Diagnostic Labs (AVDL) from 6 commercial broiler companies in Alabama. An overview of the major diseases that were diagnosed from these commercial broiler (290) and broiler breeder (190) cases have been presented here. All cases were necropsied according to the standard operating procedures developed at AVDL. In every case, a complete necropsy was performed, visible gross findings were identified, and samples were collected for further laboratory analysis. Collected tissues, blood, and swab samples were analyzed through bacterial culture, ELISA, PCR, cell culture, and histopathology. One-day to 9-weeks old broilers were submitted with a history of high flock mortality. Important diseases or lesions diagnosed in broiler necropsies were dermatitis (18 cases in 1-day-old chicks), colisepticemia (49), necrotic enteritis and coccidiosis (17), bursal lesions (37), infectious bronchitis (41), salmonellosis (2), reoviral arthritis (20), and infectious coryza (4). Major diseases diagnosed from broiler breeders were yolk peritonitis (32 cases), bacterial synovitis (20), Histomoniasis (14), Coccidiosis (13), reoviral tenosynovitis (11), fowl cholera (6), yolk pneumonia (6), infectious coryza (2), Pox (2), and mycotoxicosis (1). The distribution of disease does not reflect the actual incidence and is influenced by submission bias of owners.Mixed infections were identified in many cases especially in broiler breeders. Mortality was associated with secondary bacterial infection influenced by immune suppression in broilers and reproductive status in broiler breeders.

Necropsy Examination of Domestic Canaries from a Single Laboratory Colony

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Fifty-eight adult domestic Canaries (*Serinus canaria forma domestica*) originating from a colony researching genetics of feather coloration were individually necropsied over a period of four years. Necropsy examination included complete histology, selected aerobic fugal and enteric cultures, Mycoplasma PCR and selected virus isolation. Aerobic culture demonstrated septicemia from *Staphylococcus aureus* (7/58), *Klebsiella oxytoca* (1/58), Corynebacterium sp. (1/58), *Acinetobacter calcoaceticus* (1/58), *E. coli* (3/58) and enteritis associated with Enterococcus sp. (1/58). PCR for *Mycoplasma synoviae* demonstrated weak positive in in 3/58. One case with positive mycoplasma PCR also had septicemia with a hemolytic E. coli. A herpesvirus was isolated from the male with concurrent Corybacterium septicemia. Mycobacteriosis (2/58) was diagnosed by histology resulting in friable liver fractures (1/55) and osteomyelitis related fracture(1/2). The proventricular-ventricular isthmus often has erosion and was colonized by megabacteria (*Macrorhabdus ornithogaster*) (15/58). Enteric parasites diagnosed with histology included Cryptosporidium galli (2/58) and small intestinal coccidiosis (*Isospora* sp.) (8/58). Cases of enteric coccidiosis had segmental dilation of proximal intestine with yellow staining around the feathers of the vent.

Systemic Isospora sp. (atoxoplasmosis) was diagnosed by histology in 4/58 and demonstrated, subserosal edema, enlarged spleen, intestinal dilation and concurrent septicemia with S. aureus. Non-infectious cause of death included blunt force trauma or collision injury (2/58), hemoglobinuria nephrosis (3/58) and yolk peritonitis (2/58). Significant pathological changes observed microscopically without determination of the etiology include biliary duplication (2/58), esophageal hyperkeratosis (1/58), non-suppurative ganglioneuritis (1/58), hepatic atrophy (1/58), desiccated feet (4/58) and unilateral atrophy of the cerebrum (2/58).

Enteric Diseases

Stimulation of local immune response as crucial step in reduction of campylobacteriosis in chickens

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The aim of the trial was to follow intraepithelial lymphocytes (IEL), *lamina propria* lymphocytes (LPL) in caecum and sIgA, MUC1 and MUC2 in the intestinal flush of caecum in chickens after *Lactobacillus fermentum* CCM 7514 application challenged with *Campylobacter* spp. A day-old chickens (120) were divided into four groups (n=30): control (C), *L. fermentum* CCM 7514 (LB), *Campylobacter* (CB), and combined *L. fermentum* + *Campylobacter* (LBCB). *L. fermentum* was administrated individually *per os* to chickens during first 7 consecutive days. Chickens were infected with *Campylobacter* spp. at day 4 of age. Determination of caecal IEL and LPL demonstrated increasing of CD8+ IEL ($P<0.001$) and LPL ($P<0.01$) at 4 dpi in LBCB group, at 7 dpi the increase was observed only in LB group. Intraepithelial CD3+ and CD4+ lymphocytes raised in combined LBCB group at 7 dpi ($P<0.05$), IgA+ lymphocytes increased earlier at 4 dpi ($P<0.01$) in this group. Caecal LPL showed the highest values of IgA+ at 4 dpi ($P<0.05$) and 7 dpi together with IgM+ cells in LBCB and LB groups. The concentration of sIgA was increased in the LB group and in LBCB group compared to control ($P<0.001$) at 7 dpi. The concentration of MUC1 was not changed among groups. The decreased concentration of MUC 2 was observed in experimental groups compared to control group at 7 dpi ($P<0.001$). The obtained results suggest beneficial effect of used *L. fermentum* on improving of local intestinal immunity against *Campylobacter* spp.

Inhibitory activity of a novel triple-strain Bacillus probiotic (GalliPro Fit) towards *Escherichia coli* in a feed matrix

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By inhibiting pathogens, *Bacillus* probiotics may confer health benefits to the host. The objective was to evaluate the efficacy of reduction of *Escherichia coli* contamination by three probiotic *Bacillus* strains in a feed matrix. The added *Bacillus* probiotic (GalliPro Fit) for poultry contains two *Bacillus subtilis* strains (DSM 32324 & DSM 32325) and a *B. amyloliquefaciens* strain (DSM 25840). The feed was autoclaved prior to *Bacillus* inoculation to reduce natural contaminants. Buffer was added to control and test samples and *Bacillus* probiotic was added to test samples to reach 1x105 CFU/g feed. Samples were incubated aerobically at 37°C for 24 hours. *E. coli* (NCTC 10650) was added to control and test samples to achieve 3x104 CFU/g feed and incubated aerobically at 37°C for 8 hours. Two replicates were analysed per control and test sample. *Bacillus* spp. and *E. coli* were counted at 0, 4, 6 and 8h. *Bacillus* sp. CFUs were counted on TSA agar, E. coli CFUs were counted on selective MacConkey agar plates. In test samples, containing both *Bacillus* spp. and *E. coli*, the *E. coli* count remained stable throughout the experiment. At 4, 6 and 8 hours, respectively, the three-strain *Bacillus* probiotic GalliPro Fit inhibited the growth of *E. coli* with 3, 4 and 5 logs, respectively ($p<0.0001$).

Immunostimulatory effect of sweet chestnut extract on intestinal microenvironment during application of antihelmintic in chickens

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The goal of the experiment was to follow effect of sweet chestnut agent on sIgA, MUC2 production and on villous morphology in jejunum and ileum during application of

antihelmintic Flimabend. The experiment was conducted in a commercial broiler chickens fattening farm. Twenty four chickens 40 days old of Kalimero-Super Master hybrid were included in the trial. Chickens were randomly divided into four groups of 6 chickens each: Fli (Flimabend®), Far (Farmatan®), Far + Fli (Farmatan® + Flimabend®) and C (control). Extract of sweet chestnut (*Castanea sativa* Mill.) - Farmatan® liquid was added into water, for 6 hours *per day* (8:00 – 14:00) during 5 days starting at age of 43 days. Chickens of Fli group received individually *per os* antihelmintic flubendazol Flimabend ® - 100 mg/g of suspension (KRKA d.d., Slovenia) in 1.43 mg of active substance/kg body weight during 7 days starting at age of 40 days. The concentration of sIgA (ng/mL) in the intestinal flush increased in Far group in jejunum (P<0.05) and ileum (P<0.001) and in Far + Fli group in ileum (P<0.001) compared to control. The concentration of MUC2 (ng/mL) in the intestinal flush increased in Far group in jejunum (P<0.001) and ileum (P<0.05) and in Far + Fli group in jejunum (P<0.001) compared to control. Height of villi increased in Far group comparing to others (P<0.05; P<0.001), in the jejunum and ileum showed increase in Far + Fli group (P<0.001). Application of sweet chestnut extract demonstrated beneficial effect on intestinal immunity during application of Flimabend.

Evaluation of *Bacillus licheniformis* (DSM 17236) for protection against bacterial chondronecrosis with osteomyelitis (BCO) lameness in chickens with exposure to *Staphylococcus agnetis*

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The objective of this study was to evaluate the efficacy of a *Bacillus licheniformis* (DSM 17236)-based direct fed microbial (DFM) to reduce *Staphylococcus agnetis*-induced BCO lameness in chickens. Seven hundred Cobb surplus breeder males were placed in 14 floor pens. Three experimental groups were distributed among pens: T1, positive control challenged with *S. agnetis* in drinking water (four pens); T2, negative control horizontally seeded from T1 (five pens) and T3, 1.6 x 106 CFU DFM / g of feed from 0 to 56 days, horizontally seeded from T1 (five pens). Lameness incidence in all groups as well as ileum histopatology and transepithelial resistance (TER) in 10 birds from T2 and T3 were assessed on day 56. GLM procedure in R.3.4.2 was used to compare treatments. BCO lameness incidence was significantly higher in T1 (77%) than in T2 (64%) and T3 (48%). Lameness incidence for T3 was statistically lower than in T2 (p=0.00225). In addition, dietary supplementation with the DFM significantly increased villus length (P=0.0006) and TER in ileum (P=0.025).

