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Avian Influenza

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CRISPR-Cas9-mediated Chicken SLC35A1 Gene Knockout and Its Role in Avian Influenza Replication

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Abstract

Avian influenza viruses (AIVs) are obligate intracellular parasites that rely on host cellular machinery for replication. A comprehensive understanding of virus-host interactions could guide the development of effective vaccines and targeted antiviral intervention strategies. Genetic perturbation platforms, such as genome-wide CRISPR knockout screens in human cells have identified several host factors that are involved in AIV replication. In this study, we conducted a meta-analysis of extant literature and identified SLC35A1, a CMP-sialic acid transporter, as a candidate gene for functional investigation in chicken cells. Utilizing CRISPR/Cas9, we targeted exon 1 of SLC35A1 in chicken fibroblast DF1 cells and generated a clonal knockout (KO) cell line. Flow cytometry and confocal microscopy analyses revealed that SLC35A1 KO cells exhibited reduced 2'-3'- and 2'-6'-linked sialic acids on the cell surface, which are primary receptors for AIVs. To assess the effect of SLC35A1 KO on AIV replication, KO cells were challenged with multiple H7N7 and H5N1 AIV strains. TCID50 assays demonstrated that SLC35A1 KO cells reduced H7N7 titers significantly compared to wild-type cells, whereas H5N1 replication was minimally affected. These findings highlight the differential role of SLC35A1 in viral replication and its implications for strain-specific restriction of virus replication. Our findings provide fundamental insights into virus-host interactions in chickens and contribute to the development of host-targeted interventions to mitigate AIV in poultry.

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Surveillance for High Pathogenicity Avian Influenza in Vaccinated Poultry Flocks

Abstract

Introduction: The International Alliance for Biological Standardization (IABS) co-organized a meeting with World Organisation for Animal Health (WOAH), Paris, France, 22-23 October 2024, to assess the latest scientific advancements and discuss strategies for high pathogenicity avian influenza (HPAI) surveillance programs in vaccinated poultry.

Results/Discussion: Sensitive, practical, cost-effective and sustainable surveillance is achievable utilizing a risk-based, multi-layered program primarily based on virological testing using highly specific and sensitive qRT-PCR assays. Targeted sampling should be used based on daily mortality of up to 15 birds (birds collected over 24-hour period and maximum of 48 hours), and if insufficient mortalities are available, supplemented by birds with specific HPAI signs or, as an alternative, birds with non-specific signs. Random samples of clinically normal chickens and turkeys have low sensitivity and high cost, but such samples may still be of value for domestic duck surveillance where HPAI infections are inconsistent at producing mortality and clinical signs. Environmental surveillance may be a suitable adjunct for early and low-cost detection of HPAI virus but will require specific validation studies. Nonvaccinated sentinel birds are costly, logistically difficult to manage, a hazard for virus amplification and should not be used. Serological surveillance for DIVA (i.e. Detection of Infected in Vaccinated Populations), has limited value being a lagging, historic indicator of infection and frequently giving false positives which requires further testing to clarify. Therefore, the DIVA feature for vaccines might not be so essential as it was previously proposed.

Conclusions: The conference conclusions and recommendations are available at:

<https://www.iabs.org/~documents/route%3A/download/2542/>.

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Protection of commercial turkeys against highly pathogenic avian influenza H5 virus of clade 2.3.4.4b using an HVT-H5 vectored vaccine

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Abstract

Highly pathogenic avian influenza (HPAI) H5 virus of clade 2.3.4.4b is a global concern for several years, including in the United States, in poultry and beyond. Turkey is one of the most susceptible species to this virus. As a complementary tool to strict biosecurity and sanitation, targeted vaccination is regarded as relevant to suppress shedding, which should reduce the amount of culling and help to protect public health.

A rHVT-H5 vaccine has been extensively tested for efficacy against numerous H5 virus clades, including 2.3.4.4b clade, in chickens. Our study aimed at testing the efficacy of this vaccine in turkeys against a recent HPAI H5 virus strain of 2.3.4.4b clade.

Forty commercial turkey poults were vaccinated at day-old; twenty of them were challenged through the intra-nasal route at seven weeks of age, whereas the remaining twenty were kept as contacts. Twenty non-vaccinated controls were directly challenged (10) or kept as contacts (10) in a similar way.

Post-challenge clinical observation lasted for fourteen days. Oro-pharyngeal and cloacal swabs were collected on 2-, 3-, 4-, and 7-days post-challenge for virus detection and quantification. Blood samples were collected and tested by HI test and by H5 and NP ELISA tests.

There was a 100% mortality in controls whereas all vaccinated turkeys survived, regardless the challenge route. A significant shedding reduction was measured in vaccinated birds compared to controls. Altogether, the data showed a satisfactory efficacy of the vaccine in turkeys against a H5 HPAI virus of 2.3.4.4b clade.

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Efficacy of the Reverse-Genetics Vaccine H5N2rg_2022 Against Highly Pathogenic Avian Influenza Viruses from Clade 2.3.4.4b in Multiple Avian Species

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Abstract

Highly pathogenic avian influenza (HPAI) viruses from the Goose Guangdong lineage, clade 2.3.4.4b, are causing unprecedented losses in the poultry industry, with spillovers to other species and threats to public health. An updated reverse-genetics vaccine, H5N2rg_2022, with the HA protein derived from the HPAI H5N1 virus responsible for the current pandemic, is under development. Extensive testing conducted through a collaboration between the vaccine industry and research institutions showed high efficacy against HPAI H5N1 viruses from clade 2.3.4.4b across multiple species. In chickens, a single dose elicited protective HA inhibition (HI) antibody titers (HI ≥ 40) in 100% of the subjects. Additionally, a challenge efficacy study confirmed 95% protection of chickens against disease caused by the HPAI virus A/Bald Eagle/Florida/W22-134-OP/2022, H5N1. In geese, prime-boost vaccination induced complete protection against disease caused by the HPAI virus A/chicken/DE-NI/AI 4286/2022, H5N1, with significant reduction in viral shedding and no infectious virus recovered from respiratory and gastrointestinal swab samples. In ducks, prime-boost vaccination resulted in 100% survival and no detectable virus shedding post-challenge with HPAI virus A/Blue Winged Teal/MB/FAV-913/2022, H5N1. Vaccinated ducks did not transmit the challenge virus to co-housed vaccinated or naïve ducks, and the highly susceptible naïve chickens and turkeys. In contrast, significant shedding occurred among unvaccinated ducks, leading to transmission and mortality in contact ducks, turkeys, and chickens. These results confirm the vaccine industry's readiness to assist in controlling the current HPAI pandemic and demonstrate the versatility of reverse-genetics technology in developing effective vaccines for HPAI.

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Development and characterization of *in vitro* chicken lung organoids as a model for H5 avian influenza virus infection

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Abstract

Highly pathogenic avian influenza viruses (HPAIV) of the H5 subtype are devastating the poultry industry, damaging the global economy, and posing a serious pandemic threat. The current lack of physiologically relevant *in vitro* models limits the ability to screen the rapidly evolving viral landscape, which has recently expanded to include species previously considered resilient to clinical disease. Organoids, which are three-dimensional, miniaturized versions of organs produced *in vitro*, recapitulate key functional, structural, and biological features of their respective *in vivo* tissues. Here, we describe the development of the first adult stem cell-derived lung organoids from SPF White Leghorn chickens. The organoids' cellular heterogeneity was confirmed using single-nuclei RNA sequencing, and their transcriptomic profiles were characterized via bulk RNA sequencing. Basic morphological features were examined using light microscopy, immunohistochemistry, immunofluorescence, and transmission electron microscopy. The chicken lung organoids successfully developed into three-dimensional structures lined with a variety of epithelial cell types, including columnar, cuboidal, squamous, and mucin-producing cells. The organoids were infected in liquid suspension with two H5 low pathogenic avian influenza viruses (LPAIVs) from a wild duck and poultry, respectively. Virus growth kinetics were assessed by tissue culture infectious dose 50 (TCID₅₀) across different multiplicity of infection. In conclusion, we developed a platform capable of modeling avian influenza virus pathogenesis in chicken lung organoids, which could aid the prediction of future viral evolution and accelerate the discovery of new antiviral targets.

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Immune and Tissue-Specific Dynamics of H9Nx AIV in Chicken Embryo Primary Cultures

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Abstract

The H9 subtype of Avian Influenza Virus (AIV) is the most widespread Low Pathogenic Avian Influenza Virus (LPAIV). Wild waterfowl are natural reservoirs, but H9 AIV infects diverse species, including humans, posing significant threats to poultry and public health. In this study, we evaluated host-pathogen interactions of H9Nx AIV strains isolated from chicken (H9CK), turkey (H9TK), ruddy turnstone (H9RT), and wood duck (H9WD) in primary cell cultures from chicken embryo tissues (fibroblast, kidney, lung, liver, trachea, and duodenum). Briefly, cell cultures were infected (MOI of 0.1), samples collected daily for 7 days post-inoculation (dpi) and cytopathic effects (CPE), viral titers (Real Time RT-PCR and TCID₅₀), and cytokine expression (IFN α , IFN β , IFN γ , IL-1 β , IL-4, IL-8, IL-10, IL-12, IL-18, and TNF α) analyzed. Distinct patterns of infection were observed across cell types, and immunofluorescence confirmed tissue-specificity and virus-cell protein co-labeling. The kidney (CEK) and trachea (CET) cell cultures were the most permissive tissues for infection, with H9CK and H9TK strains showing the highest titers (above 5 Log₁₀/ml).

TCID₅₀ assays confirmed the production of infectious particles between tissues, particularly in CEK. Cytokine expression varied by cell type and dpi, reflecting diverse tissue-specific immune responses. Overall, our findings emphasize the distinct immune and tissue-specific dynamics of H9Nx strains, providing insights into pathogenesis and virus-host interaction.

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The immunoregulatory effect of blend of *Echinacea purpurea*, oregano oil and elderberry extract on chickens vaccinated against Avian Influenza

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Abstract

Echinacea purpurea (EP) has immune enhancement, anti-viral, antioxidant, anti-inflammatory, and anti-bacterial activity. Likewise, Elderberry contains flavonoids which may have immunomodulating, anti-inflammatory, antioxidant and antiviral effects and oregano oil contains basically two terpene: carvacrol and thymol, with high antioxidant and antimicrobial activity. The aim of this study was to determine the effect of using EP + elderberry + oregano oil on growth parameters and immune response in broilers chickens vaccinated against Avian Influenza (AI). A total of 140 male chicks (one-day-old) were selected. Chicks were divided in two treatments with seven replicates (ten birds/replicate). All chickens were vaccinated against AI at 10 days-old. The groups were as follows: Treatment group (vaccinated/basal feed + EP + elderberry + oregano oil 0.5 g/kg), and Control group (basal feed). Chickens supplemented with EP + elderberry + oregano oil enhanced the final body weight and reduced feed conversion ratio ($p < 0.05$) compared with control group. In regard to immunomodulatory effect, the EP + elderberry + oregano oil group showed significant higher titers of antibodies by ELISA and hemagglutination-inhibition assays. Besides, the expression of genetic markers of IL-1, IL6, TNF- α , IFN- γ , GPx and SOD was up-regulated by the treatment group compared with control group. In conclusion, the dietary supplementation of EP + elderberry+ oregano oil enhanced the growth performance and improved immune response of AI vaccination.

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The safety and efficacy of recombinant Baculovirus-H4 vaccine against low-pathogenic avian influenza (H4N4) in turkeys

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Abstract

The baculovirus expression system is a safe vaccine delivery approved by USDA against livestock. In this study, a unique construct design enabled the expression of the HA (Haemagglutinin) gene of low pathogenic avian influenza (H4N6) on the surface of baculovirus. The *Spodoptera frugiperda* 9 (Sf-9) cell line was used to grow and titrate the recombinant baculovirus-H4 vaccine. A pilot study using 40 1-day-old turkey poults was run to establish the preliminary safety and efficacy. Significant reductions in the gross and histopathology lesion scores of collected samples were seen in the vaccinated challenge group (VCG) compared to the non-vaccinated challenge group (NVCG) suggesting the efficacy of the vaccine. Additionally, the Hemagglutination Inhibition (HI) assay showed significantly higher antibody titer in the NVCG as compared to the VCG indicating that the vaccine interfered with the replication of the virus. The viral shedding in the oropharyngeal swabs at 7 days post-challenge was quantitatively measured using real-time RT-PCR. The average viral copy number is remarkably lower in numerical value in the VCG compared to NVCG but without a statistically significant difference. This study indicates that HA surface expression on the baculovirus system could be a safe and protective recombinant vaccine against LPAI-H4N4 in turkeys. Future studies with a larger sample size and other diseases will help proving the concept of recombinant baculovirus vaccine safety and efficacy in poultry.

Keywords: Baculovirus, low path avian influenza virus, hemagglutination, recombinant, vaccine safety and efficacy

Avian Metapneumovirus

Avian Metapneumovirus maternal derived antibody evaluation in serum of day-old turkey poult

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Abstract

Avian metapneumovirus (AMPv) continues to infect turkey flocks across the United States since the emergence of the disease in the fall of 2023. In turkeys, respiratory disease from AMPv is usually seen in birds between 3 to 12 weeks of age, but birds of all ages are at risk of infection. In the first weeks of life, resistance to infectious agents is ensured significantly by specific maternal antibodies (matAb). In poultry, these antibodies are transferred through the egg to the embryo and eventually to the hatching poults. The transfer of specific matAb against AMPv was previously confirmed via the egg yolk and poult serum. Antibody titers were directly related to the circulating antibody levels in the turkey breeder hens (Kowalczyk et al., 2019). Turkey breeder hens and successive poults hatched from those flocks were bled for detection of anti-AMPv antibodies using a commercial ELISA test kit. A field evaluation of poult matAb levels was compared from breeder hen flocks at various stages in production, post-live viral exposure and post inactivated viral vaccination. Early vaccination of poults using live vaccines, when matAb titers are high, may prove ineffective because of neutralization of the vaccine. Knowledge on the level of matAb transfer from laying hens to the progeny will help to develop appropriate immunoprophylaxis schedules against AMPv.

Epidemiology of the First Reported Outbreak of Avian Metapneumovirus Subtype A in Poultry in the United States

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Abstract

The first reported commercial poultry case of avian metapneumovirus type A (aMPV-A) in the United States was identified at the California Animal Health and Food Safety (CAHFS) Laboratory in October 2023. Since then, aMPV-A has not only persisted in commercial broilers, turkeys, and layers in California but in multiple States in the US. The disease causes significant upper respiratory disease and exacerbation of opportunistic pathogens. A retrospective analysis of aMPV-A chicken and turkey commercial poultry cases submitted to CAHFS from October 2023 to September 2024 was conducted. The aim of this study is to evaluate the epidemiological behavior of avian metapneumovirus type A during the first year of this outbreak, with a focus on temporal distribution, co-morbidities, and age and poultry type affected. Ultimately, analysis of these factors provides a useful summary that can help identify potential risk factors for aMPV-A infection.

Experiences with Live Avian Metapneumovirus Vaccination

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Abstract

After its first description in South Africa in 1978 from turkeys with respiratory signs, different subtypes of Avian Metapneumovirus (aMPV), have spread across the globe. Subtypes A and B are the most prevalent and widespread serotypes found throughout Europe, Asia, LATAM and Africa. Up until the end of 2023, U.S. commercial poultry flocks have been free of A and B subtypes. However, since the first aMPV detections in the U.S., both subtypes have caused severe production losses in all types of commercial poultry. Consultants from outside the U.S. shared learning experiences for effective aMPV vaccination, including the use of live aMPV vaccines. To support the industry in their combat against aMPV, imported live aMPV vaccines were approved for experimental use by the USDA at the very end of 2024. Once introduced into the U.S., U.S. poultry specialists are documenting further lessons learned with the live aMPV vaccine inside the country. This presentation will combine experiences from outside U.S. with aMPV vaccination including various vaccination schedules used in the different types of poultry along with the first U.S. experiences after the introduction of the first live aMPV vaccine into the U.S.

Phylogenetic Analysis of Recently Isolated Avian Metapneumovirus across the Genome, Fusion, and Glycoprotein

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Abstract

The re-emergence of Avian metapneumovirus (aMPV) in the United States poses a serious economic and animal health threat to the poultry industry. Avian metapneumovirus is divided into four subtypes based on different laboratory techniques, where serology suggests that subtypes A and B are of a single serotype. Only nine complete genomes have been submitted to GenBank since the outbreak began, including only two of subtype A. The Next Generation Sequencing lab at Vaxxinova US recently obtained sequences from three field isolates of subtype A. For comparison, additional genomes and coding regions were collected from GenBank. We performed a phylogenetic analysis on these field strains of aMPV-A, including historic and contemporary strains publicly available. Alignment of the entire genomes showed 99.84 % and greater similarity among the three strains; showing a maximum 21 nucleotide differences from one another. Alignment of amino acid sequences for the Fusion and Glycoproteins displayed greater than 99.44 % and 99.71% similarity, respectively. In each phylogenetic analysis, aMPV-A_turkey/USA/CA/24-003049-001/2023 was the

most similar sequence from GenBank. The high level of similarity among the three field isolates suggests they share a common source. Because the three field isolates most closely resemble an aMPV-A from the US, this analysis suggests that aMPV-A_turkey/USA/CA/24-003049-001/2023 and these field strains likely share a common outbreak source. After the aMPV-A_turkey/USA/CA/24-003049-001/2023 strain, these isolates are most similar to Mexican strains from a 2022 publication, which supports work published in the past year by Goraichuk, *et al.*

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Isolation and Attenuation of Avian Metapneumovirus Type B (aMPV-B)

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Abstract

Avian metapneumovirus (aMPV) is a highly contagious pathogen that causes an upper respiratory tract infection, egg production losses, and immunosuppression in chickens and turkeys. Secondary pathogens and environmental conditions exacerbate the disease and result in major economic losses. Of the four recognized aMPV subtypes (A, B, C, and D), aMPV-A and aMPV-B have been the most common outside the United States, and aMPV-C has only been described in the United States in 1996. However, during the winter of 2023-2024 aMPV-B was diagnosed in the Southeast of the United States, followed by aMPV-A in the Southwest. By the summer of 2024, both viruses spread and merged in the Midwest. Our laboratory isolated two aMPV-B viruses from the affected turkeys in North Carolina. One virus was isolated after two passages in Specific-Pathogen-Free (SPF) chicken embryos, and the second virus was isolated after six passages in Vero cells. Both isolates showed inconsistent propagation in the SPF chicken embryos but replicated well in the Vero cells. Additionally, both viruses were adapted to propagate in LMH cells. After numerous passages in either Vero or LMH cells, the pathogenicity of the passaged viruses was evaluated in two-week-old turkey poults and SPF chicks in comparison to the parent virus isolate. In turkeys, all passaged viruses showed loss of pathogenicity (attenuation) while the original parent virus induced respiratory signs such as nasal discharge and foamy eyes. None of the evaluated viruses were pathogenic in SPF chickens. The attenuated aMPV-B strains are potential candidates for live modified vaccine development.

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Evaluating a commercial ELISA as tool to monitor poultry flocks for Avian Metapneumovirus exposure.

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Abstract

In the fall of 2023, a marked increase in avian metapneumovirus (aMPV) cases was reported across multiple states in the United States. Unlike historical detections of subtype C in the USA, these cases were attributed to subtypes A and B. Diagnosing aMPV is particularly challenging due to overlapping clinical signs with other respiratory diseases, necessitating the use of laboratory methods such as PCR and serology for accurate identification. In this study, serum samples from PCR-confirmed aMPV-positive chicken and turkey flocks (subtypes A or B) and aMPV-negative flocks (collected before the outbreak) were analyzed. Samples were tested with a commercial aMPV ELISA (BioChek) and a virus neutralization (VN) test specific to subtypes A and B. ELISA results were compared with VN data to evaluate diagnostic sensitivity and specificity. The commercial aMPV ELISA demonstrated high sensitivity and specificity, effectively distinguishing aMPV-positive flocks from negative ones. This study underscores the utility of ELISA as a practical and reliable tool for assessing aMPV status, supporting effective management of the disease in poultry operations.

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Popcorn Pneumo Infections: A Field Study of Viral Ct and Antibody Titers for AMPV Subtype A and B Infections and Subtype A and B Co-infections

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Abstract

Avian metapneumovirus (AMPV) is an enveloped virus and belongs to the family Pneumoviridae, genus *Metapneumovirus*. This virus presents as one of 4 subtypes (A-D); subtypes A and B were introduced into the United States in the fall of 2023. This virus primarily affects the respiratory system of poultry, and birds often present with swollen heads and sinuses, mucopurulent discharge, snick, and lethargy. AMPV is often associated with secondary colibacillosis leading to severe mortality, plant condemnations, and financial losses.

In an effort to understand transmission, length of infection, and duration of serological antibodies, eight flocks will be followed from the start of infection in the brood barn through market (~5-21 weeks of age). These eight flocks had either AMPV subtype A, B, or subtype A and B co-infections in brood. Tracheal PCR swabs will be taken weekly until birds no longer test positive for any AMPV subtype. Serology will be monitored every three weeks from ~9-21 weeks of age. Length of infection and type of infection will be compared to ending mortality rates as well as feed conversion ratio (FCR) and market weight. It is expected that coinfections of subtypes A and B will generate longer infections, longer duration of serological antibodies, and higher rates of mortality with lower body weights compared to infection of only subtype A or only subtype B.

Onset of immunity and efficacy of a Live Attenuated Subtype B aMPV Vaccine in broilers after challenge, with or without maternal antibodies

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Abstract

Avian metapneumovirus (aMPV) causes a highly contagious upper respiratory infection in poultry worldwide. Maternally derived antibodies (MDAs) do not protect against aMPV infection, but may interfere with live vaccine viruses. The objective of this study was to determine the onset of immunity induced by a single dose of the vaccine, administered via drinking water (DW) to 7-day-old chicks, or via spray (SP) route to 1-day-old chicks, in response to an aMPV challenge. The study also aimed to explore the impact of MDAs on vaccine's efficacy. Two experiments with four groups were performed, one for each administration route. Groups A and C used broilers without MDAs (SPF), while groups B and D used commercial broilers with MDAs. Groups A and B were vaccinated on day 0, and groups C and D were mock-vaccinated. Three weeks after vaccination, all chicks were challenged by eye-drop with a virulent aMPV strain isolated from chickens. Respiratory signs were evaluated in 8 chicks/group. A Fisher's exact test was used for statistical analysis in R software v4.4.0. In the absence of MDAs, vaccination by both administration routes showed a significant reduction in respiratory signs (DW 87.5%, SP 75% reduction, $p < 0.05$) compared to the control groups. The results obtained in the presence of MDAs were very similar (DW 100%, SP 87.5% reduction, $p < 0.05$ compared to control groups). The results demonstrate that RESPIVAC® aMPV vaccine induced an early immunity within 3 weeks after vaccination and no interference between MDAs and the vaccine's efficacy was observed.

Interventions in Broiler Breeders Used to Reduce Mortality Associated with Avian Metapneumovirus Subtype A

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Abstract

Historically, only Avian Metapneumovirus (aMPV) subtype C was found in the United States and was limited to turkeys. But in 2024, aMPV subtypes A and B swept through the country's flocks of both turkeys and chickens. With traditional biosecurity measures seemingly ineffective and no vaccination options available at the start of the outbreak, veterinarians were limited in their ability to manage the spread and mortality associated with aMPV. This proved no less true across four broiler production complexes in Texas. Antibody titers from these complexes verified that aMPV subtype A was present in more flocks than suspected. In addition, evaluation of retained serum proved the disease had

also been present in the area for longer than believed. With prevention seemingly impossible, controlling the secondary bacterial infections common to aMPV became the best option to reduce mortality. In a world rightly concerned with the judicious use of antimicrobials, treatment was originally withheld until clinical signs appeared. This proved to be inadequate and a policy of treating the entire farm with oxytetracycline as soon as signs appeared in any of the houses was implemented for the breeder farms. A retrospective analysis of the policy found that treating the entire farm prevented the loss of up to 1.07% of breeders. This translated to a savings of \$2,140 in pullet costs per house with the potential to produce an additional 12,947 broiler chicks. An experimental autogenous AMPV vaccine was later implemented to further control clinical signs and evaluation of its effects is ongoing.

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Monoclonal Antibodies Targeting the N-Protein of Avian Metapneumovirus Subgroup B in US: Production, Characterization, and Diagnostics Implications

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Abstract

Recently, the avian metapneumovirus subgroup B (aMPV/B) has been introduced in US poultry resulting in huge economic losses to chicken and turkey sectors. This underscores the urgent need to develop reliable strategies for diagnostic and research purposes. Thus, monoclonal antibodies (mAbs) were prepared against the nucleoprotein (NP) which is highly conserved among all members of aMPV/B. After successful production and purification of recombinant NP, four successive intraperitoneal doses were conducted into SPF bulb mice at two-week intervals. Two weeks post-last immunization, the splenocytes were fused to myeloma cells to develop hybridoma. Five mAbs were screened and selected using the indirect immunofluorescent assay (iIFA), belonging to the IgG1 subclass. Further characterization was performed through neutralization test, western blot, and immunohistochemistry (IHC). These mAbs have been used successfully to develop the aMPV/B-specific IFA, allowing specific, sensitive, and time-saving detection of the viral antigen in the tissue cultures instead of relying on the cytopathic effect visualization. Additionally, these mAbs were successfully leveraged to create an aMPV/B-specific IHC, detecting viral antigens in the nasal respiratory tracts of experimentally and naturally infected cases. This provides an extended detection duration of the infection. in the respiratory tracts of the infected birds, addressing the limited detection timeframe for aMPV/B infection by conventional tests such as RT-PCR and virus isolation. In conclusion, the newly developed techniques in this study will offer a substantial way to understand the dynamics and pathobiology of this emerging infection and enhance diagnostics.

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Development and validation of RT-LAMP assay for rapid detection of Avian Metapneumovirus Subgroup B from clinical samples

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Abstract

Abstract

In 2024, an outbreak of Avian Metapneumovirus (aMPV) was reported in the USA. The virus affects turkey and chicken leading to respiratory and reproductive diseases with huge economic losses. A rapid and sensitive diagnosis constitutes a great tool for effectively controlling the disease. Meanwhile, real-time RT-PCR is the gold standard for aMPV detection, but performing this assay requires expensive equipment and qualified personnel.

We have developed a reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) which is a rapid, cost-effective, simple, and highly sensitive diagnostic for the detection of aMPV-B by targeting the G gene in a real-time fluorescence-based method. Sensitivity and specificity were assessed by using aMPV-B RNA and non-targeted pathogens. Tests were performed on 10-fold dilutions of the virus ¼ master mix and compared the results with real-time RT-PCR. The performance was also evaluated, firstly direct positive choanal swabs samples from birds with Ct value >36 were enriched in intact virus precipitation reagent, and negative samples were tested. The reaction was performed at 68°C for 30 minutes.

The specificity showed 100% for respective targets without amplification within 30 minutes. Furthermore, the assay can detect up to 35 Ct value samples, equivalent to ~30 copies per 20 µL reaction, and achieved >85% accuracy with real-time RT-PCR results. In the future, we will develop a point-of-care multiplex RT-LAMP assay to detect the three aMPV subgroups A, B, and C for field diagnosis.

Keywords: aMPV-B, RT-LAMP, point of care, field diagnostic tool, enriched.

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Using Controlled Exposure to AMPV SubType A to Increase Protection in Turkeys

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Abstract

Avian metapneumovirus (aMPV) is a globally significant respiratory pathogen in poultry, causing acute, highly contagious upper respiratory tract infections in turkeys and other avian species. Although infected birds develop local and systemic immunity, maternal-derived antibodies provide limited protection. In intensive poultry flocks, the virus spreads rapidly, with wild birds implicated as potential reservoirs and transmission vectors.

In regions where vaccines are unavailable, alternative control measures are essential. This study investigates the efficacy of a controlled exposure approach using subtype A aMPV to stimulate antibody production in breeding turkeys. The virus, harvested from a wild aMPV subtype A infection, was tested to confirm the absence of subtype B and avian influenza via environmental and paired tracheal swabs in saline medium. A trial was conducted at a site with 29-week-old breeder replacement turkeys. Fifty turkeys in a 5,000-head house on a 30,000-head farm were exposed to the virus solution, applied via facial wiping and eye drop administration. Secondary bacterial infections were treated at the time of exposure.

Within 36 hours, environmental samples from the initial house tested positive for aMPV, with subsequent houses testing positive within another 36 hours. The results demonstrate the rapid spread of immunity across the flock and highlight the potential of controlled exposure as a practical interim strategy for managing aMPV in regions lacking vaccine access. This approach provides valuable insights into mitigating the economic and health impacts of aMPV on poultry operations.

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Persistence of Maternal Antibodies in Progeny Hatched from Turkey Breeder Hens Exposed to Avian Metapneumovirus Type B

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Abstract

The purpose of this study was to determine the persistence of maternal antibodies from the progeny of turkey breeder hens exposed to avian metapneumovirus (aMPV) type B. Turkey breeder hen flocks were identified as being naturally exposed to avian metapneumovirus type B through convalescent serology via ELISA, or positive aMPV type B PCR results. During hatching, poults from these flocks were identified and marked with a wing tag. Serology samples were collected at day of hatch for aMPV ELISA using a commercially available kit, as well as choanal cleft swabs and cloacal swabs for aMPV PCR. Serology samples, choanal cleft swabs and cloacal swabs were collected weekly, through six weeks of age at which time the study was terminated. Choanal cleft swabs and cloacal swabs collected at all time points throughout the study were negative for aMPV type B PCR. All day of hatch serology samples were positive on aMPV ELISA. However, titers began to quickly decline and all samples were aMPV ELISA negative by three weeks of age. This study demonstrates that while the hen may pass maternal antibodies to the poult, those antibodies are quickly diminished. Therefore, ELISA positives on turkeys greater than three weeks of age should be attributed to field infections rather than maternal antibodies. Additionally, this study can help to guide decisions on when to boost aMPV modified live vaccines on turkeys in the field.

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To study virus evolution and optimization of challenge model for emerging avian metapneumoviruses

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Abstract

The recent introduction of avian metapneumovirus (aMPV) subgroups A and B signifies a serious threat to poultry industry. In this study, we have completed whole genome sequencing (WGS) of subgroups A and B viruses to understand the virus evolution of these viruses after circulating in the US poultry for more than one year. We have completed WGS of 50 subgroups B virus using both targeted and non-targeted next generation sequencing methods. We have completed WGS of 10 subgroups A viruses. Unique amino acid changes have been detected in both viruses over time as well as related to geographical regions. We have optimized challenge model for subgroup B in both chicken and turkeys. Upon pathogenicity study, all the challenged turkeys showed significant clinical signs and lesion scores for 7 days post-challenge. The virus was detected in the upper respiratory tract (including nasal turbinate and trachea) and in lungs with peak viral load at 5 days post-challenge. The study in chicken is ongoing by following the challenge experiment similar to turkeys. In conclusion, this isolate represents a potential candidate for a challenge model study in chicken and turkey without evidence of host specificity and for vaccine production.

Bacteriology

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Using animal challenge models to elucidate novel APEC serogroups pathogenicity

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Abstract

Colibacillosis, caused by Avian pathogenic *Escherichia coli* (APEC), leads to high morbidity and mortality in poultry. Among APEC, serogroups O1, O2, and O78 are often implicated. However, our studies identified several emerging serogroups in Georgia. Here, we evaluated the pathogenicity of ten APEC strains (O25, O91, O86, O161, O88, O115, Onovel12:H4, O45, and NT) using three animal models. We hypothesized some strains would exhibit different virulence in some assays.

We used 12-day-old SPF embryonic eggs challenged with APEC at a concentration of 300-500 CFU/0.1 ml via the allantoic fluid in embryo lethality assay (ELA). Eggs were candled daily, and deaths recorded. All APEC were virulent, with the highest mortality (100%) observed for O152 and O145, while O88 caused 50% mortality.

One-day-old chicks were inoculated subcutaneously with 100 µL (10^8 CFU) of the APEC strains. Death times and lesion scores were combined to calculate pathogenicity scores (PS). Serogroups O15, O91, and O88 had significantly lower ($p < 0.05$) PS than the positive control (APEC O18), while O25, O152, O115, and O45 had numerically higher PS scores.

3-week-old SPF chickens were challenged with 10^8 CFU/ml of bacteria via the intratracheal route. Mortality and clinical signs were observed for 5 days. Results showed APEC O91 was highly virulent, causing 80% mortality after 1 d.p.i., while O115 and O86 were moderately virulent.

These novel APEC serogroups exhibited varying pathogenicity and high virulence in embryos, chicks, and chickens and virulence varied across models, suggesting that the route of infection and immune system influence disease development.

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The status of antimicrobial use, antimicrobial resistance and flock health in sentinel broiler chicken flocks in Canada

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Abstract

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) monitors trends in antimicrobial use (AMU) and antimicrobial resistance (AMR) in sentinel broiler chickens flocks. The total AMU, measured in milligrams per kg broiler chicken biomass increased by 11% between 2022 and 2023. The distribution of flocks that were high, middle and low users of antimicrobials (based on mg/kg broiler chicken biomass) was similar between 2022 and 2023, indicating that disease occurrences and production practices were stable. The antimicrobials used in broiler chickens were largely classes intended for the control of necrotic enteritis. The prevalence of multidrug resistant (MDR) *E. coli* isolates was stable while MDR *Salmonella* increased by 9% between 2022 and 2023. Notable results included the detection of *Salmonella* isolates resistant to nalidixic acid and exhibited reduced susceptibility to ciprofloxacin (9%); isolates were mostly *S. Enteritidis*, and the occurrence of stable level of ciprofloxacin resistance in *Campylobacter*. Mortality slightly increased by 0.5% (2022: 4.1%; 2023: 4.6%) and ranged from 1 to 30%. The flock that experienced high mortality was viral disease-associated. Other key findings important to public health and flock health will be discussed including more recent findings.

