Avian Influenza

Pathobiology of classical H7N1 and Gs/GD H5N8 highly pathogenic avian influenza viruses in different chicken breeds and role of Mx 2032 G/A polymorphism in infection outcome

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The susceptibility to highly pathogenic avian influenza virus (HPAIV) infection varies among chicken breeds/lines. Local chicken breeds are thought to be naturally resistant, but experimental studies seldom support this. To date, the susceptibility of Spanish local chicken breeds to HPAIVs is unknown. We evaluated the pathobiology of two HPAIVs (a Spanish Gs/GD H5N8 clade 2.3.4.4B and a classical H7N1 HPAIV) in local Spanish (n=6), commercial (n=1), and experimental (n=1) chicken lines. Both viruses were highly lethal in chickens, but H7N1 HPAIV was more virulent based on clinical signs, mortality rates, and mean death times (MDTs). While the H7N1 HPAIV was shed to high titers by a higher number of chickens orally and cloacally, the H5N8 HPAIV was shed efficiently only by the oral route, possibly decreasing transmission efficiency. Both HPAIVs replicated systemically but displayed tropism differences. Interestingly, H7N1 HPAIV had a greater neurotropism, explaining the associated higher mortalities. Based on clinical signs, mortality, and virus shedding, 5/6 local breeds were highly susceptible to HPAIV infection, while 1/6 local breed and the commercial and experimental breeds were more resistant. The analyses of the chicken Mx gene revealed that AA and AG genotypes at position 2032 were statistically associated with longer MDTs. In summary, the HPAIV infection outcome is influenced by the virus, the genetic background of the host, and particular alleles in genes encoding antiviral proteins, underlining the complexity of HPAIV infections. Surveillance and education in commercial but also local chicken holdings are required to early detect the circulation of HPAIVs.

Vaccination Efficacy of Multivalent Recombinant Herpesvirus Turkey Vaccines Against Highly Pathogenic Avian Influenza

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Vaccination strategies are important as a control tool for high pathogenicity avian influenza (HPAI) viruses. A single vaccine given in the hatchery against multiple pathogens is advantageous for disease control and for cost savings. One-day-old SPF leghorn chickens received one of three vHVT vaccine candidates, which contained avian influenza (AI) insert alone (vHVT509-AI) or AI and additional inserts, as Infectious Bursal Disease Virus (IBDV) (vHVT522-AI-IBD) or Newcastle Disease Virus (NDV) (vHVT523-AI-ND). Four-weeks post-vaccination birds were challenged against three different H5 HPAI viruses [A/Tk/Mn/12582, H5N2, clade 2.3.4.4 (Minnesota/2015); A/Egypt/N04915/2014, H5N1, clade 2.2.1 (Egypt/2014); and A/domestic_turkey/Hungary/54433/2016, H5N8, clade 2.3.4.4B (Hungary/2016)]. All sham vaccinated birds died between 2 to 3 dpc while100% of vHVT-vaccinated birds lacked clinical signs and survived challenge against H5 HPAI viruses. The only exception was 90% protection in vHVT523-AI-ND vaccinated birds and challenged with Egypt/2014 and Hungary/2016. Independent of the vaccine and challenge virus used the OP shedding was

significantly reduced compared to the sham group at 2 dpc ($p < 0.05$). Four weeks after vaccination, most vaccinated birds had HI antibodies detected when using the HPAI viruses as antigen, and the titers significantly increased in the post-challenge sera for all survived birds. Therefore, our study demonstrated that vHVT vaccine candidates containing AI insert alone or AI in combination with IBDV or NDV had similar efficiency in protection against challenge with homologous or heterologous HPAI viruses. Moreover, these vaccine candidates in a single dose hold a great promise to provide simultaneous protection against multiple viral diseases affecting poultry worldwide.

Development of next-generation live attenuated influenza vaccines with elite interferon-inducing capabilities

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Avian influenza outbreaks continue to pose a major threat to poultry industry worldwide. There is currently a growing need to develop effective influenza vaccines against emerging viruses in the field. We previously showed the effectiveness of a live attenuated influenza vaccine (LAIV) against antigenically distant challenge viruses in chickens. The effectiveness of LAIV was linked to its ability to induce high levels of type I interferon (IFN) in primary chicken embryo cells. The aim of this study was to deconstruct the viral subpopulations of LAIV and further improve its immunogenicity by selecting the viral clones with higher IFN-inducing capacities. In addition, the genomic determinants of IFN induction were identified and this information was used to custom-design a highly efficacious master LAIV candidate for poultry. From one-hundred plaque isolates, we identified several candidates that induce significantly higher levels of IFN in cell cultures compared to the original LAIV. Vaccination of 2-week-old chickens with the top 3 IFN-inducer candidates proved their elite innate immune-stimulatory nature, while retaining their full protective efficacies against influenza challenge. Genome-wide identification of mutations revealed additional truncation in the non-structural 1 (NS1) and a single amino acid substitution in polymerase basic 2 (PB2) proteins as determinants of higher IFN response in these strains. A next-generation LAIV candidate, with highly enhanced IFN-inducing character, was reverse-genetically created by combining both NS1 and PB2 mutations in a single virus. This next-generation LAIV candidate is currently being tested for its improved protective efficacy against early and heterosubtypic influenza virus challenges in chickens.

Evaluation of the cross-antigenicity of two H9N2 vaccine strains assessed by HI-test using sixteen distinct AIV H9N2 antigens

Andreas Herrmann

MERIAL S.A.S.

Immunisation of chicken for protection against an emerging disease. Insights in chicken post vaccinal serological immune response against LPAIV H9N2 and cross-reactivity against various distinct antigens provide better understanding of cross-immunity for better protection.

T Cell Epitope Engineering Strategy to Improve the Immunogenicity of Avian Influenza H7N9 Vaccine

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The low immunogenicity of H7 hemagglutinin (HA) is a great challenge in developing efficacious vaccines against avian-origin H7N9 viruses. Previous studies suggest that this low immunogenicity could be due to the regulatory T cell (Treg)–stimulating epitopes found on H7 HAs. We hypothesized that the immunogenicity of H7 HAs can be improved by replacing the Treg–stimulating sequences with memory CD4+ T cell–stimulating sequences from seasonal H3 strains. These modified H7 HA sequences were expressed as soluble recombinant proteins and were designated as rH7 HA OPT1 or OPT2. To evaluate the efficacy of the rH7 HA OPT1 and OPT2, we designed a novel humanized mouse model (HLA-DR3) for the efficacy study to emulate the human major histocompatibility class II (MHC II) –T cell receptor (TCR) interaction. HLA-DR3 mice were intranasally pre-immunized with a H3N2 (A/HK/2014) virus in order to recall the H3 memory response by the CD4+ T cell–stimulating sequences embedded in rH7 HA OPT1 and OPT2. Twenty-four female H3 pre-immunized HLA-DR3 mice were divided into three groups and vaccinated with either rH7 HA OPT1, OPT2, or wild-type (WT) H7 HA via the intra-mauscular route (3 µg/mouse/0.05 ml X three times). The rH7 OPT1- and OPT2-vaccinated groups showed higher H7 HA–specific IgG response, and displayed a lower mortality, weight loss, and lung viral titer following H7N9 lethal challenge compared to WT vaccinated or control groups. This study confirms that T-cell engineered vaccines can improve the immunogenicity and survival against H7N9 challenge in a humanized mouse model.

Characterization of a Novel Avian Influenza Live Virus Vaccine Based on Disruption of M2/M42 Gene Expression That Protects Against Clade 0 and 2.3.4.4 Highly Pathogenic Avian Influenza

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Vaccine protection of poultry against highly pathogenic avian influenza virus will be discussed. In addition, characterization of viral ion-channel proteins (M2 and M42) on replication, morphology and transmission will be discussed. Attendees interested in influenza virus and immunology should attend this program.

Roles of the matrix and nucleoprotein genes on the virulence of H5N8 highly pathogenic avian influenza virus clade 2.3.4.4

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Highly pathogenic avian influenza viruses (HPAIV) subtype H5Nx clade 2.3.4.4. have caused numerous outbreaks in wild bird populations and in domestic poultry worldwide. Avian influenza viruses do not typically cause highly mortality in mallards and other wild waterfowl. However, some H5Nx viruses have been implicated in mass die-offs of ducks. We previously reported clear differences in pathogenicity between two H5N8 HPAIV, where one strain caused mild clinical disease in mallards whereas the other strain caused high

mortality of up to 80%. In this study, by using reverse-genetics generated reassortant viruses, we found that the matrix and nucleoprotein genes were implicated in the increased virulence in mallards. To further investigate the mechanisms behind the disparity in virulence, we examined the characteristics of the reassortants viruses in vitro, by comparing viral growth curves in avian fibroblast cells and in liver cell lines. Moreover, the role of the nucleoprotein genes in viral mRNA synthesis was examined using a polymerase reporter assay. To determine if changes in the matrix gene are associated with virion morphology, we also examined the reassortants viruses under an electron microscope. The findings of this study further our knowledge of pathogenicity of avian influenza viruses and help identify genes and mutations associated with increased virulence in avian species.

Evaluation of environmental sample collection devices and target sampling areas for Avian Influenza detection in layer cages

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During recovery from an outbreak of avian influenza virus (AIV), ensuring that the infected premises is free of residual virus is critical for releasing a quarantine and restocking. Environmental testing may also be utilized as an adjunct to testing animals when attempting to identify infected premises. Currently there is not a uniform approach to testing wire cages. Thus, establishing an optimized and validated sampling protocol to ensure the removal of residual virus in such typical environments after an outbreak is essential. Optimization of collection procedures can impact the likelihood of detecting the virus in the environment, as more sensitive methods and tests will yield higher viral loads within the same level of residual virus. The primary objective of this study was to compare sampling devices and to determine optimal locations for AIV detection on environmental surfaces in wire cages to provide general guidance for environmental sample collection and testing for residual AIV after an outbreak. Another objective was to explore the possibility of reducing the number of samples collected and yet yield the same results to make the process less laborious. For that purpose, a laboratory-based, animal trial to simulate the environment of real poultry houses containing infected birds was conducted. Three sampling devices and multiple sampling locations, were evaluated.

Infectivity and transmission in turkeys and chickens of low and highly pathogenic avian influenza viruses from the 2020 outbreak in turkey farms in North Carolina and South Carolina

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The pathobiology of avian influenza virus (AIV) changes as it circulates and adapts in different avian species. In this study we examined the infectivity and transmissibility in chickens of an early and a more recent Mexican low pathogenic (LP)AIV subtype H5N2 that has been circulating and causing losses in poultry in Mexico since 1994. We show that later viruses are better adapted to chickens which would increase the transmissibility of the virus and it's control.

Bacteriology/Antimicrobial

Trends in antimicrobial use and antimicrobial resistance in broiler chickens; impact of industry-wide antimicrobial use reduction strategy

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In light of the global and national directives to reduce the public health risks of antimicrobial resistance (AMR), the poultry industry in Canada has implemented their antimicrobial use (AMU) reduction strategy (https://www.chickenfarmers.ca/the-antimicrobial-use-reduction-strategy/; a phased approach for reduction from 2014 to 2020). This presentation aims to describe trends in AMU in broiler chickens and turkeys and to compare with trends in AMR. Data on AMU and AMR in enteric bacteria, collected from 2013 to 2018 from broiler chickens (n = 793 flocks) and from 2016-2018 from turkeys (n = 241) were used. Between 2013 and 2018 in broiler chickens, the total AMU in milligrams/population correction unit (mg/PCUBr) decreased by 11% and the number (n) of defined daily doses for animals using Canadian standards (nDDDvetCA) per 1,000 broiler chicken-days decreased by 17%. Between 2016 and 2018 in turkeys, the mg/PCUTk decreased by 11%, whereas the nDDDvetCA/1,000 turkey-days increased by 10%. In broiler chickens, the trends in resistance to ceftriaxone paralleled the quantity and frequency of ceftiofur use (decreasing trends). In both species, trends in resistance to gentamicin mirrored the frequency and quantity of gentamicin and lincomycin-spectinomycin use (decreasing trend). Integration of AMU and AMR data (combined poultry species) using a summarized AMR unit of measurement, AMR Indicator Index, (AMR Ix for ceftriaxone, gentamicin, and multiclass resistance) and AMU indicators (mg/PCUPoultry for ceftriaxone, gentamicin and total AMU) are reflective of the success of the industry-wide AMU reduction strategy. These data highlight the importance of AMU and AMR surveillance for monitoring the impact of such strategies.

Use of AMN to prevent Clostridium perfringens induced necrotic enteritis in broilers

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AMN is a natural biocide (botanical origin) effective against bacteria and fungi. An experiment was conducted to determine its efficacy to prevent necrotic enteritis (NE) in 35-day-old broilers.

160 broilers were distributed into four treatment groups: non-infected control (T1); infected control (T2); non-infected group receiving AMN as a prevention at 0.5 kg/t through feed, continuously (T3); infected group receiving AMN as a prevention at the same administration schedule (T4). Birds in T2 and T4 were challenged with toxigenic strains of C. perfringens on days 14, 15. All diets contained 23.5% crude protein and no antimicrobials or coccidiostats.

Results showed that AMN as a preventive in infected birds (T4) was effective in controlling NE (1.81% ADG increase ($P<0.10$), 4.72% feed conversion improvement, compared to T2). AMN in non-infected birds (T3) had a positive effect compared to non-infected birds (T1) as it improved ADG, feed conversion and livability ($P<0.1$). Infected birds in T2 showed the most severe unspecific lesions and the higher NE lesion score (1.7), compared to the other groups.

Infected birds with AMN (T4) showed mild unspecific lesions and the lowest NE score (1.3).

In conclusion, AMN in feed can be used as a preventive for NE in poultry farms. AMN in unchallenged birds also has a positive effect on performance, thanks to its beneficial activity towards the balance of the gut flora.

Evaluation of the Effectiveness of a Direct-fed Microbial with an AGP and an Ionophore Coccidiostat to Reduce Salmonella heidelberg in Broilers

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This study evaluated the effectiveness of modifying the intestinal flora of broilers directly with a Bacillus licheniformes direct-fed microbial (OptiBac L?) plus indirectly with the antibiotic Flavomycin? and either salinomycin or the chemical coccidiostat Narasin in a Salmonella heidelberg challenge study. This study had four treatments (Treatment 1: infected control with 60 g/ton salinomycin; Treatment 2: Flavomycin 3 g/ton plus salinomycin 60 gm/ton plus OBL ½ pound/ton; Treatment 3: Flavomycin 3g/ton plus salinomycin 60 g/ton plus OBL 1 pound/ton; Treatment 4: Flavomycin 2 g/ton plus Narasin 72 g/ton plus OBL ½ pound/ton. The challenge was on day 3 when ½ of the chicks, 25 chicks per pen of 50 chicks, were tagged and orally inoculated with 8.0x107 CFU/chick S. heidelberg (nalidixic acid resistant at 25 µg/ml). On day 43 treatment 2 had a significant reduction in bootsock S.H. enumeration by micro most probable number (MPN). At 43 days ten broilers of indirect challenge and five of direct challenge had ceca aseptically removed and cultured for S.H. prevalence and number by MPN. All treatments had a numerical reduction in S.H. prevalence versus challenge control, and treatment 2 was significantly reduced (44% T2 versus 72.6% T1). Treatments 2 and 3 had numerical reductions in S.H. numbers (MPN/g). Censoring all culture negative results -0.5 log10/g of ceca a Tobit regression model, the direct challenge of treatment 2 had significantly ($P = 0.004$) reduced S.H. numbers in their ceca (-1.05 MPN/g). In summary, the treatment with the DFM (OBL) and antibiotic (Flavomycin 3g/ton) with salinomycin (60g/ton) had a significant reduction of S.H. in the birds' environment and in the ceca. In addition, all of the DFM and antibiotic treatments had a significant performance improvement by 43 days.

Development of a Mass Administered Vaccine for Prevention of Necrotic Enteritis in Broilers

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A new generation of a Salmonella typhimurium antigen delivery vaccine strain expressing protective antigens of Clostridium perfringens type A has been developed. Multiple battery cage studies with a Necrotic Enteritis challenge model were performed to 28 days. The vaccine strain exhibited significant reduction in N.E. mortality. The challenge model consisted of E. maxima at 14 days and C.P. on days 18, 19, and/or 20. In one of the studies, N.E. mortality was significantly lower in the vaccinated group (8.3%) compared to the unvaccinated challenged group (30.2%) ($P < 0.001$). The vaccine-prevented fraction of Necrotic Enteritis mortality was 0.72 (95% CI:0.57, 0.82). Additionally, the vaccinated birds had significantly improved body weight and FCR to the placebo vaccinated cohorts. The results of each of the studies will be presented. In addition, the vaccine candidate is currently under licensing review by USDA and is expected to be field tested soon.

Characterization of bacterial chondronecrosis with osteomyelitis (BCO) in broilers in a commercial setting

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DuPont

Bacterial chrondronecrosis osteomyelitis (BCO) is a leading cause of lameness in broilers and a major cause of production losses. With overt disease visualized as "Kinky Back", the culmination of disease involves the multi-action of both micro-bone lesions and disease along with colonization from opportunistic pathogens. The micro-fissures and lesions of the bones may be related to a complicated pathway of physiological interactions, while the bacterial initiation of disease is more straightforward. To better determine dynamics of bacteria within general health and BCO scenarios, a field study was initiated using paired farms within a single complex. Initial investigations first focused on distributions of disease to demonstrate sufficient outbreaks and creating statistical baselines sufficient for classifying a "sick" farm compared to a "healthy" farm. From the field samples, a progression of general bacterial isolation/quantification techniques were used along with 16s sequencing from both sick and healthy farms. From general bacterial isolations, changes were identified in Avian Pathogenic E. coli for their densities and combination of virulence factors. Other changes were also identified in C. perfringens as well for their overall abundance when comparing healthy and sick farm samples. Using 16s sequencing for total capture of bacterial communities, it was also found that bacterial changes including Lactobacillus, E. coli, Staphylococcus, and Salmonella were significantly different. Although these species tended to change by sample, Staphylococcus cohnii was statistically the strongest correlation to disease. Altogether, generally monitored species such as APEC, Clostridium, and Lactobacillus do show some changes in response to disease; however, more specific biomarkers such as S. cohnii may provide more specific determination of disease as a supplementation to diagnostics and early detection.

Detection of Necrotic Enteritis (NE) B-Like Toxin in Biological Samples of NE-Afflicted Chickens by Capture Enzyme-Linked Immunosorbent Assay

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Necrotic enteritis (NE) is a devastating enteric disease caused by Clostridium perfringens type A/G which impacts global poultry industry by compromising performance, health and welfare of the chickens. Although necrotic enteritis B-like (NetB) toxin is considered a major virulent factor in NE development, reports on the detection of NetB protein per se in NE-afflicted chickens are lacking. Here, we first report that native NetB toxin can be detected in biological samples from NE-afflicted chickens using NetB-specific monoclonal antibody-based capture ELISA.

Necrotizing Hepatitis Associated with Clostridium perfringens in Broiler Chicks

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Five cases of necrotizing hepatitis in broiler chicks were submitted to the Poultry Diagnostic

and Research Center for histopathology and bacteriology in 2017. Three additional cases were submitted to the Georgia Poultry Laboratory Network. Elevated mortality was observed in flocks aged between 1 day to 1.4 weeks of age with or without neurological signs. In most flocks, increased 3-day mortality was followed by an elevated 7-day mortality. Gross lesions included green to dark brown discoloration of the liver, congested lungs, serosanguineous fluid in the caudoventral aspect of the abdomen, and emphysema in the yolk sacs. In birds older than a week of age, disease with neurologic signs became evident and consisted of tremors, stargazing, and incoordination. Histopathological evaluation revealed multifocal to coalescing fibrinoheterophilic and necrotizing hepatitis associated with gram-positive long rod-shaped bacteria. Other histopathological lesions observed included fibrinous splenitis, necrotic enteritis, fibrinoheterophilic pericarditis, and acute encephalitis with gliosis and cerebellar spongiosis. Samples tested were positive for Clostridium perfringens (CP) by immunohistochemistry and bacteriology, and CP isolates from liver samples harbored netB and tpel virulence genes. Broiler breeders are the suspected source of the infection, and testing revealed netB-positive CP among breeder flocks. Antimicrobial therapy was coupled with enhanced sanitation at the farm and hatchery, markedly decreasing the mortality and clinical signs.

Characterization and Antimicrobial Resistance Patterns of Pasteurella multocida Isolates from Chickens in Mississippi

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A retrospective study including sixty-seven Pasteurella multocida isolates from commercial poultry obtained from cases submitted to the Poultry Research & Diagnostic Lab (PRDL) at Mississippi State University was conducted from January 1st, 2015 to June 30th, 2019. The origin of the submissions included broilers, broiler breeders and commercial layers, being broiler breeders the most frequent. The age of the birds ranged from three days old (broilers) up to seventy-nine weeks old (commercial layers). Mixed samples from males and females were received in most of the cases with higher incidence during the winter and early spring months. Acute septicemia and chronic lesions were frequently observed in the birds. The hock joints were the samples with the highest rate of successful isolation. Thirty-one isolates were serotyped, nine isolates were classified as serotype 4 and seven isolates as serotype 3x4. Fingerprinting was carried out in fifteen isolates; seven isolates exhibited similar profiles to those observed with attenuated vaccines. Most isolates (90%) exhibited susceptibility to amoxicillin, ceftiofur, enrofloxacin, florfenicol and trimethoprim/sulfamethoxazole; whereas intermediate susceptibility was observed for erythromycin, gentamycin, neomycin and spectinomycin. Sixty-four isolates showed a

multidrug-resistant pattern and one isolate was pandrug-resistant.

Genomic Analysis of Avibacterium paragallinarum from 2019 infectious coryza cases in the United States

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Since March of 2019 infectious coryza suspect cases with symptoms including decreased consumption of feed and water, and swollen heads and wattles with catarrhal discharge were submitted from commercial layer farms in Pennsylvania and nearby states. Avibacterium paragallinarum was successfully identified and isolated from infra-orbital sinus swabs. In addition to classical phenotyping analysis of the isolates, whole genome sequencing (WGS) analysis was performed to identify genetic relationships between the isolates.

The outer membrane haemagglutinin gene (hmtP210) showed a high degree of similarity amongst all regional isolates and clustered with serotype C reference serovars. However, pan-genome analysis of core genes showed distinct clusters of the isolates and analysis of the accessory genes further separated them by states.

Genomic landscape of Ornithobacterium rhinotracheale in commercial turkey production in the United States

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Ornithobacterium rhinotracheale (ORT) is a bacterium that causes respiratory tract infections in avian hosts worldwide, but is a particular problem for commercial turkey production. Little is known about the ecologic and evolutionary dynamics of ORT, which makes prevention and control of this pathogen a challenge. The purpose of this study was to gain insight into the genetic relationships between different ORT populations through comparative genomics of clinical isolates from different US turkey producers. ORT clinical isolates were collected from four major US turkey producers and several independent turkey growers and whole-genome sequencing was performed. Genomes were compared phylogenetically using single nucleotide polymorphism (SNP)-based analysis, and then assemblies and annotations were performed to identify genes encoding putative virulence factors or antimicrobial resistance determinants. A pangenome approach was also used to establish a core set of genes consistently present in ORT, and to highlight differences in gene content between phylogenetic clades. A total of 1,457 non-recombinant SNPs were identified from 157 ORT genomes, and four distinct phylogenetic clades were revealed. Isolates clustered by company on the phylogenetic tree. However, each company had isolates in multiple clades with similar collection dates, indicating that there are multiple ORT strains circulating within each of

the companies examined. Additionally, several antimicrobial resistance proteins, putative virulence factors, and the pOR1 plasmid were associated with particular clades and multi-locus sequence types, which may explain why the same strains seem to have persisted in the same turkey operations over decades.

Effects of the in ovo Administration of the 6/85 Mycoplasma gallisepticum Vaccine on Layer Chicken Embryo Livability and Post-Hatch Performance

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Mycoplasma gallisepticum (MG) is one of the most pathogenic of the avian mycoplasma diseases in chickens. It is particularly pathogenic in commercial layers. Mycoplasma gallisepticum has a major impact on the economy of the poultry industry worldwide. It causes chronic respiratory disease in chickens, and infectious sinusitis in turkeys. It has been reported that MG affects fallopian tube inflammation, which causes egg production loss, and egg quality. The estimated cost of MG infection is around $150 million annually. In the US, commercial layers are raised in multiple-age systems and older birds can pass MG to younger birds that do not have strong immune systems. To control and prevent MG infection, MG vaccines have been released and used commercially.