Histopathology and Ultrastructural Pathology of Focal Duodenal Necrosis

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Focal Duodenal Necrosis (FDN) is an intestinal disease of laying hens, characterized by multifocal mucosal erosions in the duodenum and proximal jejunum. It has a worldwide distribution and it is considered one of the top 5 disease concerns in table egg layers in the USA. Affected flocks exhibit low egg case weights and egg production drops. We have associated this disease with *Clostridium perfringens* infection. Macroscopically, FDN lesions are characterized by reddened to greyish mucosal erosions that may be covered with yellow pseudomembrane. Microscopic lesions are characterized by necrosis and loss of enterocytes from villous tips and fibrinoheterophilic inflammatory infiltrate admixed with long Gram-positive and filamentous Gram-negative rod-shaped bacteria. The lamina propria usually has lymphoplasmacytic inflammation with heterophils in villous tips. Long rod-shaped bacteria are usually present within the luminal exudate, attached to enterocytes and bound to connective tissue of the lamina propria. Ultrastructure of FDN lesions reveals enterocyte detachment from the lamina propria with heterophilic infiltration, cytoplasmic vacuolation in enterocytes with organelle loss, widening of intercellular junctions and presence of long rod-shaped bacteria within lesions.

Immunology

Cytokine transcription profiles in turkey poults during turkey coronavirus infection

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Turkey poults infected with turkey coronavirus (TCoV) usually have acute diarrhea, ruffled feathers, and decreased feed and water consumption, resulting in decreased body weight gain. The present study was to investigate the cytokine transcription profiles (IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, IFN-α, IFN-γ, and LiTAF) in ileum, cecal tonsil, and spleen of turkey poults during TCoV infection. One-week-old turkey poults were orally challenged with 105 EID50/mL/turkey of TCoV isolate (NC20) that was isolated from a turkey flock with acute enteritis in North Carolina (NC). Clinical signs and body weights of turkey poults were recorded during experimental period. Turkey poults were necropsied at 3, 7, and 21 days post infection (dpi). The jejunum and ileum from TCoV-infected turkey poults had severe, shortened, blunted, and/or fused villi at 3 and 7 dpi. Ileum had the highest TCoV viral load at 3 dpi. mRNA expression of LiTAF, IL-1β, IL-8 in ilea and IL-6 in cecal tonsils was significantly up-regulated ($P<0.05$) in infected turkeys at 3 dpi. mRNA expression of IFN-γ and IL-10 mRNA levels in ilea were up-regulated at 3, 7, and 21 dpi. mRNA expression of IL-1β, IL-6, IL-8, IL-12 and LiTAF in ilea were decreased at 7dpi. In conclusion, transcription of pro-inflammatory (IL-1β, IL-8), inflammatory (IL-6, LiTAF), anti-inflammatory (IL-10) and T helper 1 (IFN-γ) cytokines is up-regulated in early TCoV infection and may be associated with the progression of clinical signs and intestinal pathology.

Transcription Of IFN-γ, TLR-3 And TLR-21 In Turkey Embryos After In Ovo Vaccination With Marek's Disease Vaccines

A.Turner, A.L. Cortes, E. Gonder, K. Robins, I.M. Gimeno

In recent years several recombinant vaccines using HVT as a vector has been introduced in the market. One of those vaccines (bivalent HVT and Newcastle disease rHVT-ND) is being used in turkeys in common bases. In previous work, we have demonstrated that to avoid interaction with wild type HVT, rHVT-ND has to be administered in ovo. We also demonstrated that both HVT and rHVT-ND replicated readily in the turkey embryo. In ovo vaccination with HVT in the chicken hasten the development of the immune system and increases the transcription of INF-γ and TLR-3 in embryonic tissues. The objective of this study was to determine if HVT and rHVT-ND has the same effect on the turkey's embryos when administered in ovo. Turkey embryos were inoculated with HVT, rHVT-ND, or they were sham inoculated at 25 days of embryonation. Samples of spleen and lung were collected at days 1, 2, and 3 post vaccination. INF-γ and TLR-3 transcripts were evaluated by real time PCR. Results will be discussed.

Evaluation of safety and efficacy of multivalent oil based vaccines containing different adjuvant and immunostimulant materials

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Oil based adjuvants have an important role in the efficacy of vaccines as enhancing the immune response against antigens. And adjuvants need to be considered according to several criteria, like the antigens used, the type of immune response, the route of inoculation, or the duration of immunity. For this purpose many types of emulsions, vaccine adjuvants, molecular immunostimulant materials and emulsion formulation have been developed. In this study, safety and efficacy of developed vaccine adjuvants were evaluated with laboratory and field tests. In the laboratory test, adjuvants were evaluated in a specific-pathogen-free (SPF) chickens maintained in high biosecurity isolator. Control group was not vaccinated and other 4 groups (CAWIO, CNU-1, KR-1, ISA-70) were inoculated twice with multivalent vaccine containing different adjuvant. During the test, the birds of control or vaccinated group did not show any clinical signs. No significant changes in the weight gain were observed after twice vaccination in five all groups. High hemagglutination inhibition titer against avian influenza virus and Newcastle disease virus was observed in the all four vaccinated groups with statistically significant difference ($P<0.05$). After challenge with Newcastle virus, all vaccinated groups were survived with different mean death time. Currently, we selected three farms for field test and are going to collect samples for evaluation of efficacy and safety of tested multivalent vaccines. This result will be presented at the meeting.

Immunomodulatory effects of chicken IL-10 expressed by a recombinant Newcastle disease virus

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Newcastle disease (ND) is one of the most important diseases affecting poultry. Vaccination remains the primary means to control ND; however, there is a need for adjuvants capable of increasing the efficacy of current vaccines. Interleukin-10 (IL-10) is a cytokine that mediates Th2-driven humoral immunity, while suppressing Th1 cellular responses. In the present study, a low virulence, recombinant Newcastle disease virus expressing chicken IL-10 (rZJ1*L/IL-10) was created to evaluate the ability of chicken IL-10 (chIL-10) to modulate the avian immune response. In order to confirm the expression of chIL-10 by the recombinant NDV, a new bioassay for chIL-10 was developed. We were able to express and deliver active chIL-10 using this modified virus, and show that chIL-10 modulated the immune response, specifically by inhibiting macrophage activation, without decreasing protective efficacy. As expected, rZJ1*L/IL-10 was able to induce higher levels of NDV-specific antibodies while lowering the antigen-specific cellular memory response. Birds primed with rZJ1*L/IL-10 presented no clinical signs of disease or mortality 14 days post challenge and shed significantly less challenge virus when compared to the sham-primed control. In summary, we have developed a system to study the effects of chIL-10 *in vivo*, a method to produce a cytokine adjuvant, and a new bioassay to detect chIL-10 activity. Our findings suggest increased chIL-10 levels have a positive modulatory role on chicken antibody-mediated immune responses.

Effect of the Addition of *Saccharomyces cerevisiae* Yeast Cell Walls to Diets with Mycotoxins on Performance and Immune Responses in Broiler.

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This study was conducted to evaluate the effect of *Saccharomyces cerevisiae* yeast cell walls (YCWs) in diets with low doses of aflatoxin B1 (AFB1) and ochratoxin A (OTA) alone or in combination, on broiler performance and-+ immune system. A total of 210 male broilers aged 1-21 days were used. They were completely randomized into seven treatments with five replicates of six broilers each, as follows: 1)Control diet; 2)Control + 350 µg/kg AFB1; 3)Control + 350 µg/kg OTA; 4)Control + 350 µg/kg AFB1 and 350 µg/kg OTA; 5)Control + 350 µg/kg AFB1 and 1.5 kg/ton YCW; 6)Control + 350 µg/kg OTA and 1.5 kg/ton YCW; 7)Control + 350 µg/kg AFB1, 350 µg/kg OTA and 1.5 kg/ton YCW. The broilers were housed under environmentally controlled conditions in Petersime battery cages. Weight gain, feed intake and feed conversion index were measured. The relative weights of the thymus, spleen, and bursa of Fabricius (BF) were evaluated. The local immune response was assessed by quantifying intestinal immunoglobulin A (IgA). The cellular immune response was evaluated by a delayed hypersensitivity test. Haemograms and blood cell counts were also performed. The results showed mycotoxines decreased performance and immune response (p<0.05). Weight gain and feed conversion, improved in the YCWs groups. The YCWs increased (p<0.05) intestinal IgAs, and cellular immune response (p<0.05). The addition of the YCWs also had an effect on the relative weight of the thymus, spleen, and BF (p<0.05), as well as on the leukocyte, lymphocyte, and heterophil counts (p<0.05). The addition of the YCWs can be an alternative treatment for diets contaminated with low doses of AFB1 and OTA.