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Potential of broiler barn dust as a population level diagnostic technique to routinely monitor pathogens and antimicrobial resistance

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Abstract

One of the major challenges to the Canadian poultry industry is disease outbreaks which causes significant economic losses. To address these issues active surveillance is crucial, which is not practiced routinely in broiler chickens due to the high cost of individual bird sampling. The objective of our study is to evaluate poultry barn dust as a sample monitoring microbial contamination and antimicrobial resistance. Sampling will be performed in 3 broiler barns in Alberta. Samples will be collected from the entire production cycle of broiler flock in 6 time points each with one week gap. Further we will gather oropharyngeal and cloacal swab samples from 15 randomly selected chickens. The Samples will be processed for whole genome sequencing, 16S rRNA sequencing, virome and resistome analysis. Meanwhile oropharyngeal and cloacal samples also will be processed for sequencing and viral isolation. Using the findings of the study we expect to determine whether dust sampling is a suitable method to surveillance poultry microbial infections and anti-microbial resistance. Due to the low cost and noninvasive nature of the technique the study will be a unique diagnostic tool for surveillance activities.

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Assessing the impact of breeds and types of poultry in a *Campylobacter hepaticus* challenge model.

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Abstract

Campylobacter hepaticus is the causative agent of spotty liver disease (SLD), resulting in mortality and production loss in laying hens. The disease is primarily associated with free ranging laying birds and occurs most often at peak production. Here, we used our *C. hepaticus* challenge model to assess if all layer breeds and bird types were susceptible to infection. Our assessment was based on clinical signs, spotty liver lesions on necropsy and confirmation by histopathological analysis, microbiology and PCR confirmation. For this study, five different bird types were assessed including Rhode Island White (RIW), Rhode Island Red (RIR), White Leghorn (WL), Broiler Breeder (BB) and Broiler (B). Thirty birds of each type were allocated into groups of 10 and challenged with one of three *C. hepaticus* challenge doses. All birds were housed in similar conditions and monitored. At intervals post challenge, birds were removed and euthanized to assess for liver lesions and presence of the challenge strain. When compared by bird type, RIR and RIW birds had highest liver lesion scores while WL had significantly lower scores. Liver lesion scores for BB and B were comparable to the WL. Bacteriological analysis found RIR, RIW, and BB had similar prevalence rates (>80% positive) compared to WL and B which had a lower prevalence (40-50%) but was not significantly different. SLD can be replicated in all bird types using a *C. hepaticus* challenge resulting in lesions and positive birds on culture. Further investigation of the effects of *C. hepaticus* on breeds is warranted.

Investigating the Pathogenicity and the Immunogenicity of Nonpathogenic *Avibacterium paragallinarum* via Challenge Study

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Abstract

Infectious coryza (IC) is a bacterial respiratory disease in chickens caused by the primary pathogen *Avibacterium paragallinarum* (AP). Isolation of the bacteria or qPCR detection of AP is performed for disease confirmation in clinical cases. Recently, multiple naïve-healthy layer flocks that had neither been exposed to IC nor received IC vaccination tested positive using the current IC-specific qPCR assays. Additionally, AP isolates were acquired from some of these naïve flocks, and whole genome sequencing (WGS) was performed. The WGS analysis revealed important genetic differences between these new AP isolates and the conventional pathogenic AP (pAP) isolates. Consequently, these isolates were preliminarily dubbed "non-pathogenic *Avibacterium paragallinarum*" (npAP) due to the absence of clinical signs. Seven 26-week-old laying hen groups were challenged with seven distinct npAP isolates from five different sites, alongside a positive control group challenged with a field pAP serotype C isolate and a negative control group. There were no significant differences ($P > 0.05$) in the mean clinical score between the seven npAP groups and the negative control group, while the pAP group had a significantly higher score ($P < 0.05$). Upon subsequent exposure to the challenge strain (homologous pAP) to evaluate the ability of npAP to protect against the circulating pAP serotype C, all 7 groups previously challenged with npAP isolates showed clinical sign scores that were not statistically different from the positive control group. In conclusion, the assessed npAP strains were confirmed to be non-pathogenic to naïve chickens and non-protective against pAP serotype C.

Control of *Enterococcus cecorum* through identifying its dynamics of adaptation to the chickens and its environment.

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Abstract

Enterococcus cecorum (EC) causes severe systemic disease and high mortality in chickens older than 2 weeks. EC survival time and susceptibility to disinfectants have not been evaluated under US poultry conditions. This project aims to identify EC survival times under different environments, temperatures, materials, and disinfectants.

Study #1 analyzed EC survival times under different environments, temperatures, and materials. Two groups were formed: autoclaved and not autoclaved incubation residues, litter, and feathers. Five replicates for each material and each group were inoculated with one pathogenic and one nonpathogenic EC. To determine EC survival time, each sample was cultured weekly, until no bacterial growth was observed in each inoculated material during two consecutive weeks.

Study # 2 will analyze EC survival under the effect of three disinfectants used in poultry (quaternary ammonium, glutaraldehyde, and Virkon S). Each product will be applied to three matrices: naked EC, EC mixed with litter, and EC mixed with incubation residues. The evaluated disinfectant will be considered effective if it demonstrates at least a 10^5 CFU reduction in viability within 60 minutes or less with the EC analyzed.

EC survived 42 days in all materials. Related temperature, EC survived better under cold temperatures (42 days), vs. room temperature (at least 14 days). Differences between EC pathogenic vs. non-pathogenic were not observed. Disinfectant test is in progress.

EC survives for longer periods in colder temperatures and in litter. In summary, EC has the best survival times in litter, followed by incubation residues, followed by feathers.

Identification of key mechanisms used by *Enterococcus cecorum* to adapt and survive under unfavorable conditions

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Abstract

Nowadays, *Enterococcus cecorum* (EC) is recognized as a pathogenic bacteria causing not only bone problems (vertebral osteoarthritis, joint arthritis, and femoral head necrosis) but also a severe systemic disease and high mortality in broiler chickens older than 2 weeks. Our previous Whole Genome Sequencing (WGS) results showed EC adapted to environmental changes over the years. Three key bacterial adaptation and survival mechanisms that must be researched for EC are desiccation resistance, biofilm production, and disinfectant resistance.

This study aimed to identify genes related to these three mechanisms by evaluating 40 EC isolates (pathogenic vs. non-pathogenic) recovered from chickens in 2023. A gene survey was performed to identify traits such as the presence of genes encoding efflux pumps that actively expel cationic disinfectants (quaternary ammonium compounds), genes associated with desiccation resistance such as those involved in cell envelope integrity, stress response regulation, and osmoregulatory genes, and for biofilm production genes encoding intercellular adhesins, fibronectin-binding proteins, elastin-binding proteins, and clumping factors.

Preliminary results identified genes suggesting resistance to desiccation, such as Cell wall surface anchor family protein, Cell wall-associated murein hydrolase LytA, Outer surface protein cellobiose operon, and genes related to disinfectant resistance, such as those encoding efflux pumps.

Our results will contribute to EC control by helping us identify the mechanisms these bacteria use to adapt and evade interventions commonly used to eliminate them from poultry environments. Understanding how EC can survive under unfavorable conditions and escape the disinfectant effect can be used to formulate better control programs.

Investigating Spotty Liver Lesions Persistence After Infection with *C. hepaticus*, the Causative Agent of Spotty Liver Disease in Layer Hens

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Abstract

Spotty liver disease (SLD) and its etiologic agent *Campylobacter hepaticus* causes multifocal liver lesions in layers, resulting in reduced egg production, and increased mortality. The goal of this project was to evaluate

potential persistence of liver lesions following challenge with *C. hepaticus*. We hypothesize that liver lesions do not resolve, without antibiotics. One hundred and fifteen, 24 woa, layer hens were used. An oral challenge dose of *C. hepaticus*, (10^9 cfu/ml/hen) was given three times, one day apart to 80 hens. At 16 dpch, the challenged hens were divided into two groups, and one group (n=40) was treated via feed with chlortetracycline ((CTC)/Aureomycin[®]) for 5 days while the second group was untreated. Also, 11-layer hens were added as sentinels/contact birds to both challenge groups at 16 dpch to evaluate horizontal transmission. At 16, 23, and 32 dpch, a subpopulation of hens per group were euthanized and necropsied to collect liver for histopathology, bile for bacteriology, and to score liver lesions. Results found that liver lesions persisted in birds that did not receive CTC, however in treated birds, liver lesion scores were significantly lower. CTC was unable to clear the infection in treated birds or control transmission as > 50% of treated and sentinel hens were positive on bacteriological analysis. This research confirms *C. hepaticus* persists in flocks where antibiotics are not used. Further research is necessary to understand the pathophysiology of how *C. hepaticus* causes liver lesions leading to significant losses for the layer industry.

Case Reports

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Concurrent Reportable Respiratory Diseases: Why Definitive Diagnosis Matters

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Abstract

In fall of 2024, birds from a flock of 9-10-week-old specialty broilers raised for the live bird market system were submitted to the Pennsylvania State University Animal Diagnostic Laboratory with a history of respiratory disease and elevated mortality. Respiratory signs observed on the farm included coughing and gasping. The flock service technician was concerned about infectious laryngotracheitis (ILT) and vaccinated the flock with chicken embryo origin ILT vaccine via the water. One week later, when respiratory signs persisted, and mortality reached approximately 0.5% per day for several consecutive days, the technician submitted birds to the lab for diagnostic workup. On postmortem examination, most birds had conjunctivitis, sinusitis/rhinitis, and tracheitis, consistent with respiratory disease. A few birds also had lesions consistent with bacterial polyserositis, including fibrinous pericarditis, perihepatitis, and airsacculitis. Differential diagnoses included ILT, avian metapneumovirus (aMPV), infectious coryza, mycoplasmosis, infectious bronchitis (IBV), and avian influenza. Due to the similar presentation of these differentials, ancillary testing was pursued to determine a definitive diagnosis. Samples were collected including swabs for PCR and bacterial culture, fresh tissues for virus isolation, and fixed tissues for histopathology. Results revealed that the case was complicated and multifactorial in nature, with detection of several viral pathogens reportable in Pennsylvania, including ILT, aMPV, and IBV. Additionally, a low virulence strain of avian paramyxovirus 1 of wild bird origin was isolated from tracheal samples, and *E. coli* was isolated from organ swabs. This case highlights the importance of obtaining a definitive diagnosis and implications for prevention and control.

Decreased Hatchability in Turkey Poult

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Abstract

In late spring 2024, the hatchery experienced a decrease in hatchability associated with a single breeder flock. Egg production and fertility remained consistent, while hatchability dropped over 3%, 13%, and 21% over 3 consecutive hatch days. Breakouts revealed elevated late mortality, pipped, and dead in shell embryos. The poult that did hatch were significantly smaller than poult of comparable breeder flocks. The eggs were set in different incubators in different rooms at the hatchery, and eggs in the neighboring machines were not affected.

There were no clinical signs of disease in the breeder flock, or increased titers in the period after indicating exposure to a common disease. There was nothing in the history of the machines to indicate a machine problem during incubation of the eggs.

An investigation on the farm revealed that the egg wash machine was not working. As a result employees were using a pump up sprayer to sanitize eggs. The mixture in the sprayer was 1:1, instead of the 1.5 oz/gallon, resulting in a residue of quaternary ammonia covering the shell and clogging the pores.

When the correct quat dilution was applied to eggs, hatchability and poult quality returned to expected levels. In total, about 6 weeks of hatch were reduced.

Quat is a commonly used disinfectant when sanitizing eggs, as it results in reduction in total aerobic, mold, yeast, and coliform counts on eggshell surfaces. Hatchability is typically increased on eggs that are sprayed compared with control eggs due to reduced bacterial counts.

Production Loss and Mortality in a Layer Flock Revealed Underlying Food Safety Concern

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Abstract

Six 28-week-old Hy-Line Brown layer hens from a flock of approximately 2,900 birds were submitted to the Penn State Animal Diagnostic Laboratory due to decreased egg production and increased mortality. The producer's primary concern was a drop in egg production from 88% to 67% over the last two weeks with corresponding shell quality concerns and loss of pigment. The submitter also reported elevated mortality of 1-2 birds per day. Differential diagnoses included egg drop syndrome, infectious bronchitis, and bacterial septicemia. On gross necropsy, 3 birds had moderate fibrinous salpingitis and oophoritis; 1 had severe fibrinous peritonitis; 3 had mild fibrinous airsacculitis; and 2 had mild fibrinous

perihepatitis. Eight eggs were also submitted: 4 had pale eggshells and 1 had a soft shell with multiple large cracks. Swabs were collected for aerobic bacterial culture and PCR. Infectious bronchitis virus was detected via PCR from pooled tracheal swabs. Heavy mixed growth of *E. coli* and *Enterococcus cecorum* was isolated from a peritoneal swab. Heavy growth of a group D *Salmonella* was isolated from another peritoneal swab. The *Salmonella* isolate was identified as *Salmonella* Enteritidis (SE) at the National Veterinary Services Laboratory. 1080 eggs from the flock were submitted for follow-up testing. 19 out of 50 egg pools were positive for SE on PCR. Since the flock contained fewer than 3,000 hens, the grower had not been conducting routine surveillance for SE. This case highlights the importance of continued outreach efforts to small flock owners selling eggs for human consumption.

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False Positives in Influenza A Screening of Turkey Semen: The Role of Bacteria and Diagnostic Limitations of Antigen Capture ELISA

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Abstract

In a modern U.S. turkey breeder operation, antigen capture ELISA is used to screen stud tom semen for influenza A before it is used for insemination at laying hen farms. Recently in one division, several cases of false positive antigen capture ELISA test results have occurred. Upon detection of an antigen capture ELISA positive, the positive semen sample and the pens milked to collect the sample were tested for avian influenza via PCR. All cases have tested negative via PCR at NAHLN laboratories on multiple samplings and were void of any clinical signs suggesting infection. This presentation outlines each case and gives detailed information regarding sample collection, diagnostic testing and explores possible causes of false positive tests with an in depth look at common bacteria cultured from positive samples as possible culprits. These cases are still developing, and all results will be reported.

Size Matters: The role of Feed particles in Runting-Stunting Syndrome.

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Abstract

For over 40 years, Runting-Stunting Syndrome (RSS) has been a persistent challenge for the poultry industry worldwide, causing significant economic losses. This disease, characterized by malabsorption, enteritis, growth impairment, and uneven performance in young birds, continues to impact broiler operations in Canada.

Our team was contacted by a broiler complex in Ontario, Canada with a recurrent history of RSS for investigation. Affected birds were submitted to the Animal Health Laboratory for diagnostic evaluation and feed samples were collected and sent to SGS Canada for feed testing and particle size analysis. Positive tissues for chicken astrovirus (CAstV) were sent to the Swine and Poultry Infectious Diseases Research Center (CRIPA) for Next-Generation Sequencing (NGS).

The study findings, including clinical signs, hatchery production data, economic impact, molecular analyses, and histological results, will be discussed in detail during the conference.

Histopathological Lesions Associated with an Increased Incidence of Lameness in Commercial Ducks

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Abstract

Recently, an increased incidence of lameness has been observed in commercial Pekin ducks in the United States. Lameness is noted as early as 5 to 7 days and continues throughout the life of the flock. Grossly, lame birds have soft to rubbery bones which progress to fractures, often in the mid to distal diaphysis of the tibiotarsus. These fractures fail to stabilize, due to continued weight-bearing stress, and progress to pseudarthroses. Histologically, the most common lesions observed in the legs of both lame and clinically normal ducks from the same flocks include failure of endochondral ossification with expansion of the zone of hypertrophy, tibial dyschondroplasia (TD), and osteopenia. In clinically lame birds, these lesions are often accompanied by partial to complete, mid to distal diaphyseal, nonunion fractures with variable formation of a cartilage callus. These lesions have been observed in multiple companies across multiple complexes, and in flocks fed at least three different commercial diets. The histological lesions suggest a deficiency in phosphorus which may be absolute or relative. An absolute deficiency is unlikely given that lesions are observed in birds fed three different commercial diets. Differentials for relative deficiency include elevated feed

aflatoxin levels, phytase imbalance, an anti-nutritional factor in the feed causing decreased phosphorus bioavailability, or an issue in the gut or elsewhere in the bird causing decreased phosphorus absorption and utilization. Further investigation is warranted to determine the true cause of this condition.

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Isolation of a Zoonotic Pathogen from a Pullet Residing in a Public Botanic Garden

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Abstract

In November 2024, a natural dead 6-month-old Barred Rock pullet from a flock at a public botanic garden was submitted to the Pennsylvania State University Animal Diagnostic Laboratory with a history of bloody feces, weight loss, and pallor. In early October, the pullet was treated for suspected coccidiosis with oregano and thyme oil via drinking water, which appeared to resolve gastrointestinal signs. In the following weeks, the pullet lost weight, despite normal feed and water consumption, and developed comb and leg pallor. On postmortem examination, the pullet was emaciated with vent fecal staining. Gross lesions suggested bacterial septicemia, including necrotic fibrinous hepatitis, peritonitis, myocarditis, and pancreatitis. The heart was rounded, with hydropericardium and a focal, dry, white bulging area around the left atrium. Ulceration of the gizzard mucosa and severe, focal, unilateral cecal dilation and ulceration with hemorrhage and necrosis were supportive of gastroenteritis and typhlitis. Differential diagnoses included lymphoid neoplasia, chronic coccidial or bacterial enteritis/typhlitis, and bacterial septicemia. Sampling for ancillary testing included swabs for bacterial culture, fixed tissue for histopathology, and Modified McMaster's fecal flotation. Preliminary results revealed severe coccidiosis and bacterial myocarditis. While MALDI identified nonpathogenic *Listeria innocua*, the heart lesions and hemolysis observed on culture suggested a pathogenic bacterium. Follow-up immunohistochemistry of cardiac tissue confirmed presence of *Listeria* antigen, and whole genome sequencing confirmed *Listeria monocytogenes*. This case highlights the importance of confirmatory testing in the face of equivocal results, as well as risks of zoonotic pathogens in public spaces shared with avian species.

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Effect and safety of administration of Fluralaner via drinking water in heavy broiler breeders

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Abstract

Ectoparasite infestations negatively affect commercial poultry. Fluralaner (brand name Bravecto®) is effective against ectoparasites. In the US, this FDA approved veterinary drug is only available in tablet form. The study aimed to validate the delivery of fluralaner via drinking water, determine its pharmacokinetics, and tissue

residue in heavy broiler breeders to ensure the safety of the drug and that an efficacious dose is received. Ten 60-week-old heavy breeders, from a commercial vendor, were given fluralaner at a rate of 0.03 mg/mL (equivalent to 0.5mg/kg/day), dissolved with 0.2% transcutol V, in drinking water for 6 hours. Water was collected every hour from the nipple lines. Three birds were bled at 0.5, 1, 3, 6, 8, 12, 24, 36, 48, 96, and 168 hours. No bird was bled 2 consecutive times or more than 4 times. On D9 birds were euthanized, and tissues collected. The concentration of fluralaner collected from the drinking pipes was 0.015 mg/ml and 0.008mg/ml, on D1 (1st dose) and D8 (2nd dose) respectively. Fluralaner concentration in the plasma was at an efficacious concentration against ectoparasites. This study showed that 0.2% transcutol V was useful to dissolve Bravecto in the water and that Bravecto could be used in drinking water. Constant stirring of the medicated water is important to maintain the drug in suspension and prevent its depletion over time. Based on drug concentrations and pharmacokinetics data, we expect that the administration via the drinking water twice, 7 days apart would be effective against ectoparasites.

Coccidiosis

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Research farm trial comparing three coccidiosis vaccines in broilers raised on used litter: vaccination assessment and gel vs. spray application

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Abstract

A trial was conducted at a research farm to determine the effect of three different coccidiosis vaccination programs in broilers raised on used litter. The trial consisted of three treatment groups corresponding to three different coccidiosis vaccines administered at the hatchery based on manufacturer recommendations which gave us the opportunity to compare gel versus aqueous spray application. At the farm the chicks were placed in thirty-six (36) pens, placed in a randomized fashion as to treatment assignment. Each treatment had 12 pens. Monitoring during the study included vaccination assessment via the novel use of chick isolators, twice daily observations, coccidia shedding, coccidiosis lesions scoring, feed consumption and bird weights. Livability, bird weight, and feed weight were used to determine performance parameters such as weight, daily gain and feed conversion. The trial was concluded when the birds were 57 days old. We were able to document differences in vaccine uptake, oocyst shedding patterns, and livability/mortality. Results and statistical analysis will be presented.

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Vaccination of Individual Birds Improves *E. maxima* Take & Output

Andrew Smith, Liz Turpin, John Ngunjiri, Jim Hutchins

Abstract

Introduction: Coccidiosis is a primary challenge in poultry production. While commercial coccidiosis vaccines exist, current delivery is suboptimal, particularly for *Eimeria maxima*. This research investigated how individual vaccine dosing via eye drop administration to day-of-hatch broiler chicks impacts vaccine take & oocyst output.

Methods: A 7-day grow-out model was utilized to assess the efficacy of vaccine delivery methods. Using this model, six separate trials were conducted to compare vaccine take and oocyst output in birds vaccinated using either the traditional spray cabinet method or the eyedrop method. Birds were vaccinated on day of hatch, grown for 7 days, euthanized, and entire intestinal tracts were collected from individual birds. Additionally, individual fresh fecal droppings were collected on day 7. All samples were processed and oocysts counted via the traditional McMaster chamber method.

Results: Birds vaccinated via eyedrop had significantly higher percent positive and output on day 7 for *E. maxima* compared to those vaccinated via the spray cabinet. The spray cabinet group had an average *E. maximum* take (percent positive) of 38%, with an oocyst output of 20,973 oocysts per bird. In contrast, vaccination of individual birds via eyedrop achieved 100% take, with an average oocyst output of 246,598 oocysts per bird.

Conclusion: Administration of vaccine to individual birds via eyedrop achieved a higher take for *E. maxima*, which is expected to provide earlier protection and result in improved bird health and performance.

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The relationship of litter moisture content and sporulation/resilience of *Eimeria* oocysts

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Abstract

The purpose of this study was to compare sporulation and resilience of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* oocysts in dry and wet broiler house litter. Fecal material from chickens excreting unsporulated *E. acervulina* (Ea), *E. maxima* (Em), or *E. tenella* (Et) oocysts were mixed in triplicate with dry or “wet” litter and incubated at recommended broiler house temperatures for each week of a typical growout period (8 wk). The *Eimeria* oocysts-spiked litter were incubated in an enclosed container with water added to achieve constant moisture content throughout the entire incubation period. Weekly samples were processed by floating in 1M sucrose, and *Eimeria* oocysts were enumerated and % sporulation determined by microscopy. Control litter not spiked with *Eimeria* oocysts were also included in the study and sampled throughout the entire 8 wk period to account for background *Eimeria* oocysts. Ea, Em, and Et oocyst concentrations did not decrease over time in wet litter, but either displayed no decrease (Ea) or a drastic decrease to undetectable levels (Em, Et) in dry litter. Moreover, % sporulation of Ea, Em, and Et oocysts was negligible at all time-points in dry litter, but increased

(Ea), temporally increased (Et), or was negligible (Em) in wet litter. These data provide further support for maintaining dry litter conditions to reduce coccidiosis outbreaks in broiler houses.

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Characterization of *Eimeria* Isolates from Commercial Broiler Operations

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Abstract

The seeding of poultry litter with vaccine strains of coccidia has previously been inferred from associations between vaccine use and anticoccidial susceptibility. However, the differentiation of vaccine strains from field strains of coccidia is not routinely done nor commercially available. The objective of this study was to differentiate field isolates of *Eimeria* spp. from commercially available vaccines, demonstrating the persistence or absence of field strains following multiple cycles in broiler operations. Fecal samples were collected from three broilers farms for five cycles, screened for oocysts via the McMaster method, then further processed for molecular identification. For this, oocysts were purified, genomic DNA was extracted and sequenced by Illumina sequencing. Commercial vaccines were included for comparison. Obtained sequences were aligned with the available reference genome to identify regions that allowed strain differentiation. *Eimeria* spp were consistently present in most samples throughout the study period. Next-generation sequencing technology allowed differentiation of some isolates but other isolates could not be processed due to insufficient DNA yields. Detailed results will be presented and discussed.

Diagnostics

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Detection of Infectious Bronchitis Virus On and Within Table Shell Eggs in Shell Quality Challenged Flocks

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Abstract

Infectious bronchitis virus (IBV) is regarded as a major pathogen of concern for poor egg shell quality, particularly in cases of wrinkled shells. Despite this classic lesion, there is a lack of evidence as to whether IBV remains present on egg shells and interior egg components, particularly those from flocks experiencing shell quality abnormalities. This information could present an opportunity to molecularly test for IBV in impacted flocks without the need for obtaining tracheal swabs or tissue samples on farm. To test this hypothesis, whole eggs from flocks with clinical egg shell abnormalities were washed with brain-heart-infusion (BHI) broth, which was then tested for IBV with real-time RT-PCR.

Tracheal swab pools were also obtained and similarly tested from each flock. The RT-PCR results from tracheal swabs and egg rinses were correlated for CT values and IBV serotype as identified via the IBV type specific PCR panel. Interior components of table eggs (albumen, yolk, and shell membranes) were also tested for IBV via RT-PCR. Virus recovered from egg shell rinses was inoculated into embryonated eggs to test for viability. Our work indicates that egg shell rinses may be a viable, accessible testing methodology to identify active IBV infection in egg laying flocks, particularly those experiencing egg quality abnormalities. Shell and egg contamination with IBV may present a biosecurity risk for companies cross-docking unprocessed eggs at in-line operations.

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Rapid diagnosis of *Escherichia coli* septicemia in broiler chicken using metabolomics

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Abstract

Pathogens cause a range of metabolic alterations upon entry into the host which can be effectively detected using metabolomics. The metabolome is sensitive to subtle physiological changes in the body and, are therefore useful as an early diagnostic tool to detect diseases even before clinical symptoms appear. Such metabolic biomarker-based studies are widely applied in human medicine, but are still in their infancy in veterinary medicine.

In this study, we focused on characterizing the serum metabolomics profile of broiler chickens following *Escherichia coli* challenge to identify potential biomarkers for early disease detection. *E. coli* is the major cause of yolk sac infections and septicemia in neonatal broiler chickens, which is associated with significant economic losses to the industry. Morbidity, mortality as well as the and the inability to detect disease at the acute stage poses great challenges to animal welfare and productivity.

We infected three-day-old commercial broiler chicks with *E. coli* while the control birds remained uninfected. Serum was collected at 8- and 24-hours post *E. coli* challenge and analyzed using liquid chromatography-mass spectrometry. We were able to identify a clear difference in the metabolomics profiles of the infected and non-infected groups. Significant changes were detected in carbohydrate, protein, lipid, and nucleotide metabolism in the *E. coli*-infected group compared to the control.

These findings highlight the potential use of metabolic biomarkers as an early diagnostic tool for *E. coli* infection in the broiler chicken industry.

Optimized Nanopore Sequencing Workflow for Poultry Foreign Animal Diseases Diagnosis from Clinical Samples

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Abstract

The poultry industry continually faces significant challenges from Foreign Animal Diseases (FAD) RNA viruses like Avian Influenza (AIV) and Newcastle Disease (NDV). While these are longstanding threats, emerging technologies are advancing their rapid and accurate diagnostics. Among them, Nanopore Sequencing stands out as a promising tool for real-time virus tracking, providing critical insights into genetic features essential for epidemiological surveillance and pathogenicity prediction. To establish Nanopore sequencing as a frontline diagnostic tool for AIV and NDV, validating its workflow steps is essential for successful implementation in poultry diagnostics.

In this context, our group is dedicated to optimizing Nanopore Sequencing as an important molecular diagnostic tool for AIV and NDV identification and characterization from clinical samples. Workflow validation integrated nucleic acid extraction, viral enrichment, library preparation, and data analysis. After extensive comparisons, the optimum workflow included (i) extraction with the bead-based automated method, (ii) viral enrichment with an amplicon-based approach for each target, (iii) library preparation using Native Barcoding chemistry, and (iv) data analysis enhanced by in-house real-time analysis tool. The workflow's limit of detection, accuracy, cost and time comparison to the Illumina and Sanger sequencing were assessed, with analysis ongoing.

In conclusion, this project introduces an additional tool for addressing major FAD threats like AIV and NDV. Furthermore, it lays the foundation for adapting Nanopore Sequencing to other poultry pathogens, enabling broader applications in disease surveillance and outbreak response.

Validation of a novel, innovative *Mycoplasma Gallisepticum-Synoviae* qPCR Kit

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Abstract

Introduction

Mycoplasma gallisepticum (Mg) and *Mycoplasma synoviae* (Ms) are critical pathogens affecting the poultry industry, requiring precise diagnostic solutions for their control. An innovative 4-plex qPCR test was designed for simultaneous detection of Mg and Ms and a dual control system to confirm the addition of sample matrix and proper amplification. In this design the PCR reaction mix is lyophilized directly into the PCR tubes enabling the reduction of laboratory errors and streamlining the workflow.

Procedure

The performance the novel qPCR assay was evaluated during a validation study. In the study, pure cultures of 16 well-defined Mg (10) and Ms (6) strains were tested to confirm inclusivity. Exclusivity testing was performed on a panel of 79 samples from other known micro-organisms, including 10 non-target *Mycoplasma* species. Furthermore, analytical sensitivity was assessed by spiking heat-boil and magnetic bead extracts from upper respiratory tract swabs with Mg and Ms genomic DNA dilution series. Exact copy number of *Mycoplasma* DNA, used for spiking, was verified by digital PCR. All samples were run in duplicate.

Results/Conclusion

The qPCR assay demonstrated excellent performance in this study. Inclusivity showed that the assay detects the targeted *Mycoplasma* species. Exclusivity was also confirmed. The limit of detection was assessed to be 2.5 genomic equivalents (GE) per reaction for Mg and at 5 GE per reaction for Ms. Results were neither impacted by sample matrix or extraction method. This new qPCR assay simplifies workflow and ensures precise and reliable results, representing a significant advancement in *Mycoplasma* monitoring.

Benchmarking Metagenomic Pipelines for the Detection of Foodborne Pathogens in Simulated Microbial Communities

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Abstract

Food-borne pathogens pose a significant public health threat worldwide, despite modern advances in food safety practices. Molecular detection of these pathogens in food matrices, including chicken meat, has gained attention for applications in tracking and preventing outbreaks. This study evaluated the performance of four metagenomic classification pipelines (Kraken2, Kraken2/Bracken, MetaPhlAn4, and Centrifuge) in detecting pathogens from simulated microbial communities representing three diverse, yet critically impacted food products. Specifically, we evaluated workflow performance in detecting *Campylobacter jejuni*, *Cronobacter sakazakii*, and *Listeria monocytogenes* reads in simulated metagenomes of chicken meat, protein powder, and dairy milk spiked with respective pathogen at 0% (control), 0.01%, 0.1%, 1%, and 30% abundances. Performance evaluations demonstrated that Kraken2/Bracken achieved the highest classification accuracy, with consistently higher F1-scores across all food metagenomes. MetaPhlAn4 also performed well, especially in predicting *C. sakazakii* in the protein powder metagenomes but appeared limited in the detection of pathogens at the lowest level (0.01%). Centrifuge exhibited the weakest performance, largely due to having a high proportion of unclassified reads driving overestimation of pathogen abundances. Kraken2/Bracken and Kraken2 exhibited the broadest detection range, correctly identifying pathogen sequence reads down to the 0.01% level, whereas MetaPhlAn4 and Centrifuge had higher limits of detection. Our findings highlight Kraken2/Bracken as a robust tool for pathogen detection in food safety and surveillance, with MetaPhlAn4 serving as a viable alternative except for rare/very low-abundance pathogens. The study provides important insights that can strengthen food safety and facilitate rapid, accurate pathogen detection for broader public health and disease diagnostics.

Meloxicam residue study in finishing age tom turkeys

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Abstract

Meloxicam soluble for drinking water is an NSAID (compounded 0.6% solution) that can legally be prescribed by poultry veterinarians under AMDUCA, 21CFR, federal, USP and state boards of pharmacy guidelines. Veterinarians need to establish a withdrawal period on drugs they prescribe. This study provides data to assist in understanding meloxicam residues.

Thirty 13-week-old commercial tom turkeys were provided treated (9mg Meloxicam/gallon of drinking water, equivalent to 2pints/5 gallons of stock solution, i.e. 2x common stock solution dilution for daily use) drinking water for 7 continuous days. Calculated actual average consumption was 2.5-3.8mg Meloxicam/bird (11.5kg average weight; 0.19mg/kg/day).

At six time-periods (6 hours, 2, 5, 11, 16, and 21 days after cessation of administration), five randomly selected birds/period were euthanized. Liver and kidney samples were harvested and tested with an assay with a limit of quantification of 1ng/g, 10x lower than the FSIS Minimum Level of Applicability for meloxicam in turkeys (10ng/g).

Residue concentrations at 6 hours after cessation of treatment were 2.0-7.2ng/g for liver and 7.0-42.6ng/g for kidney tissue. All samples from days 2-21 days post-cessation of administration had no detectable meloxicam.