Few studies have been done in our lab using a virulent MG vaccine (FMG) using in ovo vaccination. FMG has a promising protection and early life immunity. However, FMG is a virulent strain and resulted in negative impacts on layer hatchability as well as layer performance. Therefore, the goal of this study is to test different in ovo dosages of MG 6/85 vaccine and test its impact on the hatchability and survivability of layer chicken embryo. The 6/85 MG vaccine is applied in the commercial layer industry starting at 8 weeks of age via spray, but it requires intensive labor, results in bird stress, and provides late protections. In ovo vaccination has been effectively used to administer vaccines in the poultry industry and shows the ability of early protection, and may eliminate labor requirements and stresses associated with handling poultry. Further, in ovo application may stimulate the chick's early immune response and may result in vaccine-derived in vivo populations that may be statically maintained within vaccinated hosts.

Bacterioloty/Antimicrobial: Salmonella and E. Coli

Exploring the Fitness of Retail Poultry Escherichia coli to Cause Foodborne Urinary Tract Infections

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Retail poultry has received much attention in the recent years as a source of urovirulent E. coli colonizing the human colon. In this context, this abstract and the presentation examine various traits of retail poultry E. coli to determine if they possess the characteristics required to cause foodborne urinary tract infection.

Colonization of Internal Organs by Salmonella Enteritidis and Salmonella Kentucky in Experimentally Infected Laying Hens in Indoor Cage-Free Housing

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Salmonella Enteritidis infections in commercial egg-laying flocks are an ongoing public health concern because reproductive organ colonization leads to deposition inside eggs. Flock housing conditions affect Salmonella persistence and transmission, but the food safety consequences of different housing systems remain unresolved. The present study assessed the invasion of internal organs after experimental S. Enteritidis and S. Kentucky infection of laying hens in indoor cage-free housing. Groups of hens were housed on wood shavings in isolation rooms simulating commercial cage-free barns and 1/3 of the hens in each room were orally inoculated with 107 cfu of either S. Enteritidis or S. Kentucky. At 6 d and 12 d post-inoculation, half of the hens in each room were euthanized and samples of liver, spleen, ovary, oviduct, and intestinal tract were removed for bacteriologic culturing. Among hens inoculated with S. Enteritidis, 100% of intestinal samples, 100% of livers, and 50% of ovaries were positive for the pathogen at 6 d post-infection. Moreover, 71% of intestines, 42% of livers, and 10% of ovaries from contact-exposed hens were colonized by S. Enteritidis at 12 d post-infection. Although 86% of hens inoculated with S. Kentucky yielded positive intestinal samples at 6 d post-infection, S. Kentucky was found in other internal organs of both inoculated and contact-exposed hens significantly ($P < 0.05$) less often than S. Enteritidis at both sampling intervals. These results demonstrate the potential for Salmonella infection to disseminate among hens in cage-free indoor housing, including frequent internal organ invasion by S. Enteritidis.

Emergence of a Novel Salmonella enterica Serotype Reading Clone is Linked to its Expansion in Commercial Turkey Production

Timothy Johnson

University of Minnesota

Concurrent separate human outbreaks of Salmonella enterica serotype Reading occurred in 2017-2019 in the United States and Canada, which were both linked to the consumption of raw turkey products. In this study, a comprehensive genomic investigation was conducted to reconstruct the evolutionary history of S. Reading from turkeys, and to determine the genomic context of outbreaks involving this rarely isolated Salmonella serotype. A total of 988 isolates of U.S. origin were examined using whole genome-based approaches, including current and historical isolates from humans, meat, and live food animals. Broadly, isolates clustered into three major clades, with one apparently highly adapted turkey clade. Within the turkey clade isolates clustered into three subclades, including an “emergent” clade that only contained isolates dated 2016 or later, including many of the isolates from these outbreaks. Genomic differences were identified between emergent and other turkey subclades suggesting that the apparent success of currently circulating subclades clade is, in part, attributable to plasmid acquisitions conferring antimicrobial resistance, gain of phage-like sequences with cargo virulence factors, and mutations in systems that may be involved in beta-glucuronidase activity and resistance towards colicins. U.S. and Canadian outbreak isolates were found interspersed throughout the emergent subclade and the other circulating subclade. The emergence of a novel S. Reading turkey subclade, coinciding temporally with

expansion in commercial turkey production and with U.S. and Canadian human outbreaks, indicates that emergent strains with higher potential for niche success were likely vertically transferred and rapidly disseminated from a common source.

Transmissible antibiotic resistance genes present in Escherichia coli and Salmonella from USA poultry

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Alternatives to antibiotics, such as probiotics, are replacing sub-therapeutic levels of antibiotics in the diets of conventional poultry due to regulatory changes limiting the use of antibiotics. The premise is that this will result in a reduction of antibiotic resistance genes in the environment. Genome data from broiler and turkey Escherichia coli isolates, which we collected before 2016, indicated that the most prevalent transmissible resistance genes were to aminoglycosides, tetracyclines, sulfonamides, and beta-lactams. We developed a multiplex PCR to detect seven of the antibiotic resistance genes to monitor transmissible antibiotic resistance genes in avian E. coli over time. Our assay determined that the average number of antibiotic resistance genes per isolate was 1.7 with a maximum of five of the seven genes in any one isolate. The number of resistance genes detected per isolate dropped significantly from 2.2 genes in 2015 to 1.4 in 2019. As similar plasmids and therefore resistance genes are present in avian Salmonella strains we are also using this multiplex PCR assay to monitor transmissible antibiotic resistance genes in avian Salmonella isolates.

The Comparative Analysis of the Salmonella enterica subsp. enterica Serovar Infantis IncFIB Plasmid Genetic Sequence Would Aid to Identify Relevant Subtypes for Salmonella Control in Poultry

Roxana Sanchez Ingunza

Private Consulting - Poultry

Salmonella enterica subsp. enterica serovar Infantis human infections associated to raw chicken products has been reported in the United States in 2018. Previously, Salmonella Infantis was most commonly associated to human infections linked to contact with live poultry. The Centers for Disease Control and Prevention (CDC) currently indicates this serovar appears to be widespread in the chicken industry. An extended-spectrum ß-lactamase (ESBL) producing, multidrug resistant (MDR) clone of Salmonella Infantis was first described in the U.S. in 2017 from isolates recovered from poultry and human infections between 2014 and 2015. This megaplasmid also carries genes that provide environmental survival advantage to the bacteria such as the mer operon. Salmonella genomes from organisms of different sources in the U.S. containing the plasmid were very closely related suggesting common origin and widespread distribution. However, this analysis was made on only one PFGE pattern. Additional PFGE patterns carrying the plasmid has been identified; therefore, the plasmid characteristics on other emergent Salmonella Infantis subtypes merit further investigation. The overall significance of the presence of this plasmid on the bacteria phenotypic characteristics is not clearly understood. This study focuses on the genetic analysis of Salmonella Infantis isolates recovered from poultry and highlights differences in content and distribution of genes and nucleotide polymorphisms observed in the IncFIB plasmid. The suggested comparative approach would aid in the selection of antigens for vaccine intervention and in the description of

phenotypes of increased environmental persistence or virulence in poultry.

Impact of E. coli vaccination on production performance of turkeys and E. coli ecology over time

Katie Stumvoll[4], M M Kromm[2], E Gerken[3], T Johnson[4]

[1]Jennie-O Turkey Store, [2]Jennie-O Turkey Store, [3]Jennie-O Turkey Store, [4]University of Minnesota

Collibacillosis, a disease of poultry caused by Escherichia coli, is one of the most challenging bacterial diseases for field practitioners to address. Colibacillosis is typically a secondary disease, therefore primary mitigation strategies often address any primary viral disease challenges and/or inadequate management of the barn environment. When focus on the primary stressors is not sufficient, field practitioners look to additional tools to help support the welfare of the birds they are responsible for. E. coli vaccination has been used extensively in layer production and to some extent in broiler production, but it is less frequently applied in commercial turkey settings. This first talk on the use of E. coli vaccination in commercial turkey flocks will utilize paired barn data to present data on its effect on both performance and the overall ecology of E. coli populations in birds and within barns.

An Embryo Co-infection Model for Enterococcus faecalis and Avian Pathogenic E. coli Assesses Pathogenicity and Virulence of Polybacterial Infections

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Enterococcus faecalis (EF) and avian pathogenic E. coli (APEC) are frequently co-cultured from lesions of septicemic birds. Uropathogenic E. coli, which are genetically similar to APEC, demonstrate enhanced pathogenicity during co-infection with EF, including enhanced wound colonization and macrophage evasion in murine disease models. However, the impact of EF coinfection on APEC virulence has not been investigated. As virulence factors for both UPEC and APEC frequently focus on iron acquisition, our hypothesis is that APEC virulence is enhanced in the presence of EF in iron-limited environments. To this end, field isolates of EF and APEC obtained as infective pairs from yolk sacculitis lesions were assessed with in vitro growth assays and in vivo with embryo lethality assays for virulence. For the growth assay, single APEC macrocolonies were grown on iron-limited agar or in mixed culture with EF. APEC in mixed culture exhibited increased growth when compared to APEC in monoculture (P=0.01). For the embryo lethality assay, cultures of APEC, EF, or a mixed culture of APEC and EF were injected into either the yolk sac at day 6 or the allantoic fluid at day 12. Eggs were candled daily to determine survivability and results were compared among groups. Results of in vitro and in vivo assays will be presented which will further characterize the effect of EF co-infection on APEC virulence and shed light on the significance of mixed infection in colibacillosis.

Case Reports

Diagnosing and managing Inclusion Body Hepatitis outbreak in primary broiler breeders

Emma Castillo

Aviagen

In this case study of an Inclusion Body Hepatitis outbreak in primary broiler breeders I will begin the presentation by characterizing how the disease manifested both in terms of clinical signs and gross necropsy findings in affected male and female chicks. I will include a summary of epidemiological trends including mortality, onset of disease presentation, distribution of disease and the search of source flocks. Previous research confirms that IBH can be seen as early as 4 days of age but most typically at 3 weeks of age. In this outbreak mortality consistently occurred at the beginning of the second week of life occasionally blending in with typical first week of life mortality making detection of the disease more difficult. This presentation will discuss important trends and clinical signs the veterinary team used to train specialists for the monitoring of IBH in the face of an outbreak. Because percent mortality in this outbreak had a wide range the veterinary team focused on specific trends and how daily mortality changed into the second week of life. To conclude, I will cover the extensive vaccination assessment and protocol changes instituted to control the disease outbreak. The longer life span of primary breeders allowed the veterinary team to compare how IBH affected flocks faired in comparison to their non-affected counterparts after the onset of disease and resolution of clinical signs. Several of the diagnosed flocks were subject to subsequent disease outbreaks.

A Case Report of Erysipelothrix rhusiopathiae Infection in Turkey Breeder Hens

Ana Paula Da Silva[1] G. Cooper[2], C. Jerry[3], S. Stoute[4]

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Erysipelothrix rhusiopathiae is a Gram-positive bacterium of worldwide distribution that affects multiple avian and mammalian species, causing a disease known as erysipelas. Although erysipelas has sporadic occurrence, the disease notably affects turkeys, inducing mortality, marketing losses and decrease in fertility in turkey breeders. The usual port of entry of E. rhusiopathiae is via skin or mucosal lesions, but artificial insemination is an important source of infection for turkey breeder hens and is likely linked to male carriers that shed the bacterium in their semen.

The California Animal Health and Food Safety Laboratory system received 37 case submissions of animals affected with erysipelas from 2007 to 2019. Of those, 23 cases were in avian species and 11 were in turkey breeders. In this study, we report a case of erysipelas affecting 36-week-old commercial Broad-Breasted White turkey breeder hens from a flock presenting a slight increase in mortality. At necropsy, two out of three birds presented with hepatomegaly and bronze discoloration of livers and enlarged, mottled spleens. Hepatic and splenic microscopic lesions included fibrinoid necrosis and mixed inflammatory cell infiltration. Pure cultures of E. rhusiopathiae were isolated from all livers and spleens. Further diagnostics, epidemiology and practical aspects of erysipelas in avian species will be discussed.

Case Report: Clinical and Histopathological Findings in First Reported Case of False Layer Syndrome in Georgia

Karen Grogan[1] Monique Franca[2], Brian Jordan[3], Jenny Nicholds[4]

[1]University of Georgia, PDRC, [2]UGA Poultry Diagnostic and Research Center, [3]UGA PDRC, [4]UGA PDRC

False Layer Syndrome is currently believed to be a delayed clinical sign of infection with infectious bronchitis virus (IBV) in pullets at an early age. Viral infection is believed to impact the post-embryonic development of the oviduct, which leads to cystic left oviducts in layer species of birds at peak production, approximately 25-30 weeks of age. The DMV/1639 strain of IBV is currently suspected to cause this syndrome in the United States and Canada, but other variant IBV strains cannot be excluded. This disease has been spreading in commercial layer operations since 2017 throughout Canada and the US, and in July 2019, a submission was made to PDRC of the first reported case in Georgia in 29-week-old hens. A clinical work up was completed and tissues were collected for serotype-specific IBV quantitative RT-PCR along with sections of the uterus of affected hens for histopathology. Choanal swabs and tissues were collected from 11-week-old pullets on the source farm of the affected layers for serotype-specific IBV quantitative RT-PCR. Histopathological findings showed significant changes to the epithelium of the magnum section of the uterus in affected hens. Special stains were utilized to understand the altered function of the changed epithelium. Quantitative RT-PCR was positive for DMV/1639 IBV in the cecal tonsil of 11-week-old pullets, and a DMV/1639 virus was isolated in embryos from this tissue. Changes to early IBV vaccination program were instituted for the next pullet placement. Testing of pullets and monitoring of laying flocks has continued.

Salmonella Bacterin Induced Hepatopathy in Broiler Breeder Pullets

Eric Heskett

Case Farms

Increases in pullet mortality were observed two to three-weeks post autogenous Salmonella vaccination in two out of three divisions on this vaccine. An extensive diagnostic investigation was undertaken to discover the root cause of the mortality. The diagnostic investigation included vaccine handling practices, necropsies, priming with other Salmonella bacterins, comparison of lots of vaccine, ruling out other infectious conditions, endotoxin level quantification in the autogenous vaccine using the limulus amebocyte lysate assay, and paired house trials using different dosages or different commercial Salmonella bacterin products. Necropsies revealed large amounts of serous fluid at the injection site as early as two hours post injection. Necropsies on birds two to three-weeks post vaccination revealed birds in good body condition with friable, pale livers and a large amount of unclotted blood in the abdominal cavity. The level of endotoxin between the two lots of vaccine revealed a difference in the level of endotoxin present. Results of the limulus amebocyte lysate assay, comparisons of vaccine programs, and results of the paired house trials will be presented and discussed.

An Interesting Case of Pasteurella multocida "Like" Clinical Signs with a Slew of Organisms

Geoffrey Lossie[1] Tsan Long Lin[2], Dr. Kristen Hill-Thimmesch[3]

Purdue University College of Veterinary Medicine/Indiana Animal Disease Diagnostic Lab

A Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) positive flock of 70,000 White Leghorns in the lower level of a 3

tiered avian style house experienced a sudden increase in mortality. Mortality levels increased over a two day period and went from approximately 15 birds a day to 124 birds in a single morning. Mortality was distributed throughout the house. There was a concurrent drop in feed and water consumption with an 8% drop in production. On-site necropsy revealed non-specific "normal mortality" findings, but over time birds were found with swollen faces and wattles with some showing neurologic signs prior to death. Ten birds were submitted to the Indiana ADDL for diagnostic testing. The results of PCR tests for avian influenza, Newcastle disease, and infectious laryngotracheitis (ILT) were negative, while those for MG and MS were positive. Various bacteria were isolated from multiple tissues including: Gallibacterium anatis, E.coli, Staphylococcus aureus, and an Avibacterium sp. (most similar to Avibacterium endocarditidis). The case is difficult to interpret as there were many infectious agents isolated, with no clear inciting agent. Of particular note is that only the bottom tier house was affected, while the upper two tiers and adjacent houses remaining unaffected. Three days prior the affected house was dewormed. A house in the neighboring building was wormed with the same active ingredient, but a different serial number, and showed no signs of illness. This case is unique in its clinical presentation, the organisms isolated, and possible confounding factors.

On a Friday afternoon...

Kayla Niel

Hy-Line International

Elevated mortality was reported in a flock of 10-day old layer breeders on a summer day in the Midwest. The flock had an excellent start, with good uniformity at placement and low first-week mortality. The grower did not notice anything abnormal until the increase in mortality on day 10. Both houses of the two-house complex were affected, but loss was much greater in house one. Initial mortality appeared to be mostly females, but eventually both females and males were significantly affected. On walkthrough of a house, 75% of birds were active while 25% were laying down against the walls of the house and ultimately dying. All birds appeared stunted and many were observed eating litter. On necropsy, birds were dehydrated, and crop and gizzard contents consisted of mostly litter with some intact corn kernels in the crop. Bone development appeared abnormal, as bones would bend on manipulation rather than snap. No other significant lesions were observed. Oropharyngeal swabs were submitted for AI PCR with negative results. Feed samples were submitted for analysis, and mortality was sent to Iowa State University for histopathological examination. While waiting on these results, electrolytes were run in the water and feed was replaced. The flock seemed to be improving until they broke with coccidiosis at day 14. Around then, additional premises started demonstrating similar mortality patterns, followed by coccidiosis breaks. This case report will discuss final test results, additional investigations, tentative resolution, and lessons learned.

Case Report: A Unique Toxicity in Broiler Breeders

Eric Shepherd

University of Georgia Poultry Diagnostic and Research Center

A case of 39-week old broiler breeders with acute onset of mortality was submitted to the University of Georgia Poultry Diagnostic and Research Center (PDRC). Mortality was approximately 1.5% in the affected house over the last 24 hours. 1 of the 4 houses on the farm was primarily affected and coincided with a recent feed delivery. Birds backed off feed in the affected house and water consumption doubled overnight. Egg production fell 15% over the next

5 days and remained 5% below peak production a week later. Feed samples were taken and submitted to two separate laboratories for routine mineral analysis. Feed was removed and replaced the day that increased mortality was observed. Mortality returned to normal levels 4 days after the feed was replaced. Birds presented to PDRC both moribund and dead on arrival. Marked dehydration was observed along with pale, swollen kidneys, regressing follicles, and varying degrees of visceral gout. AI rapid test was negative and there was no growth on aerobic cultures of bone marrow. Histopathological changes were consistent with a peracute insult including skeletal muscle myofiber degeneration and necrosis, tubulonephritis and tubular necrosis, and necrotizing interstitial pneumonia with urates. Feed analysis from both laboratories consistently showed elevated levels of multiple minerals including, but not limited to, potassium, magnesium, sulfur, iron, and sodium. Feed samples were further submitted for ionophore and mycotoxin testing. Samples were saved and fed back to birds to recreate the clinical signs and lesions observed in this case submission.

Investigation of Increased Cases of Erysipelas in Commercial Turkeys

Stephen Williams

Butterball

Case report covering an increase in the number of Erysipelas cases in commercial turkeys over a one-month period in the Fall of 2019. This presentation will describe the bacteria Erysipelothrix rhusiopathiae, common clinical signs seen in turkeys and how this disease is spread. Veterinarians will gain knowledge of the clinical signs of Erysipelas, interventions and investigation into this disease challenge.

Immunology/Vaccinology

Replication of HVT Single (HVT-ILT) and Double (HVT-ND-ILT) Constructs and Efficacy against Challenge in SPF Birds.

Ivan Alvarado[1], Maricarmen Garcia[2], Daniel Maekawa[3]

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

In this presentation, the level of protection provided by two turkey herpesvirus recombinant vaccines against infectious laryngotracheitis against challenge will be compared. Other criteria used to evaluate HVT constructs will be presented.

Salmonella Strains Displaying ENhanced Innate Immunity Response Activators (ENIIRA) to Stimulate Innate Immunity to Enhance Protective Immunity

Roy Curtiss[1], Soo-Young Wanda[2], Vinicius Lima[3], Banikalyan Swain[4]

[1]University of Florida, [2]College of Veterinary Medicine, University of Florida, [3]College of Veterinary Medicine, University of Florida, [4]College of Veterinary Medicine, University of Florida

We have significantly improved attenuated Salmonella strains as vectors to deliver protective antigens or DNA vaccines encoding them. We therefore eliminated many means by which Salmonella manipulates induction of immunity and have genetically modified them to display regulated delayed attenuation, regulated delayed synthesis of protective antigens and regulated delayed lysis in vivo. As a consequence, these mucosally delivered vaccine vectors have enhanced abilities compared to the wild-type parent to invade and colonize internal effector lymphoid tissues to maximize induction of mucosal, systematic and cellular immunities

and to display complete biological containment with no persistence in vivo or survival if excreted. We refer to these strains as Protective Immunity Enhanced Salmonella Vaccine (PIESV) vectors.

We have observed that vaccination of both mice and chickens with control PIESV strains with empty plasmid vectors not encoding protective antigens has conferred low-level protective immunity to a diversity of bacterial, viral and parasite pathogens. We inferred that this was due to activation and recruitment of innate immunity. We have thus specifically designed Salmonella strains to serve as superior adjuvants to activate TLR4, TLR5, Nod1, Nod2 and TLR9. These adjuvant strains either with wild-type lipid A or designed to synthesize the non-toxic MPLA are totally avirulent at high doses by all parenteral and mucosal routes of inoculation. They are superior to other adjuvants in enhancing immune responses to subunit and live attenuated vaccines. We refer to these adjuvant strains as Enhanced Innate Immunity Response Activators (ENIIRAs). Further ENIIRA improvements are being made and new applications evaluated.

Protective Effects of a Modified Live E. Coli Vaccine Against Homologous And Heterologous Challenges In Laying Hens

Manuel Da Costa[1], U. Tri[2], CK Mah[3], A. Sidna[4]

[1]Zoetis, [2]Zoetis Inc., [3]Zoetis Inc., [4]Gadjah Mada University, Indonesia

The presentation will explore the effects of live E. coli vaccination in laying hens when an homologous or heterologous challenge is present

The Effective Use of Siderophore Receptor Proteins (SRP) as Antigens in Poultry Vaccines.

Dan Domingo

Epitopix LLC

Bacterial pathogenesis requires active acquisition of iron from host reservoirs. This is accomplished by sophisticated iron-gathering systems using secreted bacterial siderophores plus outer membrane-bound siderophore receptor proteins (SRP). In the host environment, bacterial SRP expression is amplified and ultimately provides iron for bacterial growth, colonization and disease. Given this key role, SRPs are logical targets for vaccine development and have been shown to be effective immunogens against Salmonella Enteritidis (SE), E. coli (EC), Pasteurella multocida (PM) and other pathogens of veterinary interest.

In a series of studies, SRP vaccine effectiveness (Fisher's Exact Test, all $P < 0.05$) was demonstrated using SPF pullets administered twice with vaccine containing either SRP-SE, SRP-EC or SRP-PM then challenged with a pathogenic field strain. Vaccine containing SRP-SE reduced colonization of reproductive tissues 100% in vaccinated birds (0% positive) compared to the placebo group (24% positive). Vaccine with SRP-EC prevented 100% of mortality in vaccinated pullets (0%) compared to placebo (76%). Vaccine containing SRP-PM (3x4, 2x5 serotypes) prevented mortality by 70% after heterologous challenge with serotype 7x9x10 and by 92% with serotype 8x14x15.

These studies demonstrate that vaccines using SRP antigens are effective for prevention of bacterial disease in chickens.

False layer syndrome IBV, pathogenicity insights and surveillance in layer flocks

Rodrigo Gallardo[1], Ana Paula da Silva[2], Alexandra Mendoza-Reilley[3], Corine Giroux[4]

[1]University of California, Davis, [2]University of California, Davis, [3]University of California, Davis, [4]Hickman family farms

False layer syndrome (FLS) is a condition in egg laying flocks (table egg layers, breeders and broiler breeders) in which hens do not reach peak of production due to a significant percentage of internal layers in a flock. This syndrome has been associated to early exposure to infectious bronchitis virus (IBV). We performed controlled challenges with IBV strains associated with FLS in the field and compared them with a wild type M41 IBV strain. Virus distribution and pathology was evaluated in the respiratory, urinary and reproductive tract at 2, 5, 10 and 21 days. In addition, epidemiological surveillance is being performed in flocks with an IBV induced FLS history to assess if the virus still persists after one day old vaccination was established to prevent this condition. Results of the experiments and surveillance effort will be discussed.