Infectious Bronchitis Virus

Role of S1 N-terminal domain amino acid differences among ArkDPI IBV vaccine subpopulations in differential binding to chicken tissues and selection in chickens

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Commercial attenuated ArkDPI-derived IBV vaccines contain subpopulations that are rapidly selected in and persist in vaccinated chickens. The S1 amino acid (aa) sequences of vaccine subpopulations positively selected in chickens differ by as few as four aa from the major vaccine populations negatively selected in chickens. At three of these four amino acid positions, ArkDPI-like S1 aa sequences of IBV identified in 80-98% of clinical samples match ArkDPI vaccine subpopulations selected in chickens, suggesting that these differences in S1 play a role in selection of vaccine subpopulations in chickens. Consistent with this hypothesis, we previously demonstrated that recombinant S1 protein representing the selected vaccine subpopulation C2, differing by only four aa from the major vaccine population (V), binds with higher affinity than V-S1 to chicken respiratory epithelium. We now show that the N-terminal domain (NTD; aa 19-258) of C2-S1 is necessary and sufficient for binding to these tissues. Neither a shorter NTD (aa 19-137), nor the S1-C-terminal domain (aa 258-538), nor S1 lacking aa 19-137 bound to any tissues tested. Surprisingly, S1-NTD binds chicken respiratory epithelium with higher affinity than complete S1. Like their S1s, C2-S1-NTD, which differs by only two aa from V-S1-NTD, binds with higher affinity than V-S1-NTD to respiratory epithelium. Binding assays with V-S1-NTD containing each of these two changes separately indicated that either aa change alone is sufficient to increase binding to levels equivalent to C2-S1-NTD. We continue to examine effects of aa differences in S1 proteins and their NTDs among ArkDPI-derived vaccine subpopulations.

Management

Applications of PathPro® web-based analytical service for mortality analysis in commercial layers

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PathPro® is a multi-tenant web-based flock health analytics service for analysis of mortality in commercial poultry flocks. Causes of mortality and ancillary findings can be analyzed by company, complex, farm, flock, age, date, genetics, and pullet and lay cycles, using standard and custom categories. PathPro® is built with ASP.Net and hosted on the Microsoft Azure platform, which provides scalability, high availability, data security, and monitoring. Flock health data is entered by hand or voice via quick entry screens and stored in an encrypted SQL Server instance. Health data is fed to interactive Power BI reports, as well as static tabular reports exportable to spreadsheets for additional analysis. PathPro® provided analysis of mortality over time to identify trends in factors affecting health and welfare. Eight mortality surveys were conducted at 3-month intervals during 2016-17 for layer complexes A (conventional cages) and B (aviary housing with floor access). Each bird was weighed and examined at necropsy. As a percentage of total mortality observed, the leading causes in A were, in descending order, peritonitis, tetany, tracheal casts, prolapse, and injury, and for B, peritonitis, prolapse, emaciation, tetany, and injury. Further analysis revealed a lower incidence of injury, as a primary cause of mortality in A (6.38%) compared to B (10.39%). Injury as co-existing lesion with other causes of mortality was analyzed for the two most recent surveys and showed a lower incidence in A (0%) compared to B (7.67%). Chickens that died from injury were, on average, 10 to 30% below the flock average body weight.

Development of biosecurity evaluation system in the duck farm associated with productivity

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According to FAO statistics, duck meat consumption of South Korea has greatly increased in recent years, resulted in rapid growth industry. However, compared to poultry industry, the current situation of hygiene, biosecurity and disease control system in duck industry are not well organized. Also, duck industry is very important in the transmission source of avian influenza

virus (AIV) and potential source of foodborne disease such as salmonellosis and campylobacteriosis. Therefore, we need effective evaluation system for the duck farm biosecurity to understand the current situation and to develop better biosecurity. To achieve this purpose, 10 broiler duck farms in same province were randomly selected and various samples from environment and feces were taken at two scheduled time, before and after restocking of ducks. From these samples, we tried to isolate the microorganisms such as Salmonella spp, Riemerella anatipestifer, campylobacter and Escherichia coli. Also, biosecurity levels of each farm was quantified by converting the risk assessment points and the results of laboratory tests into a score. By using this scoring system, a correlation between biosecurity levels and farm productivity index was evaluated by multivariate linear regression analysis.

Demographic characteristics of small poultry flocks in Ontario

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The recent increase in the number of small flocks throughout Ontario calls for an assessment of their demographic characteristics, in order to evaluate the potential risks they may pose to commercial poultry operations. A prospective surveillance study of small flock postmortem submissions to the Animal Health Laboratory was conducted over a 2-year period (October 2015 – September 2017). Completion of a standardized husbandry and biosecurity questionnaire was a requirement of the submission. A total of 154 questionnaires were received. Chickens were the most commonly submitted (83.8%), followed by turkeys, gamebirds, and waterfowl. The primary reason for raising birds was personal consumption (meat/eggs). Birds were acquired from various sources, including on-line classifieds, hatcheries, friends, and feed stores. Birds were often kept on the premises for less than 2 years, although longer periods (> 5 years) were reported. Birds were frequently housed in mixed groups, and in most cases had free-range access. Over half the owners reported that the birds had not been vaccinated. Biosecurity practices were inconsistent or inadequate. For instance, only 75% of owners isolated sick birds, less than 40% wore dedicated shoes or clothing when entering the coop, and less than 10% used a foot-bath. More than 60% of owners allowed visitors in the coop.
The results of this study provide a baseline characterization of small poultry flocks throughout Ontario, and will aid in detecting specific opportunities to improve the health of small flocks and mitigate the risk they may pose to commercial poultry flocks and public health.

Marek's Disease Virus

Decreasing the temperature of Marek disease vaccine preparation during application increases viral replication.

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Marek Disease and infectious bursal disease (IBD) control through vaccination is a common practice in the layer industry and hatchery application of VAXXITEK HVT-IBD® is a global trend. However, several factors may affect the efficacy of these vector vaccines, most of them during the hatchery application. High temperature during the application process may affect the ability of the cell associated vaccine to properly immunize the bird. The objective of this work was to demonstrate the benefits of cooling the vaccine preparation on the vaccine replication by Marek's disease real time PCR in the feather pulp seven days post vaccination. Vaccine preparations where applied by the subcutaneous route either cooled (4-8 Celsius) or at room temperature (20-23 Celsius). After seven days, feather pulp samples where obtained in FTA cards and processed to assess the vaccine replication. The results indicate that when during the vaccination process Marek vaccine preparation is cooled down, more of the virus infected cells are preserved and better immunization is obtained. Based on the laboratory reference values established for HVT vaccines when used together with Rispen (around 25% positives at 7 days indicate good application), the viremia in the cooled preparation vaccinated birds almost doubled the one of the room temperature preparation (36 vs 19% detection). These results suggest that keeping

the vaccine preparation between 4-8 C aids in the virus preservation, improve its replication and should be included in the standard operation procedures during hatchery application of HVT vaccines.

Effect of infectivity rate on Marek's disease vaccine titer variability

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In previous studies we have demonstrated that cell-associated Marek's disease vaccines have an inherent variability in the titers. In a study evaluating 42 vaccines, the coefficient of variability (CV) of vaccine dose ranged from 10 to 59% and was greatly affected by inappropriate mixing of the vaccines. That was expected as MD vaccines, once reconstituted, create unstable cell suspensions in which cells lose viability, flocculate and sediment. However, not all vaccines were equally affected by poor mixing suggesting that other factors were also contributing to large CV. The objective of this study was to evaluate the effect of infectivity rate (IR) on the CV. IR is defined as the percentage of cells infected with viable vaccine virus in a vaccine vial and calculated as the average PFU per number of live cells at time of titration (avg PFU/ # alive cells). To assess the effect of IR on dose variability, two approaches were used. First, commercial vaccines were titrated and CV was calculated before and after adding non-infected chicken embryo fibroblasts to decrease IR. Secondly, various dilutions of a commercial vaccine were grown in CEF to obtain various IR and then titrated to calculate CV. The correlation between IR and CV is discussed.

Mycoplasma

The Development of Real time PCR protocols to Differentiate *Mycoplasma synoviae* Vaccine and Field Strains

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Mycoplasma synoviae is a poultry pathogen of worldwide prevalence. The current approaches to *M. synoviae* control include continuous surveillance and quarantine, medication, vaccination and/or elimination of infected breeding flocks. With worldwide increase in the use of live attenuated vaccines as a control method, molecular techniques that allow the differentiation of live attenuated *M. synoviae* strains are increasingly important for diagnostics and applied research. A number of molecular techniques have been described for *M. synoviae* strain differentiation, including DNA sequencing of specific genomic targets (primarily *vlhA*) and multi-locus sequence typing (MLST). In this research comparative genomics as well as sequence analysis of specific targets (including *vlhA*) were used to identify genetic differences among the *M. synoviae* vaccine and field strains. Strain-differentiating primers and probes for Taqman® real-time PCR were developed and tested on mixtures of DNA as well as tracheal swabs from infected chickens. Application of these protocols allows rapid differentiation between *M. synoviae* strains in a quantitative manner.

Geospatial analysis of *Mycoplasma synoviae* in Georgia's commercial poultry flocks

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Georgia Poultry Laboratory Network

Mycoplasma synoviae is a significant avian pathogen and the occurrence of infection impacts the poultry industry in myriad ways, including economic loss. This project uses quantitative thematic mapping to illustrate a geospatial analysis of *M. synoviae* in Georgia's commercial poultry flocks.

Reflections on the Last Five Years of Mycoplasma Gallisepticum (MG) and Mycoplasma Synoviae (MS) Testing at the Georgia Poultry Laboratory Network in Georgia Commercial and Backyard Operations.