Under these conditions, there were no violative samples in kidneys and livers from finishing toms with this specific formulation of meloxicam at or beyond 2 days post meloxicam. Dosage and formulation of product may impact residues. The veterinarian should utilize this data to assist in developing meloxicam withdrawal periods for their flock whether for stresses related to movement, vaccination or infection.

Epidemiology

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Investigating the Diversity of Fowl Adenoviruses Circulating in the Middle East and Their Impact on Poultry Health (2022 – 2024)

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Abstract

Fowl adenoviruses (FAdVs), belonging to the genus *Aviadenovirus*, are significant pathogens in poultry, causing inclusion body hepatitis, hydropericardium hepatitis syndrome (HHS), and gizzard erosion in chickens and other avian species. These infections lead to substantial economic losses in the global poultry industry. Recently, an increase in mortality associated with FAdV-related clinical signs has been reported in the Middle East. This study investigates the diversity of avian adenoviruses circulating in this region, providing valuable insights into the prevalence, genetic variability, and potential impact of FAdVs on poultry health in the Middle East.

Around 200 PCR samples were collected from various countries in the Middle East, including Jordan, Syria, Lebanon, Saudi Arabia, Iraq, UAE, Oman, and Libya. The samples, which included trachea, liver, spleen, and cecal tonsils, were collected based on clinical signs suggesting fowl adenovirus infection. Upon receipt, DNA was extracted for PCR detection of the adenovirus, and positive samples were then sequenced to identify the FAdV serotype.

The results indicate that FAdV-E (serotype 8b) was the most frequently detected serotype across several countries, including Syria, Lebanon, Iraq, and the UAE, suggesting its widespread presence in the region. Other serotypes, including FAdV-C (serotype 4), FAdV-D (serotype 2, 11), and FAdV-A (serotype 1), were also identified, demonstrating the genetic diversity of FAdVs in the Middle Eastern poultry industry. This study highlights the diversity of Fowl adenoviruses

(FAdVs) circulating in poultry populations across the Middle East, with significant findings regarding the prevalence of various FAdV serotypes.

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Development of a quantitative PCR assay for enumerating *Clostridium perfringens* and *Clostridium septicum* in poultry litter

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Abstract

Clostridial dermatitis (CD) in turkeys is caused by *Clostridium septicum* and *C. perfringens* and remains one of the most important diseases in turkey production. A key unanswered question related to CD, as expressed by some of the veterinarians managing this condition, is: Why are some clinically affected CD farms more refractory to treatment with penicillin than others? One hypothesis is that treatment efficacy could be related to the overall microbial load and composition of *Clostridium* spp. in the turkey barn. To evaluate the microbial load of *Clostridium* spp. in poultry litter, an enumerative assay is needed. The objective of this study was to develop an assay that would allow us to enumerate *C. perfringens* and *C. septicum* in poultry litter. To develop this assay, we collected litter samples from farms that have and have not yet broken with CD. Litter was washed with 1x PBS, and DNA was extracted from the litter wash using a Qiagen PowerSoil Pro kit. We performed qPCR on extracted litter wash DNA to enumerate *C. perfringens*, *C. septicum*, as well as the overall *Clostridium* genus from poultry litter. Levels of *C. perfringens* were higher than those of *C. septicum* across all samples that were tested. Additionally, levels of *C. septicum* were higher in litter collected from barns that had clinical CD compared to those that did not have clinical CD or that had no history of CD. This qPCR assay will be an important tool in the management of turkey flocks and CD.

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Evaluation of Vertical Transmission, Broiler Performance, and Environmental Persistence of the 2022-2024 *Mycoplasma synoviae* Outbreak in NE Georgia

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Abstract

Mycoplasma synoviae (MS) is a significant pathogen in poultry, commonly causing subclinical infections. However, virulent strains can lead to upper respiratory disease and tenosynovitis. Although MS often remains subclinical, co-infections can exacerbate clinical signs, impacting bird health and performance. Transmission occurs horizontally via the respiratory tract and vertically through transovarian transmission, allowing the pathogen to infect progeny. MS control is managed through biosecurity and the use of MS-negative breeding stock.

In the last two years (2022–2024), MS-positive submissions from Georgia have more than doubled, with genotype “S-76” now the predominant strain, accounting for 38% of cases. This is the third peak of MS positive flocks in the last decade. In 2014 and 2016, 78% of MS-positive submissions from Georgia broiler-type chickens to the Poultry Diagnostic Research Center (PDRC) were identified as genotype “S-56.”

In response to the growing prevalence of MS in the southeastern United States, this study aimed to address two objectives. The first objective was to assess the vertical transmission of MS in commercial broiler breeders and evaluate its impact on broiler performance. Broiler flocks from MS-positive and MS-negative breeder flocks were compared within the same company. Mortality, performance, and ELISA titers were compared between the two groups. The second objective involved comparing the in vivo infection dynamics of two MS isolates, S-56 and S-76. A bird trial was conducted to evaluate and compare the virulence, shedding patterns, and environmental persistence of these genotypes, providing insights into their epidemiology and potential implications for the poultry industry.

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Genetic Sequence Comparison of Mycoplasma Synoviae Cases in West Central Ohio and East Central Indiana Turkey and Chicken Farms

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Abstract

Mycoplasma synoviae (MS) has been a historical bacterial disease challenge in the west central Ohio and east central Indiana poultry industry. This area has turkey and egg-laying chicken farms neighboring one another. MS appears to be endemic in the chicken layer and pullet populations without causing apparent clinical signs. In neighboring meat turkey farms, MS can sometimes result in catastrophic clinical disease such as progressive weakness, lameness, swollen hocks, increased cull birds and elevated overall flock mortality ranging from 10-40%. In analyzing recent December 2024 data from a local turkey processing plant, MS flocks resulted in the elevated need for carcass reprocessing (0.96-2.56%, goal <0.6%), trimming (0.8-1.69%, goal <1.0%), and total condemnations (0.29-2.95%, goal 1.12%). Due to the proximity between turkey and chicken farms, the turkey farmers have laid “blame” on nearby chicken operations as being the source of MS infection in their turkeys. In an effort to understand the epidemiology of MS in these poultry operations, this presentation will describe the sequencing results from the first and last quarter of 2024 examining the MS strains predominantly found in turkey versus neighboring chicken farms and their relationship to each other.

Food Safety Symposium

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Vaccination Strategies against Salmonella

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Abstract

Salmonella control is important for all segments of the commercial poultry industry from broilers, turkeys, breeders, and commercial layers. The goal for broilers and commercial turkeys is not eradication but reduction. For commercial layers, the goal is complete prevention of shed of *Salmonella enteritidis* (S.E.) to insure compliance with the FDA “Egg Rule.” To be successful, producers still need to utilize multiple interventions to lower the amount or load of Salmonella. Vaccination is one of the tools that can be utilized to increase resistance to colonization of Salmonella sp. from the birds’ feed or environment. Also, vaccination can be a very targeted tool to decrease shed of particular Salmonella serovars from one generation to the next. Additionally, Holt et al. found Salmonella vaccination to decrease the ability of S.E. to survive in eggs thus reducing the risk to consumers from commercial layer eggs. Vaccination of breeders (broiler, turkey, layer) will use a combination of commercially available live vaccine and/or inactivated vaccines. Live vaccines have been shown to provide protection to multiple Salmonella serotypes while inactivated vaccines have historically been protective to the Salmonella serotype contained in that vaccine. Traditionally, inactivated vaccines have been oil emulsion adjuvants and injected into breeder hens. The use of both live attenuated and inactivated vaccines are one of the key tools in any Salmonella control strategy. The presentation will review current research on live and inactivated vaccines for broilers, breeders and layers. In addition, data from field studies will be presented.

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Poultry Preharvest Sampling Schemes for *Salmonella* Management

Bill Potter

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Abstract

As poultry integrators consider best practices in preharvest *Salmonella* monitoring, the pros/cons of different sampling schemes must be considered. Best practices for sampling of environmental swabs, litter drag swabs, boot socks, fecal material samples, external bird rubs, feather-on rinses, breeder serology titers, breeder cloaca swabs, hatchery tray swabs, and hatchery residues must be optimized based on desired information. Lab analysis decisions are based on many factors such as number of samples needed, simplicity vs. complexity, lab facilities required, and personnel training requirements. Analytical options to consider may include a combination of plate agglutination, next-generation sequencing, serological ELISA, traditional PCR, biochemical ID, 16S sequencing, and whole genome sequencing with

bioinformatics, qPCR, and MPN's. The sampling sites and analyses selected can be used to drive intervention innovation and continuous improvement in pathogen reduction.

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Layer Industry Commercial Egg Salmonella Controls

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Abstract

Since 1989 when internal egg contamination by *Salmonella enteritidis* (SE) was implicated in cases of human illness the egg industry has been involved in developing preharvest control measures to combat this pathogen.

Presently, the preharvest controls are based on the US Food and Drug Administration's (FDA) Egg Safety Rule that was introduced in 2009 for premises of 3000 layers or more. This plan involves 1) biosecurity, 2) cleaning and disinfection of houses, 3) securing day-old chicks from SE negative sources, 4) rodent and fly control measures, 5) refrigeration of eggs within 36 hours of laying, and 6) a monitoring scheme of pullets (14 to 16 weeks) and layers (40 to 45 weeks and post molt) to detect premises at high risk for SE egg contamination.

The incentives to maintain negative flocks is 1) the requirement for quite expensive egg testing if samples of feces during grow or lay are positive for SE, and 2) required diversion from shell egg sales to pasteurization or hard cooking should egg testing find a positive.

Management inputs beyond the FDA Egg Safety Plan to assure negative flock status are 1) use of an effective vaccination scheme using both live and killed vaccines, 2) use of non-antibiotic feed or water additives that aid intestinal health and have shown effectiveness in reducing SE and *Salmonella spp.* infection, 3) drinking water sanitation, 4) effective egg sanitation during processing to avoid shell contamination with SE or other serotypes of *Salmonella spp.*

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AFIA Safe Feed/Safe Food Program

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American Feed Industry Association, Arlington, VA, USA

Abstract

Nearly two decades ago, the American Feed Industry Association (AFIA) set out to develop a program that would establish and promote generally accepted food safety guidelines that ensure continuous improvement in the delivery of a safe and wholesome feed supply for the growth and care of animals. That goal blossomed into the mission of the Safe Feed/Safe Food Certification Program, launched in 2004. Today's audit has touchpoints on all aspects of FSMA for animal

food along with biosecurity, supplier approval and food defense. Major benefits of participation include market differentiation, ease of B2B transaction and improved regulatory compliance.

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Poultry Food Safety Regulatory Update

Ashley Peterson

National Chicken Council, Washington, USA

Abstract

In April 2023, the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) proposed a new regulatory interpretation regarding *Salmonella* in not-ready-to-eat breaded stuffed chicken products. In the Agency's final determination, published in May 2024, FSIS declared *Salmonella* at or above 1 CFU/g as an adulterant in the raw incoming chicken. FSIS also provided insights into its *Salmonella* Framework in October 2022 outlining the Agency's alternative approach to reducing *Salmonella* in raw poultry products focusing on preharvest requirements, statistical process control, and an enforceable final product standard. This proposal, published in August 2024, focused on whole chickens, chicken parts, and ground chicken as well as ground turkey. It, too, declared *Salmonella* as an adulterant in poultry products through an enforceable final product standard set at or above 10 CFU/g coupled with certain *Salmonella* serotypes. The proposal also outlined requirements pertaining to statistical process control, sampling locations, and data submission. Comments on this proposal were due in January 2025.

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Feed Additives Impact on Salmonella

Tim Johnson

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Abstract

A variety of approaches are required to have effective pre-harvest control of *Salmonella* in poultry. Many of these approaches involve feed additives. This presentation will provide an overview of current approaches employed using feed additives and discuss some of the science supporting (or not supporting) their efficacy. The implications of multiple or different strains of *Salmonella* on feed additive efficacy will also be discussed.

NPIP Considerations in the Preharvest Food Safety Symposium

Denise Heard

U.S. Poultry & Egg Association, Tucker, USA

Abstract

This lecture is part of the Preharvest Food Safety Symposium, it will focus on the NPIP Salmonella Programs, their history as related to Salmonella controls, current certification options, and how the program can be valuable to the poultry industry. A review of the recent proposals given consideration at the previous Biennial Conference will be provided, and the ways in which the NPIP has been considered as an avenue for programs for preharvest monitoring will be discussed.

Gate-to-Plate: Industry-Driven Food Safety Excellence in Canadian Poultry and Eggs

Martine Boulianne

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Abstract

The Canadian poultry industry operates under a national supply management system. Independent producers coordinate production decisions at the national and provincial levels. This unique structure has enabled the creation and implementation of national, industry-driven food safety initiatives.

One such initiative is the On-Farm Food Safety Program (OFFSP) developed by the Chicken Farmers of Canada in 1998. This science-based program is designed to ensure the production of safe, high-quality chicken through standardized farming practices. The OFFSP is based on Hazard Analysis and Critical Control Points (HACCP) principles focusing on risk identification and management throughout the production chain. Key program components include biosecurity, flock health management, feed and water quality controls, and record-keeping. Producers must adhere to standard operating procedures, undergo regular audits, and maintain traceability systems. Training and certification are mandatory for farm personnel for consistent application of best practices.

To evaluate program efficacy, compliance rates and pathogen prevalence data are systematically collected and analyzed. Since its implementation, OFFSP has achieved over 95% national farmer participation. Surveillance data indicate significant reductions in on-farm Salmonella and Campylobacter levels supporting improved food safety outcomes.

Similarly to the broiler initiatives, Turkey Farmers of Canada and Egg Farmers of Canada (EFC) have also developed national, HACCP-based on-farm food safety programs. The EFC's 'Start Clean–Stay Clean®' program primarily focuses on preventing Salmonella Enteritidis for layer flocks.

All programs aim to minimize food safety risks directly at the farm level, ensuring consistent high standards across Canadian poultry production and aligning with national poultry food safety commitments.

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Preharvest Salmonella Control Strategies

Ken Macklin

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Abstract

Salmonella continues to pose a major challenge to the poultry industry, with the CDC reporting approximately 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths in the U.S. each year. In response, USDA's Food Safety and Inspection Service (FSIS) released a proposed framework that places greater emphasis on reducing *Salmonella* at the preharvest stage. While this regulatory spotlight is relatively new, the poultry industry and research community have long been exploring ways to minimize *Salmonella* on the farm. Preharvest intervention strategies include a broad range of tools—feed additives (such as prebiotics, probiotics, phytogenics, and organic acids), water treatments, dietary adjustments, vaccination, and improved flock and litter management. Among these, sound biosecurity and effective poultry house management remain essential foundations for control. While no single approach offers a complete solution, integrated strategies that combine multiple interventions are showing strong potential in reducing *Salmonella* load before birds reach processing. Continued investment in practical, scalable solutions will be key to helping the industry meet evolving regulatory expectations while protecting public health.

H5 Influenza Mini-Symposium

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H5 influenza: Leveraging public forums to advance public policy

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Abstract

The persistent threat of highly pathogenic avian influenza (HPAI) poses significant challenges to both animal and public health, yet critical policy measures to mitigate its spread often face governmental inertia. This talk explores how AAAP utilized public forums as strategic platforms to drive policy action on HPAI control. By engaging stakeholders—including other veterinary organizations, media, and public health groups—AAAP veterinarians were able to amplify scientific evidence, highlight economic and public health impacts, and create demand for effective intervention strategies.

Key discussion points include framing scientific data for non-specialist audiences, leveraging various forms of media to generate awareness, and forming coalitions with allied industries to exert political pressure. Case studies will illustrate successful use of public forums to advance neglected policies, including improved surveillance systems, addressing roadblocks to implementing comprehensive disease mitigation strategies, and funding to support expanded response activities.

This presentation will provide attendees with insights into how AAAP has engaged in the dynamics of public discourse, utilized tools available for effective information transfer, and how a coalition veterinarians are working on shaping policy conversations. By focusing on the intersection of science communication and advocacy, the talk aims to enhance understanding of how public forums can be leveraged to drive meaningful policy change for HPAI control.

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H5 influenza in dairy: the difficulties in balancing science and production with pragmatism

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Abstract

In March 2024, the world seemed to stand still when H5N1 was detected for the first time in commercial dairy cattle in Texas. A virus primarily associated with the poultry industry, H5N1 was an unexpected threat for the dairy sector and its veterinarians. The weeks following this initial discovery presented a crucial window to contain the virus, but the absence of clear guidance from industry leaders, state, and federal authorities led to a concerning lack of action. This gap in direction left both the dairy and poultry industries struggling to respond effectively to the rapid and unforgiving spread of H5N1 across multiple states and farms.

This presentation will serve as a post-mortem analysis, aiming to identify key lessons learned and critical control points that could better equip the industry for future outbreaks of unknown or foreign agents. It will also address key questions that need to be answered in order to develop comprehensive and actionable strategies for managing H5N1 in cattle and other commercial species moving forward.

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H5 influenza: California dairy vet field perspective

Blaine Melody

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Abstract

H5 influenza was introduced to California through the movement of lactating cows in late September 2024. As in other affected states, the virus spread rapidly through vulnerable dairy farms in the region, also impacting nearby poultry

operations. Data from other states indicated that dairy cows shed detectable levels of the virus in bulk milk tanks before clinical symptoms appeared. This early detection provided our team with an opportunity for proactive herd screening, allowing us to identify infections early and gain a better understanding of how the virus spreads both within and between herds through longitudinal analysis. This discussion will review the findings from our collaborative effort, which may help shape future influenza control strategies in both the dairy and poultry industries. Additionally, it highlights the importance of involving local practitioners in both preventive and control strategies for emerging diseases in production animal species

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H5 influenza: Human health risks from A(H5N1) influenza viruses

Richard Webby

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Abstract

Close to 1000 zoonotic infections with the A/goose/Guangdong/1/96-lineage A(H5) viruses have been detected globally with a mortality of over 50%. Despite these numbers, decades of circulation in birds, and sporadic detections of mammalian adaptive markers the A(H5) viruses have maintained their avian influenza virus features. Fears of this changing to include more mammalian influenza virus phenotypes were exacerbated with the detection of A(H5N1) viruses in dairy cattle in the U.S. Contrary to these fears, the ability of the bovine-sourced A(H5N1) viruses to bind to human virus receptors, transmit between ferrets, and evolve antigenically, all markers associated with elevated risk to humans, have not changed even with more than a year of continued circulation in dairy cows. The occasional spillover of virus from birds to other mammals has similarly lead to limited evidence for mammalian adaptations. While these data are reassuring from a public health perspective, the continued evolution of the A(H5) viruses requires constant assessments and strong collaboration between One-Health partners.

Immune dynamics in the long-term maintenance of infectious bronchitis virus (IBV) in the cecal tonsils of chickens

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Abstract

The prevalence of infectious bronchitis (IB), caused by infectious bronchitis virus (IBV), has risen in Canada, resulting in significant economic losses. IB is an acute respiratory disease marked by clinical signs due to viral replication in the upper respiratory tract. However, IBV can disseminate to other tissues and persist in the cecal tonsils (CT) even after being cleared from other sites. Persistently infected chickens act as reservoirs of infection and contribute to viral evolution. This study hypothesized that the natural abundance of regulatory T cells (Tregs), defined as CD4⁺ CD25⁺ T cells, and their early presence in the CT contribute to IBV persistence. Tregs suppress immune responses by inhibiting T cell activation, impairing CD8⁺ T cell function, and secreting anti-inflammatory cytokines.

The study aimed to investigate why the CT harbors higher IBV genome loads than the spleen during later infection stages, despite both being secondary immune organs, and to highlight the significant presence of Tregs in the CT of young chicks. Experimental infection of one-week-old specific pathogen-free chicks with IBV strains Delmarva (DMV)/1639, Massachusetts (Mass), and California (Cal) 1737 revealed DMV/1639 as the most pathogenic strain, causing severe clinical signs, high viral genome loads, and continuous viral shedding compared to uninfected at 3-, 8-, 10-, and 14-days post-infection (dpi). Immune cell recruitment, including B and CD8⁺ T cells, was significantly lower in the CT than in the spleen at 8 and 10 dpi. These findings will be further validated through cytokine profiling (IL-10, TGF- β , and IFN- γ) and Treg analysis

***Clostridium septicum* alpha toxin-based recombinant subunit vaccines can protect turkeys against experimental Clostridial dermatitis**

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Abstract

Clostridium septicum is one of the major pathogens causing Clostridial dermatitis (CD) in turkeys. In recent years, CD incidences in turkey flocks are on the rise. Unfortunately, there are no effective vaccines currently available. Here, two non-toxic domains of *C. septicum* alpha toxin, namely ntATX-D1 and ntATX-D2, were identified, cloned, and expressed in

Escherichia coli as recombinant proteins, and used in immunizing turkeys. Experimental groups included, negative control (NCx) that did not receive *C. septicum* challenge, while an adjuvant-only positive control (PCx) and two immunization groups, ntATX-D1 immunization (D1) and ntATX-D2 immunization (D2), received *C. septicum* challenge. Immunized turkeys received 100 µg of protein at 7, 8 and 9 weeks of age along with an oil-in-water nano-emulsion adjuvant subcutaneously, followed by a challenge at 11 weeks of age. Results showed that D1 and D2-immunized groups had reduced ($P<0.05$) mortality and gross as well as histopathology lesion scores when compared to PCx birds. Gene expression evaluation showed that turkeys in the PCx group had higher ($P<0.05$) expression of pro-inflammatory cytokine genes in the skin, muscle and spleen than the NCx group, while the D2 group had lower ($P<0.05$) transcription of these genes compared to PCx. Peripheral blood cellular analysis revealed increased ($P<0.05$) frequencies of activated CD4+ / CD8+ cells in the immunized groups, particularly in the D2 group, while both D1 and D2-immunized turkeys developed antigen-specific serum IgY antibodies. Collectively, it was evident that both subunit proteins, especially the ntATX-D2 possesses excellent vaccine candidacy potential in protecting turkeys against CD.

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Characterization of mucosal immune responses during necrotic enteritis in chickens and identifying immune genes associated with disease susceptibility

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Abstract

Clostridium perfringens, a Gram-positive, anaerobic and toxin-producing bacterium, causes Necrotic enteritis (NE), an enteric disease of chickens that negatively impacts the economy of the broiler industry worldwide. Although NE has been such an economic burden, no effective vaccines are currently available. This is perhaps largely because of a poor understanding of the host responses during NE. In the present study we used two NE predisposing models, namely the 'dietary' and 'dietary + Coccidia (a mixture of *E. maxima*, *E. tenella* and *E. acervulina*)', to evaluate the mucosal (duodenum and jejunum) and lymphoid (cecal tonsil 'CT' and bursa of Fabricius 'Bursa') immune gene expression in broiler chickens during NE. We also used two virulent strains of *C. perfringens*, Str. CP44 and Str. CP64, to reproduce NE. Results showed that Coccidia-predisposition followed by CP44 or CP64 infection induced increased ($P<0.05$) expression of IL-1 β , IL-6, IL-13 or FoxP3 genes in the cecal tonsils compared to the Coccidia-alone group. Additionally, the Coccidia+CP44 infected group had higher ($P<0.05$) IFN γ transcription in the duodenum and jejunum tissues. Furthermore, birds receiving dietary predisposition+CP44 (but no coccidia) also had elevated ($P<0.05$) expression of the IL-6 gene when compared to the negative (uninfected) control group. Collectively, our findings showed that coccidia-predisposition to NE results in an increased gene expression of pro-inflammatory cytokines (IL-1 β /IL-6/IFN γ) in both mucosal and lymphoid tissues, as well as increased immunoregulatory transcription factors such as FoxP3. Further work to devise novel vaccines against NE in chickens is currently underway.

Protection of broiler chickens from *Escherichia coli* and Necrotic enteritis through trained immunity induced by synthetic and natural CpG motifs

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Abstract

Bacterial infections by *Escherichia coli* (*E.coli*) and *Clostridium perfringens* (CP) are responsible for significant economic losses (\approx US \$6 billion/year) in the broiler chicken industry worldwide. Previously, we have demonstrated that delivery of two doses of oligodeoxynucleotides containing CpG motifs (CpG-ODN) in neonatal broiler chickens promotes trained immunity by metabolic reprogramming of immune cells to protect them against *E. coli* and *Salmonella* during grow-out period. The objectives of this study were to explore the ability of CpG-ODN to induce trained immunity using two industry-feasible delivery methods: (1) administration of CpG-ODN by the *in-ovo* route at day 18 of incubation followed by CpG-ODN or live CP via intrapulmonary (IPL) route at hatch to protect chickens against *E.coli* septicemia; (2) administration of two CpG-ODN injections via intramuscular route (IM) at days 1 and 4 of age against necrotic enteritis. Interestingly, administering CpG-ODN via the *in-ovo* route followed by CpG-ODN or live CP by IPL route induced trained immunity. The induction of trained immunity was confirmed by metabolic reprogramming of immune cells (metabolism shift from glycolysis to increased mitochondrial OXPHOS at a significant level ($p<0.0001$), which provided significant protection ($p<0.0001$) against *E. coli* septicemia at 27 days of age. Two IM administrations of CpG-ODN protected broiler chickens at 20 days of age by significantly reducing gross and histopathological lesions in the jejunum ($p<0.05$) Monocyte/Macrophage cytokine production (IL-1 β and TNF α) significantly elevated ($p<0.05$) in protected groups. Trained immunity provided broad-spectrum protection in broiler chickens against bacterial infections from the hatch to grow-out period.

Immune Responses against RNA Viruses Investigated by Transcriptome Analysis

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Abstract

For a long time, the immune response against specific viruses has been investigated with the rational that a better understanding will help in the development and improvement of prevention methods, in particular vaccines. The most recent method that has been employed is the analysis of the transcriptome in infected organs. In the last three years, our laboratory has analyzed the transcriptome after infection with Newcastle disease virus (NDV) and avian reovirus (ARV). Three animal trials using specific pathogen free (SPF) chickens of different ages and different NDV strains investigated the transcriptome in Harderian glands and tracheas, while one animal trial and two in-vitro studies in SPF chicken embryos and cell culture investigated the transcriptome after infection with ARV. Genes consistently identified

as differentially expressed after infection with NDV included USP41, OASL, IRF7, GBP1, and IFIT5; all genes that have previously been implicated in the immune response against viruses. Well-known antiviral immune genes that were identified in the experiments involving ARV included IFI6, OASL, RSAD2, SAMD9L, CMPK2, and MX1, indicating only a partial overlap with the NDV results. While some differences might be explained by different sampling time points and host systems, they are nevertheless worth consideration. In addition to these genes, numerous lesser-known genes were identified. However, the results also show the limitations of transcriptome analysis, especially the identification of certain pathways whose labels are extrapolated from human biology and may not accurately represent their role in chickens.

Immunosuppressive Viruses

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Evaluating broiler health and performance with increased Marek's protection.

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Abstract

Marek's disease is highly significant to the commercial chicken industry. To complicate this issue the traditional primary clinical sign of tumors has now evolved to an underlying state of immunosuppression which often manifests as increases susceptibility to secondary pathogens, poor performance and lack of uniformity. This change is due to the increasing shift in virulence of the Marek's disease virus. These vv+ strains severely debilitate the developing immune organs of young chicks from the first day of virus exposure, particularly T cells in the thymus, even in the face of maternal antibodies and the most current MDV vaccination programs. This case study followed key performance indicators (FCR and ADG) using statistical process control charting (SPC JMP® 18.0.1) over time, from one year prior to approximately 7 months post vaccine change. Study duration was 13 weeks. In addition, immune health surveys (histopathologic assessments of thymus and bursas) were performed before, during and after study completion. The role and value of monitoring production performance through control charts will be discussed, as well, the importance and value of immune health surveys provide as a complementary tool to understanding forward health. This study demonstrated: primary immune health organs (bursa and thymus) had prolonged damage despite no visible tumors at the onset of the project; improvement in histologic scores of bursal and thymus tissues, as well as daily gain and feed conversion improvements beyond the complexes expected seasonal improvements, were directly correlated with the use of the alternative vaccine.

An Epidemiological Study of Infectious Bursal Disease in the Canadian Commercial Broiler Industry: Geographical Variations and Vaccination Programs

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Abstract

In Canada, infection with variant strains of the infectious bursal disease virus (IBDV) in commercial broilers leads to moderate to severe bursal atrophy, contributing to immunosuppressive disease. Typically, infections occur after 20 days of flock age, though earlier infections are occasionally observed.

Depending on the age at which IBD infection occurs, affected broiler flocks may experience subclinical infections, increased condemnations at processing plants, poor growth, higher feed conversion rates, and increased mortality due to concurrent diseases such as inclusion body hepatitis (IBH), colibacillosis, and infectious bronchitis.

This review aims to investigate the prevalence of variant IBDV strains across Canadian provinces, focusing on molecular differences in the VP2 region of the virus. The evolving dynamics of these variant strains over time and across various regions will be discussed. This data is derived from diagnostic submissions of bursal tissue samples to the University of Guelph Animal Health Laboratory for PCR testing and subsequent genotyping of positive samples over the past 14 years.

We will also briefly address different vaccination programs in various geographical regions, which may include hyperimmunization of broiler breeder parents, in-ovo vaccination of broilers with recombinant or immune complex vaccines, hatchery spray vaccination, and/or on-farm vaccination with live intermediate vaccines. One successful approach has been the rotation of hatchery and on-farm vaccination regimes based on seasonal IBD pressure and/or concurrent IBH concerns.

Effects of variant infectious bursal disease virus SK09 infection on immune responses in broiler chickens with and without maternal antibodies

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Abstract

Variant of infectious bursal disease virus (varIBDV) causes severe immunosuppression which can lead to secondary infections. Our previous studies revealed that broiler progeny from varIBDV SK09 vaccinated broiler breeders were protected against varIBDV SK09 infection. However, the detailed immune mechanisms against varIBDV infection in

broilers with maternal antibodies (MatAb) against varIBDV have not yet been investigated. The objective of this study was to characterize immune cell profile and cytokine gene expressions in broilers (with or without MatAb against varIBDV SK09) challenged with varIBDV SK09. Broiler progeny from unvaccinated broiler breeders (MatAb^{-ve}) or broiler progeny from varIBDV SK09 vaccinated broiler breeders (MatAb^{+ve}) were inoculated with varIBDV SK09 at day 6 of age. The subsets of T cells, B cells, and macrophages in the bursa of Fabricius (BF), spleen, and cecal tonsils were examined by flow cytometry. A significant decrease of B cells and an increase of T cells and macrophages were observed in the BF and spleen at day 5 post-infection (dpi) in MatAb^{-ve} broiler progeny compared to MatAb^{+ve} broiler progeny. Significant upregulation of IFN-γ and granzyme A genes were detected in the BF in MatAb^{-ve} broiler progeny compared to MatAb^{+ve} broiler progeny at 5 dpi. Since the virus was not detected in MatAb^{+ve} broiler progeny at 5 dpi, the results suggest that MatAb against varIBDV SK09 in MatAb^{+ve} broiler progeny inhibited viral replication which induced subsequent immune system cell recruitment and cytokine expressions in MatAb^{-ve} broiler progeny.

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Whole genome sequencing of probe-enriched infectious bursal disease virus isolates

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Abstract

Infectious bursal disease virus (IBDV) is ubiquitous in poultry, causing immunosuppression which leads to secondary infection and mortality. Traditional viral isolation and characterization methods include cell culture and PCR to classify the bi-segmented virus based on the hypervariable region of VP2 within Segment A. However, recent publications propose a classification method using both Segment A and B of the viral genome. We sought to develop a method of IBDV classification using both viral segments by employing a probe enrichment method to allow for whole genome sequencing from viral isolates and potentially field sample material. By combining publicly available enrichment probe development tools with novel lab protocol and bioinformatic workflows, we can sequence and classify both viral segments of IBDV from sample isolates. This information can be used for IBDV genotyping, vaccine development, and further protection of poultry flocks from IBD.

Molecular characterization of Infectious Bursal Disease Virus (IBDV) variant strains circulating in Mississippi between 2017-2024

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Abstract

Infectious Bursal Disease Virus (IBDV) is a major cause of immunosuppression in chickens, causing production losses and welfare issues. Vaccination programs, though widely used, do not provide sterilizing immunity, threatening vaccinated flocks to harbor subclinical infections with field strains. These strains can evolve through antigenic drift or reassortment, producing variants that compromise flock immunity.

Mississippi is among the major poultry producers in the United States, while the last molecular characterization was published in 2003, new molecular data is required since IBDV variant strains remain as a menace. This study aimed to characterize IBDV strains circulating in Mississippi from 2017 to 2024. Over 50 bursal samples from clinical cases underwent RNA isolation, reverse-transcription polymerase chain reaction (RT-PCR), and Sanger sequencing, targeting the VP2 hypervariable region (HVR). All the IBDV sequences were classified in the US variant A2 genogroup with amino acid identities ranging between 85 to 98% compared with the prototype variant Delaware E, however, we detected increased antigenic drift mutations in the HVR: S254N/K, S317R/T/I, G322E, and E323D compared to Delaware E. Moreover, this mutation pattern was similar to the genetic signature recently reported in variant strains in the Delmarva region. We also observed conserved mutations outside the HVR loops: I264M, T269S, and I272V. These findings provide insights into the genetic diversity of IBDV variants in Mississippi and their potential role in immune evasion. A larger dataset including clinical history, prevalence, and geographical region will be analyzed to draw more robust conclusions.