Histological Evaluation of the Effects of High Incubation Temperature on Embryonic Development of Thymus in Broiler Chickens

ChangHee Lee

Poultry diagnostic and research center

Providing optimum incubation temperature is critical for successful embryonic development, hatchability, and post-hatch growth performance. High incubation temperature, especially the late stages of incubation will negatively affect embryo development, including the immature immune system. The objective of this study is to examine the influence of embryonic heat stress in the late period of incubation on bursa and thymus development. In the experiment, 100 fertile broiler eggs were incubated in individual tabletop incubators with optimum eggshell temperature (approximately 100F°) during the whole period of incubation and another 100 fertile broiler eggs were exposed higher eggshell temperature (approximately 103F°) from Days 15 to 18 of incubation. At hatch, basic chick quality parameters such as chick body weight, chick yield, chick length and yolk-free body mass are compared to see the effect of high incubation temperature on embryo development. The ratio of heterophil to lymphocyte in blood which indicate embryonic heat stress was analyzed by blood smear. The size of bursa and thymus was measured by taking digital pictures and measured its area by using Image J software. The cortical lymphocyte/parenchyma ratio was measured from thymus tissues by H&E staining and morphometric analysis using Image J software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2015). The size of bursa and thymus and lymphoid depletion on thymus will be a distinct indicator of immunosuppression induced by heat stress during the late incubation period.

Development of Poultry Immune Reagents

Hyun S. Lillehoj[1], Woo H. Kim[2], Charles Li[3]

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

The objectives of this project are 1) to identify chicken immune molecules, particularly cytokines, chemokines and cell surface markers, express them as recombinant proteins, and characterize their function, and 2) to develop monoclonal antibodies (mAbs) to the target chicken molecules. Cloning of chicken genes (23 in total) for were carried out the number of sets of primers which were designed and synthesized to amplify based on the chicken genomic and

mRNA sequence. The recombinant proteins were obtained by transformation into E. coli, transfection into mammalian cells, or expression in yeast in collaboration with Kingfisher Biotech. To develop mAbs against them, we immunized mice, collected lymphocytes, fused the lymphocytes with myeloma cells, screened, and generated single-cloned hybridoma. For functional characterization of the recombinant protein and mAbs, several assays have been conducted including ELISA, immunohistochemistry, Western blot, flow cytometry, qPCR, cell proliferation, and nitric oxide assay. All the target we selected have shown to have critical functions in host defense against pathogens and all recombinant proteins expressed have met the quality standard for immunization in mice for mAbs production. Twenty-three target proteins consist of 16 cytokines (interleukin-4, 7, 10, 12, 13, 17F, 21, 22, 23, 26, 34, IFN-a, IFN-?, TNF-a, CSF-1, and TGF-ß), 3 chemokines (CXCLi2, CCL4 and 5), 1 surface receptor (CD127), perforin and granzyme. The mAbs developed in this study represent new sets of immune reagents which are specie-specific for poultry.

Protection by a Novel Dual Recombinant HVT ND-IBD Vaccine alone or in combination of an IBD 89/03 Live Attenuated Vaccine against a Variant E Strain Challenge of Infectious Bursal Disease

Alexandra Mendoza-Reilley[1], Ivan R. Alvarado[2], Alejandro Banda[3]

[1]Merck Animal health, [2]Merck Animal Health, [3]Poultry Research and Diagnostic Laboratory, Mississippi State University

The objective of the study was to evaluate the level of protection provided by a novel dual-recombinant HVT ND-IBD vaccine (Innovax ND-IBD®) and the live attenuated 89/03 vaccine against a challenge with an infectious bursal disease Variant E strain.

Two hundred and twenty-five commercial fertile eggs were divided in five treatment groups, as follows:

a) Dual recombinant HVT ND-IBD (Innovax ND-IBD®)

b) 89/03

c) Dual recombinant HVT ND-IBD® & 89/03

d) Non-vaccinated/Challenged

e) Non-vaccinated/Non-challenged

Replication of the viral vectored vaccine in feather follicles of 14-day old vaccinated birds was evaluated by the amplification of specific segments of the HVT vector (Viral Flex Seq®). At 7, 14 and 21 days of age, twelve birds from each group were orally challenged with a Variant E strain. Five days after challenge, body and bursal weights were registered to calculate bursal/body weight ratios to determine the degree of bursal atrophy. Bursal tissues were also collected for histopathological evaluation and virological procedures. Data was analyzed by Tukey's or SNK tests. Replication of the viral vectored vaccine was detected in all the HVT ND-IBD vaccinated groups.

As expected, maternal antibody protection decreased overtime, as observed in the non-vaccinated-challenged group. When compared with non-vaccinated/challenged group, adequate protection against challenge was observed when the dual recombinant HVT ND-IBD vaccine was applied alone or in combination of 89/03 live attenuated product. Furthermore, bursae index scores showed protection starting at 14 days post-vaccination with the dual recombinant product, in the groups of broiler chicks with maternal immunity.

Parasitology/Enteric Health

Parasitological Surveillance in a Pastured Poultry Farm

Maria Tereza Bethonico Terra

Auburn University

Alternative systems for poultry rearing cater to an increasing market's demand for products with the lowest use of drugs and for environments with high perceived animal welfare. Pastured systems are one example. In this production, the animals are raised in mobile structures that are moved every two or three days in a rotation system. This practice can reduce the concentration of feces decreasing the chances of the animals to ingest them. However, there is a gap in the literature evaluating and quantifying the parasitological challenges in pastured production.

We collected fecal samples from turkeys, broilers and layers on a pastured farm in two-week intervals to determine counts of coccidia oocysts and worm eggs. For all three types of birds, the counts of coccidia and worm eggs were low compared to published numbers in conventionally reared poultry, indicating that the rotation system effectively reduced the infection pressure. We did not find obvious shedding patterns related to season and age of the flocks. Next-generation sequencing of PCR products showed the presence of most described Eimeria spp. in layers as well as in broilers. Worm eggs were identified as Ascaridia galli.

The study will increase the number of samples and complete a year worth of samples in order to clarify the behavior of those parasites in this type of production system.

Resistance to fenbendazole in Ascaridia dissimilis and its economic impact on the production of turkeys

James Collins

University of Georgia, Dept. of Infectious Diseases

Infection with Ascaridia dissimilis, the most prevalent and one of the most economically important gastrointestinal nematodes of turkeys, most often produces reduced feed conversion efficiency. Currently, fenbendazole (FBZ) is the only FDA approved treatment for A. dissimilis. We recently tested the efficacy of FBZ against 5 field isolates of A. dissimilis. 3 isolates (Wi, Ow, Po) demonstrated >99% efficacy. However, two (Sn, Ad1018), yielded efficacies of 63.9% and 76.2%, respectively, indicating FBZ resistance. Having proven FBZ resistance in A. dissimilis, we wanted to determine the impact of resistant worms on growth and productivity. 384 1-week old turkey poults were infected with the Sn (resistant) or Ow (susceptible) isolate. For each isolate, 8 replicates each of untreated and treated pens were established with 12 birds/pen. Turkeys were grown to 10 weeks, following commercial growing practices. Birds were infected with 25 eggs/bird/week in feed. At 4- and 8-weeks post infection, treated groups were administered FBZ via water (SafeGuard® Aquasol, 1.25mg/kg) for 5 consecutive days. Weight, weight gain and feed conversion were analyzed weekly for differences between groups. At weeks 7 and 8 post infection, feed conversion in Ow-treated was significantly better than the other groups, using 700 grams less feed/bird. Since this was the only group with an effective treatment, these results strongly suggest that worms infecting the other groups (due to either lack of efficacy or lack of treatment) caused a significant decrease in feed efficiency. Further work is needed to determine the full scope of A. dissimilis infections on feed conversion, but based on these data, there

appears to be a large economic impact of A. dissimilis on turkey production

Broiler Breeders: An Assessment of the Enteric Parasites Population

Steve Fitz-Coy

Merck AH

Over 400 chickens from one week of age to over 6o weeks were evaluated during a series of necropsy sessions over several months. Coccidia were prevalent in the younger age groups and the helminths were more prevalent in the older bird populations.

Dietary supplementation with Bacillus subtilis direct-fed microbials alters chicken intestinal metabolite levels

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TDirect-fed microbials (DFMs) are dietary supplements containing live microorganisms which confer a performance and health benefit to the host, but the mechanisms are unclear. Here, a metabolomics approach was used to identify changes in intestinal metabolite levels in chickens fed an unsupplemented diet or a diet supplemented with B. subtilis strain 1781 or strain 747. Body weight gains of chickens fed the B. subtilis-supplemented diets were increased, compared with chickens fed the unsupplemented diet. Compared with unsupplemented controls, the levels of 83 metabolites were altered ($p < 0.05$) (25 increased, 58 decreased) in chickens given the B. subtilis 1781-supplemented diet, while 50 were altered ($p < 0.05$) (12 increased, 38 decreased) with the B. subtilis 747-supplemented diet. Twenty-two metabolites were altered ($p < 0.05$) (18 increased, 4 decreased) in the B. subtilis 1781 vs. B. subtilis 747 groups. Changes in the levels of these intestinal biochemicals provided a distinctive biochemical signature unique to each B. subtilis-supplemented group, and were characterized by alterations in the levels of dipeptides (alanylleucine, glutaminylleucine, phenylalanylalanine, valylglutamine), nucleosides (N1-methyladenosine, N6-methyladenosine, guanine, 2-deoxyguanosine), fatty acids (sebacate, valerylglycine, linoleoylcholine), and carbohydrates (fructose). These results provide the foundation for future studies to identify biochemicals that might be used to improve poultry growth performance in the absence of antibiotic growth promoters.

In vitro inhibitory effect of a new three-strains selected probiotic on several serovars of Salmonella

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Bacillus are known to be capable of producing antimicrobial substances, including bacteriocins, able to inhibit Gram-negative bacteria such as Salmonella. The objective of the study was to evaluate the in vitro inhibitory effect of a new three-strain, bacillus-based probiotic for poultry on several Salmonella serovars using two different methods.

The first method was an agar plate-based method. Separate agar plates were poured which included selected and differing serovars of Salmonella enterica. The bacillus-based probiotic was added to wells created into the plate. After 24 hours of incubation, a zone of inhibition of up to 2mm could be measured. The

second method was a feed matrix-based methodology. The probiotic was added at 10^5 CFU/g of autoclaved feed. The pathogens S. Heidelberg or S. Typhimurium were added at 10^3 CFU/g of feed. During the 24 hours' time of incubation, samples were taken at different time points. Samples were added to plates, incubated and a counting of pathogens was conducted.

In these in vitro experiments the new bacillus-based probiotic was able to inhibit the growth of all Salmonella serovars belonging to different serogroups. When testing the probiotic vs. Salmonella spp. in an agar-based method, a radius of the inhibition zones was observed for all serovars. When testing the probiotic vs. Salmonella spp. in a feed matrix, it inhibited the growth of S. Heidelberg (1.3 log inhibition after 4 hours, 4.6 log inhibition after 8 hours) and S. Typhimurium (1.6 log inhibition after, 4 hours, 2.2 log inhibition after 8 hours). Results of this trial may be an important source of information regarding production use of probiotics.

Parasitology/Enteric Health: Coccidia

A new method for Eimeria Speciation and Quantification Using Flow Cytometry

Daniel Adams

North Carolina State University

An accurate and speedy diagnosis of Eimeria at the species level are both challenging and necessary. Using a compound microscope, the oocysts of the different Eimeria sp. can be discernible by size and shape. A flow cytometer can be described as an "automatic microscope." The objective of this study was to develop a non-antibody based diagnostic method for rapid, automated and objective speciation and quantification of Eimeria sp. in poultry, using flow cytometry (FCM). Oocysts of different species of Eimeria causing coccidiosis in chicken were evaluated. Oocysts were purified by a series of washes and centrifugations. To quantify the oocysts, a known amount of beads were added to the suspension. FCM analysis was performed using the LSRII. The number of oocyst in the suspensions were verified using the McMaster modified method. Furthermore the species of stock suspension were verified by PCR assay. The LSR II flow cytometry analyzer simultaneously identified Eimeria maxima, E. acervulina, and E. tenella oocyst populations from vaccine samples. The proportions of oocysts were similar when using the FCM application and the McMaster slide. These results suggest that FCM could be used to identify and quantify coccidia oocysts of chickens quickly. We will also present FCM results obtained from litter samples of commercial houses. A key advantage of FCM is that it can be done quickly, enabling decisions to be made immediately, whereas other diagnostic methods are slower, labor-intensive, and more expensive.

Coccidiosis Vaccine Trial in Turkey Poults

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Coccidiosis is a term given to describe an infection of the protozoa Eimeria spp. in the intestinal tract. There are four species of Eimeria that are pathogenic in turkeys: E. adenoides, E. dispersa, E. gallopavonis, and E. meleagrimitis. The objective of this trial was to evaluate the effectiveness of a commercially available anti-coccidial vaccine in preventing clinical coccidiosis in Jennie-O Turkey Store's commercial turkey flocks. Eleven farms were selected for this trial, each consisted of one control flock (received coccidiostat) and two treatment flocks (received coccidiosis vaccine). The control flocks were housed in a separate barn while the two

treatment flocks were in a barn with a shared air space but separated by a fence. The vaccine was administered in barns at poult placement via course gel droplet onto the birds prior to placement into rings. Fecal samples were collected on days 5-7, 12-14, 19-21, 26-28, and 33-35 from both the control barn and the experimental barns. Fecal samples were collected via bogus paper and submitted for fecal oocyst counts. Oocysts per gram (OPG) were analyzed and data was organized. Further analysis of the production statistics including finish barn morbidity and mortality as well as harvest weights will help determine the efficacy of the vaccine over the life of the turkey.

Typing Eimeria maxima isolates from Alabama broiler flocks using a multi-locus sequence typing (MLST) scheme

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Eimeria maxima is a eukaryotic parasite of chickens that can cause coccidiosis and predispose its host to secondary infection with Clostridium perfringens, the causative agent of Necrotic enteritis (NE). Multi-locus sequence typing (MLST) is a molecular typing tool that reflects the population and evolutionary biology of bacterial pathogens. MLST schemes have also been developed for some eukaryotic pathogens. We developed a MLST scheme for E. maxima based on six gene loci from MLST schemes of several different parasites. Using this scheme, we were able to distinguish two commercial vaccines and several types of field strains.

Over the course of one year, we sampled two commercial broiler farms in Alabama on a regular base and several further farms opportunistically. We determined oocyst counts and investigated the species composition by next generation sequencing. Isolates containing E. maxima were typed using the developed MLST scheme. The results will be useful in the development and evaluation of coccidiosis prophylaxis programs, by vaccination or use of anticoccidials.

Zoamix: A Blast from the Past

Timothy Cummings

Zoetis

Zoamix is one of the oldest feed additive anticoccidials that came to the U.S. market in the 1960's, and following a period of being off the market, it was relaunched back to the U.S. market in the fall of 2014 as an aid in the prevention and control of coccicidiosis in broiler chickens, pullets, and turkeys. Since that time, Zoamix usage in the poultry industry increased each year to the point where it has been the leading feed additive anticoccicial sold in the U.S. for past three years. Although there were some general guideline recommendations made for Zoamix when it was being relaunched, more specific recommendations for Zoamix will be offered based on what has been learned from field experiences with Zoamix on performance and cocci control.

Experiences Vaccinating with a Novel Coccidiosis Vaccine in Long Lived Chickens in the United States

Kelli Jones

Ceva

According to surveys conducted recently (2019) by the American Association of Avian Pathologists (AAAP) Research Priorities Committee, commercial chicken companies in the United States (US) indicated a “high to very high research need for improved vaccines to optimize intestinal health and combat

coccidiosis." More specifically, they would like work done looking at the most effective non-ionophore/antibiotic strategies for controlling coccidiosis, and to determine best practices for using coccidial vaccines.

As a result of this industry interest and demand, a novel coccidiosis vaccine, Immucox ® 5, for long lived chickens was released in the United States in late 2019. Leading up to its release, and immediately thereafter, efforts were made to fine tune and maximize the effectiveness of this vaccine in the industry.

Successful coccidiosis vaccination requires that the vaccine be handled and stored appropriately, and that it be administered in a way that maximizes the uptake of the vaccine oocysts. In addition to ensuring a good initial vaccine oocyst uptake, several subsequent cycles must take place successfully in the field without interruption. This "re-cycling" is entirely dependent on management factors during the first 4-5 weeks of life in a poultry barn.

This paper will focus on experiences in the field related to this vaccine's use, as well as best management practices in both the hatchery and in the field, in order to optimize application, uptake, re-cycling, and the development of immunity in long lived chickens against coccidiosis.

Lessons learned changing coccidiosis vaccines in grandparent broiler breeders

Christina Lindsey[1], Kelli Jones[2]

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When our previous supplier of coccidiosis vaccine announced a limitation in supply, we had to find a different product. In this presentation, we'll discuss how we handled this transition in the hatchery and in the field for our internal flocks of Grandparent (GP) broiler breeders. Topics will include hatchery administration, managing litter moisture in the field, managing coccidiosis vaccine reactions, and using oocyst counts (OPGs) to optimize the coccidiosis vaccine management program through the brooding stage.

The Potential Impact Of Three Phytogenic Feed Additives On Chicken Eimeria from Coccidiosis Vaccine.

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In recent years poultry producers have seen an increase in demand for an antibiotic-free product while still dealing with the need for high-quality animal protein and keeping feed costs lower. To meet consumers demands, phytogenic feed additives are becoming a seemingly more popular topic among veterinarians, nutritionists, feed manufactures, producers, farmers and scientists. Phytogenic feed additives are plant-based feed additives or botanicals that are used in animal nutrition as alternatives to antibiotics, ionophores and chemical coccidiostats. Research on phytogenic alternatives have shown some of them to have antibacterial, anticoccidial, as well as, anti-inflammatory properties. These substances can be derived from herbs, spices, other plants and their extracts, like essential oils.

The aim of this study was to determine the potential impact on coccidiosis vaccine when birds were fed three different phytogenic feed additive products: 1.) A 5% oregano essential oil products; 2.) A proprietary blend of tannic acid extract, phytogenic molecules, beta glucans and a Bacillus subtilis; 3) Hops. The potential impact of all three phytogenic products are discussed.

Identifying and Quantifying Eimeria Species in Mixed Samples with Molecular-Based Technologies

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Multiple Eimeria species can infect a chicken simultaneously with each parasite species varying in its pathogenicity and perceived commercial importance. Therefore, identification of individual Eimeria species in mixed samples is valuable for understanding this disease complex. Recently, molecular tools for identification have been exploited because morphological differences of oocysts remains unreliable.

Digital droplet PCR (ddPCR) is a sensitive quantitative method that could be applied to the identification and quantification of Eimeria species. A ddPCR-based method was developed that exploited targets that were genus-specific or species-specific. A multiplexed approach with a probe designed to provide DNA copy number for all Eimeria spp. combined, and a probe to provide DNA copy number for the seven described Eimeria species was used. Single-species samples of known identities were tested alone and in combinations to assess the sensitivity and repeatability of this experimental assay. Experimentation with this technology and unique design has demonstrated its ability to provide relative abundance of Eimeria species DNA. Samples analyzed originating from commercial coccidiosis vaccine vials and feces from flocks post-vaccination were tested with providing results consistent with expectancies. This assay provides a commercially-viable assay alternative to Next Generation Sequencing.

Obtaining relative species abundance and correlating it with oocyst per gram counts will create data needed by veterinarians, researchers, technical representatives, nutritionists and producers. Using this assay to convert uninformative OPG counts into precise measures of parasite diversity and relative abundance is prerequisite for unraveling this disease complex and thereby improve control of coccidiosis in commercial poultry industries.

Applying Metagenomics to Characterize the Eimeria species Composition in Litter from Commercial Broiler Farms

Kate Worthing

ARS-USDA

The detection of Eimeria in broiler chickens often relies on examining gut tissue for oocysts and schizont stages by light microscopy or on molecular assays such as polymerase chain reaction (PCR) that can detect and differentiate Eimeria species recovered from gut tissue or litter. These diagnostic methods are usually done when there is a spike in mortality at 2-4 weeks of age which is often due to necrotic enteritis (NE), a disease most often associated with a concurrent Eimeria infection. It is now well accepted that E. maxima plays an important role in the occurrence of NE in broilers, and control of E. maxima by either vaccination or anticoccidial drugs is crucial to preventing NE-associated mortality in young chicks. What remains unknown is whether strains of E. maxima exist that are more predisposing to NE by being more pathogenic, that is causing greater damage to intestinal tissue. In our laboratory, two strains of E. maxima were identified, one (E. maxima APU1) being highly pathogenic causes 25% weight gain depression with as few as 1000 oocysts. Another strain (E. maxima APU2) requires doses greater than 25,000 to cause a similar level of weight gain depression. A series of oligonucleotide primers were made to conserved regions of single copy orthologous genes (SCO) of Eimeria and used in metagenomic analysis (Illumina MiSeq) to characterize the relative frequencies and population structure of Eimeria recovered from

litter at various times of growout on commercial broiler farms. These primers flanked variable SCO regions and thus may be helpful in identifying different Eimeria strains of interest, such as those whose presence may correlate with mortality at 2-4 weeks of age.

Effects of Coccidiosis Vaccination With or Without the Use of a Phytogenic Feed Additive in Broilers

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The objective of the present study was to determine the effects of coccidiosis vaccination with or without a phytogenic in-feed additive (Macleaya cordata extract, MCE) on the growth performance and intestinal health in broilers. A total of 648 1-day old male Cobb 500 broilers were assigned to 4 treatments in a complete randomized block design, including 9 replicates per treatment. Treatments were: Negative Control (NC; No feed additive or vaccine), Coccidiosis Vaccine (V), V plus phytogenic feed additive at 60 ppm (VMCE60), and V plus phytogenic feed additive at 90 ppm (VMCE90). Birds were vaccinated for coccidiosis at placement, with a commercially available live vaccine administered according to the manufacturer recommendation. The litter used consisted of pine shavings after 3 cycles. Birds were fed corn-soybean meal all-vegetable diets ad libitum, and no medication was included in feed or water throughout the study. Growth performance was determined at 28, 44 and 53 days, based on body weight (BW), feed intake (FI) and feed conversion ratio (FCR). Effects on intestinal permeability (FITC-dextran absorption) and inflammation markers (serum levels of TNF-alpha and IL-10) were evaluated at 28d of age. Results showed that vaccinated birds experienced an early reduction in BW and poor FCR (1-28d, p0.05). At 28d, serum levels of FITC-dextran were significantly increased ($p<0.05$) in the vaccinated birds (V) compared to the remaining treatment groups, indicating impaired gut integrity. The negative impacts of vaccination, both on growth performance and intestinal health, were alleviated ($p<0.05$) when treatments included the phytogenic feed additive. In conclusion, live coccidiosis vaccination does have negative impact on growth performance and intestinal health in broilers. Furthermore, this study demonstrates the benefits provided by the phytogenic feed additive on the intestinal health of broilers.

Virology

Viral Hepatitis in Turkey Poults

Megan Lighty

Jennie-O Turkey Store

The scientific literature describes turkey viral hepatitis (TVH) as a generally subclinical disease of young turkeys characterized by multifocal hepatic necrosis likely caused by a picorna-like virus. Clinical signs of the disease are not well-defined, and the disease is thought to manifest when combined with other factors such as concurrent infection and/or environmental stress. Morbidity and mortality can be highly variable and the economic significance of TVH is unknown. Within the last year, turkey companies in the United States have reported an increased incidence of multifocal necrotizing hepatitis in turkey poults. Gross lesions range from subtle mottling to multifocal white/gray/tan foci in the livers. Reovirus has been consistently isolated from the livers of affected poults. Flock history, necropsy findings, and results of diagnostic testing will be reviewed.

Isolation of Egg Drop Syndrome (EDS) Adenovirus from Brown Layers in Pennsylvania

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Starting in 2018, several flocks of brown egg laying hens from Pennsylvania presented with production of pale, soft-shelled, and shell-less eggs at peak of production, without other apparent clinical signs. The hens tested negative for avian influenza, Newcastle disease, and infectious bronchitis, however, serological evaluation showed exposure to Egg Drop Syndrome (EDS) adenovirus. Avian adenoviruses can be divided into three groups. The first group comprises of adenoviruses in chickens often associated with inclusion body hepatitis, the second group is hemorrhagic enteritis virus (HEV) in turkeys, and the third group is EDS virus. EDS virus is a duck adenovirus that can infect chickens through indirect or direct contact with duck feces, untreated contaminated water, or lapse in biosecurity. The virus replicates in the uterus of hens and is transmitted in or on eggs and droppings contaminated with oviduct secretions.