Len Chappell, MS

Data from serology testing (ELISA &HI) and antigen detection (PCR:MGMS) of Georgia commercial and backyard flocks were analyzed over the last five years. Comparison tables and trends will be discussed. Yearly rates of Mycoplasma infections will be compared between commercial and backyard flocks.

Parasitology

Surveillance for *Heterakis* species in game birds in Pennsylvania

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Histomoniasis, caused by the protozoan *Histomonas meleagridis,* is a significant disease of wild and domestic gallinaceous birds. Transmission of this parasite is dependent on utilization of the cecal nematode *Heterakis gallinarum* as an intermediate host. Due to the critical role that *H. gallinarum* plays in the introduction, transmission, and maintenance of *H. meleagridis*, it is important to define the host range and distribution of this nematode. From 2015 to 2017, ceca were collected from wild and domestic game birds that were either submitted as diagnostic cases or hunter-harvested. Cecal contents were examined for *Heterakis* nematodes using a 1–mm sieve. All *Heterakis* spp. present were counted, preserved in 70% ethanol, and identified to species based on morphologic characteristics of the male worms. *Heterakis* nematodes were detected in five of the seven game bird species examined, including ring-necked pheasants (*Phasianus colchicus*; 52/53 (98%)), ruffed grouse (*Bonasa umbellus*; 48/64 (75%)), wild turkeys (*Meleagris gallopavo*; 49/65 (75%)), domestic chickens (*Gallus gallus domesticus*; 3/8 (38%)), and chukars (*Alectoris chukar*; 4/38 (11%)). Nematodes in all species were identified as *H. gallinarum*, except for ruffed grouse, which harbored *H. isolonche*. No mixed-species infections were identified in any examined birds. No *Heterakis* nematodes were identified in ducks (*Anas* sp.; n=50), American woodcock (*Scolopax minor*; n=27), or domestic turkeys (*M. gallopavo,* n=10). The results presented herein indicate that *H. gallinarum* is common in wild and domestic upland game bird species. Future research is needed to determine the prevalence of *H. meleagridis* infection in these species.

Blackhead outbreak prevention attempt-a success?

Stephen Williams, DVM

During a routine farm visit, a depressed 12 week old hen was removed from a flock of 8,100 hens. A necropsy was conducted and blackhead like lesions were identified. Other depressed birds were removed from the flock and samples were submitted to the state laboratory. Histopathology confirmed the presence of Histomonad organisms in the spleens and livers of the organ samples. Previously this flock and others like it, have had mortalities up to 40% due to blackhead outbreaks in the area. A meeting was held with the grower to create an intervention plan. A plan was designed to minimize bird stress and maintain excellent litter conditions. Through intensive monitoring, the delay of certain vaccinations, and proper ventilation the farm never experienced an increase in mortality due to blackhead.

The use of a live non-attenuated coccidiosis vaccine modifies *Eimeria* spp. excretion in commercial antibiotic-free broiler chicken flocks compared to conventional shuttle anticoccidial programs.

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Our objectives were to compare the effects of an antibiotic-free (ABF) program in commercial broiler chicken flocks using a live non-attenuated coccidiosis vaccine on fecal *Eimeria* spp. excretion and growth performances with those of conventionally raised flocks. Fecal samples were collected every 3 days for oocysts counts in 44 flocks of 7 farms. A live non-attenuated anticoccidial vaccine (Coccivac B®) was administered by spray cabinet at the hatchery in ABF flocks only. Shuttle programs in conventional flocks consisted of in-feed chemical anticoccidials from 0 to 20 days of age, followed by ionophores until slaughter. Age of the flock at the oocysts excretion peak (AGE_PEAK) and the number of oocysts at that excretion peak (OPG_PEAK) were recorded. There was a significant difference of 2.7 days (p=0.0001) for the AGE_PEAK, from 26.4 days in the conventional treatment to 23.7 days in the ABF program. There was no significant difference between treatments for the OPG_PEAK (p=0.626). There was a significant decrease of 2.28 gr in the average daily gain (p=0.004) and increase of 0.08 points for the feed conversion ratio (p=0) in the ABF program compared to the conventional program. There were no significant differences for body weights at slaughter, livability and condemnations. It can be concluded that an ABF program using a live non-

attenuated vaccine will have an earlier oocysts excretion peak compared to a shuttle program, but no significant effect was observed on the total number of oocysts at that excretion peak between the two programs.

Control of Poultry Red Mite: first Italian experiences with Fluralaner

Corrado Longoni

For many years Poultry Red Mites were one of the biggest and unsolvable problems of poultry industry. They are external parasites causing huge economic losses especially in eggs sector. In Europe the most diffused mite is Dermanyssus gallinae, and a study in 2005 estimated an economic cost of 130 million euros per year due to production losses and treatment costs. The environmental chemical treatment available until last year were only partially effective, and some illegal products were used, creating the perfect conditions for the big Fipronil scandal of last summer. A new innovative product is now available for the treatment of Dermanissus gallinae, it contains a new molecule: Fluralaner. it is not an environmental acaricide as the others product actually employed, but it is a systemic treatment that have to be administered in drinking water, able to kill mites immediately after the blood meal. In this paper the first two field experiences with this new innovative product conducted in Italy are described. These preliminary data can give an idea of the efficacy of this product and of the impact it can have on farm profitability.

The Effect of Single Species Coccidia Exposure on the Current Industry Broiler Genetic Strains When Exposed at Varying Ages – Part 2

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Coccidia exposure with the subsequent performance impact, oocyst shedding and cycling characteristics in broilers is well documented. However as the broiler genetics have evolved, the growth characteristics of the common broiler strains have evolved as well. The aim of this study was to determine how single species coccidia exposure impacts the performance, lesions severity, and shedding characteristics of the main two current male broiler strains (Cobb 500 and the Ross 708) when they were exposed to pure field strains of either *Eimeria maxima* or *Eimeria tenella* at either 7, 14, 21, or 28 days. The study was conducted in a battery cage set-up with 8 cages per treatment containing 20 birds per pen. The birds were challenged with the single species coccidia by oral gavage at either 7, 14, 21, or 28 days of age. Body weights and feed were weighed on day 0, 7, 14, 21, 28, and 35 days of age to determine body weight gain and feed conversion. 3 birds per pen were removed and coccidia lesion scores performed on day 14, 21, 28, and 35 days of age. Oocyst shedding was evaluated by collecting fecal samples at day 14, 21, 28, and 35 and OPG results calculated for those time points. Final results, conclusions, and discussion of these findings will be presented.

Reovirus

Expression and characterization of σC from two avian reovirus genotypes

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Avian reoviruses (ARV) belong to the *Orthoreovirus* genus in the family *Reoviridae.* They are widespread in chickens and turkeys and have been linked to several diseases in poultry; most notably tenosynovitis in chickens. The failure of vaccines to protect poultry against ARV diseases has been attributed to antigenic differences among strains, particularly among the viral encoded σC proteins. Therefore, to produce a vaccine which can be easily modified to keep up with viral mutations, we used a platform expression system to produce the σC protein. ARV strains were isolated from infected broilers and the σC gene was sequenced. Two σC genes, from genogroups 1 and 2, were cloned into plasmid transfer vector pVL1393 and then recombined into the genome of baculovirus under the control of the polyhedron promotor. The expression and relative quantity of the σC protein produced by these recombinants was determined using PAGE/Western blot analysis and a relative potency ELISA, respectively. Partially purified σC expressed from baculovirus cultures was used to inoculate chicks to determine the immunogenicity of the protein. Serum samples were tested in a commercial ELISA for ARV specific antibodies.

Emerging avian reovirus genotypes in free-range broilers, France

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Avian reoviruses are agents of various conditions in chicken, the most significant being viral enteritis or viral tenosynovitis. The "classical" viral tenosynovitis is an emerging condition of broilers and future layers, leading to huge economical losses worldwide. Many variants, referred to as genotypes, are emerging worldwide and challenge the efficacy of commercial vaccines. For a decade, viral tenosynovitis has been an increasing challenge for the French poultry industry, mostly for free-range broilers. A very severe emergence occurred in 2011 and was associated to a genotype 1 strain (Troxler et al, 2013). In 2016, a new emergence occurred in free-range broilers in South West France and 10 cases were included in a field investigation: All 10 affected flocks showed a dramatic incidence of lameness, reaching nearly 50 to 100% of birds and occurring as soon as 3 to 4 weeks of age. Affected birds showed a mono- or bilateral swelling of gastrocnemius tendon, leading to severe lameness. No other gross lesions could be consistently observed on affected birds. Reovirus isolation was assayed on the chicken liver cell line (LMH): a cytopathic effect (CPE) could be detected from the 2nd to the 3rd passage. Supernatants of CPE positive cell cultures were processed for RT-PCR analysis: total RNA was extracted and a positive *sigmaC* gene specific RT-PCR was performed. A 1088 bp PCR product was obtained and submitted to Sanger sequencing. A total of 5 viral isolates were sequenced and were strictly identical. All these sequences clustered within genotype 4.