Improving In Ovo Vaccination Accuracy: Understanding the Critical Role of Site of Injection (SOI)

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Abstract

In ovo vaccination has revolutionized poultry production by enabling mass immunization of chicken embryos. However, the effectiveness of this practice relies heavily on the Site of Injection (SOI). Proper SOI—injecting into the amnion or embryo—ensures strong immune responses and optimal protection, while misplacement in the air cell or allantois results in inadequate immunity and economic losses. This paper emphasizes the critical role of SOI accuracy in enhancing vaccine efficacy, particularly for Herpesvirus of Turkey (HVT) and SB-1. Additionally, the study evaluates the impact of *in ovo* vaccine delivery routes on viremia and disease protection, focusing on Marek's disease.

The findings highlight several factors influencing SOI precision, including embryo development stage, egg orientation, transfer timing, and incubation conditions. Optimal timing between 17 days and 12 hours to 19 days and 4 hours of incubation is essential to achieve accurate injections. Embryo development, inconsistent incubation conditions, and mistakes on the *in ovo* process can undermine SOI accuracy. Daily device checks and maintenance, operator training, and SOI studies are indispensable for monitoring and improving vaccine delivery performance.

This research highlights the importance of precise vaccine placement to ensure effective Marek's disease protection, and enhance flock health and productivity. By refining SOI practices, hatcheries can maximize the efficacy of *in ovo* vaccination and secure robust immune protection in poultry.

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The impact of AL2 IBD challenge at different ages on the response to a killed SE vaccination and the sparing effect of rHVT-IBD on titer suppression

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Abstract

This study was conducted to compare the impact of AL2 challenge when given at different ages on the ability of chickens to produce antibodies to a killed SE-ND-IB vaccine given at 4 weeks of age. A second goal was to see if vaccination with a recombinant HVT-IBD vaccine would have similar sparing effects not only on the bursas but on the active immune response to the inactivated Salmonella vaccine—**Study Design.** 630 SPF leghorns were divided into 6 treatments: T01 (NC), T02 (D11 AL2), T03 (D21 AL2), T04 (D28 AL2), T05 (D0 IBD vax; D21 AL2) and T06 (D0 IBD vax; D28 AL2). All groups were vaccinated at 4 weeks of age with a commercial SE-ND-IB bacterin. Bursas were measured and weights taken on Days 21-28-35-56. Newcastle disease virus (NDV) and Salmonella Group B and D ELISA titers were measured on D55. Birds with B:BWts at least 2 standard deviations below the negative controls were considered not protected—**Results and Discussion.** All three AL2 challenge ages resulted in significant atrophy that lasted until study termination. The D11 AL2 challenge significantly reduced ELISA GMT response to ND (50%), Sal-B (90%) and Sal-D (85%). The D21 AL2 challenge reduced Sal-B (80%) and Sal-D (40%) GMTs, and the D28 AL2 challenge reduced ND GMTs by 55%. The rHVT-IBD vaccine protected birds from bursal atrophy and against the depressed titer responses caused by D21 and D28 AL2 challenges.

Monitoring of breeder flocks vaccinated against Fowl Adenovirus using virus neutralization tests for serotypes 4 and 8b

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Abstract

Peru faces severe issues with Fowl Adenoviruses (FAdV), which cause diseases such as IBH and Hepatitis-Hydropericardium Syndrome. The most prevalent serotypes are 4 and 8b, which are included in vaccination programs. Vaccinating breeders is the most effective way to prevent these diseases and stop vertical transmission to the offspring. The goal of breeder vaccination is to produce a high level of maternal antibodies (MAb) to protect the chicks during their first 3 weeks of life. Vaccines used must create a strong and lasting immune response in the breeders against all prevalent serotypes. However, monitor humoral antibody levels in vaccinated breeder flocks and their progeny is challenging to due to the lack of specific ELISA kits. Therefore, a virus neutralization (VN) assay was implemented to test the effectiveness of a killed FAdV serotype 8b vaccine in Peru. In this study, two breeder flocks, (80,000 birds each), were vaccinated with the FAdV 8b killed vaccine at 9 and 20 weeks. An autogenous polyvalent vaccine was given at 4 weeks, and a specific-serotype 4 vaccine at 14 weeks. Blood samples were collected at 9, 14, 20, and 30 weeks. The day-old-progeny from breeders at 30 weeks of age was also evaluated. Seroconversion was measured using a microneutralization assay in LMH cell culture with 100-500 TCID₅₀ of neutralizing virus for serotypes 4 and 8b. The results showed strong VN titers against serotype 8b. MAb titers in the progeny were also encouraging with average VN titers exceeding 33,000. Results for serotype 4 served as reference.

Keynote Speaker

Advances in Broiler Production Efficiencies 1980 through 2025

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Abstract

The modern history of the poultry industry, whether we are talking about broiler, commercial layer or turkey production is a story of continuous improvement in many areas including production efficiencies leading to lower costs of production and increased availability of products to meet changing consumer demands.

Forty-five years ago in 1980, the leading broiler genetics company in their feeding and management guide stated that their efficiency goal for their broilers was a bird that would grow to 4.0 lbs. at 52 days of age on a feed conversion ratio of 2.1 lbs. of feed for every lb. of weight gain. In 2025 the actual production efficiency would be a bird reaching 4.0 lbs.

at 32 days of age on a feed conversion ratio of 1.52 lbs. of feed for every lb. of weight, 40% faster growth, 28% less feed to the same weight. In 1980, boneless breast meat as a % of live weight might have been 13.5? Now average boneless yields surpass 27% and reach 31% for the heaviest birds. Truly remarkable.

The purpose of this presentation to the American Association of Avian Pathologists is to share an overview of the history of U.S. broiler production over the last 45 years and to examine where changes will likely occur. We will examine the challenges producers confront in meeting the increasing consumer demand for more chicken, and why poultry continues to be the preferred protein choice throughout the world.

Reovirus

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Using primary chicken and turkey intestinal organoids to dissect the pathogenesis and host response to avian reovirus (ARV) infection.

Declan Kehlbeck¹, Sofia Egana-Labrin¹, Alex Broadway¹, Jimmy Dong¹, Jyothsna Girish¹, Megan Liu¹, Milos Markis², Younggeon Jin¹, Kristen Brady³, [Andrew Broadbent](#)¹

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Abstract

Avian reoviruses (ARVs) are an economic burden to the poultry industry, affecting chickens and turkeys. The viruses can replicate in the gut, and while the majority are asymptomatic, some cause enteritis, and others spread to the tendons, causing tenosynovitis. ARV pathogenesis is poorly understood. To address this, we generated primary inside-out floating intestinal organoids from chickens and turkeys and infected them with ARV. Chicken organoids were infected with a live attenuated vaccine strain, a strain that caused enteritis but not tenosynovitis (GF2-E), and a strain that caused tenosynovitis but not enteritis (B8-T). Disease outcome was independent of viral replication, measured by RTqPCR and TCID₅₀. Strain GF2-E replicated similarly to B8-T, and less than the vaccine strain, yet GF2-E exhibited a higher average expression of pro-inflammatory genes, and caused a more pronounced loss of barrier permeability, by Trans-Epithelial Electrical Resistance (TEER) assays. We hypothesize that strains responsible for enteritis cause differences in the intestinal response to infection compared to other strains, and we propose that the organoid cultures can be used to screen viral isolates to help determine whether they are the primary cause of enteritis, or an asymptomatic bystander infection. Moreover, ARV replicated similarly in chicken and turkey organoids, yet there were differences in the host response to infection, by RNASeq, improving our understanding of how different species respond to infection. As turkey isolates are typically passaged in chicken cells prior to sequencing and genotyping, we plan to determine if passage in different host cells alters the viral sequences.

An Insight into Avian Reovirus Evolution and Cross-Species Transmissions in Turkey Hosts

Cheng-Shun Hsueh, Michael Zeller, Amro Hashish, Olufemi Fasina, Pablo Piñeyro, Mohamed El-Gazzar, [Yuko Sato](#)

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Abstract

Avian reovirus (ARV) has been an economically important virus in turkeys, causing a variety of pathology including tenosynovitis, hepatitis, and enteric disease. Despite its ubiquity, the evolutionary history of cross-species transmission between chickens, turkeys, and wild birds—thus epidemiology of the virus, remains poorly understood. This knowledge gap severely impedes tracing viral transmission, developing effective control strategies, and mitigating the disease's significant economic impact. This study investigates ARV segment-by-segments phylogenetics, with a particular emphasis on cross-species transmission and host adaptation in turkey, utilizing whole-genome sequence (WGS) of 77 turkey cases and 1 quail case from the Iowa State University Veterinary Diagnostic Laboratory between 2019-2024, supplemented by segment sequences from GenBank (89-150, dependent on segment). Phylogenetic analyses of these 10 segments identified chickens as the common ancestral host, with initial spillovers to turkeys occurring in the mid-20th century and subsequent established stable transmissions within turkey populations. Our findings reveal that all 10 segments of ARV have a different history, with the M2 segment having a particularly unique evolutionary history with 6 distinct clades based on temporal phylogenetic relationship. Six M2 clades reveal host-specific evolutionary patterns (galliforms vs. waterfowl). Temporal phylogenies and migration analyses highlighted predominantly unidirectional transmission from chickens to turkeys, with sporadic reverse spillovers. Our findings emphasize the complexity of ARV evolution across multiple avian host species, with segment independence playing a critical role, and highlight the necessity of robust genotyping schemes to effectively trace viral transmission.

Field Surveillance of Turkey Reovirus: Infection Dynamics, Environmental Persistence, Noninvasive Sampling, and Pathogenicity in Commercial Farms

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Abstract

This study evaluated the effectiveness of non-invasive sampling as a unique way to screen turkey reovirus (TRV) in commercial farms. Five commercial farms with previous TRV infection histories from two states were recruited. Samples were collected from the farm environment (walls, litter, fans, and drinkers) in the preplacement (after cleaning and disinfection), and at regular intervals through the rearing period. Additionally, tendons, livers, hearts, and intestines were collected to confirm the infection and correlate the environmental virus's existence with clinical infection. This study found that TRV was detected and isolated in the farm environment before clinical infection detection. Specifically, TRV was identified and isolated from the farm environment during the preplacement phase, indicating that the virus can resist the cleaning and disinfection strategies commonly employed in turkey farms. Additionally, TRV was detected in the heart and liter samples during the first week of age, raising the possibility of vertical transmission. In conclusion, TRV

detection in environmental samples like litter, walls, and fans is predictive, possessing a potential diagnostic role for early detection of the infection and assessment of the decontamination processes. This study offers valuable insights into the sources of TRV infection under field conditions.

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Investigating the genomic variability of avian reovirus variants by serial passages in embryonated chicken eggs

Abdul Rehman Bilal, Rachel L Jude, Rodrigo A Gallardo

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Abstract

Viral arthritis and tenosynovitis are typical clinical outcomes of avian reovirus infection in broilers and broiler breeders in the United States since 2011. This pathology has led to significant economic losses and welfare issues to the poultry industry worldwide. Due to segmented double-stranded RNA genome, this virus is prone to point mutations, recombinations, and reassortments causing emergence of ARV variants that complicate prevention and control strategies. It is imperative to investigate regions in ARV genome that harbor variability for better characterization and associate them with changes in antigenicity or pathogenicity. This study aims to investigate variability of two plaque purified avian reovirus isolates by investigating their viral load and genomic changes along with embryo mortality they induce during serial passaging in specific pathogen-free embryonated chicken eggs. Results showed increased mortality as passages increased. Isolate 1 showed a significant increase in viral load from passage 5 to passage 10. However, for isolate 2, the viral load increase was not significant. Since the highest variability has been reported in S1, M2, and L3 genes, sequences from the 1st and 15th passage were obtained and analyzed. This analysis showed mutations in segments that might have correlation with observed embryo mortality and/or increased viral load. Clinical and genomic changes, detected in above-mentioned genes, suggest ARV adaptation to embryonated eggs. Mutations will also be explored at whole genome level. This study provides insights about variable regions in the ARV genome that can be associated in subsequent experiments with functional changes in antigenicity and pathogenicity.

Salmonella

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Effect of a phytogenic combination administered via drinking water on pre-harvest *Salmonella* in broilers

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Abstract

Salmonella in broilers remains a major challenge in the poultry industry, with significant implications for customer food safety. As a primary contributor to salmonellosis outbreaks associated with meat consumption, implementing effective control measures is paramount. This study assessed the impact of a phytogenic combination on *Salmonella* colonization in poultry ceca and carcass rinsate, aiming to enhance pre-harvest food safety. Potential to reduce *Salmonella* colonization was evaluated through prevalence and enumeration following oral inoculation with a nalidixic acid-resistant *Salmonella* Typhimurium (ST) strain. Three hundred sixty male Cobb 500 broilers were allocated to four treatments with six replicates and orally challenged with 1.0 ml of 7.0×10^8 CFU/ml ST seven days before harvest (day 28). Treatments included: (A) challenged control (CC), (B) CC with water treatment at 2 dpi (5 days pre-harvest), (C) CC with water treatment at 4 dpi (3 days pre-harvest), and (D) CC with water treatment at 5 dpi (2 days pre-harvest). *Salmonella* counts were assessed in the ceca (days 32-35) and carcass rinses (day 35). A t-test analyzed day 32 data, ANOVA with Scheffe's test was used for days 33-35, and chi-square tested the incidence of ST positive samples. Water treatment significantly ($P < 0.05$) reduced ST counts in the ceca at 4, 5, and 6 dpi, while carcass rinsate counts decreased by 2.02, 1.40, and 1.47 \log_{10} CFU/ml, respectively. These findings suggest that administering a phytogenic water supplement 2-5 days before processing may be an effective pre-harvest intervention for reducing *Salmonella* in broiler production.

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Incidence of *Salmonella* Hadar in Two Big Tom Complexes

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Abstract

Salmonella Hadar has been identified as a serotype of public health significance in raw comminuted turkey. The incidence of this serotype was tracked in two big tom complexes (299 big toms flocks) from breeders through growout for all placements in CY2024. Bootie samples were collected during early brood, post-move and pre-market in commercial toms, and early brood, selection and peak lay in breeders. The presentation will discuss several factors that may influence the incidence of S. Hadar in commercial flocks. These include those related to vertical transmission, commercial vaccine usage, and the relationship between prevalence during brood and growout in commercial big tom flocks. All samples were cultured using conventional NPIP methodologies. One hundred of these samples were also

processed using deep serotyping by CRISPR sequencing. A comparison of the results will also be presented to better understand the value of this technology to commercial turkey production.

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Development of a Multiepitope Subunit Vaccine Against *Salmonella* *Infantis* Using Immunoinformatics

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Abstract

Salmonella enterica* serovar *Infantis is a major cause of non-typhoidal *Salmonella* (NTS) outbreaks worldwide, predominantly linked to poultry products. The rise of multidrug-resistant strains complicates treatment, emphasizing the need for effective vaccination strategies. This study aims to develop a multiepitope subunit vaccine targeting *S. Infantis* in poultry, utilizing immunoinformatics approaches to enhance specificity and efficacy. We identified [\[MG1\]](#) [\[M2\]](#) a highly antigenic, immunogenic, conserved, and non-allergenic protein target suitable for epitope mapping using subtractive proteomics and reverse vaccinology tools from a dataset of 692 proteomes of ***Salmonella Infantis*** of poultry origin. A multi-epitope vaccine construct was designed and evaluated through computational analyses, including stability assessments, tertiary structure modeling, docking simulations with host immune receptors, and molecular dynamics simulations to predict immune response activation. The vaccine construct demonstrated high stability and the potential to induce both humoral and cell-mediated immunity. Its hydrophilic nature suggests compatibility with *E. coli* expression systems for scalable production and formulation in aqueous solutions. Preliminary immunoinformatics predictions indicate the vaccine's capability to interact effectively with immune cells, promising enhanced protection against *S. Infantis*. Further comparative study on the other 8 most common poultry-associated NTS serovars also identified same protein as a potential target, indicating the potential to make a pan-serovar vaccine in poultry against *Salmonella*. Although the vaccine demonstrates promise *in silico*, ongoing *in vivo* validation and animal trials are essential to confirm its efficacy and safety, representing critical steps toward developing a practical vaccination strategy against multidrug-resistant *S. Infantis* in poultry.

Effect of different litter treatments and treatment of broilers with organic acids on the litter microbiota

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Abstract

Salmonella Enteritidis (SE) is a major cause of foodborne illness, with poultry products often linked to outbreaks. This study investigated the effects of organic acids in drinking water and litter treatments on litter microbiota as strategies to control SE. Broilers in floor pens were infected with SE and assigned to one of three water treatments: citric acid (Citric), a mixture of lactic, acetic, and propionic acids (Acetic), or water (Control). After 39 days, broilers were removed, and litter from each group was composted, treated with formic acid salt, or left untreated. Two additional flocks were raised on this litter, maintaining the same treatments. Litter samples were collected before (day 0) and after the first treatment (day 7) and between subsequent flocks. DNA was extracted from all litter samples, 16S rRNA was amplified by PCR, and Illumina sequences were analyzed using a standard workflow in QIIME2 and R. Differential abundance analysis (ANCOMBC2) of day 0 revealed increased *Bacteroides*, *Erysipelatoclostridium*, *Monoglobus*, *Butyrivibrio*, and *GCA-900066575* in group Citric. On day 7, *Pedobacter* was decreased in group Acetic with untreated litter. Alpha diversity analysis on day 0 showed significant differences between Citric and Control groups. Beta diversity analysis (PERMANOVA) showed significant differences in Bray-Curtis distances between Citric and Control groups. Results of the later sampling days will also be presented. These results suggest that water and litter treatments influence litter microbiota composition. Further research is needed to confirm their role in controlling SE in broilers.

Vaccinology

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Combining Computational Immunology with Vaccine Design: Development of a Flagellin-Based Vaccine for Targeting Infectious Bronchitis Virus

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Abstract

Infectious Bronchitis Virus (IBV) is the causative agent of Infectious Bronchitis (IB), a highly contagious viral disease causing significant economic losses in the poultry industry due to immunosuppression and increased susceptibility to secondary infections. This study focused on analyzing the spike protein of IBV, which contains key antigenic determinants, to identify dominant epitopes. Immunoinformatic tools were used to predict B-cell, CD4+, and CD8+ epitopes. These epitopes were then fused to the N-terminal of flagellin, a potent TLR5 agonist, to enhance immune activation as an adjuvant in the vaccine formulation. The physical and chemical properties of the candidate vaccine, including secondary structure prediction, tertiary structure modeling, molecular docking, immune response simulation, and in silico cloning, were assessed. The results demonstrated that the candidate vaccine was antigenic, soluble, stable, non-allergenic, and formed a strong complex with the TLR5 receptor, enhancing immune stimulation. Immune simulation analysis showed that the candidate vaccine effectively triggered cellular and humoral immune responses, increasing cytokine production. Efficient expression of the candidate vaccine was achieved in the *Escherichia coli* expression system after codon optimization and in silico cloning. The flagellin-adjuvanted vaccine developed in this study offers a novel approach for preventing and controlling IB, contributing to improving poultry vaccines targeting Infectious Bronchitis Virus.

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Risk and Protection Assessment of One-Day-Old Gel-Drop Administration of the Infectious Laryngotracheitis Chicken Embryo Origin Vaccine in Broilers

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Abstract

Field experience demonstrates that Infectious Laryngotracheitis virus (ILT) chicken embryo origin (CEO) vaccines administered via drinking water (dw) at 12-14 days of age (doa) effectively protect broilers against ILTV. However, this method is no longer cost-effective due to logistical challenges. To reduce cost and facilitate the administration of CEO vaccines, broiler production companies are administering the CEO vaccine in the hatchery cabinet via oral gel drop

(OGD). This study evaluated the clinical outcomes post-vaccination and the protection efficacy post-challenge in broilers vaccinated at 1 doa with CEO-OGD alone or broilers previously vaccinated in-ovo with a trivalent recombinant Herpesvirus of Turkey (HVT) vaccine that expresses antigens for ILTV and Infectious Bursal disease (HVT-ILT-IBD+CEO-OGD 1 doa), and in CEO dw vaccinated broilers at 14 doa (CEOdw 14 doa). Results showed that post-vaccination of CEO-OGD 1 doa severe clinical signs led to euthanasia of 21% of the birds, as compared to HVT-ILT-IBD+CEO-OGD 1doa and CEOdw 14 doa, which induced moderate clinical signs and no mortalities. At 30 doa, all groups, including a non-vaccinated group (NVx), were challenged with genotype VI virulent ILTV strain. Post-challenge, all vaccinated groups exhibited a significant reduction of clinical signs and viral genome loads compared to NVx. However, CEOdw14 doa showed the lowest challenge virus genome load. These findings demonstrate that while all three CEO vaccination strategies protected broilers, significant differences in clinical outcomes after vaccination were observed.

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Day-old vaccination with a live *Mycoplasma gallisepticum* vaccine (MG304) using a novel intra-nasal delivery machine for commercial hatcheries

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Abstract

Live *Mycoplasma gallisepticum* (MG) vaccines provide the most effective method of MG control in broiler breeder and commercial layer chicken flocks globally. These vaccines are applied from 3-16 weeks of age by eyedrop, coarse-spray or drinking water. The poultry industry is seeking to advance the application of vaccines into the hatchery. However, there are limited post-hatch application methods available and eyedrop is not a practical method.

There are currently no live MG vaccines approved for use in day-old chicks, however, Vaxsafe MG304 has been shown to be safe and effective when applied to day-old chicks by eyedrop, coarse-spray and gel-spray methods (Kanci Condello *et al.*, 2024).

In partnership with a specialist engineering firm we have developed a prototype machine suitable for hatchery-based delivery of respiratory vaccines using a novel method that delivers the vaccine into the nasal passages via the choanal (palatine) cleft. The intra-palatine cleft (IPC) vaccination method was compared to eyedrop application by assessing the colonization of MG304 vaccine using qPCR of upper respiratory tract swabs. IPC was shown to be safe and as effective as eyedrop in achieving vaccine colonization across a range of doses and diluent types.

This is the first report of a novel intra-nasal vaccination method delivered mechanically via the palatine cleft. Delivery of a live MG vaccine via IPC was shown to be as safe and effective as eyedrop in day-old chicks. MG vaccination in the hatchery can advance the onset of protection and offer a more controlled delivery method than on-farm delivery.

Protection Efficacy of a Trivalent HVT Recombinant Vaccine (HVT-ILT-IBD) Against Infectious Laryngotracheitis (ILT) in Broilers Chickens

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Abstract

Infectious laryngotracheitis (ILT) is an economic respiratory disease that affects the poultry industry worldwide. Biosecurity and vaccination are needed to control the disease. ILT vaccination with recombinant vaccines is considered a first option in broilers since these vaccines are totally safe and recommended to be applied at the hatchery. Recombinant vaccines that utilize the Herpesvirus of Turkey (HVT) vector have become widely available, and more recently, a new trivalent recombinant vaccine that protects against Marek's disease (MD), Infectious Laryngotracheitis (ILT), and Infectious Bursal disease (IBD) has been developed. The objective of this study was to evaluate the protection efficacy of a new trivalent recombinant HVT-ILT-IBD vaccine when compared to a bivalent recombinant HVT-ILT vaccine against an ILT challenge in broilers. Compared to the non-vaccinated/challenged (NVx/Ch) group, both the HVT-ILT-IBD and HVT-ILT vaccines significantly reduced clinical signs, tracheal viral load, and histopathological lesions post-challenge. The probability of chicken survival was significantly higher for the HVT-ILT-IBD (96%) and HVT-ILT (93%) vaccines after challenge compared to the NVx/Ch (68%) group. The body weight gain after the challenge for HVT-ILT-IBD and HVT-ILT vaccinated groups was better maintained than the NVx/Ch group of broilers. In particular, the HVT-ILT maintained weight gains similar to the non-vaccinated/non-challenged (NVx/NCh) group. Overall, this study demonstrated that solid and comparable ILT protection is achieved with trivalent (HVT-ILT-IBD) and bivalent (HVT-ILT) recombinant HVT vaccines against an ILT challenge.

Broiler Chickens Immunized With Recombinant Non-Toxic Alpha Toxin Domain-2 of *Clostridium septicum* Can Be Protected Against Clostridial Dermatitis

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Abstract

Clostridium septicum is a major pathogen identified to cause Clostridial Dermatitis (CD) in poultry, which is an economically important disease in the current era of 'no-antibiotics-ever' farming. CD incidences are on the rise, and unfortunately, there are no effective vaccines currently available. Our previous work identified a non-toxic domain#2 of *C. septicum*

alpha-toxin (ntATX-D2) as a protective vaccine antigen for turkeys. In the present study, we evaluated the protective efficacy of recombinant ntATX-D2 immunization (via subcutaneous route) of broiler chickens against an experimental *C. septicum* challenge. The results showed that the immunized chickens had significantly higher body weight gain and

reduced gross pathology (disease severity) when compared to unimmunized birds, indicating protection against CD. Additional investigations into protective mechanisms showed that the ntATX-D2 immunization led to; 1) A significantly higher levels of antigen-specific serum IgY antibodies, 2) modulation inflammatory responses in the skin and muscle tissues, as indicated by the significant transcriptional downregulation of proinflammatory cytokine (IL-1 β , IL-6, and IFN γ) and upregulation of anti-inflammatory cytokine (IL-10 and TGF β) genes, when compared to the

unimmunized control, and 3) ntATX-D2 immunization resulted in upregulation of B and T cells systemically compared to the unimmunized birds, indicating that the vaccine was able to prime the adaptive immune system.

Collectively, our results indicated that ntATX-D2 vaccination of broiler chickens can provide protection against CD and the mechanisms of protection seem to operate through anti-ATX antibodies coupled with modulation of local and systemic inflammatory responses.

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Phylogenetic analysis of over 1,000 IBDVs from US chicken flocks since 2009 and how killed vaccines cross protect against IBDVs in each major cluster

Kalen Cookson, Po-Hsin Yu, John Dickson

Zoetis, Durham, NC, USA

Abstract

While the adoption of bursal derived vaccines dramatically improved progeny protection against Del-E and other similar variant IBDVs, by the late 1990s novel variants like AL2 and T1 had emerged that demonstrated the ability to still infect progeny containing high antibody levels at an early age. Since 2009 our diagnostic lab has sequenced the variable region of VP2 recovered from over 1,000 US flocks. This presentation will show one phylogenetic tree containing all these IBDV sequences. Results will show that the IBDVs fall into 6 general clusters: 1) Classic—16.6%, 2) AL2—28.9% (318N, 321-E), 3) Group-6—25.6% (322E), 4) Del-E—12% (254S, 323-E), Del-E—9% (254N, 323-E) and Variant Other—7.9%. The Classic cluster is made up mostly of the Faragher insert contained in most rHVT-IBD vaccines. The Group-6 cluster, named after the many IBDVs sharing the same restriction enzyme pattern, contains the most extensive branching. The Del-E prototype cluster represents about 10% of all US sequences. Over half of the second Del-E cluster containing the mutation 254N also contain 299S—making them 98% identical to the China variant type that has spread to much of Southeast Asia, Western Canada and, most recently, Middle East. The Variant Other cluster notably includes viruses from Pennsylvania that are also most prevalent in Eastern Canada and named after the South Africa variant 05SA8. The presentation will highlight IBDVs from each of these variant clusters, where they contain mutations in the 4 hydrophilic peaks and how today's killed vaccines cross protect against them.

Poster 56

Alternative method for administering commercial vaccines to turkey poults at placement

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Abstract

The purpose of the present studies was to evaluate AquaBeads® (AB), which are translucent green gel beads that can be applied as a feed top dressing at placement, as a vehicle for administering commercially available live vaccines to control coccidiosis (Immucox®-T) or *Salmonella* (Megan Egg®) in turkeys. In Exp 1, 200 d0 turkey poult hens were randomly allocated to: 1) non-vaccinated, non-treated control that did not receive AB or coccidiosis vaccine (CV), 2) only received the CV via oral gavage, 3) received AB at 1g/poult as a vehicle for CV, or 4) received AB at 1.5g/poult as a vehicle CV. In Exp 2, 400 d0 turkey poult hens were wing banded, individually weighed, and allocated to: 1) non-vaccinated, non-treated control, 2) received only the *Salmonella* vaccine (SV) via oral gavage, 3) received Gel-Pac® (via spray application) as a vehicle for SV, or 4) received AB at 1.0g/poult as a vehicle for SV. For both experiments, Groups 2-4 received the same 1X dose of the vaccine regardless of vehicle used. In Exp 1, total oocyst output for Group 3 and 4 was significantly ($P<0.05$) lower than Group 2. In Exp 2, *Salmonella* recovery from the cecum was significantly higher in Group 4, but there was no significant difference in the incidence of *Salmonella* recovered across vaccinated groups. No differences in 7-day BWG were observed between vaccinated groups in Exp 2. These results suggest that AB could be used to administer vaccines to turkey poults at placement.

Virology

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Ethiopian isolates of Newcastle disease virus whole genome sequencing reveals high genetic variation from vaccine strains

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Abstract

Introduction: Newcastle disease poses a significant threat to poultry production in Ethiopia, with several outbreaks reported in vaccinated chickens. Analyzing the genetic characteristics of currently circulating virus strains is important to understand the molecular basis contributing to antigenicity differences among vaccine and field strains. **Methods:** In this study, we examined the complete genome sequences of seven NDV strains isolated from chicken farms, including vaccinated chicken flocks and live bird market located in different districts of Ethiopia between January and December 2023, and compared them with vaccine strains. **Results:** Next-generation sequencing showed that all strains have a genome length of 15,192 bp and are composed of six genes in the order 3'-NP-P-M-F-HN-L-5'. Although the identity rates of the whole nucleotide sequences were 96.13 to 99.91% among these strains, they showed less identity (79.14 to 79.84%) when compared to the NDV vaccines commonly used in Ethiopia (LaSota and HB1). There were high genetic

variations among all genes between the vaccines and our isolates. Furthermore, various amino acid differences were predicted in the functional domains and neutralizing epitopes of the F and HN surface glycoproteins compared to those in the vaccines. Based on phylogenetic analysis of the full F gene open reading frame, all isolates belonged to genotype VII.1.1. The F gene cleavage sites had ¹¹²RRQKRF¹¹⁷, a characteristic of virulent NDVs. **Conclusions:** These findings indicate that a vaccine genetically more closely related to the velogenic NDVs prevalent in the country may be needed for effective prevention of the disease in Ethiopia.

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Turning over rocks: a case of EDS'76... or not

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Abstract

Introduction

Egg Drop Syndrome (EDS'76) is a viral disease caused by Duck Adenovirus 1 that can cause significant drops in egg production in chickens, as its replication in the egg shell gland can lead to shell-less eggs. It is important to primary breeders because it can be vertically transmitted. Infected chicks and pullets may carry the virus without clinical signs until they come into lay. This presentation will share the story of our disease investigation and response, along with an alternative explanation for so-called "positive" results on the EDS'76 serology test.

Diagnostic Workup and Disease Response

An outbreak of EDS'76 in Missouri in 2024 prompted testing of grandparent broiler breeder pullet flocks in the state before moving these flocks to the breeder farm. Serological test results were concerning, even after comparing with titers of flocks in EDS'76-negative states. We quarantined the MO flocks. Follow-up molecular testing for EDS'76 on a multitude of sample types (shoe covers, fecal samples) was negative. We moved the flocks to the breeder house under quarantine to wait for clinical signs. When the flocks came into lay, eggs tested negative by PCR. A complete lack of clinical signs reassured us that our flocks had not been exposed to the virus after all.

Conclusion

Discussion with industry colleagues led us to the conclusion that the EDS'76 serological test was reacting with one of our vaccinations due to its tissue culture origin.

Wealth of Knowledge

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The potential impact of imidacloprid and its metabolites on food safety: a toxicokinetic study in laying hens

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Abstract

Imidacloprid (IMI) is widely used in broiler houses in the United States for the control of disease-transmitting darkling beetles. IMI is moderately toxic to birds as evidenced by reports of accidental toxicosis by ingestion of high doses of IMI in commercial and in wild birds. However, there is very limited knowledge regarding the distribution and persistence of IMI and its metabolites in poultry products (meat, meat by-products, and eggs) following acute exposure to sublethal concentrations. In this study, mature hens received a dose of imidacloprid (placebo, 1 mg/kg, or 10 mg/kg) and liquid chromatography tandem mass spectrometry was used to quantify IMI and its metabolites in tissues collected on a time course. Birds did not show any clinical signs after the administration of IMI. After 24 hours at 10 mg/kg, IMI equivalents (sum of IMI and its metabolites) exceeded the regulatory threshold (determined by the code of federal regulations) for pectoral and thigh muscle, brain, liver, spleen, kidney, fat, and eggs. Similarly, after 24 hours at 1 mg/kg, IMI equivalents surpassed the threshold for liver, spleen, and eggs. This data raises concern for the potential of IMI and its metabolites to persist in poultry products destined for human consumption.

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Global Probiotic usage in Commercial Poultry: A 50-year history in 12 minutes

John Schleifer

Devenish Nutrition Inc., Fairmont, MN, USA

Abstract

Probiotic usage in commercial poultry has become common-place, particularly since the year 2015. This presentation will explore the evolution of global probiotic usage in commercial poultry; starting with the 1973 seminal paper published in the journal Nature by Nurmi and Rantala. From that 1973 paper, probiotic usage has progressed in a number of different forms, delivery methods, and usage rationale. The progression of probiotic usage will be traced chronologically with a focus on the early undefined products to the now-used, defined probiotic cultures. The difference between lactic acid-based probiotics and the commonly used Bacillus-based probiotics will be presented. Delivery methods of probiotics will be described in an historic context as well as expected efficacy of probiotic application. The influence of market forces on changes in probiotic usage and bacterial strain composition will be discussed. This historic perspective will be presented in the 12-minute time allotment.