Each subsequent flock of hens placed on this multi-age Pennsylvania farm through 2019 showed identical presentation of drop in egg production, which can be attributed to EDS virus becoming endemic on the farm. EDS adenovirus was isolated from the shell glands of affected brown hens and propagated in commercial duck embryos inoculated via chorioallantoic sac route and in duck embryo fibroblasts. The virus did not replicate in specific-pathogen-free chicken embryos, chicken embryo fibroblasts, or LMH cells. Isolated virus was inoculated into susceptible brown hens to reproduce the disease and fulfil Koch's postulates. Commercial inactivated EDS vaccines are not currently sold in the USA, therefore attempts were made at adaptation of EDS adenovirus isolate to cell lines approved for autogenous vaccine production.

Comparison Of Antibody Response Against Infectious Bursal Disease In Broiler Breeders Injected Subcutaneously In The Back Of The Neck, Inguinal Fold And Intramuscular In The Thigh Or Breast

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Broiler breeders were vaccinated as embryos with a recombinant HVT+IBD vaccine and housed in a breeder facility at the University of Georgia. The recombinant vaccine administered in ovo was the sole Infectious bursal disease (IBD) primer the flock received. All birds were raised following broiler breeder company recommendations. The pullets received a conventional live primer and inactivated vaccination program where the inactivated IBD consisted of two injections of 0.5 ml given at 20 weeks of age intramuscularly on each side of the breast. When the breeders were 48 weeks of age they were divided into 5 groups of 20 birds each and received a second inactivated IBD vaccine as follows: 1) subcutaneously in the back of the neck; 2) Inguinal fold; 3) intramuscular in the breast; 4) Intramuscular in the thigh. The fifth group did not received a second IBD vaccination to serve as negative control. Serum samples were collected from all breeders in the study on the day of vaccination to establish a baseline and then three and 6 weeks after vaccination. Antibodies against IBDV were measured with two different ELISA kits. Seroconversion was evaluated by comparing the percent increase on Geometric mean titer (GMT) and decrease in

coefficient of Variation (CV). Vaccine injection in the breast produced the highest increase in GMT and biggest decrease of CV values. Detailed results will be discussed.

Virulence assessment of chicken astrovirus (CAstV) isolated from outbreaks of White Chicken Syndrome (WCS) in Western Canada

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In the last 5 years, chicken astrovirus (CAstV), the causative agent of a condition known as White Chicken Syndrome (WCS), has emerged as an economically important disease across Western Canada. CAstVs were isolated from liver and intestinal samples of affected chicks and embryos following inoculation of samples in chicken embryo liver cells (CEL).

To evaluate the virulence of a CAstV clinical isolate, two ages of embryonated eggs, namely 14 & 18 days of embryonation (DOE) were infected by the in ovo route at three different titers 101.0, 102.0, 103.0 TCID50 per embryo. At 14 DOE, the site of deposition would be the yolk sac, while at 18 DOE, it would be embryo/amniotic sac. Once hatched, the birds were placed on floor pens and monitored daily for clinical signs for a 7-day observation period. Cloacal swabs were obtained at day of age from all birds (including unhatched embryos), and at 7 days of age. The samples of liver, gut, and kidneys were collected for quantitative polymerase reaction (qPCR) and histopathology. Clinical signs, qPCR, and histology data will be discussed in detail at the conference.

Effect of antibiotic growth promoter administration on the intestinal microbiota and virus shedding post avian influenza virus infection in chickens

Brittany Seibert

University of Georgia

Outbreaks of avian influenza virus (AIV) can have detrimental effects in poultry flocks resulting in mortality and indirect costs because of eradication efforts, control and loss of markets. The role of the microbiota in essential physiological processes and disease progression has expanded. Recently, chickens infected with low pathogenicity avian influenza virus (LPAIV) showed an intestinal microbiota population shift, with an increase in Escherichia and decrease in probiotic organisms like Lactobacillus. Further, similar studies reported that the absence of commensal gut microbiota in chickens increased virus shedding and compromised immune responses toward LPAIV infection. While it is shown that the intestinal microbiota affects AIV infection, it is not well known how altering the intestinal microbiota will affect AIV replication and shedding. Extensive literature reports showed that a variety of antibiotic growth promoters (AGPs) change the composition and diversity of the microbiota at different parts of the avian intestinal tract. While the literature is rich in the relationship between AGPs and the intestinal microbiota, little is known about the effect of AGP-induced microbiota changes on AIV shedding. Therefore, we explored the potential effect of a widely used AGP (Bacitracin methylene disalicylate (BMD)) on LPAIV shedding and weight gain in broiler chickens. Results showed that there was no significant difference in virus shedding and weight gain among groups. However, intestinal microbiota changes were observed. Understanding the relationship between AGP induced microbiota changes and AIV infection will further increase our comprehension of the

impact that the microbiota plays in AIV infection in poultry.

SARS-CoV-2 and MERS-CoV are unable to replicate in common poultry species and embryonating chicken eggs

Erica Spackman

SEPRL-USDA-ARS

The efficacy of an adenovirus-vectored Newcastle disease virus (NDV) vaccine expressing the fusion (F) NDV protein (adeno-F) was evaluated against challenges with virulent NDV strains containing heterologous and homologous F proteins. In a preliminary study, two different doses (low and high) of adeno-F were tested against a virulent NDV strain containing the homologous NDV F protein, CA02. In a second study, at three weeks post vaccination, the efficacy of the high dose of adeno-F was compared to a live attenuated NDV vaccine strain (LaSota) against three antigenically distinct virulent NDV challenge strains, one homologous (CA02), and two heterologous (TZ12, EG11) to the F in the vectored vaccine. In both experiments, clinical signs, mortality, virus shedding, and humoral response were evaluated. In the first experiment, the survival rates from birds vaccinated with adeno-F at a high and low dose were 100% and 25%, respectively. As for the second experiment, birds vaccinated with the high dose of adeno-F, had a survival rate of 80%, 75%, and 65% after challenge with the CA02, TZ12, and EG11 viruses, respectively. All of the LaSota-vaccinated birds survived post challenge no matter the NDV strain used except for one bird challenged with CA02 that died from causes unrelated to the challenge.

Comparison of the monitoring methods for following the take of immune-complex vaccine against Gumboro disease in commercial layers

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The control of Infectious bursal disease (IBD, also called Gumboro disease) is extremely important to prevent losses in layer production. The control of Gumboro disease must include not only the protection against the clinical signs, but also the prevention of replication of field IBDV strains, that can introduce and keep disease challenges for the subsequent flocks. By this way, the virus pressure should decrease cycle after cycle and the likelihood of emergence of new IBDV strains should be reduced.

A novel option to control IBD is an immune-complex vaccine (Novamune®), specifically developed for egg-type genetic lines and slow growth chickens. The vaccine strain, a live attenuated IBDV, is capable to reach the bursa, when maternal immunity has waned, it brings a high level of protection against various types of IBD viruses and it effectively prevents shedding.

To monitor the proper replication of the vaccine strain in the bursa of Fabricius, comparisons of combined methodologies were applied in parallel. A first vaccinated pullet flock was monitored by serology (ELISA) and detection of vaccine virus (RT-PCR). A second vaccinated pullet flock was followed by histology and RT-PCR.

The results of antibody response, vaccine virus detection and histological results will be disclosed, as well as the comparison between the different methodologies. These data provide evidence of an attractive, innovative, convenient tool for the control of Gumboro disease in commercial layers.

Virology: ILT and IBV

Validation of Day of Age Infectious Bronchitis Vaccination

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Infectious Bronchitis (IB) vaccination of day-old chicks in the hatchery is a common practice in commercial broilers in the USA. Live attenuated IB vaccines reconstituted in water are usually administered using different spray cabinets. Dosing and combinations of existing IB vaccine strains are often adapted to address the challenges present in each region. However, despite of vaccinating billions of broilers annually, validation of IB vaccination has not been a standard practice in the industry. This work describes how quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR) was utilized as a validation tool for IB vaccination of day-old chicks in commercial hatcheries. Several field cases utilizing different IB vaccine strain combinations, dosing, spraying techniques, and diluents (water vs. gel) were analyzed. Samples consisting of 15 individual choanal swabs were taken at 5 days post-vaccination from each case. qRT-PCR results showing virus detection and relative quantification across individual birds are presented. Opportunities for improvement IB vaccination of commercial broilers can be extracted from these results.

Molecular characterization of field isolates of infectious bronchitis virus in Mississippi from 2013 to 2019

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This is a retrospective study about the presentation of infectious bronchitis virus (IBV) genotypes in Mississippi (MS) from January 2013 to June 2019. Six hundred and twenty-three IBV isolates from seven counties (Copiah, Covington, Jasper, Jones, Neshoba, Pike, and Scott) with important poultry production were isolated and genotyped at the Poultry Research and Diagnostic Lab (PRDL) in Pearl, MS. The genotyping of these isolates was conducted by partial sequence analysis of the hypervariable region (HVR) of the S1 gene. Before winter of 2013, the most frequently detected serotype was Arkansas-Delmarva Poultry Industry (Ark-DPI) with 76% of the isolations. The second most isolated serotype was Connecticut (Conn) with 13% of the isolations. Between December 2013 and the fall of 2014, Georgia 08 (GA08) isolates emerged, especially in the counties with more dense poultry population (Covington, Jones and Scott). During 2015, isolations of this subtype came to an end. However, in spring of 2016 this subtype re-emerged with limited geographic presentation (Covington and Jasper) and has been isolated to the present time. On April 2019, the subtype Delmarva (DMV)/1639 was initially isolated in Copiah, Neshoba and Scott, counties, from samples of broiler flocks with respiratory problems. The phylogenetic analysis including several Ark-DPI isolates indicated their very close relation among themselves and distribution in

the phylogenetic tree regardless the county or year of isolation. A similar situation was observed with GA08 isolates. These phylogenetic analyses may suggest that populations of Ark-DPI and GA08 field strains circulating throughout the state are very close related.

Genotyping of Infectious Laryngotracheitis virus (ILTV) isolates from Western Canada based on partial open reading frame (ORF) a and b

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University of Calgary

Infectious Laryngotracheitis (ILT) is an acute upper respiratory disease in chicken caused by the etiological agent Gallid herpesvirus 1 (Infectious Laryngotracheitis virus, ILTV). The control of ILT relies on two types of vaccines, recombinant and live attenuated vaccines. The live attenuated vaccines can be either embryo passaged (chicken embryo origin, CEO) or tissue culture passaged (tissue culture origin, TCO). The live attenuated vaccines have proven to be very effective in disease control however, they have residual virulence which makes them able to replicate, cause disease and/or revert to the original virulent form. Canada lacks information on the molecular nature of ILTV that circulates in the country. The objective of the study was to determine the molecular nature of ILTV isolates that caused ILT in chickens raised in Western Canada. From the year 2009 to 2018, samples submitted for the diagnosis of ILT were obtained from Alberta (n=46) and British Columbia (n=9). Sanger sequencing of ORFa and b was used for genotyping. We found that most of these isolates cluster in group V (CEO revertant), some cluster as grouped VI (wild type) and a few in the TCO group. With this method, TCO related clusters (I, II and III) are grouped together since the USDA strain is likely their parent strain. Clusters VII, VIII and IX also group together, and are related to Australian strains. Further studies are under way to ascertain the virulence and transmission potential of these Canadian ILTV isolates.

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Infectious Bronchitis Challenge Studies in Broilers—a Comparison of IB Vaccine "Takes" and Protection

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Two studies will be presented. Study 1 compares different bivalent combinations of IB vaccines (Mass+GA98, Mass+GA08 and GA08+GA98) to the trivalent combination of these same vaccines (Mass+GA98+GA08) given to broilers by day of age spray and challenged at 25 days with a DMV/1639 isolate. Study 2 compares the same trivalent combination on protection against either DMV/1639 or Arkansas challenge. Vaccine takes of the various combinations will be compared by serotype specific PCR probes. Tracheal protection based on histopathology and PCR Ct values will be presented. The results will show that bivalent combinations give modest protection against heterologous IBV challenge but the trivalent combination gives significantly better IBV protection and this level of protection was similar against both DMV/1639 and Arkansas IBV challenge viruses.

Evaluation of host genetic resistance against infectious laryngotracheitis

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Infectious laryngotracheitis (ILT) and Marek's disease (MD) are caused by two closely related species, Gallid alphaherpesvirus 1 and Gallid

alphaherpesvirus 2, respectively. Despite similarity of the viruses and 70+ years of work on genetic differences in host resistance to MD, there has been very little effort in trying to control ILT through host genetic resistance. The best understood mechanism for the involvement of genetic resistance to MD involves the MHC or, as it is known in the chicken, the B complex. Using ILTV challenge strains 63140 and 1874c5, we utilized our unique poultry genetic resources (including B-congenic lines B*2, B*5, B*12, B*13, B*19 and B*21) to evaluate the effect of MHC on ILT disease incidence. In addition, we challenged Line 6 and Line 7 birds which carry the same MHC but differ in non-MHC genes. Significant differences between clinical signs and viral load were observed between genetic lines, with Lines B*2 and B*5 demonstrating the most resistance. Line 6 also demonstrated more resistance compared to Line 7. These results provide a basis for genetic selection of breeding flocks that are more resistant to ILT.

Infectious Laryngotracheitis Sequence Typing Directly from Clinical Samples

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Infectious Laryngotracheitis (ILT) is a contagious Herpes virus that causes upper respiratory disease of chicken leading to significant economic losses in form of mortality and egg production losses. Identification of ILT virus strain is important for the disease control and eradication efforts. Identification of ILT viruses was historically performed through genotyping by restriction fragment length polymorphism (RFLP) of multiple sub-genomic segments. More recently, sequence typing of ILT was achieved by amplifying and sequencing five large genetic segments, which classified ILT into nine genotypes. Given the large size of the target segments, ILT sequence typing was performed only from viral isolates or from clinical samples with high viral copy numbers. The purpose of this study is to reduce the number and size of target segments necessary for ILT sequence typing to allow for ILT the sequence typing directly from clinical samples with relatively lower viral copy number. Three targets were identified, and three sets of primers designed to amplify and sequence these targets. Amplification and sequencing was successful from clinical samples with lower copy number. Two of the original nine genotypes, namely tissue-culture vaccines and tissue-culture vaccine related genotypes, were not differentiated using this new assay. This means that the new assay sequence typed ILT into eight instead of nine genotypes. In spite of this reduction of discriminatory power, the new assay would allow for the characterization of many more ILT cases. This in turn allows for better understanding of ILT epidemiology and effective control and eradication efforts.

Efficacy of two commercial infectious coryza vaccines after challenge with a serotype C Avibacterium paragallinarum strain isolated from outbreaks in layers and broilers in California

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Infectious coryza is a bacterial upper respiratory disease caused by Avibacterium paragallinarum. In recent years, a significant increase in infectious coryza cases have been observed in California, affecting both broiler and layer chickens. The strains isolated in California have

been classified as serotype C-2. However, genotypic analysis using the hemmaglutinin genes HMTp210 and hagA demonstrated high similarities to strains that belong to serotype C-1. The aim of this study was to compare the efficacy of two commercial vaccines after challenge with a wild-type serotype C strain of Av. Paragallinarum isolated in California. Vaccine 1 is a quadrivalent killed bacterin vaccine that contains serotype A, B and C-1 strains as well as a variant of serotype B. This vaccine is not commercially available in the US. Vaccine 2 is a trivalent killed bacterin vaccine that contains serotype A, B and C-2 strains and is widely used in North America. Despite differences in serotype C strains in vaccines 1 and 2, our results indicate that both vaccines, if administered in two doses as recommended by the manufacturer, are able to induce appropriate protection and reduce bacterial shedding when compared with unvaccinated chickens. Further results from these experiments will be discussed.

Pathogenesis of the Canadian Delmarva (DMV/1639) Infectious Bronchitis Virus (IBV) Infection in Layers

Mohamed S. H. Hassan

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IBV infection may lead to reduced egg production and poor egg quality in layers. In the recent past, one of the most dominant IBV variants isolated from Canadian layer flocks with egg production problems was DMV/1639. Our main hypothesis was that experimental infection with DMV/1639 variant will result in pathological changes in the reproductive tract leading to egg production and quality issues. To determine whether the isolated Canadian DMV/1639 leads to egg production problems, we infected specific pathogen free layers at the peak of lay (26 weeks) with an uninfected control group. When the post-infection egg production dropped to 40% (10 days post-infection), the chickens were euthanized to observe gross lesions and collect samples. We found that IBV genome is quantifiable from cloacal and oropharyngeal swabs as well as reproductive tract of infected chickens. At the post-mortem examination, we found shortened oviductal length and ovarian regression in some of the infected layers. One of the infected chickens showed pale swollen kidney with the tubules distended with urates. Histological changes were also noted in the kidneys and reproductive tracts of infected chickens. We were also able to demonstrate anti-IBV antibody response in serum and locally in the reproductive tract washes. We are in the process of evaluating cytokine response and immune cell recruitment in the reproductive tract following IBV infection.

DMV/1639 type IBV transmits from vaccinated chickens to other vaccinated chickens after challenge

Brian Jordan[1], Jongseo Mo[2], Erica Spackman[3]

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Infectious bronchitis virus (IBV) is an upper respiratory tract pathogen of commercial poultry that causes relatively mild clinical signs but predisposes chickens to secondary bacterial infections which can lead to airsacculitis and carcass condemnation at processing. Because of the economic impact of condemnation, nearly every commercial chicken is vaccinated against IBV. IBV vaccines are serotype specific, however, so having a perfectly matched vaccine is not always possible. To this end, producers are combining different IBV vaccine types in attempt to achieve cross-protection. This strategy has been effective at reducing or preventing clinical signs, but field type IBVs are still isolated. Thus, it appears that field viruses can still transmit using this vaccine strategy though this has not been examined experimentally. To answer this

question, we reared vaccinated (Mass and GA08 type vaccines) and non-vaccinated chickens separately and mixed them together in different combinations after some were challenged and others were not, to evaluate the transmissibility of a DMV/1639 type field virus. We found that the DMV/1639 virus readily transmits from non-vaccinated, challenged chickens to other non-vaccinated chickens as expected. Interestingly, the DMV/1639 virus also transmitted from vaccinated, challenged chickens to vaccinated, non-challenged chickens, although it occurred a week later than in non-vaccinated chickens. As in the field experience, clinical signs were significantly reduced in vaccinated, challenged chickens, but challenge virus was still detected. This information is significant because if field virus is able to transmit in vaccinated chickens, it can replicate and mutate, leading to a potentially new variant IBV.

Safety and Protection Efficacy of a Cell Line Adapted Infectious Laryngotracheitis Virus (ILTV) BDORFC Strain Following In ovo and/or Spray Vaccination with and without rHVT-LT Vaccine

Daniel Maekawa Maeda

University of Georgia

In an effort to produce more stable live attenuated vaccines for infectious laryngotracheitis virus (ILTV), deletion of genes related to virulence has been extensively pursued. Among viral genes associated with virulence but non-essential for viral replication in vitro is the open reading frame C (ORF C). The protection efficacy of an ILTV recombinant strain with deletion of the ORF C gene (BDORFC) when administered in ovo was comparable to the tissue culture origin (TCO) vaccine. As rHVT-LT in ovo vaccination is a common practice in the poultry industry, the development of live attenuated strains that can be also administered in ovo is being considered as a possible vaccination strategy against the disease. Therefore, the objective of this study was to evaluate the safety and protection efficacy of an LMH cell line adapted BDORFC strain when administered in ovo and/or spray with and without a rHVT-LT vaccine. Vaccination with the BDORFC strain either alone or in combination with a rHVT-LT vaccine did not affect hatchability and only minimal signs of respiratory distress were recorded for groups of birds that received the BDORFC strain via spray. After challenge, all vaccinated groups showed reduction in clinical signs and comparable lymphocyte and monocyte infiltration into the trachea. However, a decrease in challenge virus replication comparable to the non-vaccinated/non-challenge group was only observed for groups of chickens that received dual vaccination of rHVT-LT (IO) + BDORFC. Consequently, compared to rHVT-LT or BDORFC when administered alone, the dual vaccination strategy improved protection against challenge.

Investigating the role of Infectious Bronchitis Virus variant DMV/1639 in incidence of False Layer Syndrome

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Infectious Bronchitis virus (IBV) is an avian coronavirus that commonly causes respiratory disease but can also affect the reproductive tract of laying type chickens. If infection occurs in young pullets, false layer syndrome can develop later in life. False layer syndrome is characterized by systemic changes in the oviduct and the development of large, fluid filled cysts by the time hens come into production. IBV strains QX and Mass have been previously found to cause

False Layer Syndrome however, IBV strain DMV/1639 in parts of Canada and the U.S. has been reported in areas where False Layer Syndrome is occurring. Because of the delayed presentation of cystic oviducts, the initial IBV infection is rarely detected and it is not clear if IBV is the sole cause or at what age infection must occur for False Layer Syndrome to develop. Our study investigates the role and timing of IBV infection on development of False Layer Syndrome, using both IBV variant DMV/1639 and Mass. For our study, eight groups of 120 SPF chickens and a control group of 50 chickens were placed separately into colony houses and challenged at either three or seven days of age, two weeks of age, or sixteen weeks of age with either DMV/1639 or M41 IBV. Post challenge, swabs were taken and necropsies were performed where tracheas, kidney, and reproductive organs were collected for histopathology and immunohistochemistry. The data collected in this experiment will provide valuable information in understanding the pathology and development of False Layer Syndrome.

Virology: Marek's Disease and Reovirus

Pathogenic and Immunogenic Evaluation of Avian Reovirus Variant Strains Isolated from Clinical Cases

Sofia Egana

University of California, Davis

Since 2015, an increase of clinical cases of viral arthritis/tenosynovitis caused by avian reovirus variant strains (ARVv) have been diagnosed by the California Health and Food Safety Laboratory (CAHFS). In order to better understand the pathobiology of ARVv, four groups (n= 182) of one-day-old SPF chickens were placed in BSL 2 rooms. Half of the chickens in each room were challenged with one of two variant ARV strains (pathogenic for tendons or heart), one classical (S1133) ARV strain or PBS in the negative control group, via footpad. The other half of the chickens remained as contact birds. Clinical signs, body weight-hock joint ratio, histopathology and viral loads in tendon and heart were assessed at different time points and compared between groups. Lymphocytic depletion in thymus and bursa was evaluated. Results will be discussed.

Protection Against 9109-Like Variant IBDV Using Dual Recombinant HVT-ND-IBD Vaccine Alone

Andres Montoya

Merck Animal Health

Infectious bursal disease (IBD) is a highly contagious disease of young chickens caused by infectious bursal disease virus, characterized by immunosuppression and mortality generally at 3 to 6 weeks of age. IBDV is ubiquitous in commercial chicken operations. IBDV causes a prolonged B-lymphocyte immunodeficiency and increased susceptibility to various viruses and parasites. Both classic and variant strains of IBDV had been isolated in the southeastern United States. 9109 is a variant strain of IBD that has been a problem in the southern states of United States. 9109 variants have been characterized as a classical, very virulent, and variant strain. 9109 had been isolated in commercial broilers that had result in performance issues. An evaluation of the protection against 9109 variants using a dual recombinant HVT-ND-IBD vaccine alone or in combination with a live intermediate standard strain and 89/03 strain of IBDV vaccine showed, based on bursa/body weight ratio and histopathology, protection in all the vaccinated groups when it was compared with the non-vaccinated/challenged group. Statistical difference was observed between the no vax/challenge group when compared to the vaccinated groups. The dual construct rHVT-ND-

IBD vaccine alone or in combination with a live IBD vaccine protected against 9109 like variant IBD virus when challenged at 18 days of age.

Differential Pathogenesis of Turkey Reoviruses associated with Arthritis, Enteritis and Hepatitis

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Turkey arthritis reovirus (TARV) has been associated with lameness in turkey flocks older than 10 weeks of age resulting in significant economic losses since 2011. The aim of this study is to design a standardized mixed-sampling scheme for early detection of viral infection. We hypothesize that use of histopathology, molecular detection and virus isolation will be helpful in detection of TARV infection at younger age which may give us an early opportunity to mitigate the problem and to alleviate animal welfare concerns. To achieve the designated aims, we recruited 10 turkey farms (6 are known with a repeated history of clinical infection and 4 are clean healthy as negative control) in 2 different states in the Midwest and the East Coast of the USA. We have collected clean litter samples and drinker swabs at zero week before the arrival of turkey poults. Additionally, we have collected meconium samples from shipping trays at the arrival time of the turkey poults. After placement, litter/fecal samples (5 pools) and drinker swabs (2 pools) were collected weekly for 10 weeks. Samples were processed for reovirus isolation using QT-35 cells. We also received legs of five birds per house at 3, 5 and 10 weeks of age to collect tendons for histopathology and reovirus isolation. The study results analyses are in progress. This study will help to determine the origin of TARV infection, to provide information about viral shedding and a sampling scheme for early detection of TARV from infected turkeys.