Molecular Surveillance of Avian Reoviruses and Efforts Towards a Comprehensive Classification Method

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Since early 2016 we have genotyped hundreds of reovirus isolates in order to surveil the occurrence of variants that might affect the broiler industry. In addition, we have studied selected variants using a full genome approach. Even though, we use as a convention the S1 gene in our genotyping schemes, other genes such as L3 and M2 show high variability and are good candidates to explore a more comprehensive genotypic characterization. Sigma C gene typing in some cases fail to correlate not only with pathotypes but also with serotype and/or antigenic type. Since the current typing is used for autogenous vaccine virus selection this situation affects vaccine effectiveness. The proteins encoded by L3 and M2 genes are capsid and outer membrane proteins, they generate neutralizing antibodies and their variability might be associated with reovirus pathology. Using this information, we will propose a strategy to better characterize reoviruses in order to understand pathogenic and serological determinants.

Serotype Analysis of Reovirus Genotypes

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The emergence of variant reoviruses from clinical cases of tenosynovitis has occurred in broilers from reovirus-vaccinated breeders in the U.S. and elsewhere around the world. Current commercial vaccines do not provide adequate protection against challenge by the new variants so many companies elect to utilize autogenous reovirus vaccines. Variant reoviruses isolated from the tendons of clinically affected broilers belong to one of 5 major genotypes based on the sequence of the Sigma C protein. The relationship between genotypes and serotypes has not been determined. Seven reovirus field isolates were plaque purified three times, to obtain clonal isolates, then used to produce hyperimmune serum in SPF chickens. The stock serum was negative for antibodies to the following extraneous avian pathogens: NDV, IBV, IBDV, and CAV by ELISA and MG/MS by HI. Each stock serum was positive for reovirus antibodies by ELISA with GMTs ranging from 9339-25530 and virus neutralizing GMTs from 512-2084. Cross-neutralization with homologous and heterologous antigen reveals major serotype differences between the isolates examined from each genotype with less than 3.1% serological relatedness observed between any of the isolates. In addition, major serotype differences were identified between isolates belonging to 2 distinct lineages within genotype 2 and 3. The results were not surprising since the Sigma C similarity was between 50-84% among the isolates but provided for the first time data to support the serotype differences between and within genotypes.

Progression Changes of Reovirus Tenosynovitis Correlated with Weigh and Immune System Histology

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Reovirus tenosynovitis is a leading cause of decreased production in broiler flocks in south Alabama. Histopathology of joints and serology are routinely used for diagnosis. From a single flock, complete necropsies with ancillary diagnostics were conducted at placement and then at 20, 27, 34 and 36 days of production for 10 birds in four houses (200 total). At sampling five normal and five "poor doers" were randomly selected from each house. Weight, hock joint synovial histology, lymphocyte depletion in Bursa of Fabricius, thymus and cecal tonsil, splenic histiocytosis and periportal hepatic lymphoid nodules were evaluated from each bird. All histology was scored 0 (normal) to 4 (severe disease) and subjected to linear regression analysis. Maternal antibodies were sufficient in all houses and rising titers indicated exposure to wild variant reovirus. Synovitis prevalence and antibody titer level were variable between houses and this allowed for statistical correlation with immune system histology. At days 20, 27, 34, and 36 of production active synovial reovirus infection affected average weight gain in broilers by 25, 16, 20 and 16% respectively and was the primary factor in culling near time of processing. Joint histology of early infection was characterized by perivascular nodular lymphocyte infiltration and progression to synovial hyperplasia, subcutaneous vascular proliferation, heterophilic infiltration and secondary bacterial synovitis. Splenic histiocytosis and cecal tonsil lymphoid depletion were correlated with reovirus tenosynovitis. Knowledge of systemic immune system response to reoviral tenosynovitis aides the diagnostician when synovial tissues are not available or coinfection with other viruses is suspected.

Salmonella

How Probiotics Can Be A Tool In Salmonella Control

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Novozymes and BI have entered into a joint agreement to find alternatives to antibiotics. Floramax is a product which has been on the market for the past several years. To better understand how Floramax works several studies have been done this past year. The results of these studies will be presented. There will also be discussion on how it is believed these products accomplish their goal.

Salmonella exposure risk of chick boxes

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For production complexes seeking to reduce the incidence of Salmonella, the hatchery is a likely source of exposure. In this project, chick boxes were evaluated for their contribution to Salmonella exposure in day-of-age chicks. At the participating hatchery, chicks are placed in "clean" boxes on Monday and Thursday, while chicks on Tuesday and Friday are placed in "dirty" boxes from the previous day. The goal of this study was to evaluate whether chicks placed in dirty boxes are at a higher risk of Salmonella exposure compared to chicks placed in clean boxes. Prior to usage, clean boxes were marked and swabbed using boot swabs enriched with milk. After chicks were placed in these boxes and delivered to the farm, they were returned to the hatchery, and the same marked boxes were swabbed the next morning as dirty boxes. All samples were submitted for Salmonella culture and serotyping. Samples collected on clean boxes were negative for Salmonella. Of the samples collected on dirty boxes, 10% yielded a positive Salmonella isolation. Based on these results, it was determined that dirty boxes represent a potential risk of Salmonella exposure in day-of-age chicks when compared to clean boxes; however, the risk is lower than expected.

Vaccinology

Assessment of the benefits provided by an IBD immune complex vaccine (BDA BLEN) in broilers under field conditions in Ecuador.

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Infectious bursal disease (IBD) control through vaccination is of paramount importance for IBD endemic countries. Herein are presented results for the use an IBD immune complex vaccine (BDA BLEN) in broilers reared under field conditions in an industrial trial in Ecuador when compared to other immune complex vaccine program. A total of 1.511.000 birds were evaluated during three runs in multiple flocks (654.000 birds vaccinated with BDA BLEN and 858.000 with other immune complex vaccine). Serology (IDEXX ELISA), histopathology (21, 25 and 28 days) and productive parameters were used to compare the two vaccination programs. No statistical differences were observed in the serological response to IBD. No evidence of IBD induced immunosuppression and no main microscopic differences within the two vaccination programs were reported. A multivariate analysis (multiple lineal regression model) was performed on the results and no statistical differences where observed in mortality among the groups. The comparison of the productive parameters showed that the flocks vaccinated with BDA BLEN showed significantly better results (P≤ 0,05) for all the productive indexes. A total of 128 grams more in body weight than the other immune complex vaccine group; lower feed conversion rate (-0,095 points); higher European efficacy index (+25,95 points); better feed efficacy (+13,05 points) and higher productivity index (+9,99 points). These results indicate the suitability of single dose BDA BLEN® in broilers under field conditions and the potential productive and financial benefits of its use.

ASSESSMENT OF THE EFFICACY OF VECTOR HVT-ND (NEWXXITEK HVT+ND) + IBD IMMUNE COMPLEX VACCINE (BDA BLEN) VACCINATION IN BROILERS UNDER FIELD CONDITIONS IN VENEZUELA.

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Newcastle disease (ND) and Infectious bursal disease (IBD) control trough vaccination is of paramount importance for ND endemic counties. Herein, we present field results for the use of a vector HVT-ND (NEWXXITEK HVT+ND) plus an IBD immune complex vaccine (BDA BLEN) in broilers reared under field conditions in Venezuela, when compared to a similar program The efficacy criteria included feather pulp PCR detection of NEWXXITEK HVT+ND at 7 days to assess the vaccine replication rate, bursal histopathology (21, 25 and 28 days) and productive parameters. Consistent to what has been reported as an indicative of proper vaccine application and replication rate for vector HVT vaccines 50% of the feather pulp samples were PCR positive at 7 days of age for NEWXXITEK HVT+ND. No main microscopic differences within the two vaccination programs: a conserved bursal architecture with scattered lympholitic areas at 21/25 days and signs of bursal recovery at 35 days were described. Statistically significant differences (P≤ 0,05) were observed in the productive parameters, the flocks vaccinated NEWXXITEK HVT+ND and BDA BLEN showed lower mortality (6,75% vs 11,94%), lower feed conversion rate (1,88 vs 2,09), higher average daily weight gain (59,40 vs 55,05) and higher European efficiency index (294 vs 231). These results indicate the suitability of single dose of NEWXXITEK® HVT+ND and BDA BLEN® for IBD and ND control in broilers under field conditions.

Benefits of the long-term use a single dose vector HVT-IBD vaccine in commercial layers.

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Early vaccination against infectious bursal disease (IBD) is a common practice in the layer industry; however, due to the high susceptibility of layers to the disease the use of live vaccines may compromise the bursal integrity in young birds generating immunosuppression and failures in vaccination programs. An alternative is the use of viral vectors for transgenic expression of immunogenic proteins that can provide adequate protection without the potential bursal damage. The objective of this work was to demonstrate benefits on the long-term single dose vaccination using VAXXITEK HVT-IBD®, a Turkey Herpesvirus (HVT) vector vaccine expressing the IBDV viral protein 2 (VP2) in layers. A total of 3.5 million pullets distributed in 36 flocks (caged and cage free) have been vaccinated over a seven years period with one dose of VAXXITEK HVT-IBD® for IBD control. The overall health status (body weight, uniformity, mortality rate and bursal histopathology) was assed and compared with

traditional live vaccinated flocks. The study demonstrated that the use of a single dose of the vector vaccine allowed IBDV control, improvements in body weight, uniformity and provided a better health status when compared with multiple live IBDV vaccines both in caged and caged free birds over the years suggesting the suitability the continuous use of a single dose of the vector vaccine to aid in the IBD and Marek disease control for commercial layers in the field.