Evaluation of Broiler Heart Activity Post-Controlled Atmosphere Stunning (CAS) to Determine Acceptability of CAS for Religious Slaughter

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Abstract

The recently updated mandatory technical regulations regarding Halal slaughter regulation (UAE.S. 993/2022 “Animal Slaughtering Requirements According to Islamic Rules”), states that methods used to induce loss of consciousness in animals must not result “in heart stop of the animal” which would impede normal blood loss during slaughter, and that death of the animal is “caused by bleeding out during the slaughter process, not because of the loss of consciousness method”. Determining that there is heart activity beyond the controlled atmosphere stunning (CAS) process could potentially make broilers eligible for Halal slaughter, which has not historically been the case. The study had two objectives:

1. Evaluate the duration of rhythmic cardiac activity of market-age broilers following CAS.
2. Evaluate whether there is sufficient duration of rhythmic cardiac activity post-CAS stunning to render broilers dead from volumetric blood loss.

Electrocardiograms of market-age broilers were evaluated following CAS system exit. Rhythmic heart activity as measured was present in male broilers for an average of 390 ± 90 s. and in female broilers for an average of 366 ± 68 s. A typical time between CAS exit and entry into the scalding tank is 265 s. We have demonstrated that rhythmic heart activity persists in market-age broilers after CAS-stunning for a period of time longer than necessary for a stunned bird to succumb from blood loss during normal stun and kill procedures, ensuring welfare and religious slaughter requirements are met so that meat from these broilers is able to enter appropriate Halal markets.

Effects of Turning Angle Deviations on Hatchability, Embryonic Development, Maternal Antibodies, and Chick Quality in Broiler Chicks

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²Professional Poultry, LLC, Mt. Olive, USA. ³Department of Poultry Science, University of Georgia, Athens, USA

Abstract

While proper egg turning during incubation is essential for hatchability, embryonic development, and chick quality, setters with inadequate turning angles are commonplace. This study investigated how turning angles outside of 38-45 degrees influenced hatchery performance, hatchability, embryonic mortality, chick quality, and maternal antibody

transfer. The project consisted of two phases. In the first phase, commercial hatcheries were evaluated to identify setters with both optimal (38-45°) and suboptimal (<38°) turning angles. Performance metrics such as hatchability, chick quality and others were analyzed to assess the impact of turning angle deviations.

The second phase was done under controlled conditions with 3 experimental groups: a control group (42°) and treatment groups with moderate and significant deviations below the recommended range (<38°, <30°). Throughout the incubation period, turning angles, temperature, eggshell temperature, and humidity were monitored. In both phases, embryonic development was assessed on days 7, 14, and 18 to detect abnormalities in vascular growth and positioning. Also, post-hatch evaluations included hatchability, classification of embryonic mortality through egg breakouts, detailed chick quality assessments using the Pasgar scoring system, body weight-to-yolk ratios and chorioallantoic membrane development. In addition, in ovo injection was evaluated and titers against Infectious Bursal Disease virus and Reovirus were measured by ELISA at hatch to evaluate if turning angle deviations impact in-ovo vaccination and passive immunity.

Previous studies highlight the importance of proper egg turning for hatchability, embryonic development, and chick quality. Evaluation of in-ovo injection and maternal antibody titers provides a new perspective on optimizing incubation turning protocols.

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Adjusting Gamefowl vaccination programs to provide protection against velogenic Newcastle disease in Southern California

Rodrigo Gallardo¹, Charlene Rivera², Alexandra Mendoza-Reilly³, Marco Solis², Jose Garcia², Theodore Derksen¹, Alejandra Figueroa¹

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Abstract

The Gamefowl Wellness Program is a quality assurance effort funded by the State of California, USDA/Aphis, and UC Davis to educate gamefowl owners in Southern California about disease prevention and biosecurity. Since 2022, we have worked with these communities, performing outreach in disease prevention, nutrition, and directing people to resources like diagnostic services provided by our state laboratory. In 2023, we introduced vaccination to achieve NDV population immunity and prevent virus dissemination in the case of a velogenic NDV outbreak. In previous years, the program has focused on live NDV vaccination, first through ocular application and then by spray, due to similar seroconversion detected after a pilot experiment. Following seroconversion inconsistencies, we decided to test three different NDV vaccination programs on three different breeder farms: a) Live La Sota spray followed by a spray boost after two weeks, b) Live La Sota spray followed by a 4-way killed SC in the leg fold, and c) Live La Sota spray followed by an HVT-F recombinant vaccine applied IM in the pectoral muscle. In addition, a fourth farm served as a negative control. Seroconversion is being measured every two weeks by ELISA in each of the farms. This project is crucial to defining the best vaccination protocol to provide population immunity to NDV-vulnerable flocks in Southern California.

Avian Metapneumovirus in a Commercial Tom Operation – We Have a Situation!

Becky Tilley

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Abstract

Avian metapneumovirus (AMPV) in the genus *Metapneumovirus*, is a member of the family Paramyxoviridae, with antigenic subtypes A-D. AMPV was first identified in the 1970's in South Africa and has since been diagnosed worldwide. Wild birds are considered the natural reservoir. AMPV infection in poultry results in respiratory disease and drops in egg production. Beginning in the fall of 2023, severe respiratory disease complicated by *E. coli* infection and drops in egg production occurred in North Carolina. This presentation will describe diagnostic challenges and capabilities, industry collaborations, clinical presentations in commercial tom flocks, management strategies, and vaccination programs.

Enhanced Gut Immunity with VG/GA Newcastle Vaccine

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Abstract

Avian cytokines have immune stimulating properties. With industry emphasis to reduce reliance on antibiotics and chemical antimicrobials, cytokines have been considered for both immunotherapeutics and as adjuvants. The VG/GA Newcastle vaccine has been used worldwide, uniquely and robustly immunizing birds against Newcastle disease because of its enterotropic nature. This study evaluated the broader immune impact of the VG/GA Newcastle vaccine, in the absence of a Newcastle virus challenge. At day of hatch, commercial broiler chicks were spray vaccinated with either or both, commercially available VG/GA and coccidiosis vaccines. Using an established Necrotic Enteritis challenge model, chicks were challenged with *C. perfringens* at 12, 13 & 14 days of age through feed. No *Eimeria* were administered to control groups. Day 15, birds were sampled and tested for intestinal permeability, tight junction proteins, and cecal tonsils and spleen were sampled for cytokine analysis. Cellular immune responses were significantly increased in the vaccinated groups. VG/GA vaccinated non-challenged and *C. perf* challenged groups did not exhibit a significant decrease in gut integrity compared to negative controls. Cecal tonsils in VG/GA groups showed significant increase in IL1, IFNgamma & IL21 mRNA levels compared to controls. IL1 & IFNgamma mRNA levels were also significantly increased in spleen compared to controls. These results highlight systemic immune stimulation by the VG/GA vaccine without a Newcastle virus challenge, and in the presence of both a live coccidiosis vaccine, and *C. perf*. The impact of this enhanced immune stimulation to bird health and performance should be evaluated

ILT Control: A Paradigm Shift

Mark Burleson

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Abstract

For years, knowledge and control of Infectious Laryngotracheitis (ILT) in chickens has been stifled by the threat of export embargos. A recent change in international disease notification has preceded a paradigm shift in how ILT is managed at the field level. The role of genotyping has played a major role as well. Are the majority of field isolations wild-type or vaccine-origin? If we know more about the virus, does that change the way we manage the disease? Does chicken embryo origin (CEO) vaccine make an outbreak worse, or does it play a role in displacing the field virus? Is the field virus hiding in plain sight? These and many other questions will be discussed and answered. The ultimate goal of this presentation is to present a new idea to managing ILT in the field and offer a break in the cycle of insanity that has become ILT control over the years.

Single Stage Incubation in the USA: Percent Relative Humidity, Machine Function, Air Temperature Role in Optimum Profile

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Abstract

Single stage incubation (SS) is increasing in the US. Often an increase in hatchability, but not overall performance is seen. Machine function, profile, and embryo demands must be considered to optimize chick quality for performance.

Chick quality for performance is determined by embryo temperature of 99.5-100.5 degrees fahrenheit and weight loss of 10-12%. Different manufacturers have different designs, which impact the profile necessary to achieve the uniform embryo temperature and weight loss necessary for chick quality.

This presentation discusses the three phases of single stage incubation: The gas sealed phase, the ventilation for moisture loss phase and the ventilation for oxygen phase. Basics, parameters, limitations and recommended profiles are discussed for each phase.

Problems with the commonly used US SS profiles are discussed, such as embryo temperature too low in the first two phases of incubation and the relationship to ventilation settings. Too high relative humidity in the gas sealed phase and too low during the ventilation for oxygen phase are discussed in relation to uniformity of embryo temperature, weight loss and bacterial challenge.

This work is the result of my experiences with single stage incubation since 2005.

Welfare

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Animal Welfare Considerations for In-Ovo Sexing

Mike Petrik

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Abstract

North America is currently considering implementing technologies to determine the sex of embryos before hatching. Several technologies can accomplish this feat, and each has advantages and drawbacks. This talk will discuss the current understanding of animal welfare implications of in-ovo sexing in general and the subtle differences between the currently available technologies. Understanding the nociceptive and pain-sensing potential of embryos will allow the industry to effectively navigate the implementation of in-ovo sexing. Understanding how in-ovo sexing protects the well-being of embryos enables the industry to enhance the overall animal welfare in egg production systems across North America, and will enhance the reputation of the industry to the public.

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Effects of spray versus gel vaccination on temperature, behavior, and performance in broiler chickens

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Abstract

Vaccination is standard practice in broiler hatcheries. The method of vaccine delivery can possibly influence chick welfare and production performances. In this field study, a comparison was done between coarse spray and gel droplet vaccination methods using Infectious Bronchitis and a Coccidiosis vaccines in 62,000 day-old chicks. Surface temperatures and huddling behavior were assessed using infrared thermography. Baseline temperatures and huddling were measured and used as a control. Thermal images were taken at multiple time points post-vaccination. Chicks vaccinated via spray method showed a significant drop in surface temperature and decreased crate occupancy, indicative of huddling—both signs of cold stress. In contrast, gel-vaccinated chicks maintained stable surface temperatures and evenly distributed crate occupancy. Production data from two identically managed houses showed that gel vaccinated birds had better livability and more efficient feed conversion rates. These findings highlight the potential of gel vaccination to improve chick comfort and welfare in the hatchery and our data shows stronger production performance in the field.

Plant-Based Choline: a potential animal welfare concern?

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Abstract

Veterinarians and nutritionists have a stereotypically fraught relationship and there is often timidity in venturing into what is seen as the others' territory. There are times, however, when the alteration of a diet formulation has impacts beyond bird performance and can also impact bird health and welfare. It is important for veterinarians to be aware of the potential implications of such changes. Choline is an essential nutrient for poultry contributing to multiple metabolic pathways and is critical for endochondral bone formation. A deficiency in choline can result in perosis, tibial rotation and other valgus-varus deformities that negatively impact poultry welfare, particularly for heavy broilers. Recent experiences in Brazil with commercial plant-based choline preparations were cause for concern. A controlled, titration trial for plant-based choline compared to choline chloride and unsupplemented diets showed a significant increase in valgus-varus leg deformities in diets containing plant-based choline. Birds who received diets supplemented with plant-based choline had leg deformities at the same rate as the unsupplemented, deficient diets raising questions about bioavailability of natural alternatives. Results of the trial will be discussed as well as a broader discussion of the impact of choline on leg health and animal welfare.

Acetylsalicylic Acid Effect in Turkey Poult with Induced Coccidiosis Enteritis

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Abstract

Acetylsalicylic acid (ASA) has anti-inflammatory, antipyretic, and analgesic effects. In poultry, ASA is used off-label for mitigating vaccine reactions or inflammation. However, research on the effect of ASA in turkeys is limited. This study evaluated the effect of ASA administered via water to turkeys with experimentally induced coccidial enteritis. Forty-eight 1-week-old turkeys were divided into four groups: NN (No Coccidia + No ASA), NA (No Coccidia + ASA), CN (Coccidia + No ASA), and CA (Coccidia + ASA). Groups CA and CN were infected with a 10X dose of a coccidial vaccine. Starting 48 hours post-inoculation and for five days, NA and CA groups were given 7.8mL/L ASA in the water via a nipple system, to achieve a dose of 50 mg/kg of ASA daily. We collected body weight, cloacal temperature, feces, blood, and water samples daily. Blood was analyzed for biochemical parameters, salicylic acid, and nitric oxide. The water was tested for ASA content. At the end of the study, all poults were euthanized, and tissues were collected for histology and gene expression of Interleukin (IL)-1 β , IL-6, IL-10, C-reactive protein, and serum amyloid A. Peak shedding of coccidia oocysts occurred on day 6 post-infection. ASA had a minimal impact mitigating the effects of coccidiosis, including weight gain and inflammatory response. No pathologic changes were associated with ASA treatment. In conclusion, ASA had minimal impact on the performance of turkeys infected with coccidia. Future research should explore whether a higher ASA dose might improve performance and therapeutic outcomes.

Women's Network

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Why Coaching Works: Cultivating Hope, Efficacy, Resilience, and Optimism

Holly Ward

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Abstract

Professional coaching has emerged as a powerful tool for personal and professional development, yielding significant positive effects on cognitive outcomes, performance, and well-being. This presentation explores the fundamental principles that underpin the efficacy of coaching, demonstrating how it fosters hope through solution-focused thinking, builds self-efficacy by cultivating confidence in one's capabilities, develops resilience to navigate challenges, and promotes optimism through positive attribution about the future. Research indicates that coaching leads to measurable improvements, including a 95% increase in positive cognitive outcomes and a 788% ROI from executive coaching through enhanced productivity and satisfaction. Furthermore, it significantly boosts self-esteem, confidence, and relationship quality.

This session will delve into practical strategies for effective coaching, including the "4 P's" framework for navigating challenging conversations (Prepare, Practice, Present, Positive Language), and the "Regulate, Relate, Reason" model for fostering constructive dialogue.

Participants will learn how coaching supports the development of growth mindsets, promotes goal attainment, and empowers individuals to overcome obstacles.

By emphasizing the benefits of coaching this presentation will equip attendees with actionable tools to enhance their professional experiences and drive transformative change in individuals, teams, and organizations.

Online: Bacteriology

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A host-specific bacteria to increase chicken gut immune maturation in early life.

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Abstract

In poultry, gut immunity is immature at hatch and relies on early microbiota to develop; however, in commercial hatcheries, chicks are hatched away from their progenitors from which they could have inherited host-specific bacteria,

such as Segmented Filamentous Bacteria (SFB). To address this limitation, our lab has developed and tested a prophylactic treatment containing avian SFB spores for newly hatched chicks.

Day-old broilers and layers orally treated with layer-derived SFB (D-SFB) and non-treated groups (CON) were housed in separate rooms. The SFB were tracked in the ileum and feces by qPCR and microscopy for 3-4 weeks. *Enterobacteriaceae* were enumerated in feces by plating. RT-qPCR measured the expression of genes associated with gut immune function over time. *In vitro* resistance to bacteria was tested using blood and ileal tissues. Statistical analyses of the data were performed using the GraphPad Prism software.

The D-SFB oral inoculum ensured maximal ileal SFB colonization in layers and broilers in early life. The treatment enhanced intestinal barrier function and homeostasis. In chickens there was no increase in IL-17 production indicative of TH17 cell activation, as previously shown in mice. This immune activation triggered broad bacterial killing on both intestinal and extraintestinal pathogens *in vitro* and *in vivo* against bacteria relevant to chickens and humans.

We developed a unique live prophylactic for newly hatched chickens to improve animal health and food safety. The ability of layer-derived SFB to cross-colonize in broiler chickens eliminates the need for multiple SFB-based products to be derived from both layers and broilers.

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The BIG Es in Poultry: A New Approach to Tackling *E. coli* and *Enterococcus* in Broiler Operations

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Abstract

Avian Pathogenic *E. coli* (APEC) and *Enterococcus* are challenging for poultry veterinarians as they are known to cause comorbidities and have been implicated in reduced hatch rates and poor livability. Interventions to date have focused on direct-fed microbial strategies for either the broiler flock or broiler breeder hens. A novel egg-based approach alters the eggshell microbiome effectively as the embryo develops, thus halting the vertical transmission route independent of the broiler breeder health status.

This new approach leverages a novel formulation with specific prebiotics and probiotics to outcompete pathogens of concern on the eggshell. Specifically, the formulation optimizes the out-competition of *Enterococcus* and APEC. Providing an intervention at the eggshell level allows for a more uniform microbiota on the shell surface and for the developing embryo. Small scale trial results demonstrated that the egg coating was capable of altering the microbiota on day of hatch in broiler chicks. This alteration in the microbiome persisted from Day 1 to Day 42 and resulted in increased abundance of Firmicutes. Furthermore, large scale trials demonstrated a reduction in the incidence of APEC and *Enterococcus* disease breaks on farm, indicating a protective effect of the microbiota alterations.

As we consider advances in poultry health and antimicrobial stewardship, we need to also evaluate novel approaches that allow us to mitigate problems as early as possible in the life cycle of our flocks. This session will provide answers and real-world data that can help the industry be successful when confronted with *Enterococcus* and APEC challenges.

Identification of *Enterococcus cecorum* reservoirs in the environment of broiler chickens

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Chair in Poultry Research, Faculty of Veterinary Medicine, University of Montreal, St.Hyacinthe, Canada

Abstract

Enterococcus cecorum osteomyelitis is an emerging disease affecting broilers and broiler breeders worldwide. Mainly characterized by lameness and septicemia, economic losses associated with this disease are considerable. Unfortunately, once the disease is present on a farm, the infection seems to recur. However, the source of these strains, where and whether they survive in the poultry house environment is unknown.

E. cecorum (EC) is a Gram-positive, non-spore forming, facultative anaerobic bacterium. It is ubiquitous in the environment and used to be considered part of the normal gut microbiota of chickens. Some virulence genes have been identified in pathogenic strains.

We hypothesised that one of these genes, *cpsO*, could be used as a marker for pathogenic EC strains. Our objectives were to identify in-barn reservoirs and to assess the persistence of EC strains isolated from chickens and their environment.

We developed qPCR assays for the *sodA* (commensal EC strains) and *cpsO* (pathogenic EC strains) genes. Twenty-one EC-affected barns were visited at 3 weeks of age in two consecutive flocks. Swabs from osteomyelitis lesions, dust (fans), biofilm from drinking water lines, flies and darkling beetles, cecal contents of normal and lame birds and feces were collected. DNA was extracted and qPCR was performed.

The results showed that pathogenic EC strains were present in all sample types at varying levels, even in healthy chickens. The highest EC counts were observed in dust samples. These results underline the importance of cleaning, disinfection and pest management to control EC infection in broiler barns.

Does all disease begin in the gut? Or, does a healthy gut improve long-term health & productivity?

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Abstract

While Hippocrates may have stated, "All disease begins in the gut" we know that the composition of the GI tract microbiome has been associated with improved production outcomes for poultry flocks. To date, nutritional

interventions, including direct fed microbials (DFM), have been utilized to modulate the GI tract microbiome in poultry. Recent research indicates that the eggshell microbiome has a large influence on the microbial composition of the GI tract and provides a novel opportunity for improving production metrics in both the hatchery and on the farm.

An novel egg-coating product was utilized in the trials. The formulation was applied to the eggshell prior to setting the eggs and resulted in an improved hatch rate in commercial broiler hatcheries. In addition to improving hatch, the product altered the microbiota of the intestinal tract of the newly hatched chick leading to improved livability. Livability improvement was mainly associated with decreased pathogenesis on farm which also resulted in improved weight gain on trial farms.

In this session, we will review production data that highlights the specific changes in microbial composition that allows broilers to be more resilient to disease challenges, such as *E. coli* and *Enterococcus*, and also allows for improved livability and weight gain. Data shared will highlight the importance of starting early to leverage the eggshell microbiome thereby facilitating favorable changes in the embryo and broiler chicken. This one-time treatment proposes a novel solution for companies that aim to improve hatchability, flock livability and overall health status of broiler flocks.

Online: Case Reports

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Infectious Laryngotracheitis with cloacitis in broiler breeders.

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Abstract

A 25 week old broiler breeder flock was investigated for an acutely elevated hen culling rate, exceeding 1% per day, in one of eight houses. These hens had been culled due to dullness and had severe cloacal congestion with urate staining. Most of the remaining birds appeared bright but a mild snick was apparent and over 10% of the flock had poor cloacal tone with varying degrees of urate staining. Necropsy revealed cloacitis and tracheal congestion.

Two weeks later another house became affected with similar findings, though with more severe respiratory signs. Necropsy revealed a more severe tracheitis than the first house and conjunctivitis. All other houses became affected throughout the next two weeks, with affected birds showing excess urates with cloacitis at the same time as respiratory signs. One house also had a temporary drop in egg production.

Histopathological examination of tracheas revealed typical changes for infectious laryngotracheitis. Cloacal changes varied between birds and included severe necrotising cloacitis with intralesional urate deposition, granulocytic cloacitis or acute hemorrhage. No significant changes were noted in intestinal or kidney samples.

ILTV genotyping, based on sequencing, grouped the sample isolate with chick embryo origin vaccine strains. The flock's vaccination programme included a hatchery-administered HVT-vectored ND and ILT vaccine, with no live ILT vaccination.

Paired serology showed no titre changes for Avian Metapneumovirus, Reovirus, Infectious Bronchitis and Group 1 Adenovirus.

The authors are unaware of previous reports of vent gleet or cloacitis as a sequel to ILT.

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Malignant melanoma in a duck with widespread metastasis

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Abstract

An approximately 6-year-old adult female fancy breed of duck was diagnosed with malignant melanoma via light microscopy and special staining with Fontana-Masson. Several nodular, black masses were present multifocally throughout the liver. Histologically, the neoplasm was composed of packets and aggregates of melanocytes that were negative for S-100 and Melan-A. Intracytoplasmic granules stained black with Fontana Masson, consistent with melanin. Metastases were identified in the lung, omentum, and kidney. Malignant melanoma has been reported in various avian species, but reports in ducks are rare.

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From Cough to Cheeseballs: Aftermath of Avian Metapneumovirus Type B in Turkey Breeder Hens

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Abstract

The first case of Avian Metapneumovirus (aMPV) Type B in the United States was detected in turkey breeders in Eastern North Carolina during Fall 2023. This presentation demonstrates the reproductive sequela of turkey breeder hen flocks infected with aMPV Type B and subsequently developed secondary bacterial infections in rearing. Several turkey hen breeder flocks between 7 and 9 weeks old subsequently contracted aMPV Type B and developed secondary colibacillosis resulting in elevated morbidity and mortality. Gross lesions on necropsy revealed pneumonia, pericarditis, airsacculitis, perihepatitis, peritonitis, and salpingitis. Bacteriology of lungs and livers confirmed *E. coli* as the causative agent. When these hen flocks entered production around 30 weeks old, there was elevated mortality directly after the first several inseminations, which initially presented as typical uterine prolapse. However, gross necropsy revealed a chronic, residual colibacillosis causing severe salpingitis and peritonitis with ascites coupled with a uterine prolapse. Bacteriology of the oviduct revealed *E. coli*. Histopathology of affected reproductive tracts and all findings will be reported. This mortality pattern continued for the first several weeks of lay, then declined with no further complications in production.

Notably, hen flocks that did not contract aMPV Type B between 7 and 9 weeks old in rearing did not experience residual colibacillosis and salpingitis during production.

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“Does that head look swollen to you?” An Analysis of 32 aMPV Cases in 2024

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Abstract

Introduction

Avian Metapneumovirus (aMPV) subtype A is an emerging viral disease in the US. On February 1, APHIS confirmed the presence of aMPV subtype A in turkeys in California. While aMPV subtype C was known to exist in the US, subtypes A had not previously been identified. This presentation will review two waves of aMPV cases in Oklahoma grandparent broiler breeders during 2024.

Review of aMPV Subtype A's Varied Clinical Picture

This presentation will describe the range of clinical signs, severity of mortality, duration of shedding, and effects on egg production of aMPV subtype A in grandparent broiler breeders. Factors such as age and gender of birds and age of housing will be discussed. The first wave occurred during May with 9 cases confirmed with PCR. The second wave of cases was from September to November with 23 cases confirmed with PCR. There were also numerous exposures to aMPV based on ELISA testing; however, these flocks were never PCR positive. No personnel, equipment, or bird movements corresponded to the cases seen. Additionally, no biosecurity differences were found between affected and nonaffected farms.

Conclusion

After experiencing these two waves of aMPV, the complex better understands the impact of aMPV. What to expect from positive farms in regards to severity and duration of clinical signs, mortality, and drops in egg production can help the grower and company better understand the possible impacts this virus will have. This is very important when considering enacting heightened biosecurity measures or adding vaccination for aMPV.

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Management of Mixed Bacterial Infection and Newcastle Disease in Isa Brown Layers

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Abstract

On October 31, 2024, a flock of 1,812 Isa Brown layers, aged one-and-a-half years, was presented to the Poultry and Fish Clinic, University of Jos Veterinary Teaching Hospital, Nigeria. The flock exhibited a history of daily mortality (4, 20, and 12 deaths over three days), a drop in egg production from 74.5% (45 crates) to 62.9% (38 crates), and passage of greenish-white stool. The birds were managed on a deep litter system, sourced from Zartech, fed commercial layer feed, and had their last Newcastle disease vaccination (Lasota booster) three months prior. Drinking water was treated with sodium dichloroisocyanurate (NaDCC). Postmortem findings included pale combs and wattles (3/3), jaundiced abdominal fat (3/3), friable bronze livers with diffuse petechial hemorrhages (3/3), fibrinous pericarditis (3/3), peritonitis with cloudy abdominal air sacs (3/3), pedunculated degenerate ovarian follicles (3/3), congested and severely edematous lungs (3/3), and pale kidneys (3/3). Histopathological examination showed bile duct hyperplasia in the liver, proliferative glomerulonephritis with lymphocytic infiltration in the kidneys, pulmonary edema with fibrin strands, and degenerative ovarian follicular cells. Microbial culture of tissues identified *Salmonella spp.* and *Klebsiella spp.*, with antibiotic susceptibility testing revealing sensitivity to enrofloxacin and four other antibiotics. Treatment involved desliming the water system with sodium dichloroisocyanurate and administering enrofloxacin (20%) at a dose of 1 milliliter per 2 liters of water for 5 days, along with povidone-iodine (10%) at 1 milliliter per 2 liters of water for 5 days. Mortality ceased within five days of treatment, and egg production improved to 42 crates (69.9%).

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Elevated zinc levels in a neurologic domestic goose

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Abstract

A nine-year-old female goose presented to an exotic animal clinic with complaints of weakness, lethargy, and anorexia. Physical exam findings included pallor, ataxia, limber neck, and emaciation. The bird was euthanized and submitted for necropsy.

Gross findings included poor muscle condition but fair body fat stores. The pancreas contained hundreds of tiny white foci. Histology findings included demyelination and vacuolation in sciatic nerves and hypocellular bone marrow. Based on the neurological signs and histopathology, a heavy metal screen was run on the liver. Zinc levels were 246 ug/g, outside the normal reference range but below the toxic range.

After consulting with the owner, the elevated zinc levels were hypothesized to be from old, galvanized chicken wire the goose would chew on. A previous radiograph was re-examined and small metal opacities were identified. Blood was drawn from three other flock members (two chickens and one goose). Zinc levels were slightly increased in one bird and exceeded the reference range in another. After environmental modification both had reduced zinc levels at a three month recheck.

While clinical signs, history, and histopathology support zinc toxicosis the level detected was below toxic levels previously reported. Most cases described severe toxicosis leading to death shortly after ingestion. In contrast this case was suggestive of a chronic low-level toxicity, indicating zinc toxicity should be considered a differential in neurological waterfowl and reported levels and reference ranges interpreted with caution.

Online: Food Safety

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Horizontal transmission of experimental *Salmonella* Enteritidis infection among egg-type pullets in cage-free housing

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Abstract

Horizontal transmission of *Salmonella* Enteritidis (SE) within cage-free laying flocks presents an egg safety risk. In one experiment, 1/3 of layer pullets were orally inoculated with SE at either 9 wk or 15 wk of age. 1-2 wk later, samples of liver, spleen, and intestine were collected from uninoculated (contact-exposed) birds and cultured for SE detection. SE was isolated significantly more often from internal organs after infection at 15 wk than at 9 wk (64 % vs 22%). In another experiment, different proportions of 15-wk-old pullets (1/3, 1/6, and 1/12) were orally inoculated, internal organ samples were collected from uninoculated birds 2 wk later, and 5 types of environmental samples (wall dust swab, nest box swab, perch swab, flooring substrate drag swab, and flooring substrate composite) were collected at intervals. The frequencies of SE recovery from both environmental samples and internal organ after inoculation of 1/3 of pullets (97% and 75%, respectively) were significantly greater than after infection of 1/6 of pullets (78% and 58%), and further decreases in SE recovery were observed for inoculation at a 1/12 proportion (10% of environmental samples and 18% of organs). Flooring substrate composites were the most efficient environmental samples (72% positive) and flooring substrate drag swabs (53% positive) were least efficient. These results suggest that a environmental contamination may contribute to horizontal transmission of SE infections among pullets in cage-free housing and infections introduced into flocks during the later stages of pullet rearing may have greater potential to spread before egg production begins.

Bacterial communities and the resistome in layer barns; a significant One Health concern

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Abstract

Bioaerosols in poultry houses are composed of a wide variety of microorganisms including bacteria, archaea, virus and fungi. Bacteria having antimicrobial resistant genes (ARGs) can be airborne within the poultry barn, can be transmitted to animals and poultry workers and can be a serious one health concern. The current study was aimed at characterizing the resistome and microbiome from bioaerosols of layer chicken barns across Alberta. A total of 15 barns (9 cage housed and 6 floor housed) were sampled in this study with a microbial air sampler, followed by shotgun metagenomic sequencing. *Bacilliota*, *Actinomycetota*, *Bacteroidota* and *Pseudomonadota* were the top four phylum in bioaerosols of cage housed poultry barns, while *Bacilliota*, *Bacteroidota*, *Pseudomonadota* and *Actinomycetota* were top phylum in floor housed systems. *Klebsiella pneumonia*, *Corynebacterium ulcerans* were significantly more in cage housed barns as compared to floor housed barns. Potential pathogenic bacterial species including *Staphylococcus aureus*, *Salmonella enterica* were more in floor housed as compared to cage housed barns, *Campylobacter jejuni* were more in cage housed as compared to floor housed ones. Resistance to tetracycline, lincosamide's and macrolides were more in cage housed as compared to floor housed bioaerosols while aminoglycosides resistance was found more in floor houses ones. Both microbiome and resistome were found more in cage housed as compared to floor housing air. Presence of potential pathogenic microorganisms and ARGs of various classes of antibiotics in bioaerosols can be a serious one health concern

Salmonella Surveillance at CDC for Public Health Action: Applications for Industry

Marisa Hast, Hilary Whitham

CDC, Atlanta, USA

Abstract

The US Centers for Disease Control and Prevention (CDC) have collected national *Salmonella* data since 1962, with the goal of detecting and preventing cases and outbreaks. This review will examine the strengths and purposes of each CDC surveillance system, what kinds of questions can be asked of surveillance data, and how industry can access this information efficiently to inform interventions and practices. CDC *Salmonella* surveillance includes systems that track cases and outbreaks or provide information about individual isolates. These systems monitor *Salmonella* burden and trends by serotype, identify patterns in antimicrobial resistance (AMR), and determine genetic relatedness between *Salmonella* samples, which can provide early signals of emerging serotypes and strains that may warrant special attention. Tracking *Salmonella* outbreaks can also help identify patterns in food or animal sources and inform interventions. In the past 15 years, for example, CDC has identified 163 *Salmonella* outbreaks linked to consumption of chicken and turkey, comprising nearly 6,000 illnesses, 955 hospitalizations, and 6 deaths. For industry and other food

safety partners to access this information, each CDC surveillance system has an online dashboard to view surveillance summaries and request detailed data. Examining this information can be helpful for partners to compare trends in *Salmonella* data collected on farms or in production to national human health patterns, including serotype distribution, food vehicle trends, AMR patterns, outbreak occurrence, and genetic relatedness to strains causing human illness. Working together, public health professionals and industry can take steps to prevent human morbidity and mortality from *Salmonella*.

Online: Infectious Bursal Disease

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Host genetics and infectious bursal disease virus vaccine efficacy

Julia Blakey, John Dunn, Huanmin Zhang

USDA, Athens, USA

Abstract

Current infectious bursal disease virus (IBDV) control focuses on biosecurity, vaccination, and maternal antibody protection during the first weeks of life. While studies have shown that host genetics have significant impact on the development of several avian diseases, including IBDV, the impact of host genetics on IBDV vaccine responses are unknown. B-congenic lines of chickens (chickens which are genetically identical except for their major histocompatibility complex [MHC]) were used to investigate the effect of host MHC on IBDV vaccine efficacy utilizing a commercial HVT-VP2 recombinant vaccine. A pilot study revealed that a variant IBDV challenge induced a greater range of bursal damage compared to a very virulent IBDV challenge in one line of vaccinated B congenic chickens. An expanded study was performed using seven lines of B congenic chickens and line 6 and line 7 chickens (chickens sharing the same MHC but differing in background immune genes), which received the HVT-VP2 vaccine at 1 day of age and a variant IBDV challenge at 3-weeks of age. Results demonstrated that both the MHC and non-MHC background genes effect bursal protection induced by the HVT-VP2 vaccine after a variant IBDV challenge.