Dual ND-IBD HVT Construct Vaccines: Efficacy and Onset of Immunity When Challenged with IBD Variant Strains

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HVT construct technology has advanced to a point where multiple commercial dual-construct HVT vaccines (ND-IBD) are now available to the poultry industry. These vaccines differ in how each construct was developed. The onset of immunity against classical IBD is generally 2 to 3 weeks for HVT construct IBD vaccines. In the Americas, variant strains of infectious bursal disease (IBD) are of significant concern, and they often break through maternally derived antibody at an early age.

Groups of SPF birds were vaccinated with commercially available dual construct HVT-ND-IBD vaccines via subcutaneous route. Vaccinates were challenged with Delaware Variant E, 9109-like IBD variant or AL-2-like IBD variant viruses at 14 or 18 days of age. Spleen and feather follicle samples were submitted for next generation sequencing to determine dissemination of the vaccine virus, and bursal histopathology, bursa: body weight ratio and serology were used to evaluate protection following challenge. Three commercially available dual HVT construct vaccines are compared to see if vaccine construction results in significant differences in vaccine dissemination, protection against

challenge with variant-type IBD or onset of immunity.

Turkey Breeders Perspective : How to Achieve Reovirus Maternal Antibodies in Poults through Vaccination of Parents

Cynthia Philippe

Hendrix Genetics Ltd

Reovirus is a major concern in commercial turkeys. To reduce its impact, all partners in the industry need to work together: commercials, breeders and researchers. Each entity has its role. Dr Rosenberger presented in September 2019 data showing that poults are more susceptible to reovirus during their first 14 days of age and that reovirus can spread through contact from infected to naïve birds. A more recent unpublished study from Dr Rosenberger showed that maternal antibodies can significantly reduce clinical signs in young poults. As breeders, we can help our customers by vaccinating against reovirus, to protect poults during their most susceptible time and to mute potential vertical transmission. To achieve it, breeder companies have to look into: vaccine quality and vaccination protocol. Since no commercial vaccine is available, turkey breeders have to rely on killed autogenous vaccines, which come with some limitations, such as no proof of efficacy. Also, without live priming, it is unknown how titers hold during production. This is why, to ensure maternal antibodies in poults, we compared different vaccination programs. Titers (CARV ELISA) will be taken 2 weeks post-vaccination and at 15, 34, 46 and 58 weeks of age. Maternal antibodies from progeny will also be monitored. Results will be compared to our baseline, which are the titers collected for 3 years prior to vaccination. This will enable to select which vaccination program is the most effective to protect hatching poults during their most susceptible period in our operations.

Evaluation of Attenuated Variant Reoviruses as Modified Live Vaccines

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Avian reoviruses are causative agents of tenosynovitis and viral arthritis in chickens and turkeys. The incidence of clinical disease continues to cause economic and health issues for commercial poultry. Historically, commercial modified live and inactivated reovirus vaccines have provided protection against vertical transmission from breeders to broiler progeny and clinical disease in breeders and broilers. However, little protection is provided against challenge with contemporary reoviruses, thus the use of custom inactivated reovirus vaccines has increased dramatically over the past seven years. Modified live reovirus vaccines contain strains that are genetically similar (>97% Sigma C amino acid similarity) and were isolated over 2 decades ago. The lack of similarity between the current field isolates and vaccine strains is a primary reason for lack of adequate protection. Two variant reoviruses belonging to genotype 1 and 5 were plaque-purified and passaged successively in chicken embryos until they became attenuated. The attenuated variant strains were found to be safe, pure and efficacious per guidelines outlined in the 9 Code of Federal Regulations. In addition, breeders were vaccinated with a combination of commercial, modified live and inactivated variant vaccines. Protection against clinical disease was evaluated in broiler progeny from the vaccinated breeders in a series of homologous and heterologous challenge studies with reovirus variants belonging to genotypes 1-

7. A high level of protection was observed in homologous challenge studies, as well as in some of the heterologous challenge studies providing evidence that the attenuated variant strains contribute to cross protection between antigenically dissimilar reoviruses.

Evaluation of Antibody Response to Autogenous Reovirus and Infectious Bursal Disease Virus Vaccines

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Avian reovirus and infectious bursal disease virus (IBDV) both cause diseases in poultry species that have very substantial economic consequences. Currently, there are commercial vaccines for broiler breeders on the market for both reovirus and infectious bursal disease virus. However, as both of these viruses undergo frequent genetic mutations, these commercial vaccines, made up of primary isolates collected decades ago, do not always protect against clinical disease. Broiler companies, therefore, will typically send current field isolates to different biological companies in order to develop autogenous vaccines to immunize future broiler breeder flocks. USDA veterinary biologics regulations require safety and sterility testing for these custom vaccines, but efficacy and potency testing prior to use is not performed. Our study will analyze, using ELISA and virus neutralization assays, whether autogenous vaccines used for both reovirus and infectious bursal disease virus lead to the production of sufficient antibodies to protect against subtypes of these viruses found in the field. A smaller portion of this study will also entail a comparison of antibody titer production with the same vaccine but injected in two different locations in the bird (inguinal fold and intramuscular in the breast muscle). Titer levels will determine immunization efficacy. This information will allow broiler companies to know if changes need to be made to the autogenous vaccination processes in order to increase coverage and decrease disease prevalence.

Turkey Reovirus Hepatitis: An Emerging Disease from a Known Etiological Agent.

Andrew Smith

Butterball

Reoviruses have been known etiological agents of tenosynovitis for many years. While many of these reoviruses are species-specific, they can all produce similar disease in poultry. In turkeys, lameness is classically seen late in the growout phase leading to excessive mortality and production losses.

In early 2019, periodic cases of poult hepatitis were observed in brood hubs across the production complex. Initially believed to be cases of Turkey Viral Hepatitis (a picornavirus) based on gross lesions and histopathology, further diagnostics and clinical signs in the field ultimately led to a diagnosis of reovirus hepatitis. This case report will cover the timeline of the initial diagnostic process as well as current knowledge of an emerging presentation of turkey reovirus.

Case Report: Runting and Stunting Syndrome in a Flock of Commercial Brown Layers

Jim Stockam

Merck Animal Health

Runting and Stunting Syndrome has been described in commercial brown laying chickens and characterized by severe stunting, increased

mortality, immunosuppression, and severe cystic enteritis. A flock of commercial brown layers, housed in a building with a history of early stunting, was clinically evaluated from 3 to 10 days of age. Daily clinical observations and diagnostics, such as electron microscopy, histopathology, virus isolation, and PCR on intestinal and lymphatic tissues, were conducted. When compared with a similar clinical case in a previous flock housed in the same farm, a pattern of comparable pathogens was identified, including a novel Reovirus and a novel Astrovirus. Isolated viruses were administered to brown chickens alone or in combination to fulfill Koch's postulate.

Wealth of Knowledge 1

2020 Research Priorities of the American Association of Avian Pathologists

Natalie Armour

Mississippi State University, CVM, DPPM

The Research Priorities Committee (RPC) of the American Association of Avian Pathologists (AAAP) exists to advance the application of science-based knowledge in the poultry industry, by ensuring that the practical research needs of the industry are communicated to researchers and research funding agencies. The RPC will determine the 2020 AAAP research priorities using a three-pronged approach. Firstly, as in previous years, surveys will be conducted of veterinarians in broiler, egg and turkey production in North America, facilitating the generation of ranked listings of research priorities for each commodity in the categories: Health/Disease, Vaccines & Pharmaceuticals, Diagnostic Tools, Food Safety, Welfare, Management/Environment, and Top Overall Research Priorities. Secondly, various AAAP task force committees will be surveyed, to determine research needs in particular fields of poultry health and medicine (e.g. animal welfare, food safety, enteric diseases, respiratory diseases, small flocks, etc.) from the perspectives of subject matter specialists. Finally, input will be sought from federal agencies that need research to establish science-based policies relating to poultry. The resulting research priorities will therefore represent the multifaceted perspectives of poultry veterinarians and specialists involved in poultry production, technical service, extension, research, teaching, diagnostics, and government, and will provide researchable and specific guidance to researchers and research funding agencies.

Using geospatial methods to produce a Georgia risk map for avian influenza

Louise Dufour-Zavala

GPLN

We are using geospatial methods to determine the likely interface between waterfowl habitat and poultry growing areas. The final product is intended to use in grower and industry education meetings.

Post Vaccination Monitoring and Respiratory Disease Monitoring in Broiler Flock by On-Side Fully Automated POCKIT Central PCR system

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Respiratory disease causes economic losses in broiler industry. Vaccination at day old of age for prevention from field challenge is common practiced. Serology method is often used for post vaccination evaluation. However, monitoring serologically at harvesting age is not suitable because sero-conversion is still on-going and results often inconclusive. POCKIT Central provides an efficient way to monitor vaccination effectiveness/uniformity in the early stage with direct evidences for vaccine strain replication. 6

batches of 500,000 broiler DOCs were monitored from 2018. Tracheal swab samples were collected and tested individually with POCKIT Central IBV reagent by weeks. S1 gene sequencing was used to confirm the presence of the vaccine strain. Generally high positive rate (>80%) were observed on day 7 which indicated successful replication of the vaccine strain. Varied positive rate among different suppliers and batches (from 20% to 100%) provided good indicator for vaccination quality and vaccination improvement.

Mycoplasmosis is common around the world which causes down-grade of meat quality, increases the risk for secondary infection and results in economic losses in broiler industry. People often use antibiotics for treatment, but antibiotics resistance now is a problem because of inadequate use. In order to better management of antibiotics use, POCKITTM Central is an effective method for pathogen monitoring and antibiotic treatment evaluation. Mycoplasmosis monitoring was done by detection of M. gallisepticum and M. synoviae with specific reagents. 6.67% houses were mycoplasma positive at day of age and antibiotics were treated immediately. Effective treatment was found in 75% houses after 1 week. Early detection reduced antibiotics use in comparison to treatment in late stage.

In summary, POCKIT Central allows the managers to evaluate the vaccination uniformity and disease monitoring on site and in time which is a health management system to reduce the risk of respiratory diseases, improve vaccination, and cut down the use of antibiotics.

Analytical epidemiology of Spotty Liver Disease

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Since the identification of the causative agent Campylobacter hepaticus, the Australian poultry industry and researchers have been focussing on the understanding of the pathogenesis of Spotty Liver Disease (SLD) and the development of C. hepaticus vaccines.

It has been found in several field situations that presence of C. hepaticus in the animal is not always associated with the clinical cases of SLD. It has thus been hypothesised that there are potential farm and/or bird risk or protective factor(s) to the onset of SLD, which is the focus of this analytical epidemiology study.

Surveys and observational studies will be used in this study to examine free-range layer farms across different Australian states over a two-year period. A case-control study in the first year will aim to identify one or more putative risk factor(s) of SLD, using a broad questionnaire will encompass biosecurity, environment, management, nutrition, bird health and physiology factors. This will be followed by a cohort study in the second year which is designed to confirm the importance of identified factor(s) in the development of the disease. At the time of reporting, the first-year study would have been completed.

The identification of risk/ protective factor(s) of SLD would lead to better understanding of the pathogenesis and control of the disease, improved welfare for the animals, reduced antibiotic use and potentially improved response to future SLD vaccines.

Egg Layer Biosecurity in 2020 – An Assessment of Daybreak Foods, Inc. Biosecurity Program

Rosemary A. Marusak[1], Caitlin McKenzie[2]

[1]Daybreak Foods, Inc., [2]Daybreak Foods, Inc.

The 2015 highly pathogenic avian influenza outbreak in the upper Midwest had a devastating effect on the egg layer industry, resulting in a loss of approximately 43 M egg layers and pullets and total national cost expenditures of approximately $950 M. This outbreak led to rigorous company biosecurity plans, a focus on the new 14 NPIP biosecurity principles, as well as immediate implementation of demanding on-site biosecurity measures. At Daybreak Foods, Inc., three levels of biosecurity - standard, elevated, and critical - were established, and accompanying company plans to address these levels were written and implemented. A considerable amount of time and resources have also been invested in structural and operational measures and procedures, including bio-centers for shower-in/out facilities and truck wash bays, dedicated company clothing, and strict worker site policies. The lack of an avian influenza introduction suggests these new precautionary policies have been successful, to date.

This talk will take a critical look at our company biosecurity program five years post outbreak as we begin to evaluate our practices and procedures from a cost, risk, and compliance standpoint. We will address the following questions: What has been effective and what has not? What procedures seem necessary and what needs revision? Why does non-compliance occur and how can we address this? What new, emerging biosecurity matters should we start to address?

Common Misconceptions in Assuming a Product is a Viable Alternative to an Antibiotic

Greg Mathis

Southern Poultry Research, Inc.

With the demand for poultry 'Raised Without Antibiotics' flocks, the number of alternative products has greatly increased. This has led to the development and use of several products such as probiotics, prebiotics, essential oils, organic acids, saponins, and tannins. One question we must consider in determining if the product is a viable alternative; does it provide the same or better disease control as an antibiotic? Research often demonstrates that most of the alternatives are not as effective as an antibiotic. Due to this, poultry producers should moderate expectations. However, alternatives do currently play a major role in maintaining bird health. Unfortunately, not all provide the same benefits. Just as each alternative category varies greatly, within each category there can be large differences. It would be an error to believe that one product is the same as another. For example, there are many types of Probiotics: feed or water based, non-heat stable or heat stable spore form, single species or mix, one-time or continuous administration, etc. Even strain variation for one species can be tremendous. Consistency and production of Plant based products have a similar issue. Just because a plant extracts claims to be the same product the extracting of active ingredients can vary from plant source to production facility. Like probiotics, dose levels vary with each plant-based product. Another misconception is that encapsulation processes provide the same degree of resistance to acid and enzyme breakdown. Thus the main misconception is that products are the same and will provide similar benefits.

New Features of 14th Edition of Diseases of Poultry

David Swayne

USDA/ARS/SEPRL

The first edition of Diseases of Poultry was published in 1943 with 13 additional editions published to date with over 20,000 copies sold worldwide. The 14th edition has been extensively revised:

•The beginning of each chapter contains a short concise summary of the most important aspects of the etiology, clinical features, diagnostic criteria, and prevention and treatment strategies

•Provides more clinically relevant information on management of specific diseases, contributed by seven clinical poultry veterinarians

•Much of the historical information and antiquated or historical diagnostic tests were removed, and readers are referred to prior editions for in-depth coverage

•A new subchapter on Disease Prevention and Control in Antibiotic-Free Production was added

•New subchapters were added to Chapter 33: (1) White Chick Syndrome, (2) Focal Duodenal Necrosis in Table Egg Layers, (3) Wooden Breast and Other Muscle Abnormalities, and (4) Idiopathic Egg Production Drops in Brown Layers.

•Major revisions were accomplished for Chicken Infectious Anemia and Circovirus Infections in Commercial Flocks, Avian Reovirus Infections, Marek's Disease, Salmonella Infections, Mycoplasmosis, and Coccidiosis.

•Many figures were updates to color in the electronic version

•The paper copy is published in 2 volumes, thus reducing the breakage of the binding as seen in prior editions published as single volume

The electronic version is available in Adobe Digitals format and has word search function. The electronic version has many more color photos than the paper copy. AAAP members will receive 25% discount.

Wealth of Knowledge 2

Technical and Analytical Considerations Performing Retrospective and Prospective Studies Using Clostridial Dermatitis in Turkeys as a Model

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[1]FSTAATS, [2]Cargill Turkeys

Investigating diseases with multiple risk factors require study designs that can encompass all the possible variables with the least amount of error. The planning and design of such an investigation has to be thorough and comprehensive. Retrospective studies allow investigators the ability to establish possible associations of a number of risk factors to the outcome. The strength of these associations can be evaluated. Risk factors with the strongest associations can be tested for causation in prospective studies. This paper seeks to review both technical and analytical considerations when planning and designing epidemiological investigations based on an actual, ongoing, field research.

What Happens When You Feed Layer Ration to Chickens From 8 to 14 Weeks of Age.

Richard Fulton

Michigan State University

Three (3), reportedly 14-week-old chickens from a flock with birds that were unable to walk were submitted for necropsy. One bird stood with difficulty. One bird was unable to stand, and

another bird had valgus angular limb deformity. All the birds had atrophy of the breast muscles, "S"-shaped keel bones, and collapsed ribs. The bones were easily bent. Growth plates of the proximal tibia and proximal metatarsus were thickened grossly. Microscopic examination of the affected epiphysis revealed elongated hypertrophic zone of cartilage with normal vascular invasion. Alterations were consistent with those produced by phosphorus deficiency or calcium:phosphorus ratio imbalance. From the case history, birds were fed starter ration until 8 weeks of age at which time they were switched to an egg layer hen ration. Thus, this was a case of rickets due to a calcium:phosphorus ratio imbalance.

Development And Validation Of Two Diagnostic Real-Time PCR (TaqMan) Assays For The Detection Of Bordetella Avium From Clinical Samples

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Each sector of the poultry industry (especially Turkey) in USA and worldwide is challenged by many respiratory pathogens. One of the most important Pathogens is Bordetella avium (BA). In 2018, BA was ranked to be the sixth in a list of the most threatening health issues for the American turkey industry. Interestingly, despite its major impact and importance, BA laboratory diagnosis still depends on Bacterial isolation and identification. It is noteworthy, that very few real-time PCR assays had been developed for the diagnosis of BA. Lack of specificity limits the use of these assays as a diagnostic tool. In our work, we developed two TaqMan real-time PCR assays targeting unique specific genes only for BA. These assays showed high level of sensitivity and specificity, that , would allow rapid and accurate detection of BA directly from clinical specimens and would help in the differential diagnosis of avian respiratory diseases.

Comparison of Selected Blood Biochemistry Values in Meat-Type Chickens and Turkeys at Critical Ages using the i-STAT and VetScan2

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Clinical pathology is not commonly utilized to assess the health status or evaluate the effect of environmental manipulations or treatments of poultry in the field. Our previous studies showed that most biochemical parameters obtained with handheld analyzers are comparable to the values obtained by traditional benchtop analyzers. The goal of this study was to provide preliminary blood reference values for broilers and meat-type turkeys, using the i-STAT and VetScan2. We analyzed a total of 60 blood samples from chicken and 60 from turkeys, at 7, 21, and 35 days of age. Using the i-STAT analyzer, we measured pH, total CO2, partial pressure CO2 and O2, bicarbonate, oxygen saturation, glucose (GLU), hematocrit (HTC), ionized calcium (iCa), sodium (Na), and potassium (K) from whole heparinized blood. We used a VetScan VS2 to measure aspartate aminotransferase (AST), bile acids (BA), creatine kinase (CK), uric acid (UA), total calcium (Ca), phosphorus (P), total protein (TP), albumin (ALB), globulin (GLOB), potassium (K) and sodium (Na) in whole heparinized blood. All samples were analyzed on-site within 15

minutes of collection. Descriptive statistics and graphic representations of the data were used to summarize the values obtained for each species by age. The two most significant differences when compared to other avian reports are 1) higher pH in younger birds (Mean pH= 7.5 for both chickens and turkeys, at 7 and 21 days of age), and 2) CK values significantly higher at all ages, particularly in the older group of chickens (Mean CK (U/L) = 1104, 4241, and >14,000 in chickens and 1051, 1051, and 1073 in turkeys; at 7, and 35 days of age respectively). While values for gases and plasma ions are published in laying and breeder chickens, this is the first study for these parameters in growing meat-type chickens and turkeys. In addition, this is the first report of full serum biochemistry values in this type of birds.

The Mission and Goals of AAAP's Diversity and Inclusion Taskforce

Valerie Marcano

University of Georgia

The mission of the AAAP's Diversity and Inclusion Taskforce is to foster and embrace a climate inclusive of all AAAP members regardless of age, gender, race, sexual orientation, socioeconomic or educational backgrounds to encourage full participation in the opportunities the organization has to offer. We strive to ensure that those in leadership positions, participating in committees and receiving awards, scholarships, and research support truly reflect our membership base. Additionally, the diversity and inclusion taskforce will maintain a protocol to adapt as the AAAP membership evolves.

Goals:

1. To collect and provide demographics on the AAAP members at large and relay that information to the AAAP Board, while following appropriate guidelines for member privacy.

2. To evaluate current AAAP procedures and identify areas of strength and weakness that need to be altered/addressed to promote diversity and inclusion.

3. To assist in the development of AAAP's position on diversity and inclusion.

4. To encourage and guide policies to promote diversity and inclusion as the demographics of AAAP change.

Comparison of Turkey Biochemical Test Results Between a Handheld Blood Analyzer and a Conventional Laboratory Blood Analyzer

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A reason for the underutilization of clinical pathology in poultry is that rapid and proper sample handling is critical to obtain reliable measurements. Since many farms are far from a diagnostic laboratory, proper sample processing presents a significant challenge to poultry veterinarians. Therefore, the use of handheld blood analyzers could be a good and cost-effective option for on-farm testing. The objective of this study was to a) determine the effect of time on blood chemistry values and b) evaluate the accuracy of i-STAT and VetScan handheld blood analyzers to conventional clinical pathology benchtop analyzers. We collected blood from 60 healthy turkeys. Each sample was tested in triplicate by either a) the cartridge CD8+ in the Handheld Analyzer i-STAT or b) Avian/Reptilian Profile Plus rotor in the VetScan2. Blood samples tested with the i-STAT were then analyzed once with a GEM Premier 3000 benchtop analyzer, and samples tested with the VetScan2 were analyzed once with the

Cobas benchtop analyzer. All samples were analyzed within 60 minutes of collection. Pearson's Correlation Coefficient and Mountain Plots were used to determine whether there were significant significance between i-STAT results. A Bland-Altman assay was used to determine whether results from the handheld blood analyzers and traditional benchtop analyzers were comparable. Preliminary results indicated high (r= 0.7 – 0.89) to very high (r= 0.9 – 1.0) correlations in blood electrolytes and other biochemical parameters, but a poor correlation for gas analytes. In conclusion, the i-STAT and VetScan2 were reliable and easy to use and had an overall acceptable accuracy in turkeys.

Is Aspirin a Useful Adjunct in the Management of Vaccine Reactions and Respiratory Disease in Broilers?

John Smith

Alectryon LLC

Salicylates were the first non-steroidal anti-inflammatory drugs (NSAIDs) to be used in any species. In poultry medicine, aspirin (acetylsalicylic acid, ASA) and sodium salicylate (SS) have been used for their antipyretic, anti-inflammatory, and analgesic properties, particularly in the management of vaccine reactions and respiratory diseases. ASA is not approved by the FDA for use in poultry, including in feed. ASA is only poorly soluble in water (3 mg/mL at 25 C (77 F)), and is rapidly hydrolyzed to salicylate, so it is not amenable for use in drinking water. SS is relatively soluble in water (1246 mg/mL at 25°C), and preparations are marketed in the US for use in poultry drinking water. Since neither drug is approved by the FDA, withdrawal times have not been established. SS is estimated to have only about 60% of the antipyretic potency of aspirin in humans, on a molar basis. ASA is reputed to be a better analgesic than SS. The anti-inflammatory activity of SS seems to be comparable to that of aspirin in humans with rheumatoid arthritis.

The dose of both ASA and SS in poultry is not well-established, and has been largely extrapolated from other species, which is a risky proposition, as the metabolism of salicylates varies widely among species, and even among different species of birds. The plasma concentration of salicylic acid (SA) above which effective antipyretic, anti-inflammatory and analgesic activity can be achieved has been reported to be 50 µg /mL for several species. This level was sustained for 8.62 hours after bolus oral administration of 50 mg/Kg in chickens. One would therefore need to give two to three bolus doses per day to maintain levels about the target of 50 µg/ml, or a total daily dose of about 100-150 mg/Kg/day. Toxicity was not observed at 100 mg/Kg/day, but was observed at 200 mg/Kg/day given as two oral boluses of 100 mg/Kg each.