Comparative Study of The Safety and Efficacy of Three Inactivated Tetravalent Vaccines in Commercial Broiler Breeders

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The efficacy and safety of AviPro® 431, Breedervac® IV plus and Maximune® 8 administered by the subcutaneous route was compared. One-day old commercial broiler breeders were divided in three vaccinated groups and one unvaccinated control group. Serum samples were tested at 2, 4, 6 and 10 weeks post-vaccination (wpv) for IBD, Reovirus, ND and IB antibody titers with ELISA kits (Idexx Laboratories and BioChek Smart Veterinary Diagnostics). Additionally, ND sero-response was measured by HI test, and serum samples from 6 wpv were tested for IBD by virus neutralization. At 2 and 6 wpv 10 birds per group were euthanized for macroscopic and histological observation to evaluate local damage in the inoculation site. AviPro® group presented significantly lower birds with visible vaccine compared to Breedervac® and Maximune® groups, which is indicative of faster degradation and higher absorption rate. Macroscopic score lesion was lower in AviPro® than Breedervac® and Maximune® groups, indicating lower tissue reaction at injection site. The biggest differences in antibody response were in IBD and Reovirus, AviPro® group presented the highest titer of Reovirus antibody, which was significantly higher than Breedervac at 10 wpv. AviPro® stimulated significantly higher IBD antibody titers than Maximune® at 4, 6 and 10 wpv, when tested with the BioChek´s kit, and 6-10 wpv if tested with either, the conventional or XR IBD kits from Idexx. The results of this study indicate that vaccination with AviPro® 431 induces lower tissue reactivity and higher peak of IBD and Reovirus antibody titers than other tetravalent vaccines.

Efficacy And Safety Of A Tetravalent Inactivated Oil Adjuvant Emulsion Vaccine Applied Intramuscularly

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AviPro® 431 ND-IB-BD3-REO is an oil-emulsion tetravalent vaccine that has proved to be effective and safe in broiler breeder pullets when injected via subcutaneous. This study assessed the efficacy and safety of AviPro® administered via intramuscular, as this is the most widely used route for this kind of inactivated vaccines. Day-old Ross broiler breeder pullets were housed, individually identified and separated in two groups. Negative control group was left unvaccinated and treatment group was vaccinated intramuscularly. The efficacy of the vaccine was determined by detection and quantification of IBD, Reovirus, ND and IB antibody titers with ELISA test kits, from BioChek Smart Veterinary Diagnostics and Idexx Laboratories, at 2, 4, 6 and 10 weeks post-vaccination (wpv). The development of ND antibody titers was also studied by HI test and blood samples from 6 wpv were tested by virus neutralization for IBD. Body weight was documented weekly and at the end of the trial for each individual bird. All birds were observed for any potential adverse effects or macroscopic lesions due to the vaccine administration at the inoculation site. No clinical signs nor macroscopic local damage were recorded in the vaccinated group during the observation period. Results of the different serological analysis showed high antibody titers against IBDV, Reovirus, NDV and IBV in all sampling ages indicating effective vaccination response. Conclusively, vaccination with AviPro® 431 ND-IB-BD3-REO administered via intramuscular induces high antibody levels against IBDV, Reovirus, NDV and IBV without local adverse effects at the inoculation site in broiler breeder pullets.

A vaccine of Saccharomyces cerevisiae expressing two adenovirus capsid-proteins against Hepatitis-Hydropericardium Syndrome

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Fowl adenovirus serotype 4 (FAdV4) is a common infectious agent which cause Hepatitis-hydropericardium Syndrome (HHS) in young bird with high mortality in Korea and worldwide. To control this disease, inactivated vaccine was used in some country with the risk of pathogen dissemination and limited efficacy. Recently, several subunit vaccines based on virus proteins were developed using *E.coli* and Baculovirus system to address inactivated vaccine problems. Among these recombinant viral protein, fiber2 and penton were demonstrated the ability against effect of virus on chickens as vaccine candidates while the hexon loop1, a part of the major capsid protein, were tried without protection. To improve the protection as well as reduce cost, a new construct of partial hexon, penton and fiber2 recombinant proteins were expressed using the yeast strain *Saccharomyces Cerevisiae* and combine into two groups including hexon-penton and penton-fiber2. In this study, we expressed immunogenic proteins of FAdV4 by yeast system and determine their ability to protect SPF chickens from HHS infections in combination.

Successful Immunization following 18.5 ED In Ovo Application Of rHVT-F Vaccine Using Egginject® In-Ovo Injector.

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In ovo vaccination is becoming more and more widespread around the world. In addition, more and more *in ovo* equipments are being marketed by different companies. Egginject® is an *in ovo* dual pressure injection machine produced by Ecat ID Company. Preliminary assessment studies using blue dye test and breakout sessions enabled to define the optimal transfer injection window to 18.5 to 19 days of embryonic development. The aim of this trial done in field conditions was to compare the immune response and the performances of broiler flocks immunized against ND at the hatchery by conventional live ND vaccine administered by spray with flocks vaccinated with rHVT-F vaccine (Vectormune ND) by the *in ovo* route. Commercial broiler chickens from the same parent stock flock were vaccinated in the hatchery either with rHVT-F *in ovo* using Egginject® equipment or with a live ND vaccine via spray. Immunization was assessed using several ND serology tests (HI and several ELISA kits) at 3, 4, 5, and 6 weeks of age. Overall performances of the flocks were recorded too. Detailed results will be presented. In summary, the robustness and reliability of this *in ovo* equipment in delivering accurately and efficiently an immunizing dose of rHVT-F vaccine was demonstrated.

LONG-TERM USE OF A SINGLE DOSE VECTOR HVT-IBD (VAXXITEK® HVT+IBD) VACCINE IN BROILER BREEDERS.

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Infectious bursal disease (IBD) control in breeders can be achieved using Turkey Herpesvirus vector vaccines that can provide adequate protection without the potential bursal damage of traditional live vaccine programs. VAXXITEK® HVT+IBD have been extensively used by the poultry industry for the last 10 years in layers, broilers and broiler breeders for Marek disease and IBD control. The ultimate goal of an IBD vaccination program in breeders is to allow them to finish the rearing period without the disease; this to be followed by inactivated IBD vaccines in order to provide with maternal antibodies for the progeny. The objective of this work was to demonstrate benefits on the long-term single dose vaccination using VAXXITEK® HVT-IBD in broiler breeders and their progeny. A total of 3.690.000 breeders (194 flocks) have been vaccinated with VAXXITEK® HVT-IBD, over a seven years period in a broiler integration in Colombia. Mortality rates, uniformity and health status were compared with the genetic line standard and traditional live vaccinated flocks. The results showed a consistent improvement in productive parameters suggesting healthier birds and no deleterious effect of using the program over the years. The transmission of IBD maternal antibodies also benefited from the vector vaccine priming strategy yielding a higher percentage of maternally derived antibodies for the progeny. These results indicate the suitability of single dose of the vector vaccine for IBDV control in this important segment of the poultry production chain.

Evaluation Of Newcastle Disease Live Attenuated Vaccine Take By In Vivo Replication And Local Immune Response Using Tear Drops

Andrea Delvecchio

Several live attenuated vaccines are available on the market for the control of Newcastle Disease Virus (NDV) in the field. The efficacy of these vaccines has to be tested in laboratory conditions in order to validate their use in the field. Furthermore it has already described that mucosal antibody production is closely related to local antigenic stimulation induced by viral replication hence the pathogenesis and tissue tropism of the viruses used for vaccination is to be considered to assess live vaccine efficacy. In our studies SPF and conventional day-old chicks were vaccinated with a commercial live attenuated vaccine against NDV (VG/GA AVINEW) by different route under controlled conditions. VG/GA AVINEW strain has already been described to be able to replicate both in the respiratory and intestinal tract. Our aim was to identify reliable and easy-to-use analytical tools for use in the field to monitor vaccine take in chicken. In order to evaluate the vaccine take both mucosal and systemic responses were assessed at different intervals: NDV-specific IgY antibodies were quantified by ELISA and HI test. Target organs for vaccine virus replication were collected at different days post vaccination (DPV) in order to evaluate the vaccine replication. The detection of the NDV vaccine was compared between the different routes of administration at different intervals. Finally, a velogenic NDV challenge study was performed and the protection in commercial broiler chickens evaluated and correlated with the serological response.

Protection Against California 2002 NDV Strain Afforded by Adenovirus Vectored Vaccine Expressing Fusion or Hemagglutination-Neuraminidase Genes

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Vectored vaccines expressing the combination of the hemagglutinin-neuraminidase (HN) and fusion (F) genes generally have better clinical protection against Newcastle disease virus (NDV) than when either the F and HN genes are expressed alone. The present study aimed to compare the protection induced by adenovirus vector expressing the F or HN alone, at two different doses, and the combination of both proteins against a velogenic NDV challenge, California/2002, at 3 weeks post vaccination (wpv). Clinical signs and mortality were monitored for 14 days after challenge. Swabs were collected and blood was collected before and after challenge from surviving birds. Birds vaccinated with adeno F and adeno F/HN with the highest dose had 100% survival rate with no clinical signs. The survival rate ranged from 0 to 25% in the HN alone or any groups at the lower vaccination dose other groups showing characteristic vNDV clinical signs. No specific antibody could be detected by HI at 3 wpv in any group whereas all surviving birds had high HI titers at 14dpc. Birds vaccinated with adenovirus F or F/HN had statistically significant lower RNA shedding compared to other groups at 2 and 4dpc in OP swabs and 4dpc in cloacal swabs. Comparisons with vaccine given as a booster will also be discussed.