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Study on live and recombinant vaccines given subcutaneously to protect against AL2 and a variant 99% identical to a prevalent IBDV in Canada and Asia

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Zoetis, Durham, NC, USA

Abstract

A variant IBDV isolated last year from a U.S. broiler flock is 99% identical to China variant SHG19. The China variant contains 4 additional mutations compared to the Del-E prototype and has spread throughout Southeast Asia, Western

Canada and, most recently, to the Middle East. In this study SPF leghorns were vaccinated subcutaneously at hatch with either recombinant Procerta HVT-IBD (T01), live vaccine Bursine-2 (T02), or both (T03). Birds were challenged at 22 days with AL2 or the novel variant at 3.0 EID50. Bursa lesions, size (atrophy) and IBDV loads (real-time PCR) were recorded at 29 days of age to compare efficacy of the different vaccination strategies. None of the vaccine treatments showed any indications of bursal atrophy on pre-challenge at Day 18 with treatments 1/2/3 having mean bursameter scores of 3.8/4.0/4.2, respectively and controls having a mean score of 3.8. Post challenge, each vaccine either alone or in combination provided significant protection from bursal atrophy while the controls were fully susceptible to both these variant IBDVs. Percent protection based on atrophy was 90-97% against AL2 and 87-100% against the novel variant. Histological and PCR results are still pending but will be included in the presentation. While Bursine-2 would likely not stimulate 90+% protection given subcutaneously to chicks all having high maternal antibodies, this study demonstrates how valuable live IBD vaccine injection can be for protecting those chicks starting with marginal antibody levels. Live vaccination is thus useful to fill the protection gap before significant recombinant immunity is attained.

Online: Miscellaneous

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Pathological Findings of Avian Reovirus in Commercial Broilers: Seroprevalence and Molecular Detection of Arthritis/Tenosynovitis in Kaduna Metropolis

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Abstract

Avian Reovirus (ARV) is a significant pathogen in poultry, contributing to arthritis and tenosynovitis (AT), which results in considerable economic losses in the poultry industry. This study aimed to assess the seroprevalence and molecular presence of ARV in commercial broilers exhibiting clinical signs of AT in Kaduna Metropolis, Nigeria. Blood samples were collected from 200 broilers across 10 farms, and serological analysis was conducted using enzyme-linked immunosorbent assay (ELISA) to determine the presence of ARV antibodies. Additionally, real-time reverse transcriptase polymerase chain reaction (RT-qPCR) was employed to detect ARV RNA in joint tissues from affected birds. The serological results revealed a 41% prevalence of ARV antibodies, indicating widespread exposure among the sampled population, with maternal antibody transfer evident in day-old chicks (98% positivity). Molecular detection confirmed ARV RNA in 15% of birds with clinical signs of AT, establishing active viral circulation. The study documented gross pathological lesions, including joint swelling and exudate accumulation, consistent with ARV-induced AT. These findings highlight ARV as a prevalent pathogen in broilers, significantly contributing to lameness and production losses. The results emphasize the need for enhanced surveillance, targeted vaccination strategies, and improved biosecurity measures in the poultry industry. Further research is recommended to identify circulating ARV genotypes in Nigeria and

investigate other potential causes of AT, ultimately optimizing poultry health management and mitigating the economic impact of ARV.

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Quantification of Marek's disease virus serotype 1 in feathers as a tool for monitoring the vaccine take and field challenge in Vietnam.

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Abstract

Feather quantitative PCR for Marek's Disease (MD) - viral load in number of the virus genome copies / 10,000 copies of the avian genome – can indicate the level of protection against MD. Early challenge and vaccine replication might be evaluated in the feather pulp 7-10 days of age. Whilst protection at 21 days of age. The earlier the replication in feathers the better the protection. Vaxxon® MD HVT CVI-N is a combination of HVT/Rispens frozen vaccine. The CVI-N is considering low passage strain with earlier virus replication. A high MD challenge layer farm in Hanoi was selected for the trial. 96 day-old commercial Lohmann Brown were divided into 3 groups: **G1** : 32 vaccinated with Vaxxon® MD HVT CVI-N ~3000 pfus /dose ; **G2** : 32 vaccinated with vaccine B - HVT CVI ~3000 pfus /dose; **G3** : 32 non-vaccinated .Birds of each group were placed in different cages at the same house. On 10 and 21 days-of-age, pulp of feather were collected in FTA cards. At 10 days, all birds from group 1 were positive for serotype 01(vaccine) whilst, 16 and 1 for G2 and G3 respectively. The majority (93%) of G1 and 53% of G2 samples were ≥ 132 copies of CVI detected. At 21 days of age, 3.3%, 34% and 71% bird from G1, G2 and G3 respectively were detected positive to field MDV. Under high MD challenge area in Vietnam, Vaxxon® MD HVT CVI-N demonstrated earlier onset of immunity and significantly less MD field virus detection in comparison with vaccine B.

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National Animal Health Monitoring System Backyard Animal Keeping Study 2024: National and City Survey Results Overview

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Abstract

Introduction

The USDA's National Animal Health Monitoring System Backyard Animal Keeping 2024 Study included a national and a city survey. The national survey provided estimates on the percentage of adults who kept poultry at their home,

property, or a community coop. Participants who kept poultry were asked to answer questions on animal management and biosecurity practices. The city survey estimated the percentage of adults who own chickens in Denver and Miami and was a follow-up to the NAHMS 2010 Urban Chicken Study.

Methods

The national survey was carried out using the AmeriSpeak survey panel at NORC at the University of Chicago, which was supplemented by an opt-in panel. The city survey used an address-based sample and was multi-modal.

Results

The national survey had 808 backyard animal keeper responses. The percentage of adults who kept any poultry in the national survey was 6.7%—of those adults, 24.1% kept 11 to 25 poultry on the day the questionnaire was completed, 18.5% used in-person services of a veterinarian for their poultry in the last 12 months, and 93.8% kept poultry for any agricultural production, which was primarily for eggs or meat. The city survey had 1,347 respondents from Denver and 848 from Miami. For the city survey, preliminary results showed that 2.5% of adults in Denver and 2.5% in Miami reported chicken ownership.

Conclusions

This study improves our understanding about backyard poultry keeping and provides information on owner knowledge gaps and opportunities for outreach, as well as poultry health and management practices used.

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Viral Tenosynovitis in Poultry: Integrating Histopathology, In-Situ Hybridization, and qRT-PCR for Avian Reovirus Diagnosis

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Iowa State University, Ames, USA

Abstract

Avian reovirus (ARV) is the causative agent of viral arthritis in poultry. Diagnosing ARV poses challenges due to non-specific lesions, the virus's ubiquitous nature, and the dearth of comprehensive and correlative understanding of avian reovirus pathogenesis and pathology. In this study, we investigated the correlative features between the severity of histologic features and viral RNA levels detected by qRT-PCR and incorporated spatial RNAScope *in situ* hybridization (ISH) to support our findings. Fifty-one cases (chickens and turkeys) from 2016 to 2024 were retrieved from Iowa State University Veterinary Diagnostic Laboratory. Case selection criteria included: 1) reported clinical history of lameness, 2) complete histopathologic examination of gastrocnemius and digital flexor tendons, and 3) qRT-PCR performed on tendons. Histologic features scoring criteria using previously published manuscript included inflammation severity, synoviocytes proliferation, lymphoid nodules, neovascularization, and fibrosis. qRT-PCR positive cases (n=38; Ct 20.9 – 35.9) exhibited significantly more severe lesions than negative cases (n=13). A subset of qRT-PCR positive (n=33; Ct 20.9 – 35.9) and negative (n=8) cases with severe histologic lesions were selected for ISH. ISH detected viral transcripts in synoviocytes and subintimal fibroblasts (11/41, 26.8%; Ct 20.9 – 32.3). Positive ISH results correlated with lower Ct values (P<.05), with a defined cutoff of Ct 28.4 linking inflammation severity to viral RNA levels. This study addresses

diagnostic challenges by correlating histological lesion severity with viral transcription levels, and we identified synovial subintimal fibroblasts as ARV-target cells. These findings provide novel insights, expand the frontiers of ARV pathogenesis, and aid in diagnosing suspected ARV cases.

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Downstream benefit of Marek's disease vaccination and dosage effect

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Abstract

Marek's disease (MD) is currently controlled by highly protective live-attenuated vaccines. Recently, we found that vaccination not only benefits chickens that have received the vaccine, but also reduces disease severity in naïve contact chickens. The goal of this experiment was to observe and compare the effect of vaccination in a large-scale experiment assessing the effect of natural transmission through chickens receiving full- and partial-dose vaccination. We used a shedder-sentinel challenge model to naturally passage MDV through 10 successive groups. Each group consisted of 10 birds kept in an individual isolator and replicated 3-6x. Birds infected in Passage 1 transmitted virus to recipients in Passage 2, and so on. Variables included vaccination status and dosage (full or 1/10 dosage). MD incidence and feather virus load were assessed for each bird. Statistical analyses of the experimental data showed that the infection and transmission dynamics of birds that have been inoculated with MDV (Passage 1) differed substantially from those of birds that were naturally infected through contact with infected shedder birds, highlighting the importance of mimicking modes of transmissions representative of field conditions in vaccination experiments. Furthermore, we demonstrated MDV transmission through all 10 passages in both vaccinated groups. Vaccination with the full recommended HVT dose was found to not only provide direct protection from MD and death to the vaccinated birds, but also indirect protection for non-vaccinated contact birds. This indirect benefit was lost in partial-dosage vaccine groups, highlighting the importance of proper dosage for downstream benefits.

Online: Mycoplasma

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Development and Evaluation of Mycoplasma gallisepticum Challenge Model in Layer Pullets.

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¹Iowa State University, Ames, USA. ²Animal Health Research Institute, Cairo, Egypt

Abstract

Manufacturers' recommendations for the administration of MG modified live vaccines is usually a single application at 6 – 9 weeks of age. This makes 12 – 16 weeks old layer pullets a suitable age for challenge studies intended to evaluate

these vaccines. The aim of this study is to develop a suitable challenge model in 12 weeks old layer pullets. MG R_{low} strain was used as the challenge strain and its ability to induce clinical signs and lesions in 12 weeks old Hy-Line W-36 layer pullets was evaluated. Three different doses (low 7.95×10^4 CCU/bird, medium 7.95×10^6 CCU/bird and high 7.95×10^8 CCU/bird) via three different routes (eye drop, spray, and contact infection) were compared and evaluated using different parameters. At 14 days post-challenge, there were no mortalities in any of the groups throughout the study. Layer pullets directly challenged with the high dose via the spray route showed the clearest and most consistent results (clinical signs, positive qPCR, seroconversion, air sac scoring and histopathological changes of the tracheal mucosa). Medium and low challenge doses applied via spray or eye drop did not show consistent results. R_{low} strain spread to the contact infection birds as confirmed by the positive qPCR results; however, no contact-infected birds showed any clinical signs, gross or microscopic lesions. Our results suggest that a high dose (7.95×10^8 CCU/bird) administered through a spray route is the model of choice in any future MG vaccine evaluation trials in 12 weeks old layer pullets.

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Evaluation of vaccination programs in layer pullets using recombinant Fowl Pox-*Mycoplasma gallisepticum* vaccine in comparison to F strain live vaccine

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Abstract

This study assesses the efficacy of a recombinant fowl-pox vector vaccine (rFP-MG) in conferring protection against *Mycoplasma gallisepticum* in different prime-boost combinations in Hy-line W-36 layer-pullets. Group 1 served as unvaccinated/unchallenged ($n=28$). Group 2 ($n = 30$) received a single dose of rFP-MG at eight weeks of age and group 3 ($n= 31$) received two doses of rFP-MG, one at day of age and again at eight weeks. Group 4 ($n= 30$) received a single dose of F strain at eight weeks and group 5 ($n= 32$) received rFP-MG at day of age and F strain at eight weeks. The last group is the unvaccinated/challenge ($n=29$). Five groups were challenged using MG R_{low} strain at a dose of 3.11×10^8 CCU/bird via the Fine Spray route. Clinical scoring and tracheal mucosal thickness at 21 days post-challenge were used as the parameters for the evaluation of each vaccination program. Groups that received only rFP-MG showed clinical signs as early as 7 days post-challenge, while the F strain groups did not show any clinical signs. The tracheal mucosal thickness of the F-Strain vaccinated groups was not significantly higher than that of the unvaccinated/unchallenged. However, the tracheal mucosal thickness of the rFP-MG vaccinated was significantly lower ($P < 0.05$) than that of the unvaccinated/challenged. Results from this trial showed that rFP-MG vaccine provided partial protection against the clinical signs compared to the unvaccinated/challenged positive control; however, it was less protective than the F-strain vaccine.

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Field Assessment of Coccidiosis Vaccine Uptake and Early Replication of Two Different Products Applied by Gel or Spray

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Abstract

Vaccination is a valuable tool for the control of coccidiosis in poultry, however there is inherent variation in uptake of commercial vaccines due to different application methods and variations in viable oocyst numbers that can exist with long shelf-life vaccines. The objective of this study was to evaluate two coccidiosis vaccines applied according to the manufacturer's recommendations, one in gel and the other by spray. This field study was done in collaboration with an integrator in the southern United States. To evaluate field vaccine uptake and early replication, we selected 20 chicks vaccinated with one coccidia vaccine by gel and 20 chicks vaccinated with a different coccidia vaccine by spray. The birds were placed in individual housing units and feces were collected from days 5 to 8 post-vaccination. Real time qPCR and oocyst counts were used to detect *Eimeria* species in the feces. Results show that the product applied using gel application is advantageous versus the product given by spray as determined by better qPCR detection and oocyst counts. Results from other similar studies will also be shared.

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Impact of Winter Coccidiosis Programs on Broiler Performance and Sustainability

Francene Van Sambeek

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Abstract

A recent research study conducted by Elanco has revealed the impact of various winter coccidiosis programs in broilers over 42 days, focusing on performance, lesion reduction and sustainability.

All programs examined significantly reduced coccidial lesions, with certain ionophores showing substantial impact on bird weight and feed conversion ratios. Certain programs examined by Elanco also demonstrated cost-efficiency, offering a lower feed cost per pound of adjusted gain. Coccidiosis control strategies also demonstrated an improved feed efficiency, potentially reducing greenhouse gas emissions by 4-7%.

The study highlights the importance of selecting appropriate anticoccidial programs to enhance broiler performance, reduce production costs and improve environmental sustainability in poultry production by seeking to optimize their winter coccidiosis management strategies.

Do we need to rethink the way we vaccinate birds at the hatchery if they have access to feed and water in the hatcher?

Katherine Schaefbauer

Jennie-O Turkey Store, Willmar, USA

Abstract

Do we need to rethink the way we vaccinate birds at the hatchery if they have access to feed and water in the hatcher?

K.R. Schaefbauer

Jennie-O Turkey Store, Willmar, MN

Hatching poults with access to feed and water is new technology that allows poults to have access to feed and water the second they pip out of their eggs. This ability to get on feed and water, sometimes more than 48 hours sooner, than poults that have to wait until they are delivered to the farm, causes the poults to behave and react differently. We have seen many differences in behavior and the way we manage the poults hatching from hatcheries with access to feed and water equipment compared to conventional hatcheries, which brought up the question around vaccinating day old poults. Do the birds that have access to feed and water require different dosing than birds hatched at a conventional hatchery? These trials were looking at a cocci vaccine using the standard drip bar and comparing the preening rate of the birds hatched from the new hatchery technology to conventionally hatched birds. These trials looked at different volumes, application timing, Osteoprotegerin (OPG) counts, and mortality in order to achieve acceptable uptake rates.

Effects of Time of Day and Sex on Helminth Egg Shedding in Nigerian Local Chickens

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Abstract

This study investigated the effects of time of day and sex on helminth egg shedding in local chickens. Forty chickens (19 males, 21 females) were obtained from Dankure live bird market, Sokoto, Nigeria, and screened for helminths. The birds were housed in a battery cage system for three weeks, with one bird per cell for identification, and acclimatized for one week. Faecal samples (2 grams per bird) were collected at four distinct times of day—06:00, 12:00, 18:00, and 24:00 hours—on August 14, 2023. The samples were stored at 4°C until analysis. Egg shedding was analyzed using the flotation

technique, and egg counts per gram (EPG) were calculated. EPG data were converted into categorical data, with "positive" categorizing birds with a count and "negative" those with no count. Statistical analysis was performed using binary logistic regression and Pearson correlation analysis to examine the relationship between EPG and worm count. Time of day significantly influenced egg shedding ($p < 0.001$), with shedding increasing from morning to afternoon. At 06:00 hours, 80% (32 birds) of the samples tested positive for helminth eggs, while at 12:00 hours, 90% (36 birds) tested positive. The lowest detection rate occurred at 18:00 hours (30%, 12 birds), recovering to 70% (28 birds) at 24:00 hours. Sex did not significantly affect egg shedding ($p = 0.1456$). A positive correlation ($r = 0.642$, $p < 0.001$) was found between EPG and worm count. These results highlight the importance of timing in egg detection and show minimal impact of sex on shedding patterns.

Online: Respiratory Viruses

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Unsung Heroes: The unseen impact of HPAI outbreaks on private poultry vet practices, the view from British Columbia.

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Abstract

Sustained HPAI outbreaks devastate local poultry producers, their community and all those in response. The Fraser Valley of British Columbia has been particularly hard hit, enduring 6 waves of the clade 2.3.4.4 HPAI circulating in Pacific Flyway migratory birds leading to significant environmental contamination in a densely populated multi-commodity poultry growing region. In peacetime, private poultry vets support the health, welfare and productivity of commercial poultry flocks through field visits, extension, diagnostics, treatments and retail sales. During an outbreak, with its concurrent movement restrictions, intense surveillance activities and enhanced biosecurity, the call for routine veterinary services are significantly reduced.

A private poultry practice is a business delivering specialized medicine to a finite number of clients. This is accomplished by financial investment in real estate, equipment, overhead, personnel and professional competency. Client loyalty is attained by dedication and engagement. When a livestock industry is facing the economic uncertainty of an FAD outbreak, the vet-client relationship is altered as the calls for service are significantly reduced. Enhanced industry-wide biosecurity measures discourage farm service visits and clinic drop-ins (considered high risk due to traffic). The practice's diagnostic service is reduced as producers are encouraged to direct submit to the local diagnostic lab. Flock depletions and extended downtimes prior to repopulation reduce the need for vaccines and treatments. There is increased hesitancy to spend so retail sales are reduced.

Meanwhile the practice must withstand the uncompensatable business losses and still maintain accreditation, personnel, overhead, inventory and client relationships. No easy feat.

Serological survey of avian metapneumovirus in broiler breeders for the state of Georgia (USA)

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Abstract

Avian Metapneumovirus (aMPV) is a known virus to the poultry industry with a costly impact on egg production for layer type poultry production and high mortality from secondary bacterial infections for all poultry commodities. At the end of 2023, early 2024, two novel groups, A and B, spread rapidly across the United States of America. The objective of this study was to investigate the evolution of flocks' exposure to aMPV in the state of Georgia for one year by serological testing as the outbreak unfolded. A total of 289 broiler breeder flocks in the state of Georgia were randomly selected from previously saved sera submitted for health monitoring. Flocks were stratified by months and region (Northeast, Northwest and South). Ten sera per flock selected were plated and tested for aMPV using a commercial serology kit. Threshold for negative, suspect and positive flocks were established on overall flock Geometric mean titers (GMT). When all flocks were combined and stratified by month, there was a significant increase in the proportion of positive flocks over time ($R^2 = 0.74$, P -value < 0.001) and a reduction in the proportion of negative flocks. Comparison of flocks median GMT sampled before and after the first confirmed case revealed an increase in titer level by region, with a marked difference in Northeast Georgia. The epidemiologic picture of aMPV in broiler breeder flocks for the state of Georgia indicated an increase of exposure in all regions over time despite fluctuations in positive detections by molecular testing.

Molecular Epidemiology of Infectious Bronchitis Virus (IBV) in Backyard Poultry in São Paulo, Brazil

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³Unicamp, Campinas, Brazil. ⁴CDA-SP, Campinas, Brazil. ⁵CDA-SAA-SP, Campinas, Brazil. ⁶Konkuk University, Seoul, Korea, Democratic People's Republic of

Abstract

Infectious Bronchitis Virus (IBV) variants belonging to the GI-23 lineage, initially identified in the Middle East in 1998, have gradually spread globally, with their first reported occurrence in commercial flocks in Parana, a state in the South of Brazil, in 2022. Before that, the common Brazilian genotypes were viruses belonging to GI-1 and GI-11 lineages. The present study evaluates the circulation of IBV in backyard poultry in São Paulo state. Active surveillance of 742 backyard poultry birds across 15 municipalities in São Paulo, Brazil, was conducted by the Official Veterinary Service from the São

Paulo Agricultural Defense Coordination from December 2022 to April 2024. A total of 742 birds (718 chickens, 17 ducks, and seven geese) from 140 backyard flocks were sampled. All birds were clinically healthy and had no vaccination history. Cloacal and tracheal swabs were collected and subjected to RT-qPCR for IBV UTR detection, followed by S1 gene sequencing of positive samples. Molecular screening of 304 cloacal and tracheal swabs using RT-qPCR detected IBV in 14.1% of samples, with the highest prevalence observed in Ilha Comprida and São Sebastião. Ten samples were successfully sequenced, and viruses were clustered within GI-23 viruses found in Brazil, with nt identity higher than 95%. Complete genome sequencing has been carried out to characterize the IBV strain. Our data indicates the introduction of the GI-23 lineage in backyard poultry from the São Paulo state, Southeast Brazil.

Funding: FAPESP (2022/08528-3 and 2023/08501-0)

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Mapping the genetic diversity of avian metapneumovirus isolated from chicken flocks in the state of Georgia (USA).

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Abstract

Avian metapneumovirus (aMPV) is an important global respiratory pathogen for poultry. Until very recently, subtype C was the only aMPV detected in the United States (U.S.), considered an aMPV-free country since the eradication of the last cases of aMPV-C in the early 2000's. Unfortunately, aMPV subtypes A and B were introduced into the U.S. in 2023-24, with outbreaks reported in many states. The rapid spread of aMPV in poultry, despite increased biosecurity practices driven by the avian influenza threat, highlights a gap in our epidemiological knowledge regarding these viruses. To improve our understanding of the genetic characteristics and the transmission of aMPV strains circulating in the U.S., 68 RT-qPCR positive samples collected between February and June 2024 from 36 farms belonging to 17 poultry production companies across the state of Georgia (U.S.) were subjected to next-generation sequencing analysis. 22 complete or nearly complete genomes were obtained from the sequencing data, all identified as subtype B, and sharing high genetic similarity among themselves and to other American aMPV-B viruses (<99% similarity at nucleotide level). The combination of a phylogenetic analysis and the available metadata revealed genetic clustering by company, with isolates collected from different farms under the same company exhibiting the highest sequence similarity. Additionally, a comprehensive single nucleotide polymorphism (SNP) analysis showed enough SNPs between aMPVs from different companies to suggest difference sources of viral introduction, likely from another U.S. source. Overall, this work will serve to inform the affected companies helping them adapt their biosafety measures for aMPV containment.

Avian metapneumovirus subtype A & B isolation and studies on vertical transmission in turkeys

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¹ARS, Athens, USA. ²Select Genetics, LaGrange, USA

Abstract

Avian metapneumovirus (aMPV) is a widespread avian disease capable of causing respiratory disease and contributing to secondary infections resulting in disease and mortality with serious economic losses and productivity primarily in chickens and turkeys. In 2023, cases of subtype A and B aMPV were reported in commercial chicken and turkey flocks for the first time in the U.S. The virus has been difficult to grow in using standard cell culture methods, but we successfully isolated both A and B viruses on baby hamster kidney (BHK) cells. Through coculture of infected BHK cells and Vero cells we were able to adapt the virus to replicate in Vero cells with observable CPE. The isolated virus was sequenced and made available to APHIS as reference reagents. Currently, a concern for the turkey industry is the possibility of vertical transmission of the virus from hen to poult. In these studies, we confirmed the presence of aMPV in the respiratory tract of infected commercial turkeys and then examined eggs from aMPV-positive laying hens for presence of viral RNA that might suggest vertical transmission of the virus. Eggs from seven different aMPV-positive commercial turkey flocks were harvested and tested for aMPV subtype A and B via RT-qPCR. Results demonstrate no evidence of viral RNA in any of the egg/embryo samples tested, either inside or on the shell surface. These results provide direct evidence that vertical transmission does not appear to contribute to virus dissemination in commercial turkey flocks.

Desperate Times Call for Desperate Measures: A Sentinel-based Experiment with Avian metapneumovirus type A in Commercial Turkey Poults

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Abstract

Avian Metapneumovirus (aMPV) also known as Turkey Rhinotracheitis is an acute respiratory virus in turkeys and chickens. Since the identification of AMPV type A and B in the Fall of 2023, the disease has spread across all major poultry producing states causing heavy economic losses to poultry production in the United States.

This presentation describes one field veterinarian's experience in the Midwestern states with AMPV type A (AMPV-A). With limited to no effective tools or vaccines available for use in the United States to aid in controlling this disease, a sentinel-based study was developed to attempt to expose newly hatched turkey poults to other poults on-farm that were acutely sick with AMPV-A. The results of this sentinel-based experiment were used to provide early exposure to AMPV-A and potential protection to newly hatched poults. Additionally, further information was obtained on the time to exposure and short latency period of AMPV-A within a commercial turkey flock. Both flocks, older and younger, were

tested prior to initiation and throughout the study to maintain knowledge of what agents were present and status of seroconversion.

Online: Virology

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Multiple Field Experiences: Impact of SRP E. coli-SE Vaccine on Broiler Breeder Hen Mortality

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Abstract

Broiler breeder hen mortality is difficult to control. Success requires prevention of specific disease conditions combined with a mastery of complex poultry husbandry programs during the rearing and egg laying periods of the hen flock. A major disease contributor to broiler breeder hen mortality is E coli peritonitis. A vaccine utilizing SRP proteins from E coli and Salmonella enteritidis antigens (Vaxxon® SRP® E. coli-SE) is the first and only commercially licensed inactivated E coli vaccine. This vaccine was evaluated for control of E coli peritonitis via field use in multiple broiler breeder complexes in the USA. In addition, in some companies a commercially licensed vaccine containing SRP antigens to Pasteurella (Vaxxon® SRP® Pasteurella) was administered in combination with the E. coli-SE SRP vaccine.

Four broiler breeder complexes changed to vaccination programs containing E. coli SRP antigens as follows: One company: Two doses of SRP E. coli-SE (12 and 18 weeks) and three companies: Two doses of SRP E. coli-SE + SRP Pasteurella (12 and 18 weeks). Weekly hen and male mortality data was compared in sequential flocks before and after the change to SRP containing vaccination programs. In all four field evaluations weekly hen mortality rates were significantly reduced in flocks receiving SRP vaccines. The positive response was consistent in flocks of different genetic lines (Ross vs Cobb) and whether SRP Pasteurella vaccine was also used. Data from hen mortality surveys showed a reduction in the incidence of peritonitis observed during necropsies of naturally dead hens in flocks receiving SRP vaccines.

Evaluation of in lay boosting with a commercial *Pasteurella multocida* modified-live vaccine in organic, free range layers

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Abstract

Pasteurella multocida (*PM*) vaccines are a valuable disease prevention tool for the egg layer industry due to limited antibiotic availability and restricted allowance particularly in speciality egg production (organic, antibiotic-free, etc.). Industry standard is to vaccinate pullets twice during the rearing stage with a combination of killed and modified-live vaccines with the expectation that protection lasts the duration of a hen's life. However, in late lay, *PM* remains a concern even among vaccinated hens. Specialty hens with outdoor access have continuous and evolving pressure, including introduction of unique serotypes not in autogenous or commercial vaccine programs. In-lay boosting with a commercial *PM* modified-live vaccine may be a feasible option to layer producers to help mitigate mortality due to *PM*. Little work has been done in the layer industry to evaluate the use of water delivery of *PM* modified-live vaccines while in lay, and handling birds in lay for wing web application is not viable from a welfare or economic standpoint. Field trials are currently underway evaluating the use of in lay boosting via water with a modified-live commercial *PM* vaccine. Two different vaccine strategies are being evaluated in this study: in lay boosting on a schedule at historical *PM* sites before a break occurs and in lay boosting after a break occurs. Results of the field trials are still ongoing, but preliminary results have indicated that there are no adverse effects on production or mortality when boosting in lay with a commercial modified-live *PM* vaccine.

Measuring Herpesvirus of Turkeys (HVT) Recombinant Vaccine Virus Takes

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Abstract

Measuring vaccine takes after hatchery application has become commonplace in the US poultry industry for vaccines that are mass applied to chicks post-hatch. However, recombinant vaccines that utilize the herpesvirus of turkeys (HVT) as a backbone are not applied en-masse but are injected into embryos at transfer. The process of in-ovo injection is quite intricate and closely monitored, but failures can still occur. For this reason, a real-time quantitative PCR test targeting the HVT-Sorf1, which is conserved across HVT's, was adapted to our diagnostic laboratory for recombinant vaccine take testing. To determine the best tissue sample and age of collection, a trial was conducted at the Zoetis Avian Research Center (ARC) where selected treatment groups were vaccinated with Poulvac Procerta HVT-IBD, and feather pulp and spleens were collected at 13- and 21-days post-hatch. Results showed that 100% of spleens were positive at both 13- and 21-days-of-age, with very similar mean Ct values (28.2 and 27.3, respectively). Feather pulp samples were 100% and 94% positive at 13- and 21-days-of-age, but mean Ct values were reduced compared to spleens (32 and 34.1, respectively). This data shows that using qPCR to measure HVT vaccine takes is a useful tool, and that spleens seem to

be a more sensitive tissue sample to collect than feather pulp. The next step for optimization of this protocol will be to collect spleens at 5-7 days post hatch, which is standard for respiratory vaccine take sampling, to determine if one collection timepoint is sufficient for all vaccine types.

Online: Wealth of Knowledge

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Multi-mycotoxin biomarker analysis in blood to assess mycotoxin exposure risk and performance impacts of a novel mycotoxin intervention in broilers

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Abstract

An UHPLC-MS/MS method targeting 36 mycotoxin biomarkers via dried blood spots (DBS) was used to determine actual mycotoxin exposure in samples obtained from commercial poultry flocks in several USA locations. Feed consumed at the time of blood collection was also analysed for 16 mycotoxins by LC-MS/MS. The combined results showed a persistent exposure of USA poultry to multi-mycotoxins.

This methodology was used in a floor pen study to evaluate performance impacts of a Mycotoxin Intervention (MI), Novin P® (Innovad), a proprietary multi-component feed additive. A study using Ross YPM x 708 male chicks on a coccidial vaccine program was conducted in 10 randomly placed pens per treatment, 52 birds per pen and grown to 45 days of age. Pens were randomly assigned to 2 treatments: T1) Control (no additives) or T6) MI: 2.00 lbs/ton during starter (0-14 days), 1.50 lbs/ton during grower (15-32 days) and 0.50 lbs/ton in finisher feed (33-45 days). Feed and DBS samples were collected for mycotoxin analysis at the end of each feed phase. MI fed birds had significantly better performance. DBS analysis for biomarkers of mycotoxins showed less numerous and lower concentrations of mycotoxins in blood from MI fed birds.

The MI has been widely used in commercial poultry operations to control deleterious effects of mycotoxins in the USA and abroad. Multiple comparisons of before and after use of the product in blind testing of blood samples analysed for mycotoxins clearly show the systemic detoxifying properties of the product.

Expression of the Avian Reovirus Sigma C Protein and a Chicken Cytokine Using the NDV TS09 Vector as a Vaccine for in-ovo Vaccination

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Abstract

Avian reoviruses (ARVs) are economically significant pathogens that cause viral arthritis syndrome and tenosynovitis in young chickens and turkeys. These viruses are also associated with various disease syndromes that negatively impact poultry production. Vaccination combined with enhanced biosecurity measures has been a common strategy to control ARV-related diseases. However, as ARVs evolve, commonly used vaccines have become less effective against the infections caused by virulent ARV variants. In this study, we aimed to develop an *in-ovo* vaccine using the Newcastle disease virus (NDV) TS09 vaccine vector to enhance protection against a prevalent ARV variant. The TS09 vaccine strain has previously been proven safe for *in-ovo* vaccination. We synthesized the antigenic sigma C (σ C) protein gene derived from the virulent ARV AVS-HMM strain, which is prevalent in the U.S. This gene was inserted into the TS09 vector, either alone or with the addition of a chicken cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF), as an independent transcription unit located between the P and M genes. Two recombinant viruses, rTS/ARV- σ C and rTS/ARV- σ C-GM-CSF, were successfully created using reverse genetic technology. Biological characterization revealed that both recombinant viruses retained a non-virulent pathotype and exhibited growth kinetics similar to their parental virus. The expression of ARV- σ C was confirmed in DF1 cells infected with these recombinants through immunofluorescence assays using anti-ARV chicken serum and an anti- σ C specific antibody. Currently, we are evaluating the safety and protective efficacy of these vaccine candidates against the ARV variant and NDV challenges through *in-ovo* vaccination trials.