Oral dosing of 50 mg/Kg SS failed to decrease systemic IL-6 levels, had no significant effect on the LPS-induced increase in prostaglandin E2 plasma concentration, and did not influence the elevation in body temperature after LPS administration. SS at 100 and 200 mg/kg had an effect on temperature in the period from 1 to 5 h after LPS administration, whereas at 6 to 8 h after LPS administration the effect was reduced. So, it appears that administering about 100-150 mg/Kg/d could be relatively safe and may have some benefit. However, in an intra-articular sodium urate crystals model of nociception in domestic fowl, no analgesic effect was found after oral administration of SS at 100 mg/kg, whereas intramuscular (IM) injection at 500 mg/kg provided analgesia for 1 hour only.

Solutions of 10-12.5% ASA in alcohol (in which the ASA is soluble) are marketed for swine and poultry as unapproved drugs. The manufacturer's instructions call for an initial dose of 4 ounces per gallon of stock solution

metered at 1 ounce per gallon of drinking water (1:128) for one day, followed by 1 ounce per gallon of stock for up to 7 days. The maximum dose of 4 ounces of 12% product per gallon of stock yields a concentration of 3.75 mg/ml in the stock, which exceeds the solubility of 3 mg/ml at 25 C (77 F). Disregarding the fact that some of the dose will be lost to precipitation, this yields a final concentration of about 111 mg/gallon of drinking water when metered at 1:128. If we assume a 5-week-old bird weighing about 1.5 Kg will drink about 0.073 gallon of water a day, this bird will receive roughly 8.1 mg ASA per day, or 5.4 mg/Kg/day, about 1/9th of the suggested effective dose.

The manufacturer's instructions for administration of SS recommend 0.8 to 1.6 ounces per 1000 pounds of body weight per day, which results in 25 to 50 mg/Kg of SS per day, in line with suggested doses in the literature. The 50 mg/Kg dose is intended as a loading dose for one day, followed by 1 ounce per 1000 pounds or 30/mg/Kg. These doses are closer to the levels recommended in the literature, but may still be insufficient. A two or three-fold increase in the dose may be necessary to achieve significant effects, and these doses may be approaching toxic levels.

In summary, salicylates may provide some analgesic, antipyretic, and anti-inflammatory effects if given at sufficient doses. It appears that such doses may be approaching toxic levels, and considering the questionable efficacy at recognized safe doses, the lack of regulatory approval, and the lack of established withdrawal periods, these products should be used with caution if at all.

Evaluation of Factors Correlated with the Development of Hemorrhagic Hepatopathy in Broiler Breeder Pullets

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Hemorrhagic hepatopathy, also known as "bacterin-associated hepatopathy", is an uncommon but severe syndrome of chickens characterized by the development of increased mortality and a disseminated intravascular coagulation (DIC) -like syndrome in chickens up to 6 weeks post-vaccination. Lesions associated with the syndrome consist of a variable combination of severe injection site reactions, granulomatous hepatitis and splenitis, visceral amyloidosis, intravascular thrombosis, grossly mottled, darkened, or friable livers, splenomegaly, coagulopathies, and, most dramatically, occasional accumulation of noncoagulated hemorrhagic fluid in the coelomic cavity. While this syndrome has been qualitatively associated with bacterin administration in primary breeder technical notes and a handful of case reports in table egg layer flocks, the literature lacks information concerning the syndrome and its risk factors in broiler breeders. Nevertheless, personal communication with broiler veterinarians suggests that the syndrome occurs at a basal rate in the industry and is often resolved by modifying vaccine protocols in pullets. In this study, details of reported hemorrhagic hepatopathy cases in broiler breeder pullets were collected via veterinarian survey, anonymized, and statistically analyzed to identify risk factors for the syndrome. This evaluation is the first and only quantitative analysis of the syndrome's risk factors and the first report of hemorrhagic hepatopathy in broiler breeder pullets in the scientific literature. Results may lead to management changes by

integrators to decrease pullet mortality associated with this condition.

Posters

Bacteriology/Parasitology

Efficacy of a Hops Extract Phytobiotic Administered in the Feed for the Control of Necrotic Enteritis Caused by Clostridium perfringens in Broiler Chickens

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[1]BV Science, [2]Dr. Bata, [3]Dr. Bata

Necrotic Enteritis (NE) is a common sequela to Eimeria infection caused by intestinal dysbiosis and Clostridium perfringens (Cp) overgrowth. With the rise of antibiotic free broiler production, plant extracts are becoming commonly used therapeutics. Study set up was a randomized complete block design comparing two doses of a phytobiotic feed additive (Herbanoplex) to a non-medicated feed, to evaluate its efficacy at relieving the negative symptoms of NE caused by Cp. Groups included T1: control feed, no Cp challenge; T2: control feed, Cp challenge; T3: 750 g phytobiotic/metric ton, Cp challenge; T4: 1kg phytobiotic/MT, Cp challenge (n=64). Chicks were orally gavaged with 5,000 oocysts of E. maxima, followed by administration of 108 Cp CFU/mL on days 19, 20 and 21 to elicit NE. To assess performance parameters, feed and bird weights were taken on days 0, 14, 21 and 28 of age; and lesion scoring (scale= 0-3) was performed on d21. Non-challenged negative control birds had no NE-associated lesions or mortality. Compared to the CP challenged, control fed birds, the phytobiotic reduced mortality by 46.6% at both doses with T2 having 23.4% mortality and T3 and T4 groups both having 12.5% mortality. The reduced mortality is supported by significantly reduced NE lesion scores in T3=0.83 and T4=0.75 compared to T2=1.21 ($p<0.05$). The ability of the phytobiotic to reduce NE symptoms (mortality and lesion scores) resulted in improved gut health and improved feed conversion ratios with the T3=1.563b and the T4=1.455c compared to T2=1.788a, and T1=1.367d ($p<0.05$).

Whole Genome Analysis of Mycoplasma gallispeticum Isolates from the United States

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Despite attempts to control avian mycoplasmosis through management, vaccination, and surveillance, Mycoplasma continues to cause significant morbidity, mortality, and economic losses in poultry production. Advances have been made in the high throughput sequencing technology, making sequencing full genomes more affordable but unfortunately, the genetic basis for virulence, transmission, host adaption etc., have not been completely determined for avian Mycoplasma spp. The goal of this research was to compare 51 M. gallispeticum isolates from 1990 to 2019 from the United States in order to identify genome differences. The isolates analyzed were from 12 states and different production systems (including broilers, layers, chicken breeders, turkeys and turkey breeders). Whole genome sequencing was performed using Illumina and the isolates compared to M. gallisepticum Rlow reference genome and M. gallisepticum live-attenuated vaccine genomes. The collective contigs for each strain were annotated using fully annotated Mycoplasma reference genomes. The analysis revealed genetic differences among the isolates including

presence and absence of genes with potential roles in virulence.

Coccidia dynamics in litter and feces of chickens in a floor pen trial

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Counting oocysts in feces or litter is one method to monitor infection levels with Eimeria spp. in chickens in commercial flocks or after experimental infection. Oocyst counts shed in feces are thought to follow a typical pattern with clear peaks representing infection cycles, while oocyst counts in litter are representative for a longer time. The objective of the study was to compare oocyst counts in fresh feces and in litter of broilers for 42 days. The birds were vaccinated against coccidia in the hatchery and were kept in pens. Each pen was 150 cm in width, 200 cm in length and had 25 birds. The birds were fed a standard three-phase diet without anticoccidials. Every two to three days on Mondays, Wednesdays and Fridays, the same seven pens were sampled by collecting fresh feces and litter from at least three sites per pen. Oocysts were counted using a McMaster chamber. Median oocyst counts were higher in litter samples than in fecal samples. The variance was lower in litter samples. There were significant differences between cages in oocyst counts in feces as well as in litter, and there were no obvious shedding patterns. These results show that monitoring oocyst counts in feces and litter is of limited value in floor pen trials.

Salmonella Investigation: Finding the Needle in the Haystack

Genevieve Huard

Hendrix Genetics Ltd

As veterinarians, we fix problems. After our diagnostic, we use our resources to prevent further spreading and save birds, if possible, by implementing quarantine procedures, treatments, environment changes, etc. However, a more challenging aspect of our intervention plan is to be able to link the disease event to an infection source with facts. This is problematic, because without facts we can only presume and if the origin isn't fixed, the probability of the problem coming back is high. This presentation describes an investigation approach to a few Salmonella contaminations identified during the summer of 2019. With time and resources being limited, being able to focus our efforts is essential. After identifying Salmonella, we listed all the possible sources of contamination: air, water, feed, supply, human, pest and decontamination process. We based our investigation approach on all those factors to thoroughly screen each case and used visual tools to enhance accuracy of our sampling. The visual tools were also useful to increase the level of understanding when results were shared with farm personnel. The sampling results showed us each locations' hot spots which allowed us to focus efforts and enhance biosecurity procedures in these specific areas. Those results in combination with auditing allowed us to identify the sources of contamination and address them accordingly on most locations. We also learnt that timing is paramount. The sooner the investigation takes place from the time of identifying the contamination, the higher the chances of findings good answers.

Control of Northern Fowl Mite (Ornithonyssus sylviarum) on Egg Layer Chickens using a Fluralaner Solution Administered to Birds through Drinking Water

Faris Jirjis

Merck Animal Health

The northern fowl mite (NFM), Ornithonyssus sylviarum infests a wide variety of domestic fowl and wild birds. These mites are one of the most

important and common external parasites of commercial poultry in the United States. Mites feed on blood, and heavy mite infestations can irritate and stress poultry, reducing egg production. Fluralaner [(Carbamoyl-Benzamide-Phenyl-Isoxazoline (CBPI)] is in development by Merck Animal Health as an ectoparasiticide for treatment and control of mites in poultry (including both northern fowl mite (Ornithonyssus sylviarum) and red mite [Dermanyssus gallinae]). The objective of this study was to evaluate the field effectiveness and safety of fluralaner given orally at two single doses of 0.5 mg/kg BW, when administered 7 days apart to control northern fowl mite in commercial layers.

Genomic Analysis of Avian Pathogenic Escherichia coli (APEC) Associated with Colibacillosis using Long Read MinION Sequencing.

Catherine Logue

University of Georgia

Colibacillosis caused by Avian Pathogenic Escherichia coli (APEC) is a significant cause of loss to the poultry industry resulting in morbidity, mortality or carcass condemnation at slaughter. In broilers, the pathogen can cause a range of syndromes including cellulitis, peritonitis, colisepticemia, perihepatitis, omphalitis etc. Here, long read MinOIN sequence-based technology was used to analyze the genome of an E. coli strain isolated from the peritoneum of a chicken diagnosed with colibacillosis. The strain was identified as a lactose positive E. coli of phylogenetic group A, serogroup O:24 and was positive for 5 genes of the minimal predictors multiplex (iroN, aerJ, iss, ompTp and hlyF), suggesting it is a well-developed pathogen.

Resistance to nine antimicrobials were detected by phenotype analysis using the national antimicrobial resistance monitoring system (NARMS) panel including amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftriaxone, ceftiofur, gentamicin, streptomycin, sulfamethoxazole, and tetracycline. Plasmid replicon analysis detected the presence of a W type replicon.

Whole genome sequence analysis using MinION yielded 57,998 reads generating 146.5 MB of data with average read length of 2,525 bases and average quality score of 10.14. Preliminary screening of the sequenced strain using the comprehensive antibiotic resistance database (CARD) pipeline identified 132 genes associated with antimicrobial resistance including genes for multi-drug efflux pumps, beta-lactamase genes, tetracycline, spectinomycin, sulfonamides, fosfomycin, kanamycin and others. This strain is of interest as it is a novel APEC due to its serogroup, possession of a novel plasmid replicon, phylogenetic group and high-level prevalence of antimicrobial resistance by genotype and phenotype analysis.

Identification, serotyping and molecular characterization of Avibacterium paragallinarum isolates obtained from outbreaks of infectious coryza in commercial poultry in the northern area of Mexico, Sonora

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Avibacterium paragallinarum (AVP) is the causative agent of infectious coryza (IC), a severe respiratory disease in chickens. Moreover, AVP causes egg production losses and increased culling rates (Blackall and Soriano-Vargas, 2013). This study describes the investigation of 8 AVP isolates obtained from 8 different epidemiological unrelated cases of IC in

commercial chickens in Sonora and. some farms had a history of vaccination with trivalent vaccines.

The isolates were identified by colony growth and biochemical characteristics and conventional PCR (Chen et al., 1998). Additionally classical serotyping (Blackall et al., 1990; Soriano et al., 2004) and molecular characterization of the partial HPG2 region (Feberwee et al., 2019) was performed.

All AVP isolates were NAD dependent, catalase negative, oxidase positive and PCR positive. Five isolates belonged to serogroup C, two to serogroup A and one to serogroup B. Sequence analysis showed the presence of 3 different HPG2 genotypes, with the absence of the single point mutation at position 1516 (Feberwee et al., 2019).

These results confirmed the presence of NAD dependent AVP strains with different HPG2 genotypes in the outbreaks of IC in commercial chickens in Sonora. Moreover, the isolates belonged to different serogroups. There was no correlation between serogroup and genogroup. In previous studies, serovar B-1 was the most prevalent in Sonora (Soriano et al., 2001; 2004), now serogroup C seems more relevant. Vaccine-challenge studies are important to investigate the effectivity of available vaccines in the control l of IC in Sonora.

Spotty Liver Disease in the U.S. layers and genetic analysis of Campylobacter hepaticus

Ha-Jung Roh

Ceva Animal Health

Campylobacter hepaticus is a causative agent of Spotty Liver Disease (SLD). SLD causes significant health issue and productivity losses in layers but often overlooked.

From 2018-2019 we have received clinical samples from suspected cases of SLD. Its characteristic whitish-grey spots on the surface of livers were identified during necropsy and liver and bile were collected aseptically.

Campylobacter hepaticus was successfully isolated and identified from the samples. After confirmatory bacteriological tests, whole genome sequencing (WGS) analysis was performed to identify multiple putative virulence factors and to characterize the isolates. Sequence analysis showed six clusters of the isolates separated by geographical regions.

A couple of the isolates were selected for in-vivo virulence evaluation using an animal challenge model and in-vitro virulence evaluation using LMH cell invasion assays.

The effect of saponins on the cycling of commercial coccidiosis vaccines

Luis Gomez

Phibro Animal Health

The objective of this floor-pen study is to determine the effects on performance of a feed additive containing two saponins and fed to coccidia-vaccinated broiler chickens during the fall in the southeastern United States. The effect on oocysts per gram (OPG) was measured at different intervals with some modulation occurring at all stages where there was oocysts cycling (up to an average of 36%). The relative degree of acquired coccidial immunity was compared amongst vaccine treatments with no statistical differences between vaccine groups and the groups vaccinated and fed saponins. There was a statistical significant difference on regards to feed conversion at all stages but specifically at processing age, being better for the saponin-fed group.

Genetic and Gender influences in a Necrotic Enteritis Challenge Model in Broiler Chickens

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Necrotic enteritis is a multifactorial disease which causes a broad range of mortality and effects on performance. Infection with Eimeria sp. paired with exposure to Clostridium perfringens is well documented to recreate gross pathological lesions and mortality, but other factors are also reported to influence disease severity, including host factors, environmental conditions, and dietary composition. Genetic line and gender are host factors which have been implicated in necrotic enteritis pathogenesis. Males are suspected to be more susceptible due to their faster growth rate compared to females. Others have suggested that genetic differences also influence resistance or susceptibility to necrotic enteritis. As genetics are continually changing, these observations are likely to change over time. The objective of these studies is to evaluate susceptibility of broiler gender and genetics in a controlled necrotic enteritis challenge. In a pair of 2 x 2 factorial design experiments, each host parameter was compared separately. In the first experiment, genetic line x challenge was assessed. In the second experiment, gender was evaluated under the same challenge conditions as the first experiment. Birds in the challenged group received Eimeria oocysts at 14 days and were subsequently inoculated with Clostridium perfringens. To minimize variation in exposure due to differences in feed and water intake, each bird receiving these pathogens was inoculated by oral gavage. Mortality associated with gross necrotic enteritis lesions was documented within each group. The percent mortality served as the primary metric for the experiments in assessing susceptibility to necrotic enteritis between groups. These experiments were designed to offer poultry producers and researchers current information on how host parameters (gender and genetics) influence severity of necrotic enteritis.

Case Reports/Pathology

Update on Variant Reovirus Infections and Lameness in Ontario Broilers

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Prior to 2012, reovirus-associated lameness was only seen sporadically in Ontario broilers. In 2012-2013 and 2017-2018, outbreaks of reovirus-associated lameness resulted in significant animal welfare issues related to pain and immobility in addition to high economic losses due to flock depopulation. Birds were splay-legged and had swollen hocks impairing their ability to walk normally. Flocks tended to be of variable age and interestingly, younger flocks had more severe clinical signs, higher mortality, and cull rates. Histologic lesions included non-suppurative epicarditis and tenosynovitis with infiltrates of lymphocytes and plasma cells with formation of lymphoid nodules. Molecular testing identified several “variant” strains of avian reovirus . Clinical signs and severity of histologic lesions were often strain dependant, and cull rates ranged from 2-3% to 50%. In the spring of 2019, autogenous reovirus vaccination of Ontario broiler breeders was instituted and had a positive impact on broiler leg health in Ontario.

An Unusual Case of IBD in a Commercial Pullet Flock

Jessica Higgins[1],Eva Wallner-Pendleton[2], Linda Michel[3], Daral Jackwood[4]

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An unusual field case of Infectious Bursal Disease (IBD) was observed in eight week-old caged pullets. Enlarged, diffusely hemorrhagic bursas were observed on gross necropsy examination. This lesion has been frequently described in cases of very virulent Infectious Bursal Disease Virus (vvIBDV). A five-week-old caged pullet flock, housed in an adjacent building did not display hemorrhagic lesions in the bursa. Samples from both flocks were submitted for further testing, including histopathology, serology, virus isolation, molecular diagnostics and challenge studies. Molecular diagnosis included RT-PCR and nucleotide sequencing of the hypervariable region of the VP2 gene (hvVP2). The predicted amino acid sequence of the hvVP2 region indicated the IBDV observed in both flocks was identical and was not a vvIBDV. Their sequences were similar to an IBDV from Ontario Canada (EF138967). No mortality was observed when the virus was orally inoculated into specific-pathogen-free, four-week-old pullets. Gross and microscopic lesions in the bursa tissue were typical of classical IBD. The hemorrhagic lesions observed in the original field case were not reproduced in the challenged SPF pullets.

Histopathologic Investigation of Poultry Processing Quality Problems

Frederic Hoerr

Veterinary Diagnostic Pathology, LLC

This presentation describes a practical application of histopathology to define and resolve issues in the quality assurance of processed poultry.

Case Study – Can We Accurately Predict Bird Weights at the Plant?

Albert Payne

1010 Consulting Group

As the degree of automation increases in the broiler processing plant, bird uniformity and weight predictability are vital. The dynamic world of poultry sales puts very stringent requirements on bird size as they compete in the market place. The ability of production professionals to accurately plan availability and maximize production efficiency is dependent on a number of factors. These include but are not limited to individual producer profile, disease status, nutrition program, season of the year, as well as multiple production considerations.

Broiler chickens do not grow in a linear progression each day. As factors combine with genetic variation, the ability to accurately predict broiler live weights on a consistent basis can prove to be very allusive. The use of simple averages across the producer profile often leaves a large number of farms/houses outside the acceptable weight range. This narrow window may be as small as +/- 0.10 to 0.15 pounds. Given the inherent variation in live animals, new methods must be used to meet the ever-important requirement of bird weight accuracy. This case study will review the use of advanced analytics to assist in this production dilemma.

Adventures In Seroconversion: Initial Failure To Respond to Chicken Infectious Anemia Virus Vaccination

Ian Rubinoff

Hy-Line International

Chicken Infectious Anemia Virus (CIAV) is a non-enveloped Gyrovirus characterized by marked anemia and immunosuppression due to thymic atrophy. While all chickens can be infected by CIAV, only chicks between 2 and 4 weeks of age usually show the clinical signs. Ensuring 100% seroconversion to CIAV in breeder flocks is a global standard to provide protection to chicks. Usual vaccination practices around the world involve one application of live CIAV vaccine via wing-web (WW), intramuscular (IM), or subcutaneous (SC) injection between 8 and 15 weeks of age.

In the subject flock, the brown egg breeder hens had 60% seroconversion from wing web CIAV vaccination at 56 days. There was one sister flock of white leghorn parent stock reared on the same farm and vaccinated with the same serial of vaccines. This flock seroconverted 100% after one wing-web inoculation. The low seroconversion in the brown PS flock prompted an IM injection at 91 days. After the second injection, the seroconversion dropped to 30%. At 174 days and 5 vaccinations, the flock was up to 55% seroconversion. Currently at 52 weeks, the flock has been vaccinated 9 times by 4 methods and 4 different CIAV vaccine strains while demonstrating titers between 75% and 90% positive. Interestingly, all other vaccine titers (bronchitis, Newcastle, avian encephalomyelitis) are completely normal indicating there is no issue with immunosuppression.

Please Keep Your Copper In Pennies, Not My Birds; A Field Case Report Of Copper Toxicity In 10 Day Old Poults

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Case report of three (3) turkey farms experiencing a severe and sudden increase in mortality. All affected flocks were approximately 10-days old at the time of onset. The initial clinical presentation of enteritis suggested Salmonellosis, however poults did not respond to appropriate antibiotic treatment. Further necropsies and diagnostic testing, as well as feed sample results showed a toxic level of copper in the feed and the poults. This case report will elaborate on this atypical presentation of copper toxicity, as well as demonstrate the importance of communication and teamwork amongst all levels of the company.

Histopathologic and Bacteriologic Characterization of Femoral Head Separation, Femoral Head Necrosis and Bacterial Chondronecrosis with Osteomyelitis in Broilers

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Histologic and bacteriologic features for groups of average 31-day-old broilers manifesting with 3 gross categories of femoral head alterations were documented. Categories included normal, simple femoral head separation (FHS), transitional changes (FHT), and femoral head necrosis (FHN). Groups having normal femoral heads and “cull-birds” having FHN with gross signs of sepsis (FHN-cull) respectively provided negative and positive control groups. There was a 9% occurrence of positive bacterial cultures for all birds tested. However, while only a 12%

occurrence was seen for the FHS group, and no positives in the FHT or FHN groups, 29% of the samples were positive for the FHN-cull group. A 11% total occurrence of femoral bacterial chondronecrosis with osteomyelitis or simple osteomyelitis (BCO-O) was observed. A progressive increase in the group inclusive occurrence of BCO-O was apparent progressing from normal (0%), FHS (4%), FHN (13%), and reaching a maximum of 36% in the FHN-cull group. A 24% overall occurrence and clear relationship between microscopic pathology and gross diagnostic category was also seen for hip synovitis-arthritis. Synovitis was not seen within the normal group, was present only at a modest occurrence within the FHS group, but at a high occurrence within the FHT, FHN and the "cull" groups. The study underlines the importance of documenting histologic and bacteriologic status in addition to gross features when conducting studies on femoral head disorder.

Vaccinology/Immunology

Immune responses to avian influenza virus infection in two genetically distinct, highly inbred chicken lines

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Avian influenza virus (AIV) causes an economically important viral infectious disease affecting both wild and domestic birds. Infection results in disease ranging from subclinical infection to respiratory disease and reduced egg production to severe, systemic disease approaching 100% mortality. MHC class I and II are involved in antigen presentation following AIV infection, and AIV-induced downregulation has been demonstrated previously. Furthermore, transcriptomic analysis has revealed different levels of downregulation of MHC class I antigen and several related genes between two genetically distinct, highly inbred chicken lines that differ in AIV resistance. In our study, we evaluated antigen presentation markers and compared innate and adaptive components of the immune response to AIV infection in Fayoumi (more resistant) and Leghorn (less resistant) chickens.

Three-week-old Fayoumi and Leghorn chickens were inoculated intranasally and intratracheally with 107 50% embryo infectious dose (EID50) of low-pathogenic AIV H6N2. At 4 days post-infection (dpi), clinical signs were recorded, tracheal swabs were collected for qRT-PCR, and gross pathology was evaluated. Trachea and lung were collected for histopathology and cytokine mRNA expression, and flow cytometry and immunofluorescence were performed to identify MHC class I and II expression. At 7 and 14 dpi, tears and serum were collected to determine local and systemic antibody responses.

Fayoumis had significantly lower AIV titers and higher AIV-specific serum IgG titers compared to Leghorns. Other samples are currently being processed.