Challenge study against conventional and emergent serovars of *Avibacterium paragallinarum* in SPF vaccinated chickens

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The spectrum of protection of a polyvalent coryza bacterin containing serovar A-1 (Strain 221); serovar B-1 (Strain 2671) and serovar C-2 (Modesto strain) was evaluated against conventional and emergent serovar C-1 (Strain ESV-135) and serovar B (Strain ESV-185) of *Avibacterium paragallinarum* (AP). SPF birds were vaccinated at 5 weeks of age (Via sub-cutaneous route) and at 9 weeks of age (Via intramuscular route) with 0.5 ml dose/bird. A total of 88 vaccinated birds vaccinated were randomly distributed into 5 groups and challenged either with the following homologous or heterologous strains. Conventional strains A-1 (2.14 x 108 ufc/ml); B-1 (3.4 x 109 ufc/ml); C-2 (1.15 x 108 ufc/ml) and emergent strains C-1 (3.25 x 108 ufc/ml) and B (2.53 x 108 ufc/ml). Serological response previous to the challenge was performed using HI test 48 hours before the challenge. A total of 28 non-vaccinated SPF birds were equally challenged with one of the 5 serovars used in this study and observed for clinical signs during 7 days. Results in the percentage of protection against each serovar was as follows: A-1 (82%); B-1 (88%); C-2 (78%); C-1 (78%) and B (78%). Corresponding non-vaccinated birds had A-1 (0%); B-1 (20%); C-2 (17%); C-1 (17%) and B (17%). The protection achieved after double vaccination and challenge demonstrated the effectiveness of this bacterin against conventional and emergent strains of *Avibacterium paragallinarum*.

Virology

Identification of infectious bursal disease viruses in backyard poultry from California USA.

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Backyard poultry are becoming increasingly popular in the US. These flocks can cause disease problems for the commercial poultry industry however. The initial outbreaks of acute infectious bursal disease caused by the very virulent infectious bursal disease virus (vvIBDV) in California were traced back to backyard poultry flocks. Very little is known about the pathogenicity of IBDV in backyard poultry breeds and the potential for these flocks to be reservoirs for IBDV. In this study we examined and characterized IBDV strains from backyard poultry samples submitted to the California Animal Health & Food Safety Laboratory System. Viruses were detected using RT/PCR and characterized using sequence analysis of the hypervariable region of the VP2 gene (hvVP2). A phylogenetic analysis was conducted and viruses that fell into the vvIBDV genogroup were confirmed to be true vvIBDV or reassorted viruses by sequencing of a portion of genome segment B (VP1). Strains of IBDV belonging to the endemic variant genogroup, vvIBDV genogroup and reassorted vvIBDV were identified. These viruses were found in several backyard poultry breeds. Studies on the pathogenicity and shedding of IBDV strains in different backyard poultry breeds are needed.

Description of clinical signs, gross and histological lesions in chickens inoculated *in ovo* with chicken astroviruses associated with "white chick disease".

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Chicken astroviruses (CAstVs) were isolated from liver and intestinal samples of unhatched embryos and chicks from flocks experiencing moderate to severe reduction in hatchability, embryonary mortality during the second week of incubation, and the presence of poor quality chicks "white chicks". The lesions observed in chicks and unhatched eggs obtained from clinical cases included, hepatocellular vacuolar degeneration, glycogen accumulation and heterophilic and lymphocytic interstitial nephritis. Chicken astroviruses were isolated by inoculation in chicken embryonated eggs, and they induced severe congestion, hemorrhages, and edema of abdominal muscles of chicken embryos. Chicken astrovirus associated with "white chick" condition in Southern United States are genetically related and they grouped in a separate cluster in group B. Chicken astrovirus isolates were inoculated into chickens *in ovo* and the eggs were hatched to evaluate the clinical signs and lesions. Analysis of the clinical signs, the lesions and comparisons with field clinical cases are presented.

Fatal hemorrhagic tracheitis by *Aviadenovirus* in Alagoas Curassows (*Pauxi mitu*)

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The Alagoas Curassow (*Pauxi mitu*) is a gallinaceous bird (Aves: Galliformes: Cracidae) kept in captivity and extinct from the small natural habitat in the Atlantic Forest of the northeastern Brazil (states of Alagoas and Pernambuco). Extinct from the wild since the 1970's, mostly due to habitat loss for sugar cane plantation, very few individuals were rescued. The captive population (~200) includes hybrids and the largest pure bred numbers (~40). Juvenile birds are kept together in common enclosures and adult couples are paired for reproduction. The A species of *Aviadenovirus* (formerly serotype 1 of Group I), has been described in inclusion body hepatitis in chickens, parrots and falcons, and respiratory infections, mainly subclinical, in chickens, although severe in quail (quail bronchitis). Our objective was to describe an outbreak of fatal hemorrhagic tracheitis in four captive young Alagoas Curassows. They were found agonizing or dead with respiratory disease. Grossly, birds had fibrinohemorrhagic tracheitis with large cylindrical blood clots in the lumen. There were diffuse and marked infiltration of lymphocytes and macrophages expanding the lamina propria of the tracheas. Basophilic and eosinophilic intranuclear inclusion bodies were found in the epithelia of trachea

associated with marked lesions. An *Aviadenovirus* was obtained in chicken eggs and by molecular characterization was genetically grouped with 99% identity to the fowl adenovirus A, based on the hexon protein gene. This is the first report of a respiratory disease by *Aviadenovirus* A in any cracid bird in Brazil, recommending for stricter biosecurity.

Field evaluation of vaccine take of two IBD complex immune vaccines in broiler flocks

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Immuno complex (IC) vaccines have been used extensively for Infectious Bursal disease (IBD) control because they can be applied in the hatchery and they are not affected by MDA. The efficacy of IC IBD vaccines depends on the vaccine strain and the balance of the amount of virus and its specific antibodies used for the assembly of the ICs present in the vaccine. In order to determine the vaccine take and consequently the onset of immunity under field conditions, flocks vaccinated with two IC vaccines formulated with Winterfield 2512 and V877 Plus intermediate strains were monitored. Sera and bursa samples from eight flocks were weekly collected up to slaughter time for histological, molecular and serological analysis. At 30 days of age, W2512 and V877 vaccine viruses were detected in 100 and 67% of bursas from chickens vaccinated with these vaccines, respectively. Histological evidence of vaccine virus replication in Bursa was higher and more consistent in W2512 flocks than V877 ones at 30 days of age. At 35 days of age, chickens receiving W2512 vaccine strain had higher ELISA titers than those receiving V877 vaccine (statistical difference). In summary, the two vaccine strains showed different replication patterns according to the three evaluated parameters showing that W2512 virus colonized bursa earlier and faster than V877 vaccine strain.

Pathogenicity And Length Of Infection Studies Of Avian Reovirus Field Variants In Experimental Chickens

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The newly emerging ARV variants and/or novel strains have been causing major poultry disease and economic losses in PA and other states in recent years. Severe arthritis/tenosynovitis and up to 70-80% morbidities were seen on ARV-affected young broiler flocks. In addition to conducting genotype characterizations on sequencing ARV Sigma C genes and full genome analysis on ARV field isolates by Next-generation sequencing, we have been conducting chicken experiments for pathogenicity, length of infection, immune responses and vaccine challenge studies on selected ARV field variants in our recent ARV research studies. Our chicken experiment results have confirmed a number of the newly emerging ARV variant strains being highly pathogenic to chickens and showing no protection by the classic ARV vaccine strains (e.g., S1133). Affected chickens less than 6-7 weeks old had heavy virus shedding during the first week post infection (pi), decreased virus shedding in the 2nd week pi and were barely shedding in or after the 3rd weeks pi. In adult layer chickens, virus shedding occurred only at the first week pi, and the ARV affected layer chickens did not show any observable clinical signs. The immune antibodies were detectable after 2 weeks pi in both young broiler chickens and adult layers.

Detection of chicken proventricular necrosis virus (CPNV) from frozen tissue homogenates derived from proventriculitis experimental studies

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In a previous study, transmissible proventriculitis (TP) was experimentally reproduced in our laboratory using an organ homogenate from broilers that did not contain IBDV, reovirus, IBV, or NDV. Lesions in the experimentally infected birds were identical to those seen in commercial broilers. The TP agent(s) in the homogenate, believed to be a virus, was never characterized. The etiology for TP identified to date is a birnavirus, referred to as chicken proventricular necrosis virus (CPNV). For the present study, frozen tissue homogenates from our experimental studies as well as material from embryonated chicken eggs inoculated with the homogenate were investigated for presence of CPNV by PCR using primers that amplify a 171bp segment of CPNV. CPNV was detected in the proventriculus, allantoic fluids, CAMs, and yolk sac of birds or embryonated eggs infected with the

homogenate. Sequencing confirmed the identity of CPNV in these tissues. TP remains to be an important disease affecting chickens worldwide. Studies to elucidate the cause and pathogenesis of TP are needed so that effective prevention and control strategies can be developed.