Understanding the Poultry Health Professionals

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Abstract

The poultry industry is rapidly growing across the globe, creating a demand for poultry health services. In the United States, the number of veterinarians and animal specialists is increasing, but there is little understanding of why poultry professionals aren't keeping up with industrial growth. We surveyed individuals within the American Association of Avian Pathologists as established members of the poultry community to determine common factors and influences in their educational background and careers. We found that many participants had some kind of rural upbringing and interaction with animals while growing up. Additionally, many participants first interacted with poultry through university classes or mentors. While more research is needed to compare to professionals who chose non-poultry specializations, our results indicate that more mentorship opportunities may aid in fostering interest in the poultry field for students and early-career individuals

Evaluation of Marek's Disease Virus-Induced Immunosuppression induced by very virulent plus Marek's disease virus in Meat Type Chickens

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Abstract

We have previously demonstrated that very virulent plus (vv+) Marek's disease virus (MDV) strains induce severe immunosuppression in commercial meat-type chickens. They have reduced splenocytes livability by 3-4 weeks post-infection, induced drastic changes in spleen immunophenotypes, reduced splenocytes proliferation ability when contacting concanavalin-A ex-vivo, and induced severe immunosuppression during reactivation of the virus (MDV-IS-R) that jeopardized protection conferred by infectious laryngotracheitis vaccines. We have also demonstrated that maternal antibodies do not confer protection against those changes, and HVT protects only against the reduced splenocytes lymphoproliferation. Furthermore, we have demonstrated that two recombinant vaccines, rMd5-BACΔMeq (experimental) and CVI-LTR (commercially available), but not conventionally used vaccines (HVT, HVT+SB-1, CVI988, or CVI988 + HVT), protect against vv+MDV-induced MDV-IS-R. The objective of the present study was to evaluate protection conferred by three MDV-1 vaccines (rMd5-BACΔMeq and CVI-LTR that protect against MDV-IS-R and CVI988 that does not) against the negative effect of vv+MDV strain 686 in the spleen (cell death and immunophenotype changes). The three evaluated vaccines induced strong activation of cytotoxic T-lymphocytes (CTLs) at 6dpi and conferred great protection against MDV-induced tumors. They also rescued splenic immune cell death at 25dpi, however, there were statistically significant differences among treatment groups in the frequency of splenic immunophenotypes. Groups vaccinated with rMd5-BACΔMeq and CVI-LTR but not CVI988 maintained a high frequency of B-cells at 25dpi and had a reduced percentage of activated CD4+ T-cells at both 6 and 25dpi. The significance of those features in the protection against MDV-IS-R needs further evaluation.

Mycotoxin mitigation strategies: yeast cell wall extract supplementation in broiler and layer performance, productivity and sustainability

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Abstract

Mycotoxins (MT), secondary metabolites produced by fungi, can cause disease in various species of poultry. Two published meta-analyses have assessed the benefits of adding yeast cell wall extract (YCWE) to decrease the impact of mycotoxins on poultry performance and profitability. The first meta-analysis assessed the impact of mycotoxins without

or with YCWE on broiler production performance, livability and environmental sustainability. Data from 25 research trials involving 10,307 broilers indicated that YCWE supplementation during mycotoxin challenge improved ($p < 0.001$) body gain weight (59 g), feed intake (-0.05), and reduced mortality by 1.74%. European Production Efficiency Factor (EPEF) increased when including YCWE versus mycotoxins alone through benefits that supported productivity. This research also indicated that the carbon footprint of production may be lower with YCWE inclusion rather than mycotoxins alone, thereby improving environmental sustainability. The second meta-analysis investigated the use of YCWE on laying hens challenged with mycotoxins. A total of 8 trials for a total of 1,774 birds were evaluated. Mycotoxin challenge had a negative impact on egg production and weight, while including YCWE during mycotoxin challenge resulted in significantly ($p < 0.0001$) higher egg production and egg weights by 4.2 percentage points and 1.37 g, respectively. Together, the recently published meta-analysis supports that supplementation of YCWE in the feed may alleviate the effects of mycotoxins, improving farm efficiency and profitability.

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An investigation into the risk factors associated with recurrent Fowlpox cases

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Abstract

A 12-week-old pullet flock presented with a co-infection of Gangrenous Dermatitis and Fowlpox. During the diagnostic investigation, a trend was detected. The simultaneous diagnosis of Gangrenous Dermatitis and Fowlpox was recorded in previous years on this farm. All diagnoses had been made in January of different years. Further research was conducted to determine the relevant risk factors associated with Fowlpox at this location during winter. Whilst conducting farm visits, insects were captured and subsequently confirmed as mosquitoes (Diptera, Culicidae) by a veterinary entomologist. The recurrent nature of this disease and heavy environmental mosquito burden was indicative of mosquitoes acting as an important mechanical vector. Subsequently, a topographic survey and review of environmental temperatures was conducted to understand the mosquito population. Finally, a nutritional analysis was completed on feed to determine if there was a relationship to skin integrity. The risk factors identified in this study were linked via geospatial analysis to 11 other farms with historical cases of Fowlpox. Investigators concluded that Fowlpox was most likely to be associated with key topographic and climate factors that create an ideal habitat for mosquitoes. Additionally, the most severe Fowlpox lesions are likely to be associated with a pre-existing dermatologic condition and high humidity. An understanding of geographic factors associated with high mosquito burden could influence insect control regimes and guide the location of future construction of poultry farms.

Poster: Avian Influenza

Poster 11

Avian Influenza Virus Surveillance in Wild Waterfowl in the Northern Sacramento Valley, California

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Abstract

Avian influenza viruses (AIV) circulate naturally in wild waterfowl, typically without causing disease. However, waterfowl are important vectors spreading AIV to susceptible populations where infection often causes severe disease or fatality. For example, a highly pathogenic avian influenza (HPAI) H5N1 virus emerged in North America in 2022 and has affected > 130 million wild aquatic birds, and commercial and backyard poultry. Early detection of AIV through surveillance is one measure to prevent or limit the extent of disease in humans or economically important poultry flocks. Surveillance efforts designed to identify or account for host species and environments that correlate with greater susceptibility to AIV infection are likely to be most effective. We monitor AIV prevalence in hunter-killed waterfowl in the Sacramento Valley of California. Overall virus prevalence in waterfowl between 2014-2015 and 2021-2022 was 9.8%. Virus prevalence rates are highest in northern shovelers (20.9%) and lowest in wood ducks (1.3%). HPAI H5N1 prevalence was 1.8% and 4.7% during the winters of 2022-2023 and 2023-2024, respectively. To date, we have not detected HPAI H5 in 2024-2025. In contrast to our data prior to 2022, the emergence of HPAI H5N1 correlates with a shift in AIV prevalence trends. While AIV prevalence remains ~20% in northern shovelers, American wigeon represent the species with the highest virus prevalence (19.6% in '22-'23 and 28.5% in '23-'24). Future studies will focus on sequencing HPAI H5-positive samples to assess the risk to humans and poultry.

Poster 12

Compatibility and Production Performance in Commercial Layers Immunized With rHVT-H7 and rHVT-F Vector Vaccines Applied Simultaneously

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Abstract

In Mexico, the main diseases affecting the poultry industry include Newcastle disease (ND) and Highly Pathogenic Avian Influenza (HPAI) subtype H7N3. Disease control and prevention were carried out using vaccines approved by the authority. Recently, animal health companies had developed different new technology vaccines, such as vector vaccines where a virus is used as a vector to carry the genetic information for the protective proteins of the inserted gene from another virus. Vector vaccines using the Marek herpesvirus of turkey (rHVT) vector have different advantages, among

which are: there is no interference with maternal antibodies, applicable in the hatchery, and provides long duration of immunity.

There are already publications by different authors demonstrating the compatibility of two HVT-vector vaccines, namely, Vectormune® ND (rHVT-ND) and Vectormune® AI (rHVT-AI H5) are effective against Newcastle Disease and Avian Influenza respectively when given simultaneously. In Mexico, we have demonstrated the compatibility and advantages of using the combination of the two HVT-vector vaccines, namely Vectormune® ND (rHVT-ND) and Vectormune® H7 (rHVT-AI H7) in commercial layers under field conditions with the evidence of improvement in production parameters.

Poster 13

Development of a Real-Time qPCR to Identify Genotype B3.13 High Pathogenic Avian Influenza Virus.

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Abstract

Highly pathogenic avian influenza virus (HPAIV) poses a significant threat to global animal health, leading to severe consequences for the poultry industry and spillover events into other species. Recently, spillover of the avian influenza H5N1 virus into dairy cattle has been reported, raising concerns about the economic and epidemiological implications of such cross-species transmissions has further heightened public health concerns. All infected dairy herds have been infected with clade 2.3.4.4b, genotype B3.13 highly pathogenic avian influenza, that had only rarely been seen in wild birds previous to this outbreak. In this study, we have developed a highly specific and sensitive qRT-PCR assay that targets two genes of interest—nucleoprotein (NP) and polymerase basic 2 (PB2)—each uniquely identifying the B3.13 lineage of avian influenza H5N1 virus. We also conducted a comparative analysis with alternative ThermoFisher chemistries, including TaqMan™ Fast Virus 1-Step Master Mix and VetMax Fast Multiplex Master Mix. AgPath kit offers comparable sensitivity and specificity, with no significant differences in test performance across the three chemistries. Using 10^{-3} dilutions of RNA samples taken from dairy cattle and poultry, the average Ct value for all three chemistries was 27. Furthermore, the detection limit of this assay is up to 10^{-6} endpoint dilution. The assay represents a valuable diagnostic tool for identifying and monitoring avian influenza H5N1 infections in poultry and dairy cattle. Its ability to identify B3.13 lineage AIV infection will facilitate implementing of targeted control measures to try to minimize economic losses and enhance surveillance of cross-species transmission events.

Poster 14

Early infection of chicks less than 10 days old with Avian Influenza (H9N2)

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Abstract

We report in this article on early infection of chicks less than ten days with AIV H9 in a broilers farm in Senegal.

An increase of mortality linked to respiratory distress in a flock of 25552 broiler was related. Mortality in the first week was normal with 0.85%. But from D8 to D19, mortality will increase with a daily rate of 3.74% and a peak at D16 of 2909 deaths. During these 12 days of illness, 12731 deaths were recorded, representing a rate of 44.91%.

Necropsy of birds revealed :

- hemorrhagic tracheitis with mucus ;
- Caseous plug at tracheal bifurcation ;
- airsacs with fibrin deposits and nephritis .

These lesions are consistent with AIV H9 infection in broilers ; therefore, the involvement of the virus in this outbreak was strongly suspected.

For diagnosis, samples of spleen, tonsil, trachea and lung on FTA card were collected and sent to two laboratories (Deventer & Anicon) for PCR to detect AIV H9 and IBV. In addition, sequencing of the isolates was performed and a differential diagnosis with other respiratory pathogens done.

Blood samples were collected at D32 and assayed for H9N2 and IBV by ELISA by using two serological kits ; a classic kit and a nucleoprotein kit which allows the detection of a wild passage.

Laboratory results showed the presence of :

- **AIV type H9** by PCR and Elisa with NP kit ;
- **IBV 100% homology with vaccine strain 1/96.**

In conclusion, the AIV H9 is responsible for this outbreak. The precocity associated with co-infections could explain this high mortality.

Poster 15

Influenza A virus protein PB1-F2: molecular signatures linked to viral pathogenesis in the context of low and highly pathogenic avian influenza

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Abstract

The highly pathogenic avian influenza (HPAI) of the H5N1 subtype is having devastating effects on the poultry industry. Millions of birds have been culled across the U.S., with economic consequences still to be determined. Most influenza subtypes contain an accessory protein known as PB1-F2, which is 86 to 90 amino acids (aa) long. PB1-F2 acts as a pro-apoptotic factor that plays a crucial role in influenza pathogenesis. Specific motifs (inflammatory motif; im and cytotoxic motifs; cm) within PB1-F2 have been identified. The amino acid change N66S has also been noted as a position that modulates influenza virulence. Interestingly, contrasting effects related to PB1-F2 have been observed in mammalian and avian species. In this study, we performed multiple aa alignments of PB1-F2 sequences derived from H1N1, HPAI H5N1, and LPAI H3N8. The results show that H1N1 viruses circulating in humans have a shorter version of PB1-F2 (11 aa), while H5N1 viruses possess a full 90 aa version. Avian-origin H3N8 viruses isolated from poultry have a 90 aa version of PB1-F2, whereas human isolates contain 35 aa. PB1-F2 sequences derived from H5N1 strains include S66, which may contribute to higher virulence. Moreover, the impact of prevalent residues at positions 70 (cm), 75 (im), and 82 (im) in the context of H5N1 strains remains to be explored. The results suggest that PB1-F2 is relevant to influenza pathogenesis in poultry. This underscores the need for further investigation into the roles of different PB1-F2 isoforms and molecular signatures in influenza pathogenesis concerning poultry species.

Poster 16

Molecular characterization of a clade 2.3.4.4b H5N1 high pathogenicity avian influenza virus from a 2022 outbreak in the Philippines

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Abstract

H5 subtype high pathogenicity avian influenza (HPAI) viruses continue to devastate the poultry industry and threaten food security and public health. The first outbreak of H5 HPAI in the Philippines was reported in 2017. Since then, H5 HPAI outbreaks have been reported in 2020, 2022, and 2023. Here, we report the first publicly available whole genome sequences of an H5N1 HPAI virus from a case in Central Luzon, Philippines. Samples were collected from a flock of layer

chickens exhibiting signs of lethargy, droopy wings, and ecchymotic hemorrhages in trachea with excessive mucus exudates. High mortality of about 95% was observed within the week. Days prior to the high mortality event, migratory birds were observed around the chicken farm. Pooled lung samples and oropharyngeal-tracheal swabs were taken from two chickens from this flock. These samples were positive in quantitative RT-PCR assays for influenza matrix and H5 hemagglutinin (HA) genes. The same samples were subjected to whole virus genome amplification and sequencing. Phylogenetic analysis of the HA genes revealed that the H5N1 HPAI virus from Central Luzon belongs to the Goose/Guangdong lineage clade 2.3.4.4b viruses. Other segments also have high sequence identity and the same genetic lineages as other clade 2.3.4.4b viruses from Asia. Collectively, these data indicate that wild migratory birds are a likely source of the 2021 H5N1 virus that caused outbreaks in the Philippines. Thus, biosecurity practices and surveillance for HPAI viruses in both domestic and wild birds should be increased to prevent and mitigate future HPAI outbreaks.

Poster 17

Monitoring H9N2 virus shedding from vaccinated birds post challenge: A key factor for vaccine production and successful control strategy

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Abstract

H9N2 is a challenging threat that poses hazards for the poultry industry and public health.

Despite being effective in controlling clinical signs, vaccines fail to prevent shedding resulting in leaky vaccines. These leaky vaccines allow the continual evolution of the field viruses that could yield more pathogenic viruses or at least antigenically drifted escape mutants. In addition, there are increased chances of zoonotic infections as a result of continuous shedding in apparently healthy infected flocks.

In this paper, we investigate whether the antigenic payload in inactivated vaccines play a role in shedding level post challenge. Also, we assessed the shedding following different vaccine platforms including the recombinant HVT and inactivated vaccines at 3, 5, 10 and 14 days post challenge.

The results showed a considerable shedding level in all vaccinated groups. Remarkable decrease in shedding was observed within the groups with higher antigenic payload. Different vaccine platforms can vary remarkably in the virus load in lung and trachea, where recombinant HVT vaccine showed lower virus loads from 5th day post challenge. However, the shedding assessed from recombinant HVT and inactivated vaccines showed insignificant differences in the shedding from challenged birds. In conclusion, several measures can be implemented by current vaccine manufacturers that can help in decreasing shedding of H9N2 virus. Thereby, limiting the evolution of field viruses and improving the control of H9N2 virus in the field.

Poster 18

Optimized competitive ELISA for the detection of H5 antibodies including new clades

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Abstract

Influenza viruses belong to the family Orthomyxoviridae. There are four types of influenza viruses: A, B, C and D; which are defined by the nature of their internal nucleocapsid antigen. Type A is the most conserved genus and can be further divided into subtypes based on their Hemagglutinin and Neuraminidase antigens. Some subtypes containing H5 or H7 are associated with highly pathogenic forms of the disease and high rate of morbidity and/or mortality. Since 2004, a new clade of H5 HPAI has been circulating worldwide in poultry flocks, leading to important losses. Recently, H5N1 infection in dairy cows has been identified in the United States, leading to different symptoms like milk production, reduced rumination or nasal discharge. Given the need for rapid and reliable serological tool, IDvet has developed a new H5 competitive ELISA able to detect anti-H5 antibodies including clades 2.2 and 2.3.4.4 in poultry and mammals. This document presents preliminary results obtained with this multi-species ELISA, ID Screen® Influenza H5 Antibody Competition 3.0 Multi-species (FLUACH5V3).

Poster 19

Replication kinetics of H9Nx and H4Nx Avian Influenza Viruses in Avian Cell Lines

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Abstract

Avian influenza virus (AIV) threatens wildlife, food security, and public health. The H9 and H4 AIV strains circulate in wild waterfowl but can potentially cause cross-species transmission. To better understand AIVs and host interaction, our primary goal was to assess the susceptibility and permissiveness of H9Nx and H4Nx AIV strains from different avian origins. AIVs from H9Nx isolated from turkey (H9TK), chicken (H9CK), wood duck (H9WD), and ruddy turnstone (H9RT) and H4Nx from blue-winged teal (H4BWT), turkey (H4TK), and mallard (H4ML) were inoculated in chicken (DF-1), quail (QM5), duck, and MDCK cell lineages at MOI of 1. Viral replication kinetics were performed, and titers were measured at 24, 48, 72, 96, and 120 hours post-inoculation (hpi) by Real-time RT-PCR and plaque assay. All cell lineages were susceptible to H9Nx and H4Nx infections. H4BWT showed the highest titers among strains and cell lineages, reaching peaks of 7.8 Log₁₀/ml in DF-1, 8.3 Log₁₀/ml in duck, 8.0 Log₁₀/ml in QM5, and 7.8 Log₁₀/ml in MDCK cells. In general, H4BWT and H9CK replication reached the highest titers in the DF1 cells, followed by H9TK in late hpi. In duck cells, H4BWT and H4ML had higher titers than H9 strains, which had similar patterns throughout the replication cycle. The QM5 cells were less permissive to the H9 AIVs replication, with low titers observed for H9CK and H9TK. Therefore, replication dynamics varied among cell lines and strains. Further tests are underway to better understand which factors can influence AIV subtypes and host interaction.

Poster 20

Serology for assessing vaccination quality against highly pathogenic avian influenza with a self-amplifying RNA vaccine in French mule ducks

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Abstract

Highly pathogenic avian influenza (HPAI) has become a global concern in poultry production and even beyond. France was the only European country to include the mandatory duck vaccination as a complementary tool for controlling HPAI.

The RNA technology represents a breakthrough in animal health. Beyond efficacy and viral shedding reduction expectation, this fully synthetic vaccine aligns perfectly with the differentiating infected from vaccinated animals' strategy (DIVA). To provide guidelines of such a vaccine to the users in the field, it is essential to establish serological response standards. Enzoootic disease control through vaccination can only be ensured if the vaccination quality is optimum. Ten commercial mule ducks flocks (totaling 60,000 ducks) were included in a field serological monitoring study. Ducklings were vaccinated with a self-amplifying RNA vaccine (Respons® AI H5, Ceva Animal Health) at day 1 and between day 21-28 by intra-muscular route.

Blood was collected from 20 random ducks every two weeks; sera were tested using a commercial indirect H5 Elisa test kit. Post-vaccination serology tests consistently showed a strong and quick antibody response detection 1 to 2 weeks after the booster injection. After this peak, a slow and steady decrease in antibody detection was observed until the end of the ducks' lifespan.

Thanks to this field investigation, an optimal blood sampling window has been set to assess the whole vaccination program quality. This will provide standards for the control and evaluation of vaccination uptake from now on in mule duck flocks.

Poster: Avian Metapneumovirus

Poster 1

Administering an overdose or repeatedly giving a single dose of a Live Attenuated Subtype B aMPV Vaccine to one-day-old chickens is safe

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Abstract

Overdose testing is required for live vaccines, as they may retain residual pathogenicity in some cases. However, there is limited data on the safety of administering an overdose or additional doses of avian metapneumovirus (aMPV) live vaccines. The purpose of this study was, therefore, to evaluate the safety of administering an overdose and the repeated administration of a single dose of the commercial live aMPV vaccine in chickens at the minimum age recommended for vaccination (1 day old) via the oculonasal route (spray). A total of 10 SPF chickens were vaccinated with an overdose (10X, $10^{6.4}$ CCID₅₀/dose) at 1 day of age and revaccinated with the same route and method fourteen days later with one dose (1X at a maximum titre, $10^{5.4}$ CCID₅₀/dose). General and local clinical signs, as well as mortality, were monitored daily for 28 days. At the end of the study, the animals were euthanized to determine the presence/absence of macroscopic lesions related to vaccination, with particular focus on the upper respiratory tract. No relevant safety concerns were observed following the administration of the vaccine. None of the animals exhibited abnormal local or systemic reactions, nor signs of illness attributable to the vaccine. Additionally, no animals died from vaccine-related causes, and no lesions were observed in any animal during necropsy. Thus, administering a 10X overdose of RESPIVAC® aMPV via oculonasal route (spray) is safe in chickens. Similarly, repeated dosing of the vaccine was also proved to be safe.

Poster 2

Development and Validation of a Universal Screening Real-Time PCR Assay for the Diagnosis of A, B, and C Subtypes of Avian Metapneumovirus.

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Abstract

Avian metapneumovirus (aMPV) is a highly contagious virus that causes acute upper respiratory disease, characterized by sinusitis and swollen heads in turkeys and chickens. aMPV is classified into four subtypes (A–D) based on nucleotide sequence of G protein. Subtype C was first reported in the US in the late 1990 and quickly became endemic in turkey flocks in the upper Midwest. aMPV subtypes A and B had not been reported in the US until the recent emergence of

aMPV-A in California and aMPV-B in North Carolina in turkeys and broilers in late 2023. These subtypes have since spread rapidly to commercial poultry in 29 states. The high sequence variability among aMPV subtypes hindered the development of a universal qPCR, requiring three separate subtype-specific assays per sample. This approach is labor-intensive, costly, and time-consuming for both clients and diagnostic laboratories. In the present study, our team was able to find a conserved region within the M gene that served as a target for a universal screening qPCR for aMPV subtypes A, B & C. Comprehensive *in silico* and wet lab validation of the newly developed assay demonstrated high specificity and sensitivity for the identification of the three subtypes compared to the available subtype specific assays from known positive clinical samples. In conclusion, the newly developed assay represents a more streamlined diagnostic tool for sensitive and efficient diagnosis of aMPV subtypes that have emerged in the U.S.

Poster 3

Development of a new model for testing aMPV vaccines in chickens using tracheal ciliary activity after an experimental challenge

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Abstract

Avian metapneumovirus (aMPV) infects both turkeys and chickens, primarily replicating in the upper respiratory tract and causing respiratory disease. Despite the high morbidity and often mortality associated with aMPV in the field, its pathogenicity has been difficult to assess in the laboratory. Therefore, the aim of this study was to develop a simple and reproducible aMPV model in chickens for testing vaccines. It was hypothesized that evaluating the ciliary activity of tracheal explants would be suitable for demonstrating the efficacy of aMPV vaccines after an experimental challenge. Fifty chickens of commercial origin were divided into two groups: one group was vaccinated with an attenuated aMPV subtype B strain 1062 isolated from chickens at one day of age by spray, while the other group received PBS. Animals were challenged at 17 days of age with a virulent aMPV subtype B strain isolated from chickens, administered via eye-drop ($10^{5.8}$ CCID₅₀/ml). Ciliary activity and clinical signs were evaluated. For ciliary activity assessment, 8 animals per group were euthanized on day 10 post-challenge. Tracheas were extracted and sectioned transversely. A chicken was considered affected if more than 1 ring presented ciliostasis. The Mann-Whitney test and Fisher's exact test were used for statistical analysis in R software v4.4.0. Both the proportion of affected animals and the clinical signs were significantly higher in the control animals compared to the vaccinated group ($p < 0.05$). Thus, the new methodology (ciliary activity evaluation) was found to be as suitable as clinical signs for assessing the efficacy of vaccines against aMPV.

Poster 4

Evaluating Live AMPV Vaccine Strategies in Turkeys

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Abstract

Avian metapneumovirus (aMPV) is a globally significant respiratory pathogen in poultry, causing acute, highly contagious upper respiratory tract infections in turkeys (turkey rhinotracheitis) and other avian species, including chickens. Despite the development of local and systemic immunity in infected birds, maternal-derived antibodies offer limited protection. In intensive poultry operations, aMPV spreads rapidly, with wild birds acting as potential reservoirs and vectors. Clinical manifestations in turkeys include nonspecific symptoms, acute upper respiratory inflammation, reduced egg production, and poor eggshell quality, resulting in substantial economic losses for the industry.

Recently, a modified live vaccine for aMPV received approval for use in the United States, presenting a promising avenue for disease management. However, initial supply shortages and a lack of regional application history necessitate an exploration of effective vaccination strategies. This study evaluates three vaccination approaches: full-dose vaccination per label instructions, half-dose vaccination, and partial flock vaccination at placement. Immunity development will be assessed through environmental sampling and serial blood tests conducted at 3, 4, 5, 6, and 7 weeks of age. Additionally, the feasibility and impact of booster vaccinations will be investigated, contingent on vaccine availability.

The findings from this study will provide critical insights into optimizing vaccination protocols for aMPV under conditions of limited supply, supporting effective disease control and mitigating economic losses in the poultry industry.

Poster 5

Evaluation of Safety Profile of a Live Attenuated Subtype B aMPV Vaccine Strain

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Abstract

Safety of live vaccines is crucial for maintaining the health and productivity of poultry flocks. While live attenuated vaccines effectively control diseases, there is concern regarding the potential for vaccine strains to revert to virulence, posing risks to poultry populations. In the case of live avian metapneumovirus (aMPV) vaccines, some outbreaks have been linked to vaccine-derived viruses. This study was conducted to evaluate the potential for the subtype B aMPV live attenuated vaccine strain 1062, to revert to or increase in virulence during a controlled five-passage reversion-to-virulence test in chickens. For this purpose, 1-day-old SPF chickens were used (5 animals for the first 4 passages and 10 animals for the final passage). In the first passage, the animals were inoculated with the vaccine strain via the oculonasal route ($10^{5.7}$ CCID₅₀/animal). For subsequent passages, a pool of samples collected 5 days post-inoculation from oropharyngeal swabs was used to inoculate the chickens of the next group with 0.1 ml of the pooled virus samples. The pooled samples recovered from each group were also analyzed by an immunoperoxidase monolayer assay (IPMA) to

verify the presence of the virus. After the second passage, the vaccine strain was no longer recovered. Hence, the second passage was repeated in 10 animals, but the material recovered from this repeated passage did not contain the virus either. These results indicate that the aMPV vaccine strain does not have the potential to increase in virulence or revert to a more pathogenic form.

Poster 6

Evaluation of the dissemination of a live attenuated avian metapneumovirus vaccine strain in vaccinated SPF chickens under experimental conditions

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Abstract

Replication of avian metapneumovirus (aMPV) during natural infection appears to be limited to the upper respiratory tract. However, under experimental conditions, it has also been detected in other tissues, such as the reproductive tract. Therefore, this study aimed to investigate the dissemination of the aMPV 1062 strain in vaccinated animals. Thirty-six SPF chickens of one-day-old were vaccinated with the subtype B aMPV vaccine strain 1062 contained in RESPIVAC[®] aMPV vaccine ($10^{5.7}$ CCID₅₀ per animal). These animals were necropsied at different time points (3-, 5-, 7-, 10-, 14- and 21-days post-vaccination) to determine the presence of the virus in different tissues (nasal turbinates, periorbital sinus, trachea, lungs, harderian gland and oviduct) and secretions (oropharyngeal and cloacal swabs). Virus detection was performed with qRT-PCR. In summary, the vaccine virus disseminated to respiratory tissues and secretions, primarily affecting oropharyngeal secretions, nasal turbinates, periorbital sinus, and, to a lesser extent, the trachea at very low levels. The peak viral replication occurred on day 5 in oropharyngeal swabs, day 3 in nasal turbinates, and day 7 in the periorbital sinus. Nevertheless, the vaccine strain was almost eliminated from the respiratory system by day 21 post-vaccination. The vaccine virus was not detected in the oviduct at any time point. The results on the cloacal swabs were also negative at all studied time points. Hence, the dissemination of the vaccine strain is restricted to the respiratory tract following oculonasal vaccination in one-day-old chickens.

Poster 7

Safety and efficacy study of inactivated vaccine against newly emerged avian metapneumovirus subgroup B

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Abstract

Avian metapneumovirus is one of the major poultry pathogens, causing considerable economic losses in the poultry industry worldwide, particularly in turkeys and chickens. Avian metapneumovirus subgroup B (aMPV/B) has been

recently introduced in US poultry farms and jumped to the forefront as a major concern affecting the poultry industry in 2024. Fundamentally, vaccination is the best method to control aMPV, mitigating the widespread infection and producers' suffering. Herein, we describe the development of an inactivated aMPV/B vaccine using the Chicken/NC/USA/ADR DL-6 virus isolated from a chicken farm experiencing respiratory disease. This isolate showed >99% identity based on whole genome sequence, G, F, and SH proteins, with the North American aMPV/Bs circulating in the American turkey and chicken farms. The virus suspension was inactivated and tested for sterility, purity, and safety. The vaccine was produced by mixing the inactivated virus suspension with Montanide ISA™51 to improve the efficacy of the vaccine. Two immunization doses were conducted within two weeks and the serum samples were collected every week for five successive weeks after the priming dose for ELISA and serum neutralization. Two weeks after the booster dose, the chicks were challenged by aMPV/B to detect the efficacy of the developed vaccine to protect the birds against the infection. The data analysis is in process. The outcome of this study should provide a better vaccine to protect chickens against aMPV B to reduce production losses.

Poster 8

Safety of a Live Attenuated Subtype B aMPV Vaccine in hens during lay

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Abstract

In laying hens, the avian metapneumovirus (aMPV) mostly causes upper respiratory tract infections but can also impact the reproductive system. Aim of this study was to assess the safety of RESPIVAC® aMPV vaccine administered in hens during lay ($10^{5.4}$ CCID₅₀/ml, maximum dose). For that purpose, fifteen SPF hens were vaccinated at the peak of lay (26 weeks of age) and monitored daily for clinical signs, mortality, and egg production and quality 4 weeks before and after vaccination. Eggs were collected on days 4, 7, 14, 21, and 28 post vaccination for albumen and yolk quality evaluation, as well as vaccine virus detection. Necropsies were performed at the end of the study to assess macroscopic lesions, with particular focus on the reproductive tract. Statistical analysis was performed using Mann-Whitney or T-tests in R software v4.4.0. No general clinical signs, local reactions, or mortality related to the vaccine administration were observed throughout the study period. No effects were observed on the percentage of normal or abnormal eggs laid. In this sense, egg production remained consistent before and after vaccination (81% vs. 84%, $p = 0.350$), as well as egg quality (2% of abnormal eggs before and after, $p = 0.933$). No significant alterations in the albumen and yolk quality were detected, and the vaccine virus was not detected in egg-content in any sample at any time-point. No lesions attributable to the vaccine were found during necropsies. The results demonstrate the safety of the administration of the maximum dose of the vaccine during lay.

Poster: Bacteriology

Poster 23

Antimicrobial Resistance profiles of emergent *Enterococcus cecorum* causing systemic disease in chickens

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Abstract

Enterococcus cecorum (EC) has been associated with vertebral osteoarthritis in chickens aged five weeks and older and recently to severe systemic disease with high mortality in chickens as young as two weeks. Whole Genome Sequencing (WGS) was performed to characterize antimicrobial resistance (AMR) in 40 EC isolates from breeder and broiler chickens. WGS was conducted using MiSeq and phylogenetic analysis was performed using RAxML SNP trees, utilizing the classic EC-SA3 (causing spondylitis in chickens), non-pathogenic CE1 strain as references, and *E. faecalis* as a control. AMR prediction was documented for various classes of antibiotics. EC isolates clustered in distinct phylogenetic clades that separated commensal from pathogenic isolates. 68% EC isolates causing systemic disease grouped in one single large phylogenetic clade, which is characterized by resistance to four or five classes of antibiotics, including virginiamycin which is shared with the isolates in the EC-SA3 clade, and to streptomycin and tiamulin. Resistance to tetracyclines was predicted only in 32% of the isolates. Resistance to erythromycin, neomycin, clindamycin or gentamicin was not detected in this clade. 7.5% EC isolates carry resistance to ionophores, commonly used for coccidian control and were genetically closely to the non-pathogenic CE1 strain. The cadDX operon, associated with cadmium resistance, oxidative stress resistance, and virulence, was detected in the isolates from the non-pathogenic clade and the large clade identified in this study. In conclusion, these results indicate the emergence of EC isolates capable of causing systemic disease in younger chickens and sharing specific AMR profiles suggesting a niche adaptation.

Poster 24

Characterization and antimicrobial susceptibility of *Gallibacterium anatis* isolates from Mexico

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Abstract

Gallibacterium anatis is a Gram-negative bacterium of the *Pasteurellaceae* family that colonizes the upper respiratory tract and lower reproductive tract of healthy chickens. However, it has also been associated with respiratory disease, salpingitis, peritonitis and septicemia in chickens. In this study, we report the phenotypic characterization by carbohydrate fermentation tests and hemagglutinating activity against four types of erythrocytes (chicken, rabbit, quail

and pig) and the molecular characterization by ERIC-PCR. In addition, the antimicrobial susceptibility was evaluated by disk diffusion method against 9 antimicrobials corresponding to 5 antimicrobial groups. A total of 33 *G. anatis* isolates obtained from commercial birds in Mexico from different organs with and without lesions. All isolates were biovar haemolytica and biotyped in four biovars. The typing of the isolates by ERIC-PCR showed 15 distinct patterns. Of the total isolates evaluated, 23 of them did not show hemagglutinating activity, while the other isolates showed hemagglutinating activity at least to one type of erythrocyte. The isolates showed resistance to antimicrobials from the group of lincosamides, macrolides, sulfonamides and tetracyclines. In addition, all isolates showed resistance to at least one antimicrobial from 3 or more antimicrobial groups. In conclusion, *G. anatis* isolates from Mexico showed phenotypic and genetic variability and were resistant to different antimicrobials. Furthermore, the tools used allowed the typing of *G. anatis* isolates obtained from Mexico.