Safety of Fowl Poxvirus-based Vector Vaccines in Commercial Layers

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Mycoplasma gallisepticum (MG) and Infectious Laryngotracheitis virus (ILTV) cause severe respiratory disease in commercial farms. Normally, biosecurity measures are not enough to control MG and ILT in multiple-age

commercial farms. In addition, early infections demand the establishment of immunity against these pathogens from the first week of age. Then, the safety of Fowl Poxvirus-based vector vaccines against MG and ILT was tested in one-day old commercial layers. Two groups of 70 one-day-old-layers were immunized separately with Fowl Poxvirus-based vector vaccine expressing key immunogenic antigens of MG and ILTV. One non-vaccinated group was maintained as control. The safety of vaccinated groups was evaluated based on clinical aspect, viability, body weight and microscopic changes. In addition, seroconversion against MG and ILT was verified using commercial indirect ELISA kits (Biocheck and IDvet, respectively). Mortality was not observed till four weeks post vaccination. Vaccinated groups presented body weight similar to the non-vaccinated one, with uniformity of weight between 83 and 90%. The histopathological evaluation confirmed the safety of the Fowl Pox vector vaccines because very mild microscopic changes in trachea, liver and spleen were found in the vaccinated groups at 10 days post vaccination. Seroconversion detected at three and four weeks post vaccination demonstrates the vaccine poxvirus replication (until 100% of antibody positivity). The results of these controlled trials show the safety of live Poxvirus-based vector vaccines that can be used from the first day of age in order to stimulate early immunity against MG and ILT in commercial birds.

Effect of a complex antibody antigen vaccine on the decay of Infectious bursal disease maternal antibodies in broilers

Alfredo Condemarin

Hipra Peru SAC

Evaluate the decay of maternal gumboro antibodies in broiles with the aim to improve the determination of the age of vaccination with live vaccines and to knoe if a cmplex antigen antibody complex vaccine impacts on this decay

Comparison of Serological Response, Persistence and Serological Profile in Progeny against Chicken Infectious Anemia Virus in Three Flocks of Broiler Breeders

Bruno Garcia[1], Eliana Icochea[2], Josue Sanchez[3]

[1]San Marcos University, [2]School of Veterinary Medicine, Nacional University of San Marcos, [3]IDEXX Laboratory

Chicken Infectious Anemia Virus (CAV) has a worldwide distribution in poultry industry which is knowledge for its clinical and subclinical effects. It is knowing the clinical disease has a low prevalence with current measurements of control, but the subclinical immunosuppression is more complicated for evaluate and control. A practical way for monitored the status of protection is the serology. For this reason, the serological response against (CAV) and the serological profile in progeny were compared in three flocks of broiler breeders vaccinated with a commercial vaccine containing live low-attenuation strain (CUX-1) applied orally at 14 weeks old. This study showed that under field conditions with the application of a single live vaccine against CAV to broiler breeder flocks was possible the control of clinical disease but humoral response in the breeders and progeny was nonuniform, indicating that it did not induce high and uniform levels of antibodies in the progeny. Poultry industry professionals interested in discussing one of the most used programs of control in CAV will find this study very interesting.

Evaluation of a Novel Autogenous Bacterin Strain for Controlling Escherichia coli Infections in Poultry

Karen Grogan

University of Georgia

Escherichia coli is an important opportunist pathogen of poultry, causing significant economic loss from carcass condemnations, air sacculitis, septicemia, peritonitis, and cellulitis. Avian pathogenic E. coli (APEC) exhibit significant O serogroup diversity and as protective immunity is serogroup specific; vaccination has been a moving target for this pathogen. The O-antigen represents the terminal part of the LPS surface molecule and APEC do possess conserved antigens in the LPS. The LPS core structure is not diverse and only two types are found among APEC. Our central hypothesis is that the core LPS structure will produce cross-reactive, protective antibodies.

A culture method was developed that inhibits O-antigen production, providing an easy method to remove the O-antigen from APEC field isolates. This technique offers the industry the ability to acquire cross-protective bacterins from autogenous strains in a short period of time. Autogenous E. coli bacterins were produced by a licensed biologics company for evaluation in a safety study and a field efficacy study. The safety study was performed to evaluate vaccine reactivity. After the completion of the safety study, the vaccine was evaluated in commercial production systems, both layer and broiler breeder pullets. Pullets were vaccinated during standard handling periods and followed throughout production to assess post-vaccination reaction and production parameters. Paired pullet/hen houses were used, with the unvaccinated house acting as the control. The presentation will present data from the both the safety and field efficacy study.

Early Onset of Immunity and Duration of Immunity of A Recombinant HVT-IBD Vaccine against Virulent, Variant, and Very Virulent IBD in SPF Birds

Angela Hartman[1], Amy Brown[2], Megan Bosserd[3], Lauren Taylor[4]

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

A recombinant HVT-IBD vaccine was developed as a bi-valent vaccine for protection against infectious bursal disease (IBD) and Marek's disease, two highly contagious diseases causing fatality, condemnations, and immunosuppression in broilers. Data supporting an early onset of immunity against four IBD challenges predominant in different global regions and duration of immunity will be presented. SPF chickens were vaccinated with the HVT-IBD vaccine in ovo or subcutaneously at hatch and challenged with either classical virulent, variant E (Del-E), variant AL-2 IDB challenges at 14 days of age or with a very virulent IBD challenge at 12 days of age. Duration of immunity was established by challenging HVT-IBD in ovo vaccinated SPF birds at 63 days of age with a classical virulent IBD challenge. The vaccine showed 96-98% efficacy against classical virulent IBD moratlity and gross lesions, 58% and 78% protection against variant AL-2 and DelE challenges based on bursa to body weight ratios, and 90-92.5% protection against very virulent IBD mortality, clinical signs and histological lesion scores. The HVT-IBD vaccine also showed 100% protection against classical virulent IBD mortality and gross lesions following a Day 63 challenge.

Development and characterization of novel mouse monoclonal antibodies against chicken chemokine CC motif ligand 4

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Chemokine (C-C motif) ligand (CCL) 4 is a CC chemokine subfamily member defined by the sequential positioning of conserved cysteine residues. Upon the binding of G-protein-coupled receptors on the cell surface, CCL4 mediates a diverse set of biological processes including chemotaxis, tumorigenesis, homeostasis and thymopoiesis. Although the physiological roles of mammalian CCL4s were elucidated > 20 years ago, there is limited information on the biological activities of chicken CCL4 (chCCL4). In the present study, we developed and characterized mouse monoclonal antibodies (mAbs) against chCCL4 to characterize better the immunological properties of chCCL4. Out of initial screening of > 400 clones, two mAbs detecting chCCL4, 1A12 and 15D9, were identified and characterized using western blotting and chCCL4-specific antigen-capture ELISA, and their neutralizing activity was validated by chCCL4-induced peripheral blood mononuclear cell chemotaxis assay. Furthermore, the intracellular expression of chCCL4 in various chicken cells by immunocytochemistry and flow cytometry was confirmed using 1A12 and 15D9 mAbs. These results collectively indicate that 1A12 and 15D9 mAbs specifically detect chicken CCL4 and they will be valuable immune reagents for basic and applied studies in avian immunology.

Duration of immunity of ts-11 Mycoplasma gallisepticum vaccine

Chris Morrow

Bioproperties

It seems to have become conventional wisdom that the duration of immunity from MG ts-11 vaccination is forty weeks. As far as I can determine there is no studies to show this. The original duration of immunity experiments look at immunity for upto 40 weeks post vaccination but there was no end points demonstrated. Often serology from vaccination increases around 40 weeks of age but wild strains or problems are often not demonstrated. Revaccination with F strain has been advocated but is it necessary? Antibiotic salesmen in some countries are reluctant to see sales disappear. Certainly some studies have shown protection to 57 weeks (Barbour et al 2000) and the ability to detect ts-11 populations increasing after 40 weeks in many flocks.

Experience in the field using molecular detection does not support a 40 duration of immunity but rather field strains are excluded if ts-11 is present.

Compatibility of Procerta HVT-ND Vaccine with Magniplex to Provide Protection Against Velogenic NDV and Virulent Classical IBDV Challenge in SPF Birds

Sing Rong[4], Kelly Turner-Alston[2], Megan Bosserd[3], Lauren Taylor[4]

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Due to vaccination routines in the field, there is a strong desire of administering HVT-ND vaccine and immune complex IBD vaccine (Magniplex) at the same time. Studies were conducted to determine the compatibility of HVT-ND and Magniplex when administered together,

followed by either an infectious bursal disease virus (IBDV) or Newcastle disease virus (NDV) challenge. Excellent IBDV and NDV efficacy were observed.

Development of a New Trivalent HVT-vectored Vaccine Against Marek's Disease, Infectious Bursal Disease and Infectious Laryngotracheitis

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Infectious Laryngotracheitis (ILT), Infectious bursal disease (IBD) and Marek's disease (MD) are important diseases in commercial poultry, currently controlled with vaccination. This control routinely includes the use of recombinant HVT vaccines, and the use of this vector technology has proven to be an effective tool for several diseases of poultry, with a well-known safety profile, hatchery application and long-term protection. However, due to interference, administering two or more HVT vaccines together creates compatibility issues when designing a vaccination program. Aiming to provide a solution to control MD, ILT and IBD with a single vaccine, a new and unique recombinant vHVT317-IBD-ILT construct has been developed. This dual-insert construct has a vHVT013 (VAXXITEK HVT+IBD) backbone with one additional insert expressing a glycoprotein D (gD) gene from ILTV. Both IBDV and ILTV genes are expressed from a single promoter. The vHVT317-IBD-ILT construct was shown to be safe for chickens, non-target avian species and mammalian species. The construct was also genetically stable after five in vitro passages, and it did not revert to virulence after five back passages in chickens. The construct had similar tissue tropism and environmental safety as the HVT parental virus. Efficacy was demonstrated after either subcutaneous or in ovo vaccination, against virulent MD, IBD and ILT challenges. The unique vHVT317-IBD-ILT vector vaccine provides an additional tool for poultry veterinarians, allowing for control of MD, IBD and ILT with a single vaccination, and providing flexibility when designing the optimal vaccination program adapted to their specific epidemiological situation

Reverse genetic infectious bursal disease virus in turkeys

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Infectious bursal disease virus (IBDV) can infect turkeys and induce neutralizing antibodies but not causes clinical disease and bursal atrophy. The purpose of the present study was to determine whether reverse genetic (RG) IBDV could potentially serve as a viral vector for delivering the genes of interest in turkeys. One-day-old turkey poults were orally inoculated with 2x107 egg infectious dose (EID50) of wild-type variant E (VE) IBDV, 2x107 tissue culture infectious dose (TCID50) of reverse genetic VE-IBDV (RG-IBDV), or 2x105.6 TCID50 of IBDV VP3-deleted RG-IBDV fused with green fluorescence protein (GFP) (GFP-RG-IBDV). Turkey poults were observed for 21 days and clinical signs and lesions were recorded. Tissue (thymus, spleen, liver, ileum, bursa of Fabricius, and cecal tonsil) were collected at 1, 3, 10, and 21 days post inoculation (DPI) for histopathology and immunofluorescence antibody (IFA) assay. Sera were obtained at 3, and 21 DPI for virus neutralization (VN) tests. Clinical signs and grossly visible bursal atrophy were not seen in any of turkey poults in each group. Histopathologic examination of various tissues did not reveal any microscopic alterations, including bursal atrophy, in the turkey poults

from each group. The highest percentage of IBDV antigen in the bursa detected by IFA from each group was 100% for VE-IBDV group at 10 DPI, 60% for RG-IBDV group at 3 DPI, and 50% for GFP-RG-IBDV at 10 DPI. Varying degree of green fluorescence for IBDV in bursal cells was presented in the bursae of turkey poults in each group until 21 DPI. The highest VN titer obtained from the sera of turkey poults in each group was 1/16384 for RG-IBDV group at 10 DPI, 1/1024 for VE-IBDV group at 10 DPI, and 1/16384 for GFP-RG-IBDV group at 10 DPI. The results indicated reverse genetic IBDV caused infection to turkeys without significant clinical signs and lesions and elicited neutralizing antibody to IBDV, suggesting that reverse genetic IBDV could potentially serve as a viral vector for delivering the genes of interest in turkeys.

Virology: Non-Respiratory Viruses

Vaccine Take Monitoring in Broilers Flocks Immunized against Gumboro Disease with Immune Complex and Vector Vaccines and its Relationship with Productivity

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Immune complex (IC) and HVT-based vector vaccines are used extensively worldwide to protect farms broilers against infection by Infectious Bursal Disease (IBD) viruses (IBDV). These vaccines can be applied in ovo or at day one of age in the hatchery since they are not affected by Maternal Derives Antibodies. In order to evaluate the performance of IC and HVT-IBD vector vaccines in broiler farms and under field conditions, vaccine take and productivity of 36 broiler flocks were investigated. The study was conducted in two farms using IC vaccine (W2512 strain) and HVT-IBD vector vaccine (14 flocks); and IC vaccine (W2512 strain) and HVT-IBD-ND vector vaccine (22 flocks), respectively. Bursa size and weight, microscopic changes, seroconversion, and molecular detection and characterization of IBDV were used to evaluate vaccine take and field virus infection whereas feed conversion, daily body weight gain, mortality and slaughterhouse condemnations were calculated and compared to evaluate protective performance of the vaccination programs. 92% of flocks receiving HVT-IBD vector vaccines showed evidence of field virus infection and replication in bursa according to the macroscopic and laboratory evaluation (low bursa weight and size, lymphoid depletion and high seroconversion). The IBD field viruses were not able to cause clinical disease but they affected sub-clinically the productivity because the lower productive performance in the farms and the lower carcass yield in the slaughterhouse compared to flocks immunized with IC vaccine. This work shows the relevance of the vaccine take monitoring that is related to productivity of the flocks.

Update on IBD Viruses (IBDVs) Infecting Broilers Today and How Inactivated Vaccines Protect Against Different Viruses in the AL2 and T1 Families

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

This presentation will summarize the findings and trends in IBDV isolations from broiler flocks over the last several years (2014-19). In addition, results will be compared to a similar survey conducted between 2009-11 (Western Poultry Disease Conference, 2012) to see how the field challenge picture has changed over the past decade. Compared to the 2009-11 survey, AL2

viruses remain the most prevalent type—making up over half of all field virus isolations. T1 viruses were again the second most common type recovered. New-types saw a big jump from 2% to 15%, perhaps at the expense of Del-E types, which fell from 21% to 9%. Finally, two bird studies will be presented that compare protection levels of inactivated vaccines against prototypes AL2 and T1, respectively, and their related subtypes. The results will show that AL2 protection levels varied by commercial vaccines and their antigenic makeup. The T1 results are pending but will be presented at the meeting.

A Serologic Survey for Astrovirus Antibody in Georgia Broilers

James Davis

Georgia Poultry Laboratory Network

Astroviruses have been associated with poor growth performance in broilers. Our survey was conducted to see how prevalent Astrovirus infections were in broilers.

Post Marek's Disease Vaccination Monitoring with Feather Pulp and Spleen in (Layer/Breeder) by On-Side Fully Automated POCKIT Central PCR system

Fu Choong Keat[1], Wei-Fen Tsai, Ping-Han Chung, Chuan-Fu Tsai, Nina Chen, and Simon Chung

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Marek's Disease (MD) causes serious economic problems especially to the long-living birds (layers and breeders) and vaccination with CVI988 (Rispens) is considered the "gold standard" for prevention. MD vaccination is applied by subcutaneous route at day of age at the hatchery. Inappropriate storage/reconstitution and incorrect administration of vaccine lead to vaccination failure, which results in sub-optimal vaccinal protection of flocks. Post MD vaccination monitoring provides a good indicator for vaccination effectiveness/uniformity and re-boost vaccination might be required as early as possible if the vaccination effectiveness/uniformity is poor.

On-Side Fully Automated POCKIT Central PCR system is suitable for timely detection of MD vaccination effectiveness/uniformity. In this study, spleen and feather pulp were collected for evaluation from day of age, 3, 7, 10, 14, and 21. The MD positive rate was up to 100% at day 7 both in spleen and feather pulp. Comparing the Ct value between 2 sample types from the same chicken showed the MD titer in spleen was higher than in feather pulp. Most sample got a Ct value >31, which indicates < 100 copies/reaction and a high sensitivity method is needed for evaluation.

In summary, POCKIT Central allows the farmers to evaluate MD vaccination effectiveness/uniformity on-site with simple steps and gets the results in 85 minutes. Both spleen and feather pulp are ideal sample type, but feather pulp is even better because of less invasiveness. Re-boost vaccination can be applied as early as possible if the vaccination effectiveness/uniformity is poor.

Pathogenicity of infectious bursal disease viruses isolated from commercial poultry with immune suppression related disease.

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Infectious bursal disease virus (IBDV) can be isolated from most commercial broiler and pullet flocks in the US once anti-IBDV maternal antibodies wane. Genetic characterization of these viruses is typically conducted using RT-PCR followed by sequence analysis of the VP2 gene. There are specific amino acids in VP2 (253Q and

284A) that have been linked to virulence and the VP1 polymerase also plays a role in the severity of disease caused by very virulent (vv)IBDV but a complete understanding of the genetic basis for pathogenicity has yet to be realized. Testing isolates of IBDV for pathogenicity can be done in susceptible specific-pathogen-free (SPF) chicks but there are some variables that are difficult to control in these studies. In this experiment, we examined the pathogenicity of 7 new IBDV isolates in SPF pullets and compared them to the 1/chicken/USA/T1/2001 (G2) isolate (AF281238.1) from Georgia, USA. Clinical signs of disease, bursa/body weight ratios and histopathology of the bursa were used to assess pathogenicity. The results indicated that all 7 isolates were pathogenic in SPF chicks. This study highlights the problematic issues and variables related to comparing virulence among IBDV isolates.

Investigation of the virulence of Marek's disease virus with virulence-associated gene modification

Taejoong Kim

USDA-ARS-USNPRC

Marek's disease virus (MDV) has evolved toward greater levels of virulence, and three virulent pathotypes, virulent, very virulent, and very virulent plus, are currently recognized. Since no current vaccines provide sterilizing immunity and the implementation of different vaccines parallels the evolution of the higher virulent pathotypes, the specific mutations associated with these changes has been the subject of comparative genomic studies. These studies have mainly focus on the genes within the repeat long region of the MDV genome and its role in the evolution of MDV toward greater virulence. We recently characterized 70 MDV genomes with known virulence by full-genome or targeted region DNA sequencing and identified single nucleotide polymorphisms (SNPs) that showed association with virulence in 8 genes UL22, UL36, UL37, UL41, UL43, R-LORF8, R-LORF7, and ICP4. Using the BAC containing the genome of very virulent plus MDV strain 686, specific base pair changes were introduced in loss-of-function experiments to investigate the effects of these SNPs in the MDV virulence. Nine SNPs in eight genes within a single BAC genome were introduced and verified by targeted sequencing. The pathogenicity of the reconstituted virus from the modified 686-BAC recombinants and that of the 686-BAC-derived parental virus will be discussed.

Genomics and pathogenicity of an Indiana turkey coronavirus isolate

Tsang Long Lin

Purdue University, CVM

Turkey coronavirus (TCoV) causes atrophic enteritis and uneven growth in the turkey flocks. A TCoV isolate was isolated from a turkey flock without significant clinical signs in Indiana (IN) in 2009. The purpose of the present study was to analyze and compare the 3'-end genomic sequences of TCoV isolated in IN in 2009 (IN-287/09) and in 1994 (IN-540/94) and to determine the pathogenicity of TCoV isolate IN-287/09. The 3'-end 7.4kb genome of IN-287/09 was sequenced. The two IN TCoV isolates from different time periods shared the same 3'-end genomic organization. The nucleotide identity of 3'-end 7.4kb genomic sequences was 95.1%, 92.5%, and 93.5% between IN-287/09 and IN-540/94, MN-ATCC/76 (TCoV isolated in Minnesota in 1976) and IN-540/94, and MN-ATCC/76 and IN-287/09, respectively. The spike (S) genes of two IN TCoV isolates shared the highest nucleotide and deduced amino acid homology. IN-287/09 and IN-540/94 belonged to the same serotype based on the cross-neutralization assay. One-week old turkey poults challenged orally with 105 EID50/ml/turkey of IN-287/09 did not have watery diarrhea and

other gross lesions. Body weight gain in IN-287/09-infected turkey poults was not significantly different ($p>0.05$) from that in the negative control. The jejunum and ileum of IN287/09-infected turkeys only had mild segmental multifocal villous blunting and irregularity at 3 and 7dpi. The enterocytes lining the jejunum and ileum of turkey poults challenged with IN287/09 were positive for TCoV by immunofluorescent antibody assay (IFA) at 3dpi only. In conclusion, the TCoV isolates IN-287/09 and IN-540/94 shared high genetic homology in the 3'-end genome and had high degree of antigenic relatedness. The TCoV isolate IN-287/09 did not cause significant clinical signs and severe intestinal pathology.

Next Generation Sequencing (NGS) of chicken astrovirus (CAstV) isolated from outbreaks of White Chicken Syndrome (WCS) in Western Canada

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In the last 5 years, a condition known as White Chicken Syndrome (WCS), has emerged as an economically important disease across Western Canada. The etiological agent of WCS is chicken astrovirus (CAstV). The affected flocks had a history of drop of production in the breeder flock at the time of lay, very poor hatchability of eggs and poor viability of hatched chicks. These affected chicks are very white in colour with green livers. The present study was directed to answer one research question: 1) What is the genetic diversity of the CAstV obtained from outbreaks of WCS in Western Canada? To address this question, we isolated CAstVs from liver and intestinal samples originated from affected chicks showing clinical signs. Then, the extracted nucleic acids were subjected to NGS. The whole genome sequences obtained in Canada were compared to available whole genome sequences available in public domain and results will be discussed in detail at the conference. The outcomes of the present project will help clarify the molecular nature of field CAstV-challenge in Western Canada facilitating sustainable control measures.

Molecular and pathological characterization of avian reovirus from clinical cases of tenosynovitis in poultry flocks from Costa Rica

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Avian reovirus (ARV) is the mayor cause of viral arthritis/tenosynovitis in young broiler chickens. It´s characterized by swelling of the foot pad and the hock joint, which leads to lameness, causing an economic impact in the poultry industry.

ARV belongs to the Orthoreovirus genus in the family Reoviridae. The Sigma C protein is expressed by the S1 gene, which is the most variable. This gen is used as a genetic marker to be characterized and classify reovirus isolates into different genotype.

During an eleven months period 32 samples of tendon and joint tissues of broiler chickens with lameness, tendon swelling, and inflammation were analyzed by RT-PCR for the S1 segment using published P1/P4 primers. A wide variety of ages were affected ranging from 1 to 42 days.

Five samples were sequenced by Sanger technique. A phylogenetic analysis was performed on partial segment of S1. Neighbor-joining tree were generated, and robustness was tested with 1000 bootstrap replicates. All analyses were carried out using MEGA 7. Four of them were classified as genotype 5 and one as genotype 2.

Histopathological analysis of tendons and synovial bands showed synovial epithelial hyperplasia and an inflammatory infiltrate composed of heterophils, lymphocytes, plasma cells and histiocytes with foci of fibrin. A fibrino-heterophilic tenosynovitis with different degrees of severity was diagnosed in all cases. Additionally, lymphoid depletion was found in the spleen.

The aim of the study was to characterize the lesions and to detect/genotype ARVs from an outbreak of tenosynovitis in broiler poultry flocks in Costa Rica.

Serological Comparison Between Different Vaccination Programs against IBD in the Field

Elisa Russo

MSD Animal Health Srl

Infectious bursal disease (IBD) is one of the most widespread immunosuppressive poultry pathogens. In broilers IBD is controlled through vaccination, with live attenuated vaccines or turkey herpesvirus construct vaccines expressing IBDV VP2 protein.

Among a broiler integration serological monitoring program (weekly blood samples) we compared serological titers of 3 farms vaccinated with a traditional live attenuated vaccine at 21 days (according to Deventer formula, group A), 5 farms vaccinated with rHVT-ND-IBD half dose (group B) and 5 vaccinated with rHVT-ND-IBD full dose (group C).

All birds came from the same breeders and the level of maternal antibodies was alike.

At 1 and 2 weeks of age groups A and B reported a similar drop in serological titers, but group C had significantly higher titers ($p<0.01$). At 3 weeks group A had lower titers with some negative samples in all farms, group B and C had similar titers. At 4 weeks group A had lower titers than groups B and C, but titers were rising, at 5, 6 and 7 weeks all groups had similar titers.

The groups vaccinated with rHVT-ND-IBD never had negative samples, whereas the group vaccinated with traditional live attenuated vaccine had a period of susceptibility to IBD.

The group vaccinated with full dose had an earlier onset of immunity compared to half dose, this is very important for groups with weak maternal immunity, and can lead to vaccination failure in birds vaccinated with partial dose.