Evaluation of the efficacy of a vaccine based on Liposomes against Newcastle Disease in broiler chickens

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Newcastle disease (ENC) is one of the diseases of main economic importance in the poultry industry due to the mortality and impact on the productive performance that it causes in birds. Therefore, new vaccine alternatives are necessary to protect the birds from the highly pathogenic viruses that circulate in different countries. The objective of this study was to evaluate the protection of an inactivated vaccine against ENC developed on the basis of liposomes in broilers. Were used 150 broiler one day old, Cobb Vantress 500, distributed in two vaccinated groups of 50 birds each and one unvaccinated group of 50 birds. The vaccinated groups will receive a program that includes one or two applications of an inactivated vaccine based on liposome, applied at day 1 and / or 14 of age. At 25 days of age, all birds were challenged with a velogenic viscerotropic strain virus of the ENC Genotype XII, with an ICPI 1.88, following the protocols described (OIE, 2012). The evaluation of the protection of the inactivated liposomal vaccine was carried out considering the mortality and nervous sequelae. Clinical signs and mortality were recorded daily until 42 days of age. The results are being analyzed.

Wealth of Knowledge

The Big Red Biosecurity Program for Poultry Develops Drone Technology For Biosurviellance.

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In an effort to advance and enhance current biosecurity practices, there is a growing need for fine-scale responsive data that can be used to assess and rank current biosecurity threats so that effective threat management plans can be developed. It may be difficult to collect the data needed by using conventional methods such as land vehicles (or on foot), satellites or piloted aircraft, in a cost-effective way. Lightweight, portable small unmanned aircraft systems (sUAS), or drones, are poised to become an important, relatively low-cost, tool in biosecurity programs. This study explores the development of an initial protocol for deploying unmanned aircraft (i.e. drones) in support of biosecurity surveillance for poultry operations and discusses their usefulness and future applications.

Histologic Findings In Long Bones & Vertebrae Of Hatchling Commercial Chickens & Turkeys

Floyd Wilson

Histologic examination of bone was part of a poultry lameness and bone health survey study involving commercial turkeys and broiler chickens. Several bone lesions in hips, long bones and vertebra were observed in poultry in the hatchling to early age groups, which included both clinically normal birds and those manifesting with lameness. The changes in long bones (femur and tibia) included the occurrence of focal cortical defects (or microfractures) as-well-as more routine fractures. Hip lesions consisted of hemorrhage and thrombosis in the coxofemoral joint and in the adjacent perirenal sites. Vertebral lesions consisted of hemorrhage into thoracic air sac vertebral diverticula, and vertebral subluxations associated with focal malacia and hemorrhage of the adjacent spinal cord. Variable osteochondrosis and osteomyelitis were also seen. There was variable expression in the occurrence of the osseous lesions between chickens and turkeys in our limited survey, but no clear association with clinical disease. Most lesions appeared traumatic and acute, for which the investigation of causality is ongoing. Examples of lesions are presented and described along with occurrence findings for the two species.

Raising Chickens In Urban Areas: What Citizens And Legislators Need To Know Before Saying “Yes” or “No”

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In recent years, there has been a growing interest in raising chickens in urban areas across the United States. Some cities already allow a limited number of chickens per household (usually 5 hens but no roosters). Having a regular supply of eggs from home-raised hens is major reason for owning backyard chickens in an urban area. Some people consider this practice "green" and an essential component of a healthy lifestyle while others simply consider it a hobby. Whatever the reason for raising chickens in an urban area, citizens and lawmakers must weigh the risks and benefits of this practice that continues to gain popularity while generating a fair amount of controversy such as foodborne illness, poultry disease outbreaks, noise, and odor complaints. Citizens and regulatory authorities must weigh the risks and benefits of raising chickens in urban areas and consider both poultry and public health risks associated with this practice before voting on any proposed legislation to allow chickens to be raised in such areas. Any city resident who plans raise chickens must gather science-based information on poultry health and production and check current regulations in the city or county where he or she resides before starting a backyard chicken flock. The checklist/quick reference guide shown in this poster may be useful.

Backyard Poultry as a Reservoir for Respiratory Diseases and the Seroprevalence of *Salmonella sp.* in Backyard Poultry Flocks

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Five hundred fifty-four chickens from 41 small flocks were sampled. ELISA kits were used to detect antibodies against avian influenza (AI), infectious laryngotracheitis (ILT), Newcastle disease (ND), infectious bronchitis (IB), *Ornithobacterium rhinotracheale* (ORT), *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), and paratyphoid *Salmonella.* In addition, plate agglutination assay was used for the detection of antibodies against poultry specific *Salmonella.* Flock owners answered a questionnaire that assessed biosecurity measures and the distance to the nearest commercial poultry facility. ORT, ND, IB, MS, MG and ILT were the most seroprevalent in backyard poultry flocks with 97% (41/42), 77.5% (31/40), 75% (30/40), 73% (31/42), 69% (29/42), and 45% (19/42), respectively. Poultry specific *Salmonella* was found in 82.9% (34/41) and paratyphoid *Salmonella* was found in 12.2% (5/41) of the sampled flocks. The questionnaire revealed that backyard poultry owners rarely use simple biosecurity measures such as use of dedicated shoes, their chicken sources are unreliable, and few of them benefit from veterinary oversight. Simple measures, use of dedicated shoes and clothes and buying chicks from NPIP hatcheries, showed to reduce the presence of antibodies against respiratory diseases and salmonella in these flocks. This research shows the continued need to examine backyard poultry flocks and educate owners on practical management and biosecurity.

Sentinel Birds: An old strategy that can enhance current diagnostic tools

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The use of specific-pathogen-free (SPF) leghorns has been used extensively to learn about local presence of pathogens in farms, either used as an initial isolation biological system or by filtering antigens that were not used in a current vaccination program. The strategy of placement of these birds has varied across regions or companies or in time, to increase the chances of capturing relevant antigens in order to establish control methods to decrease its effects on the general population. These variations in the strategy used has also been key for non-relevant findings. During the past 4 decades the use of these birds has varied from coccidia monitoring to viral antigens detection (IBV, IBDV, etc.) and seroconversion to specific endemic antigens (WNV, AIV, etc.). In 2017, we have used sentinel birds to aid in the selection of live respiratory vaccines to decrease aerosacculitis condemnations in the processing plant, in the selection of antigens for autogenous vaccine production, in understanding that not all causes of poor health derived from antigens but less than desirable environment management, amongst others. For example, vaccinated SPF leghorns can aid in discerning the pathogens that are relevant clinically while at the same time discerning the ones used in the vaccination program. This way a field veterinarian can add or substract antigens as part of the strategy for reducing condemnations.

Disease surveillance in small poultry flocks in Ontario

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Small poultry flocks may act as potential reservoirs of avian and zoonotic pathogens. The recent increase in the number of these flocks throughout Ontario calls for a better assessment of baseline disease prevalence in this sector. To this aim, a prospective surveillance study of Ontario small flock (non-quota/non-commercial) postmortem submissions to the Animal Health Laboratory was conducted over a 2-year period (October 2015 – September 2017). Upon the owner's consent, a full postmortem examination and a pre-set array of tests for infectious agents were conducted. A total of 160 submissions, corresponding to 246 birds, were received. Chickens were most common (82% of birds), followed by turkeys, game birds, and ducks. The most common primary etiologic diagnoses were bacterial (23%), viral (14.5%, including Marek's disease), and neoplastic (10%). Pre-set microbiological tests detected *Campylobacter* spp., *Brachyspira* spp., *Mycoplasma synoviae*, *Mycoplasma gallisepticum*, and *Salmonella* spp. in 35, 33, 36, 27, and 3% of tested submissions. Infectious bronchitis virus, fowl adenovirus, infectious laryngotracheitis virus, and reovirus were detected in 39, 35, 16 and 6% of tested submissions. Non-virulent avian avulavirus-1 was isolated from one chicken submission, and avian influenza virus (H10N8, LPAIV) from one turkey. In conclusion, infectious agents were the most common cause of death/signs for submitted birds. In contrast to other studies, prevalence of *Salmonella* spp. isolated from ceca was low. The information provided by this study will contribute in determining the baseline prevalence of infectious agents among small flocks in Ontario, and will aid in developing disease prevention and control measures.

How do you engage small and large animal practitioners with poultry veterinarians and educate them about chickens?

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Transient phenomenon? Current craze? Foodies' desire for fresh eggs? Baby boomers or young families wanting to get closer to nature? Whatever the reason, we have been observing in recent years an increasing number of phone calls for backyard birds and urban chickens. The most frequent comment from callers is: 'I can't find a veterinarian to look at my chickens!'. To poultry veterinarians, these birds, of unknown health status, do represent a biosecurity risk for commercial poultry yet they need proper care, diagnosis and treatment. In order to help colleagues interested in backyard flocks and urban chickens, a network was created under the umbrella of the Quebec Ministry of Agriculture (RAIZO-basse-cour) with the support of a dynamic group of poultry veterinarians. Any veterinarian of the province with an interest in feathered species can join the group by contacting the ministry. Twice a year, a webinar on a specific topic is given by a poultry veterinarian to interested veterinarians and allows for direct interaction. An online discussion forum is also available to any participant who has a question on a case, a dosage… to which one or more members of the group can reply to. The Chair in Poultry Research has also been actively involved in giving training conferences to veterinarians on poultry physiology, husbandry, common diseases, as well as workshops on how to perform a clinical examination, take samples and anesthetize chickens. A few articles and videos have been published and are readily accessible to the veterinary community.