These data discourage the use of reduced doses of the rHVT-ND-IBD vaccine.

Virology: Respiratory Viruses

Unfavorable Consequences of Lung Macrophage Recruitment Following Infectious Bronchitis Virus (IBV) Infection

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Infectious bronchitis virus (IBV) infection in chickens is important economically for layer and broiler chicken industries. Macrophages play an essential role in innate immune responses. The information is scarce on macrophage recruitment to the respiratory tissues. The interaction between macrophages and IBV and the mechanisms which lead to virus clearance

and disease pathogenesis are also poorly understood. Our main hypothesis was that IBV infection will result in increases in macrophage numbers in respiratory tissues and the recruited macrophages become targets for IBV replication with negative consequences. In this study, first, we investigated the ability of IBV to recruit macrophages in trachea and lungs of chickens. Second, we investigated whether IBV establish productive infection in macrophages recruited to lungs and trachea in vivo. Then we confirmed our data in an in vitro system. When chickens were infected with IBV at 6 days of age, we found that IBV infection increase macrophage numbers in both trachea and lungs as determined by flow cytometry assay. Using a double immunofluorescent technique, we recorded that IBV establish productive infection in lung and tracheal macrophages. This in vivo observation was substantiated by demonstrating IBV antigens and viral particles in macrophages following in vitro IBV infection. Evaluation of the functions of macrophages following IBV infection of macrophages revealed that the production of antimicrobial molecule, nitric oxide is inhibited. Further studies are underway to uncover the significance of macrophage infection of IBV in the pathogenesis of IBV infection.

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Comparison of the protection and viral elimination of a vaccination program against Newcastle disease virus using homologous vaccines of genotype XII and a conventional vaccination program in broilers.

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Newcastle disease (EN) in Peru is detected in poultry flocks despite of vaccination and biosecurity. Conventional vaccines protect against mortality and clinical signs, but do not prevent or decrease the elimination of viruses. In Peru, the majority of ND outbreaks are caused by strains of genotype XII. This paper aims to compare the protection and viral elimination of a live and inactivated attenuated homologous vaccine program based on genotype XII and a conventional program of live and inactivated genotype II heterologous vaccines, La Sota strain. Percentage of mortality, oral and cloacal viral elimination, antibody response by HI and clinical signs were evaluated. The statistical analysis was performed with logistic regression analysis, Kruskal Wallis test and Fisher's exact test, with a significance level of 5%. On the 1st day of age, two groups of birds were vaccinated with live and inactivated La Sota and homologue of GXII, respectively. A control group was not vaccinated. The challenge was performed at day 30 of age with a virulent VEN genotype XII. Groups with the homologous vaccine suppressed or reduced the viral elimination of the challenge virus. Protection against mortality and clinical signs was more than 94% in both groups. However, the levels of antibodies specific for the homologous vaccine were not increased. These results demonstrate that homologous vaccines are capable of controlling the elimination of virulent virus, in a homologous challenge, to the environment.

Molecular Characterization of Newcastle Disease Viruses Isolated from Chickens in Tanzania

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Newcastle disease is a highly transmissible disease that affects many avian species, including domestic poultry. Infections with velogenic Newcastle disease virus (vNDV) have been reported worldwide. In Africa, vNDV is endemic and a major burden in poultry production. To investigate NDV genotypes circulating in Tanzania, the goal of this study was to molecularly characterize NDV isolates originated from village indigenous flocks and chickens obtained at live bird markets.

Twenty-four vNDV strains were isolated between 2011 and 2017. A RT-PCR reaction was performed to amplify a 788-bp fragment that included the 3' end of the M gene (173 bp) and the 5' end of the F gene, which includes the F gene hypervariable region (615 bp). Sequencing was performed on a MinION. This third-generation sequencing method allowed us to amplify the F gene hypervariable region of all isolates.

Phylogenetic analysis of the partial F gene sequences showed that, out of the 24 isolates sequenced, 15 clustered with sequences from genotype XIII, 8 with genotype V and one with genotype VII. These results will be further discussed.

Evaluation of the impact of the temperature of water used for the reconstitution of Infectious Bronchitis (IB) live attenuated vaccines

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Infectious Bronchitis is one of the major diseases in the poultry industry at global level. Vaccination is widely used in order to protect the chickens from clinical disease and reduce economic losses due to primary or secondary infections. In recent years, the temperature of water used for the IB vaccines reconstitution has been questioned and temperatures superior to 20°C have been correlated with a consequent and significant loss of IB vaccine titres. The objective of our trials was to evaluate, under experimental conditions, the impact that water temperature could have on the Boehringer Ingelheim IB live vaccines.

In the present trial two commercial IB vaccines belonging to different genotype were tested: H120 strain (Mass-type vaccine) and CR88 strain (793B-type vaccine). Each vaccine was reconstituted with drinking water at different temperature from 5 up to 30° C and the titers of the vaccine solutions were measured on Chicken Kidney Cells (CKC) immediately after the reconstitution. Furthermore, the stability of the vaccine solution titers for each vaccine were evaluated up to 120 minutes after the reconstitution on CKC.

The results showed that vaccine titers over time were comparable and at the expected level whatever the vaccine used and the temperatures tested: the temperature of water up to 30°C had a no significant impact on IB vaccine titers after reconstitution and its stability during at most 2 hours. However, it is recommended to use any vaccine in a period of time as short as possible after reconstitution as expected by best practises.

Live Attenuated Vaccine-like Viruses are Involved in the Evolution of the Canadian Delmarva (DMV/1639) Infectious Bronchitis Virus

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Infectious bronchitis (IB) results in significant economic losses in the global poultry industry. The causative agent, infectious bronchitis virus (IBV), shows marked genetic diversity due to mutations and genomic recombination. Control of IB relies on vaccination. The impact of genetic diversity of the IBV is failure of vaccination. Live attenuated vaccines belong to two serotypes, Massachusetts (Mass) and Connecticut (Conn), are commercially available in Canada. In the past few years, a number of US variant-like IBVs, predominantly DMV/1639 strain, have been increasingly isolated from IB vaccinated broiler and layer flocks in Eastern Canada. We received five archived clinical samples that had been genotyped based on partial S1 gene sequences as DMV/1639 variants for further virus isolation and detailed molecular characterization. In order to understand the evolutionary origin of the Canadian DMV/1639 isolates, we examined the whole genome sequences from the five IBVs retrieved after serial passages in specific pathogen-free (SPF) chicken embryonated eggs. Phylogenetic analyses, nucleotide sequence identities, and SimPlot analyses confirmed that recombination events involving a Conn vaccine-like strain, a 4/91 vaccine-like strain, and one strain that is yet-unidentified have led to the emergence of the Canadian DMV/1639 isolates. We are in the process of studying the pathogenesis of Canadian DMV/1639 variants both in young and adult laying chickens and optimizing a vaccination strategy to control IB caused by this IBV variant.

Infectious Bronchitis Surveillance via RT-PCR

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Animal disease surveillance is the systematic collection, management and analysis of health status data. It serves as a tool to monitor trends and perform a risk analysis.

A wide range of infectious bronchitis virus (IBV) variants circulate globally and understanding their geographical prevalence is a key factor for successful prevention. While prevalence data obtained from clinical cases submitted to diagnostic laboratories is useful, additional active monitoring for circulating IBV in non-clinical flocks can provide a baseline status to compare.

During the last 3 years, we have performed surveillance to monitor circulating IBV in sick and healthy broilers and layers, using highly sensitive real-time PCR genotyping assays. The information generated has been helpful to document the spread of certain IBV strains such as DMV1639 and GA13 into new areas and evaluate the effectiveness of vaccination programs. Active surveillance also has the potential to help detect novel strains, which is critically important for rapidly changing viruses such as IBV.

Examples and data from our IBV surveillance will be presented.

Highly pathogenic avian influenza H5N8 virus infection: pathological presentation of game bird cases during the 2016/2017 epizootic in Britain.

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Thirteen outbreaks of high pathogenic avian influenza virus (HPAIV) H5N8 were detected in poultry in England and Wales from December 2016 to June 2017. Amongst the outbreaks in England were a cluster of three infected game bird rearing farms. The initial infection of pheasants on one of these farms most likely came from the large populations of wild birds in the surrounding area, especially migratory waterfowl. Following serological and virological testing, it was determined that the virus was spread to another two linked premises by the transfer of birds between sites, a common practice in the rearing process. Disease suspicions were reported to the Department of Environment, Food and Rural affairs, which obtained official samples in according with standard EU protocols. These samples were submitted to the Animal and Plant Health Agency for diagnosis as official statutory disease investigations. H5N8 was confirmed with the molecular detection of viral RNA and virus isolation. Additional carcases from the three premises were submitted to APHA for an extensive investigation of gross pathology, histopathology and viral distribution by immunohistochemistry. Whilst gross observations in some birds were consistent with HPAIV, these finds were inconsistent within and between premises . The investigation yielded important information regarding the tropism of the virus and discovered a novel presentation of diphtheric plaques on the oropharyngeal mucosa, which was associated with a nercrotising stomatitis and viral tropism for the oropharyngeal mucosa and salivary glands. This presentation aims to increase awareness of the potential pathological presentations of HPAIV in pheasants for future disease recognition in the field. Improved detection could help prevent the distribution of virus between farms via the movement of birds and mitigate the substantial economic impacts for farmers, the wider poultry industry and trade.

The Effect of Eurasian Lineage H9N2 Low Pathogenicity Avian Influenza Virus on Chicken Egg Production and Contamination of Eggs with Virus

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The effects of infection of hens in production with an unusually virulent strain of low pathogenic avian influenza virus will be presented.

Reconstitution and Mutagenesis of Avian Infectious Laryngotracheitis Virus from Cosmid and Yeast Centromeric Plasmid Clones

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Infectious laryngotracheitis is an economically important pathogen of chicken with morbidity of

100% and mortality rates as high as 70%. Factoring in decreased production, mortality, vaccination and medication, a single outbreak can cost producers over a million dollars (1). Current live attenuated and vectored vaccines lack safety and efficacy, respectively, leaving a need for better vaccines. Since infectious bacterial artificial chromosome-based clones of infectious laryngotracheitis virus (ILTV) containing intact origins of replication are not feasible, we present the reconstitution of infectious ILTV from a collection of both yeast and bacterial clones and the identification of a nonessential insertion site within a redundant packaging site (ipac2). These constructs and the methodology necessary to manipulate them will facilitate the development of improved live virus vaccines through modification of genes encoding virulence factors and establish ILTV based viral vectors for the expression of immunogens of other avian pathogens.

Detection and Molecular Characterization of Strains of Avian Influenza Virus Isolated From Migratory Birds of The Coast of Peru, 2018-2019

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The main objective of this study was the identification and molecular characterization of strains isolated of the Avian Influenza virus (AI) in migratory birds from 04 wetlands of the central coast of Peru, during the 2018-2019 period. A total of 400 samples were taken from stool swabs and were processed by viral isolation in SPF chicken embryonated eggs and confirmation by RT-PCR test. Six samples were positive for influenza A virus, which are being processed for characterization by sequencing. This study will allow us to determine and analyze the viral subtypes circulating in the migratory birds present in the Peruvian territory and to determine the variability by comparison with the strains previously detected in our environment, as well as in Latin America.

Avian influenza virus surveillance in columbiform birds of Huarmey, Ancash-Peru

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The avian influenza (AI) virus has been isolated in many species of birds around the world. In wild birds the infection is normally asymptomatic, but H5 and H7 subtypes of virus can become highly pathogenic when they are transmitted from wild birds to domestic birds. In Peru the presence of any type of avian influenza virus in birds was never detected, however, the introduction of the virus in many areas has been demonstrated, principally due to wild bird's migration. The objective of this study is to detect the presence of AIV in wild birds that live surrounding of poultry farms in Huarmey, Ancash-Perú, located 300 km north of Lima, where there are several flocks of broiler breeders and where are prevalent columbiform birds. Fresh fecal samples are being collected since october 2019 to march 2020 because during this time occurs greatest bird migration in Perú. The samples will be processed in the Avian Pathology Laboratory at the Veterinary School, San Marcos University by virus isolation in SPF embryonated eggs. Samples will be analyzed in pools of five samples, according to specie and date and following the standard methods. The allantoid fluid will be tested by hemagglutinating inhibition assay. All positive samples will be molecularly characterized.

Characterization of variant infectious bronchitis virus from a small scale poultry holding in Egypt

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Attending of the AAAP meeting will help me to discus my work about infectious bronchitis virus characterization and evolution in Egypt through full genome sequence of IBV isolate that was isolated in 2016 from small scale production and comparing the sequence with other related sequences in the gene bank. For better understand and comparison, we did full genome sequence for an IBV isolate from 2012 isolated from large scale production. Moreover we followed up the evolution in GI-23 IBV viruses in Egypt over time period of seven years through identification of a recombination break point including gene 3ab in 26 samples from 2012 until 2019

Protection against different genotypes of Newcastle disease viruses (NDV) afforded by an adenovirus-vectored fusion protein and live NDV vaccines

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Avian metapneumovirus (AmPV) is an infectiou RNA virus associated with respiratory disease in chickens and turkeys. Three unique subtypes, A, B, and C have been recognized to cause clinical disease in poultry, but only subtype C has been identified in the United States. Detection of subtype A in Mexico it was reported in 2015. Next generation sequencing using a random detection approach was used on samples from chicken flocks in Mexico in 2019. Although this random sequence approach has limits in sensitivity, both subtype A and B AmPV was detected was samples. The use of real-time RT-PCR targeted to either A or B subtypes confirmed detection of both viruses. Sequence analysis showed the subtype A virus was highly similar to the published 2015 virus. The subtype B virus was a new detection and sequence analysis is ongoing. The presence of AmPV in commercial poultry in Mexico represents an additional respiratory pathogen of concern and a potential threat to U.S. poultry.

Wealth of Knowledge/Epidemiology/Diagnostics

Assessing perceived risks and benefits for participation in poultry disease monitoring projects

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The development and implementation of disease monitoring projects is useful for rapid communication of outbreaks and improvement of the understanding of endemic and epidemic diseases that affect animal health and producer profitability. However, producers have traditionally been reluctant to share information related to animal health, challenging the implementation of such programs.

The objective of this study was to develop a national survey aimed at poultry professionals in order to describe their beliefs toward participation in poultry disease mapping and monitoring projects; and to identify main perceived benefits and risks for participation. An anonymous online survey was developed using Qualtrics software and distributed to poultry professionals through industry associations.

A total of 60 participants filled out the survey, representing 25 states for poultry production. Layers, breeders, and pullets were the most represented commodities; followed by broilers, turkeys and game birds/backyard/small flock poultry. The majority of responders (75%) reported being familiar with such projects, with 30% stating already participating in one. Approximately half of the responders were poultry veterinarians, with the other half being represented by system owners, production managers, and administrative positions. The main perceived benefits by responders were up-to-date visualization of disease outbreaks and planning of truck routes; and the main risks were the potential for increased attention and misinformation from animal rights groups and the release of premise locations in the event of a public records request. Results from our survey highlight areas that need to be considered for future development of these programs.

West Nile Virus Infection in Commercial Duck Breeder Flocks

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A commercial duck company had a recurring problem with drops in breeder flock egg production over the past few years. The egg production drop occurred around September. Egg drops had been dramatic with 10, 20, 35, and 40 percent drops in some flocks. Four ducks from a breeder flock that experienced a recent drop in egg production from 1,700 to 1,000 eggs produced per day were submitted for necropsy. During the drop, the mortality rate increased, and some birds appeared sick. All four hens were out of egg production. One bird had a pale cardiac ventricle. Two ducks had fungal airsacculitis and pneumonia. Microscopic examination of lungs and air sacs revealed fungal hyphae that were consistent with Aspergillus sp. Cerebral blood vessels were cuffed by lymphocytes with lymphocytes within the vascular walls. The heart had multifocal necrosis with random foci of lymphocytes and mononuclear cells within the myocardium. Polymerase chain reaction (PCR) testing revealed the presence of West Nile virus RNA.

Comparative Analysis of Two New Multilocus Sequence Typing (MLST) Schemes for Characterization of Mycoplasma gallisepticum strains

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Mycoplasma gallisepticum (MG) is the most pathogenic avian mycoplasma species. It affects commercial, non-commercial poultry and wild birds. MG strain identification is essential for epidemiological outbreak investigation, prevention and control programs. Recently, two new MLST schemes have been developed independently for typing MG isolates and clinical samples. Both schemes are available for public use at pubMLST database. No direct head to head comparison has been performed to evaluate how their results are compared. Moreover, one of the two schemes has not been evaluated against a reference method. The aim of this study was to compare the characteristics of the two schemes to make a recommendation about their usage. In order to compare the

results of these two schemes and evaluate their results, we have typed a diverse collection of 81 isolates. In addition, we have compared the results of the two schemes to a whole genome based typing approach core genome multilocus sequence typing (cgMLST). All schemes were assessed based on the discriminatory power index and degree of congruency with the epidemiological information of the typed strains. The results of this study have confirmed that cgMLST had a superior discriminatory power to both MLST schemes and both MLST schemes are valuable tools for population-based studies. In addition, the higher discriminatory power of the cgMLST scheme is better and recommended for outbreak investigation. We recommend using only one MLST scheme for typing isolates globally in order to build the global database for MG and maintain its usefulness for understanding the global MG population dynamics and epidemiology.

MinION Sequencing for Selective and Rapid Detection of Avian Influenza A Virus

Iryna Goraichuk

USDA/ARS/SEPRL

Until recently, sequencing technologies have been providing data through short-read technique. A relatively new sequencing technology, the MinION nanopore sequencer, provides a platform that is much cheaper than other existing sequencing platforms with the potential of rapid sequence and analysis. The MinION allows the sequencing of individual strands of DNA and can produce millions of reads which places it in the Next Generation Sequences category. The Nanopore MinION sequencer can provide a useful and inexpensive method to obtain influenza virus genomic sequences for screening, diagnostics, and research purposes.

Design and evaluation of a device to teach euthanasia of chickens by cervical dislocation

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In conjunction with Engineering students enrolled in the University of Delaware's (UD) Senior Design Capstone Program, a device was developed to train Animal Science students to perform cervical dislocation (CD) on avian species. This method of euthanasia is a humane and approved (AVMA) method to dispatch many types of research animals. Institutional Animal Care and Use Committees require training and proof of proficiency prior to granting approval to perform the procedure. UD's Avian Virology group currently trains students on animals that have been euthanized by other methods rather than a trial by fire approach that can be traumatic to both the animals and student. The goal of this project was to capture the forces used by experienced scientists while performing CD to euthanize chickens. The data was then used to design a training device that would prevent the sacrificing of additional animals strictly for training purposes. The training device simulates the mechanics of cervical dislocation, including proper location and use of force, and provides visual feedback to the trainee and instructor as to the successful execution of CD to humanely euthanizing the chicken.

The Mission and Goals of AAAP's Diversity and Inclusion Taskforce

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The mission of the AAAP's Diversity and Inclusion Taskforce is to foster and embrace a climate inclusive of all AAAP members regardless of age, gender, race, sexual orientation, socioeconomic or educational backgrounds to encourage full participation in the opportunities the organization has to offer. We strive to ensure that those in leadership positions, participating in committees and receiving awards, scholarships, and research support truly reflect our membership base. Additionally, the diversity and inclusion taskforce will maintain a protocol to adapt as the AAAP membership evolves.

Goals:

1. To collect and provide demographics on the AAAP members at large and relay that information to the AAAP Board, while following appropriate guidelines for member privacy.

2. To evaluate current AAAP procedures and identify areas of strength and weakness that need to be altered/addressed to promote diversity and inclusion.

3. To assist in the development of AAAP's position on diversity and inclusion.

4. To encourage and guide policies to promote diversity and inclusion as the demographics of AAAP change.

Expression and Characterization of Avian Reovirus SigmaC-MBP Fusion Proteins as ELISA Antigens

Linda Michel

The Ohio State University

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. ARV strains form at least six distinct genotype clusters. The ARV protein primarily responsible for this diversity is sigma C, which induces the formation of neutralizing antibodies that protect chickens from disease. The presence of these antibodies can be detected with commercial ELISA kits. Increasing antigenic diversity of the virus, however, may decrease the effectiveness of this assay for detection of ARV antibodies. To circumvent this problem, we used a platform expression system to produce the sigma C protein fused to a maltose binding protein (MBP) tag to facilitate purification. ARV strains were isolated from infected broilers and the sigma C gene was sequenced. Genes from different genogroups were cloned into a MBP fusion plasmid transfer vector and then transfected into the genome of baculovirus under the control of the polyhedron promotor. Sigma C-MBP expressed from baculovirus cultures was purified over amylose resin and used to coat ELISA plates. An ELISA was performed using serum from chickens infected with ARV from different genogroups. The results indicate the different sigma-C MBP antigens reacted stronger to homologous ARV antibodies.

Trends in Mycotoxin Contamination in the United States Corn Crop

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Mycotoxins are secondary fungal metabolites that are detrimental to animal health and performance even at low levels. The classic signs such as reduced feed intake and oral and intestinal lesions used as indicators for exposure often underestimate other costs of mycotoxicosis, including increasing frequency and severity of disease via immunosuppression, inflammation, and modulating the gastrointestinal environment. Corn samples were submitted and analyzed for aflatoxins (Afla), type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin-A utilizing LC-MS/MS. The 2019 data were compared to previous years to examine contamination trends. Preliminary results are limited due to harvest delays reducing sample submission, but suggest a high proportion of samples contain at least one mycotoxin (96%), similar to 2018 (97%). The co-occurrence thus far is lower than 2018 (2019: 51%, 2018: 75%). Currently, B-Trich is the most prevalent mycotoxin group and appears to have lower prevalence, but similar average contamination levels compared to 2018 (2019: 75%, 1,376 ppb; 2018: 83%,686 ppb). FUM has lower prevalence and similar average levels compared to last year (2019: 54%, 2968 ppb; 2018: 68%, 2,905 ppb), while ZEN levels and prevalence have decreased (2019: 42%, 386 ppb; 2018: 54%, 604 ppb). To date, no Alfa has been detected; however, corn stored with higher moisture has increased risk for storage toxins. Thus far, the 2019 harvest has a continued risk due to mycotoxins; however, harvest delays have influenced sample submission and the risk profile of the harvest is likely to shift as additional samples are analyzed.

Characterization of Reoviral Hepatitis and Splenitis in Commercial Turkey Poults

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For the past year, a spiking mortality (2-8% loss in less than one week) affecting 10 to 25-day-old commercial poults has been observed in flocks throughout the Midwest. Poults are generally found dead without premonitory signs. The most consistent gross lesion is hepatosplenomegaly. The enlarged livers are often pale and contain numerous 1-3 mm white foci. Crops and intestines are often devoid of ingesta. Gizzards are filled with litter and have linear erosions in the koilin lining. Histopathology reveals necrotizing hepatitis and splenitis. Liver and spleen have discrete, multifocal to coalescing islands of necrosis with infiltrates of plump macrophages containing cellular debris, and varying numbers of lymphocytes, plasma cells and heterophils. The lung often contains interstitial infiltrates of mononuclear cells. Viral inclusion bodies are not observed. There is no significant bacterial growth from liver, spleen, intestine and lung. Negative contrast electron microscopy of ground liver and spleen reveals virus particles resembling reovirus. Livers are positive for reovirus by RT-PCR and by inoculation of embryonated chicken eggs and LMH cells without any indication of picornavirus or adenovirus. Initial Sanger sequencing of the reoviral gene fragments indicates identity with turkey enteric and arthritis reoviruses. These

findings indicate a novel presentation for reovirus in turkeys.

Fecal Parasite and Select Pathogen ELISA Testing on Wild Turkeys from Middle Tennessee

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This study is part of an on-going 6-year investigation on the health of wild turkeys in middle Tennessee. Fecal exams and ELISA screening on wild turkeys was preformed to determine the prevalence of fecal parasites and select pathogens and to determine the baseline health of the wild turkey population in middle Tennessee.

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

Nicki Smith

Georgia Poultry Laboratory Network

Each month, Georgia Poultry Laboratory Network (GPLN) produces a Bronchitis Early Warning System (BREWS) report to keep Georgia's commercial poultry industry informed about cases of infectious bronchitis virus (IBV) within the state. Provided via email to key individuals, a map of Georgia showing county-level IBV isolations serves as an important component of the monthly BREWS report. Today's web-mapping technologies make it possible not only to deliver this map product in an online environment, but to encode additional information using interactive elements. This project explores the development and utilization of an interactive BREWS web map as a tool to support Georgia's commercial industry.