Poster 25

Comparison of antibody titers and egg production of layers vaccinated with oil emulsion or aluminum hydroxide-based adjuvant 3-way *Salmonella* vaccines

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Abstract

The adjuvant in a *Salmonella* bacterin can directly influence the immune response of birds. Different adjuvants will induce different levels of circulating antibodies. The goal of this study was to evaluate the serological response induced by 2 bacterins against *Salmonella*, containing different adjuvant technologies and the possible impact on egg production. Six hundred 1-day-old Lohman Brown layer chicks were housed in a completely randomized design, divided into 2 treatments: T1 – an oil emulsion bacterin containing strains of *S. Enteritidis*, *S. Typhimurium* and *S. Infantis* (0.3 ml/bird – Salmin Plus®); T2 – an inactivated vaccine containing strains of *S. Enteritidis*, *S. Typhimurium* and *S. Infantis* (0.5 ml/bird) in an aluminum hydroxide-based adjuvant. All groups were vaccinated at 10 and 14 weeks of age, by intramuscular injection in the pectoral muscle. Twenty blood samples of each treatment were collected for *Salmonella* Enteritidis (O:9) ELISA at 10; 14; 17; 27; 33; 40; 48; 56 and 65 weeks. The percentage of egg production was evaluated between 19 and 68 weeks. Antibody titers and performance data were submitted to Kruskal-Wallis test by Jamovi® software. The antibody titers of the two groups were statistically similar at 10 weeks ($P=0.932$). At the ages of 14; 17; 27; 40; 48; 56 and 65 weeks, birds from T1 demonstrated higher circulating antibody titers than T2 birds ($P<0.05$). No statistical difference was found on egg production. Birds vaccinated with an oil emulsion vaccine had higher levels of serum antibodies up to 65 weeks without any negative effect on egg production

Poster 26

Comprehensive characterization of *Castellaniella ginsengisoli* clinical isolates– an emerging pathogen in chickens?

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Abstract

Since 2018, uncommon bacterial infections have been identified in broiler breeders at the Poultry Diagnostic and Research Center (PDRC), University of Georgia. Birds from 22 cases exhibited increased mortality, lameness, and swollen wattles, resembling fowl cholera. However, 16S rRNA gene sequencing revealed 99% homology with *Castellaniella* spp., suggesting a different causative agent. Historically regarded as non-pathogenic environmental bacteria, *Castellaniella* lacked characterization as animal pathogens. This study addresses this research gap by providing the first comprehensive genomic and phenotypic characterization of chicken-origin *Castellaniella* isolates. All of the isolates exhibited genome sizes of approximately 2.9 million base pairs and were phylogenetically closest to *C.ginsengisoli*. Antimicrobial susceptibility testing revealed low minimum inhibitory concentrations (MICs) for tetracycline, oxytetracycline, enrofloxacin, neomycin, and gentamicin, suggesting these as potential treatment options. Conversely, high MICs were observed for β -lactams and macrolides, indicating that they should be avoided for clinical use. Elevated MICs for sulfonamides and aminoglycosides were linked to the detection of the *sul2* and *aph* antimicrobial resistance (AMR) genes, respectively. Despite high MICs for β -lactams, no acquired resistance genes or resistance-associated mutations were found, suggesting an intrinsic resistance mechanism may exist. Virulence factor analysis revealed that *C.ginsengisoli* possesses genes involved in several pathogenic mechanisms, including secretion systems, fimbriae, flagella, biofilm, and capsule formation. Our comprehensive study provides a vital foundation for advancing diagnostics, guiding treatments, and driving future *Castellaniella* research.

Poster 28

Gut antimicrobial mechanism of an avian host-specific bacteria

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Abstract

Chickens are a greatly important food producing animals, thus the robustness of their immune systems is a key factor many producers are interested in bolstering. Our lab has previously demonstrated that inoculating day-old birds with a host-specific Segmented Filamentous Bacteria (SFB), leads to gut immune modulation, which decreases *Enterobacteriaceae* shed in feces. This study seeks to investigate the link between the SFB-related antimicrobial activity with mechanistic target of rapamycin (mTOR) pathway..

Specific-pathogen-free layers were inoculated with SFB at day-of-hatch in isolated control and inoculated rooms respectively. At 2 weeks post inoculation (14-DPI), ileal explant tissues were harvested, cleaned, and incubated with penicillin, streptomycin, gentamycin, and amphotericin B for 2 hours prior to treatment with mTOR activator, inhibitor,

or carrier respectively for an additional 2, 4, and 6 hours. Following treatment, supernatants and explants from the assay were snap frozen for antimicrobial assays/HPLC and RT qPCR respectively.

Significant decrease in *IL-10* expression was observed in the explants of SFB-inoculated birds treated with mTOR inhibitor relative to non-inoculated birds treated with mTOR inhibitor. *IL-17* expression was significantly higher in mTOR-inhibited SFB-inoculated birds and trending lower in mTOR-inhibited non-inoculated birds. No significant difference in *IL-6* expression was observed across all comparisons.

Decrease in *IL-10* expression in the inhibited group but not the control group indicates some degree of crossover between SFB-inoculation and mTOR. Elucidating the pathway mechanics by which SFB and mTOR affect the immune response of the ilea is vital to future understanding and development of immune-bolstering treatments for chickens.

Poster 29

Persistence of multi-drug resistant *Escherichia coli* causing lameness on a broiler chicken farm

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Abstract

Multi-drug resistant (MDR) *E. coli* was isolated from three successive flocks on a broiler farm in Great Britain. Carcasses were submitted to Animal and Plant Health Agency (APHA) for investigation of lameness, which began at 17-days-old. By depopulation at 33 or 38-days-old, culls due to lameness reached 17% in the first flock and 6% in the following flock. There was no response to amoxicillin. Response to doxycycline was slow but considered effective. Post-mortem findings included femoral head necrosis and septic arthritis. Histopathology detected vertebral osteomyelitis. Bacteriology isolated *E. coli* from various tissues. Whole genome sequencing (WGS) determined that *E. coli* isolates exhibiting MDR shared the same MLST type (ST-1564) and flagella antigen (H21). This genomic profile was similar to a strain of avian pathogenic *E. coli* previously identified by APHA, which was associated with high mortality and possessed an array of virulence and antimicrobial resistance (AMR) genes. Prior to placing the third flock, changes were made to cleaning and disinfection protocols during turnaround. Chicks were vaccinated with a commercial *E. coli* vaccine at placement. Cull rates for lameness in this crop were much lower (0.7% at 38-days-old). *E. coli* was isolated from tissues in vaccinated culled birds at 21-days-old, but WGS identified different strains, predominantly ST-117 and serotype O11:H4. These isolates carried fewer AMR genes and only one exhibited MDR. Overall, this case highlights a new clinical presentation for an emerging strain of virulent *E. coli* with MDR and demonstrates potential for management interventions to reduce its prevalence between flocks.

Poster 30

Phenotypic and genotypic characterization of Avian Pathogenic *Escherichia coli* (APEC) isolated from chicks and embryos in the hatchery.

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Abstract

The zootechnical performance of broilers depends on proper embryonic development. Avian Pathogenic *Escherichia coli* (APEC) is associated with omphalitis due to contamination of the embryo and can cause serious problems in birds, especially in the first few days of life. The aim of this study was to determine the occurrence of APEC in the yolk sac of embryos, pecked eggs and 1-day-old chicks. A total of 254 samples were collected (113 from yolk sac of 1-day-old chicks, 85 pecked eggs and 56 embryonated eggs), and 60.63% (n=154/254) were positive for *E. coli*. The isolates were subjected to PCR to identify the APEC minimum virulence genes. A total of 35.71% (n=55/154) strains were classified as APEC. An increase in the frequency of APEC was observed with the progresses up to hatching: 3.89% (n=6/154) of yolk sac of embryos were positive for APEC, 10.38% (n=16/154) of pecked eggs and 21.43% (n=33/154) of 1-day-old chicks. Regarding phylogroup evaluation, the most common was B2, with 36.36% (n=20/55), predominating in the final stages of embryonic development and after hatching. Predictive identification of the clonal complex ST 131, ST117 and ST95 was also carried out, with ST131 being the most prevalent (23.63%; n=13/55), followed by ST117 (14.5%; n=8/55) and ST95 (3.6%; n=2/55). Phenotypic analysis of antimicrobial resistance showed a total of 25.45% multidrug-resistant strains, with the highest resistance rate to amoxicillin (43.64%). In conclusion, APEC is one of the more important pathogens during the incubation period, highlighting the MDR, B2-ST131/ST95 and G-ST117 high risk lineages.

Poster 31

Salmonella spp. Characterization by using Next Generation Sequencing from a Breeder Farm

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Abstract

In this study, *Salmonella* spp. was first isolated from cloaca swabs in a remote breeder farm adjacent to cattle rearing areas. Since immediate characterization and serotyping was not possible, the cultures from the agar were scraped on an FTA card and sent to Genereach Biotechnology Corp. for whole genome sequencing. Genomic DNA was extracted by using the taco DNA/RNA Extraction Kit and next generation sequencing (NGS) libraries were constructed with the Illumina DNA Prep Kit followed by sequencing on an Illumina NovaSeq X Plus platform to generate paired-end reads. Raw sequencing data were processed by using a customized bioinformatics pipeline. Initial quality control and adapter trimming were performed by using fastp v0.23.4. High-quality reads were assembled into scaffolds with SPAdes v3.15.5 and minimap2. For serotyping analysis, SeqSero2 v1.3.1 was used to classify samples based on the predicted O and H antigen gene sequences. All the isolates were found to belong to *Salmonella enterica* subsp. *enterica* serovar Kentucky and sequence type ST198 (cc56) based on the whole genome sequencing data. The isolates are also found to harbor various antimicrobial resistance genes which confer resistance to aminoglycosides (tobramycin, amikacin), beta-lactam

(amoxicillin and ampicillin), folate pathway antagonist (piperacillin, ticarcillin and cephalothin) and tetracycline (tetracycline and doxycycline). Moreover, the *Salmonella* Pathogenicity Islands (SP-1 to SP-5 and SP-9) were also found in all the isolates. This study highlights the need for continuous monitoring and implementation of proper biosecurity management programs in poultry farms to prevent the dissemination of multidrug-resistant *S. Kentucky*.

Poster 32

The Impact of a 3-strain *Bacillus* Probiotic on Broilers in a Naturally Occurring *Enterococcus* Challenge Model

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Abstract

Enterococcus cecorum (EC), an intestinal bacterium, can cause lameness, mortality, and economic losses in poultry. *Bacillus*-based solutions may support gut health and potentially reduce EC-related burdens. We aimed to evaluate the efficacy of Microsaf®, a 3-strain bacterial probiotic (*Bacillus amyloliquefaciens*, *Bacillus licheniformis*, and *Bacillus pumilus*), in mitigating the effects of naturally occurring *Enterococcus* infections. A natural challenge model was used at an experimental farm with a high *Enterococcus* burden. A total of 960 one-day-old Cobb 500 chicks were raised on used litter and divided into two groups (Control and Test) with 12 pens/group. The Control group received a standard commercial diet, while the Test group received the same diet supplemented with the *Bacillus* probiotic at an inclusion rate of Log 5 CFU/g of feed. Birds were weighed on days 1, 14, 28, and 42 for evaluation of feed conversion ratio (FCR) and body weight gain (BWG). *Enterococcus*-associated culls and mortalities were necropsied, and thoracic vertebrae swabs were analyzed for bacterial counts using the Most Probable Number (MPN) method. Data were evaluated via two-way ANOVA, with means separated by Duncan's MRT ($p < 0.05$). The test group showed significant improvements on performance, including reduced mortality (5.42% vs. 8.96%, $p=0.033$), better FCR (1.691 vs. 1.735, $p<0.001$), higher final BWG (2.72 kg vs. 2.27 kg, $p=0.001$), and fewer positive samples for EC (45% reduction, $p=0.02$). The probiotic reduced *Enterococcus*-associated lesions, improved performance and survivability in broilers under a natural challenge, demonstrating its beneficial effect as a practical solution for commercial poultry production.

Poster 33

Typing and antimicrobial susceptibility of *Pasteurella multocida* isolates associated with fowl cholera obtained from Mexico.

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Abstract

Pasteurella multocida is the etiological agent of avian cholera, a septicemic disease that leads to production losses. In this study, we identified 15 isolates of *P. multocida* obtained from commercial poultry farms in Mexico using specific PCR and MALDI-TOF MS. Additionally, the typing of the isolates was performed by phenotypic tests, capsular typing by PCR, ERIC-PCR and phylogenetic analysis based on the 16S rRNA gene. The antimicrobial susceptibility to 9 antimicrobials was evaluated using the disc diffusion method. By PCR, the isolates were positive for *P. multocida* and belonged to the capsular serotype A. All isolates were identified by MALDI-TOF MS. By phenotypic tests, all isolates belonged to subspecies *multocida* and biovars 3 and 13. Two ERIC-genotypes were identified (13 isolates were ERIC-genotype I and 2 isolates were ERIC-genotype II). Phylogenetic analysis based on the 16S rRNA gene classified the isolates into 2 previously reported genetic groups. In terms of susceptibility to antimicrobials, 100% of the isolates were susceptible to amoxicillin/clavulanic acid, tilmicosin and tetracycline; as well as resistant to erythromycin. In addition, all isolates were resistant to at least one antimicrobial from 3 or more groups of antimicrobials. These results confirm the presence of *P. multocida* isolates obtained from poultry from farms in Mexico, showing little variability by both phenotypic and molecular tests. The differences in susceptibility to antimicrobials show the importance of performing this evaluation in diseases associated with *P. multocida* in Mexico.

Poster 34

Typing based on HMTp210 and antimicrobial susceptibility of non-typeable NAD-independent isolates of *Avibacterium paragallinarum* obtained from Mexico

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Abstract

Avibacterium paragallinarum is a Gram-negative bacterium belonging to the *Pasteurellaceae* family and is the etiologic agent of the disease called infectious coryza. In Mexico, the presence of isolates belonging to serovars A-1, A-2, B-1, C-1 and C-2 has been identified by hemagglutination inhibition (HI). In addition, the presence of NAD-independent isolates has been reported. However, in some cases, the presence of isolates that cannot be serotyped by HI has been

reported. Recently, a molecular typing technique based on region 1 and 2 (HVR) of the HMTp210 gene was proposed, which has been useful for identification the serovar of isolates. In this study, the presence of 4 NAD-independent isolates of *A. paragallinarum* obtained from cases of infectious coryza outbreaks in laying hens from Puebla, Mexico in 2022 and 2023 is reported. The isolates were identified by *A. paragallinarum*-specific PCR (HPG-2) and could not be typed by the HI test. The HMTp210 gene was sequenced and analyzed, as well as the determination of antimicrobial susceptibility to 15 antimicrobials. The analysis of region 1 and concatenated regions 1 and 2 of the HMTp210 gene allowed the identification of three of the isolates as serogroup A, serovar A-1 and one of the isolates as serogroup C serovar C-1. 100% of the isolates were resistant to doxycycline, enrofloxacin, erythromycin, oxytetracycline and tetracycline. Analysis of the HMTp210 gene allowed the typing of independent non-typeable NAD isolates from Mexico, and evaluation of antimicrobial susceptibility could be useful for the treatment of infectious coryza in Mexico.

Poster 35

Use of in-feed MiXscience Products for control of *Enterococcus cecorum* in Broiler Chickens

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Abstract

Enterococcus species can cause septicemia and residual lameness observed in broiler flocks. MiXscience DFMs and fatty acid esters-based solutions have antibacterial activities *in vitro* against *E. cecorum*. This study evaluated these solutions in an *E. cecorum* challenge model in broiler chickens. The 42-day trial was composed of a challenged control and three groups supplemented with a DFM (Mixguard B, MB), Glyceromonolaurate (GML) or a blend of fatty acid esters (Lumigard Most, LM). Each treatment had six replicate floor pens of 25 male Ross broilers. On day 3, all chicks were challenged with 2.0×10^7 CFU/chick of *E. cecorum* strain SA3 by oral gavage. On day 42, spleens and FTV swabs were collected from 100 birds per treatment. *Enterococcus* prevalence data were subjected to a GEE and means were separated using Bonferroni procedure (P -value = 0.05). Birds and feed were weighed on 0, 14, 36, and 42 days. Broiler performance data were analyzed using ANOVA and means were separated using LSD procedure.

There were no significant differences in the *Enterococcus* prevalence outcomes. At 42 days, the spleen samples were 54% positive in the challenged control with MB, LM, and GML products at 46%, 48% and 48%, respectively. FTV samples were 11% positive in the challenged control. MB had significantly lower overall mortality (1.33%) than the control (6.0%). The numerically lower septicemia combined with performance and mortality improvements suggest MiXscience products supported broilers during a strong pathogenic *E. cecorum* challenge.

Poster: Case Reports

Poster 36

Co-Infection of Avian Reovirus, Marek's Disease, and Mycoplasma synoviae in a Gamefowl Flock in Ibaan, Batangas, Philippines

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Abstract

Avian reovirus (ARV) affects nearly all domestic poultry species. ARV is commonly associated with severe viral arthritis and it can lead to immunosuppression. This predisposes the birds to viral and bacterial infections such as Marek's disease (MD), and Mycoplasma synoviae (MS). The gamefowl farm reported that approximately 25% of the birds started to exhibit swollen shanks, exudation on the keel bone, and nasal discharge. Blood and organ samples were collected from ten (10) morbid birds for serology, histopathology and PCR/RT-PCR to confirm the diagnosis. Necropsy findings showed lesions in the tarsometatarsal, keel, and tracheal regions. Serum samples tested positive for ARV and MS using ELISA. Initial PCR testing revealed negative results for ARV and MD. However, RT-PCR confirmed the presence of ARV and MD DNA/RNA in the tissue samples. Follow-up necropsy and PCR was also conducted post-vaccination. In the follow-up, the samples tested positive for MD which was sent for sequencing. To the best knowledge of the authors, this is the first documented case of co-infection of ARV, MD, and MS in free-range chickens in the Philippines.

Poster 37

Investigation of Post-Water Vaccination Reactions in a Broiler Complex: A Case Report

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Abstract

In this case report, an on-farm investigation occurred in a broiler complex after reports of numerous farms having increased respiratory symptoms and mortality following water vaccination with a live chicken embryo origin laryngotracheitis vaccine. Two farms total were visited. At the first farm a water vaccination audit was performed, and oropharyngeal swabs collected for diagnostics. At the second farm, post vaccinated birds were observed and necropsies performed on reported reactions. A rule-out causation list was compiled after visiting the farms and obtaining more information on the complex's current vaccine protocol and vaccination process. The rule-out list functioned to help investigate and explain the complex's excessive respiratory symptoms. It identified birds were not adequately clearing their day-of-age hatchery respiratory vaccination before receiving their next field respiratory vaccination in the water. Thus, making it impossible for them to process and clear this vaccination properly. This, paired with excessive water starvation, challenges in vaccine application, and improper amount of water and vaccine used by vaccination crews,

caused the undesirable respiratory reactions seen post-field vaccination. A case report on discovering and assessing misapplication of water vaccination in the field. This case report delivers the message that vaccine application and assessment is crucial and key for proper vaccination and protection from disease.

Poster 38

Meningitis associated with *Salmonella* Typhimurium in quail : a case report

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Abstract

On August 2024, a flock of 78000 seven-days-old quails experienced high mortality. Total mortality since the introduction of quails was 5.9%, after a hatchery mortality of 13.3% in the hatchers. Affected birds showed neurological signs including torticollis and opisthotonos. No macroscopic lesion consistent with neurological disorders were described in the post-mortem examination. Bacterial culture, without enrichment, of the brain, isolated *Salmonella* Typhimurium and *Escherichia coli*. In addition, *E. coli* was isolated from liver and heart. At histopathological examination, brain section exhibited severe heterophilic meningitis consistent with a bacterial infection. It is, as to the author's knowledge, the first report of neurologic disorders associated with a bacterial meningitis caused by a co-infection with *Salmonella* Typhimurium and *Escherichia coli* in quails. The fact that *Salmonella* grew in direct culture alongside *E. coli*, an easy-growing bacteria which tends to mask other pathogens present, shows that the *Salmonella* brain infection must have been massive. *Salmonella* Typhimurium is indeed able to cross blood-brain barrier and can cause neurologic disorders notably on pigeons. Moreover, a Xba1 PFGE (pulsed-field gel electrophoresis) was performed to compare this *Salmonella* strain with a *Salmonella* Typhimurium previously isolated, a few months earlier, in another barn on this farm (pheasant production). The pulsotype was different, suggesting two different introductions of salmonella in each production units, rather than a transmission between pheasants and quails. The pulsotype-based comparison of strains was essential here for the epidemiological investigation, in order to explore the source of contamination.

Poster 39

Salmonella arizonae septicemia in chukar chicks

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Abstract

This case report describes an outbreak of *Salmonella arizonae* causing septicemia in a flock of 7-day-old chukar chicks in California. The birds were presented to due an elevated first week mortality. The birds had been treated with penicillin and oregano however these were ineffective in controlling the disease. Postmortem examination revealed primarily

enlarged yellow livers and dark enlarged spleen as well as some congestion of the lungs, consolidated yolk material in yolk sacs, and dark mottled kidneys. Bacterial culture revealed the presence of *Salmonella enterica* spp *Arizonae* from liver, spleen, and yolk sac tissues. Despite *Salmonella arizonae* is rarely isolated but has been shown to be a primary pathogen in poultry and this case emphasizes that the bacterium can still be a major pathogen in young birds. This case also highlights the need for good control of pathogens in breeder flocks as well as thorough cleaning and disinfection in the hatchery.

Poster 40

Three outbreaks of suspected vaccine-related avian encephalomyelitis in broiler chickens in Great Britain

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Abstract

In 2024, three outbreaks of avian encephalomyelitis were detected by Animal and Plant Health Agency. Two outbreaks occurred on farms with conventionally reared broilers, and affected houses were sourced from the same parent flock. A third outbreak occurred in an unrelated organic broiler flock. Clinical signs were first observed between 1 and 12-days-old. These included ataxia, drooping wings, and inability to stand. Gross pathology was limited. Histopathology detected Purkinje cell necrosis and gliosis in the cerebellum, perivascular cuffs and necrotic neurons in the cerebrum, and chromatolysis in the brainstem. Brain samples from the first two outbreak were submitted to a commercial lab for PCR testing, which detected avian encephalomyelitis virus (AEV). A detailed history from all outbreaks revealed definite or possible exposure of parent flocks to a vaccine strain shortly before or during lay. This led to suspicion of vertical transmission to affected chicks. In the first two outbreaks, the parent flock was administered a live commercial AEV vaccine at 22-weeks-old, after transfer to the laying farm. In the third outbreak, the parent flock was kept on a multi-age free-range site. Their range access during lay had close proximity to another flock recently vaccinated in-rear, raising the possibility of horizontal transmission of a live AEV vaccine strain. Next generation sequencing is being utilised to interrogate the strain of AEV involved in all outbreaks, and results will be presented at the annual meeting. This case highlights the need for care and consideration when administering live AEV vaccines to broiler breeder flocks.

Poster 41

Unmasking Fungal Pneumonia in 15-week-Old Pullets: A Case Report from the Caribbean.

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Abstract

Fungal infections, particularly aspergillosis, continue to pose a significant health challenge to the global poultry industry, resulting in economic losses and adversely impacting animal welfare. On October 1st, 2024, six live 15-week-old Isa Brown pullets from a flock of 3,500 birds were submitted to the School of Veterinary Medicine, University of the West Indies, Trinidad and Tobago, for necropsy with a history of respiratory distress and increasing mortality over two weeks (5%). Postmortem findings revealed grossly emaciated carcasses with numerous pale-yellow nodules in the air sacs and lungs. Microscopic findings included multifocal to coalescing pulmonary granulomas with intralesional fungal hyphae, multifocal air saccular granulomas, and focal necrotizing heterophilic encephalitis. *Aspergillus fumigatus* was cultured from the lungs, and periodic acid-Schiff (PAS) staining of affected tissue revealed clusters of narrow-angle-branching fungal hyphae consistent with *Aspergillus*. This report describes a case of fungal pneumonia and encephalitis in a 15-week-old commercial pullet flock in Trinidad and Tobago. Poultry producers in the Caribbean and other developing regions are more at risk as factors such as the tropical climate, limited resources, and inadequate husbandry practices exacerbate fungal growth and proliferation. These challenges make it difficult to effectively manage and prevent aspergillosis outbreaks, further compounding the economic and welfare impacts on poultry production.

Poster: Coccidiosis

Poster 43

Comparative analysis of broiler chicken productivity following vaccination with coccidiosis vaccines versus essential oil in a commercial farm

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Abstract

Avian coccidiosis remains a major challenge in Malaysia's poultry industry. Anticoccidials are widely used but face increasing resistance issues and concerns over drug residues, particularly for exporters and organic farms. Alternatives like phytogenic products and attenuated coccidiosis vaccines are being explored. This study compared the performance of broiler flocks vaccinated with a live attenuated coccidiosis vaccine to those treated with oregano essential oil (EO) over three consecutive cycles on a Malaysian farm.

The study included six broiler flocks, totalling 163,000 chickens, from May to September 2024. Of these, 82,000 chickens were vaccinated with EVANT[®] (Group A), while 81,000 received EO in drinking water (Group B) (from Day 12, 5 days

weekly until harvest). Performance metrics included slaughter age, mortality, body weight (BW), average daily gain (ADG), feed conversion ratio (FCR), efficiency performance indicator (EPI), and total production cost (TPC).

Broilers of Group A showed significantly higher BW (+9.59%) and ADG (+9.56%) than Group B. Mortality, FCR, and TPC differences were not statistically significant, though Group A showed consistent improvements over cycles. In contrast, Group B yielded variable results and higher costs.

In conclusion, vaccinating provided more efficient performance and consistent protection against *Eimeria* challenges over three cycles compared to EO, making it a cost-effective alternative for coccidiosis management in broiler production.

Poster 44

Comparative field analysis of attenuated by precociousness coccidiosis vaccines vs. non-attenuated coccidiosis vaccines in Argentina

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Abstract

One of the main challenges facing modern poultry farming is avian coccidiosis. Comparative field trials are often the most representative method for evaluating the effectiveness of two products. However, the lack of control over all variables can introduce biases related to batches, feed, and climate. For this reason, it is recommended to conduct trials with a large number of animals and at different stages to minimize such biases.

This study is a continuous comparative trial conducted between October 2021 and October 2024 in Argentina, comparing the productive outcomes of 8,287,358 broilers (167 farms) using EVANT[®], an attenuated precocious vaccine (Group A), with 21,145,464 broilers (456 farms) using a non-attenuated vaccine (Group B).

The performance data used for evaluation included: slaughter age, slaughter weight, mortality, feed conversion ratio (FCR), daily weight gain, and production efficiency index.

Significant improvements were observed within Group A in terms of slaughter weight and daily weight gain. Numerical, but not statistically significant, improvements were noted in slaughter age, FCR, and production efficiency index.

The results suggest that the administration of attenuated coccidiosis vaccines over time can generate a positive impact on commercial production compared to other vaccination strategies.

Poster 45

Effect of Anticoccidials in Controlling Intestinal Damage in Broilers Challenged With *Eimeria maxima*.

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Abstract

Eimeria maxima infection is a well-known predisposing factor to necrotic enteritis (NE) in broilers. Ionophores and nicarbazin-potentiated ionophores are important tools to control coccidiosis. In addition, ionophores are reported to have low Minimal Inhibitory Concentration (MIC) to *Clostridium perfringens*, especially narasin, thus there is a perception that it has a superior effect in preventing NE in the field. The study investigates the effects of different anticoccidials – Nicarbazin+Semduramicin/Semduramicin (NS/S), Nicarbazin+Narasin/Narasin (NN/N) and Nicarbazin+Monensin/Monensin (NM/M), compared to uninfected untreated control (UUC) and an infected untreated control (IUC) on weight gain (WG), ISI score (histopathology technique that evaluates 8 parameters in the intestinal mucosa microscopically) and *E. maxima* lesion score (6 days post-infection) in a floor pen where birds were challenged with field strains of *E. maxima* (70,000 oocysts/bird) on d18 to predispose birds to NE. The potentiated ionophores were used from 0 to 21 days and the ionophores from 22 to 42 days. ANOVA was applied. Anticoccidial programs tested were able to significantly improve WG (kg) at 42 days ($P < 0.05$, SNK test) - UUC 2.950a; IUC 2.542b; NS/S 2.933a; NN/N 2.974a; NM/M 2.936a. ISI score was similar in challenged groups – UUC 7.59b; IUC 17.72a; NS/S 17.48a; NN/N 17.76a; NM/M 17.73a as well as *E. maxima* lesion score - UUC 0.4a; IUC 1.63b; NS/S 1.40b; NN/N 1.60b; NM/M 1.57b. Anticoccidials tested were equally capable of controlling *E. maxima* infection and consequently reducing the predisposition to NE.

Poster 46

Effects of monoglycerides fatty acids on the performance, body composition, and immune response in coccidiosis-vaccinated W-36-layer pullets

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Abstract

Glycerides of short and medium-chain fatty acids are recognized for their benefit in intestinal integrity and health, which is critical for optimal nutrient absorption and digestion. Controlled dose of coccidiosis vaccine applied to prevent coccidiosis infection in birds, may inadvertently create poor gut health and impaired production performance during cycling. This study aimed to investigate the effects of a monoglyceride blend of short and medium-chain fatty acids (SMCMG) on growth performance, body composition and jejunal mRNA expression of pullets vaccinated with COCCIVAC-D2 in-feed. 600-day-old Hy-Line W-36 pullets (10 replicates/treatment) were cocci-vaccinated and allotted to 3 dietary treatments (T1=No SMCMG; T2= 0.05% SMCMG, T3= 0.10% SMCMG). Diets were fed in four phases (P).

Performance was recorded weekly. Birds were sampled for jejunal gene expression and body composition measurement. There was a significant effect ($P<.005$) of SMCG on BW in P2, P3, and P4 with birds in T3 showing higher body weight gain compared to T1 and T2. Additionally, FCR was significantly and tended to be significantly improved in birds belonging to T3 in comparison to T1 and T2 at week 16 ($P=0.068$). Bone mineral content tended to be significant ($P=0.084$) at d 21 and total tissue weight was numerically higher at d 21, 42 and 112 in T2 and T3 compared to T1. MUC-2 expression numerically decreased in a linear manner with increasing SMCMG. In conclusion, the study showed that in pullets vaccinated against coccidiosis, SMCMG supplementation at 0.10% can improve gut health and performance over an extended time.

Poster 47

Evaluation of Broiler Performance and Level of Coccidia Protection in Anticoccidial Drug and Quillaja Saponin Combination Programs

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Abstract

Coccidiosis is an intestinal disease of chickens with devastating economic impacts on broiler production due to impaired nutrient absorption and poor performance. This study evaluated the performance and coccidiosis control effects of anticoccidial and *Quillaja* saponin combination programs compared to anticoccidial programs alone. Male Cobb 500 chicks were randomly assigned to seven treatments (7 pens/treatment): T1) No additive; T2) Salinomycin (55 ppm); T3) Salinomycin (55 ppm) + *Quillaja* saponin (250 ppm); T4) Narasin (79 ppm); T5) Narasin (79 ppm) + *Quillaja* saponin (250 ppm); T6) Clopidol (125 ppm); T7) Clopidol (125 ppm) *Quillaja* saponin (250 ppm). Performance parameters were measured on D14, 21, 28, and 42. A mixed inoculum of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* was administered to all groups via feed on D21. On D27 and D35, four birds were removed from each pen, euthanized, and gross coccidia lesion scores determined for *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* using Johnson and Reid (1970). Microscopic *Eimeria maxima* scoring was also determined. The raw data were analyzed using LSD test was used to separate means when ANOVA F values were significant ($p\leq 0.05$). The addition of a *Quillaja* saponin to the respective ionophore resulted in decreased mortality adjusted feed conversion (maFCR) beginning at D21 and for the remainder of the study. *Eimeria tenella* scores at D27 were decreased for the ionophore + *Quillaja* saponin treatments when compared to the ionophore. Based on zootechnical performance, a *Quillaja* saponin feed additive was effective as support for the anticoccidial programs.

Poster 48

Evaluation of performance in commercial broilers with the use of coccidiosis vaccine against anticoccidials in India

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Abstract

Coccidiosis, caused by protozoa of the genus *Eimeria*, compromises both the productivity and welfare of poultry. Anticoccidial drugs in feed have long been used as an effective control method; however, increasing drug resistance is leading to subclinical coccidiosis in broilers, resulting in significant economic losses. This challenge has prompted the development of newer solutions. Live coccidiosis vaccines have proven effective in restoring sensitivity to anticoccidial drugs and controlling field strains of *Eimeria*.

The objective of this study was to compare the performance of broiler flocks administered with EVANT[®], a live attenuated coccidiosis vaccine and without anticoccidials in feed (n = 90,048) against unvaccinated flocks receiving anticoccidials in feed (n = 41,427). The commercial flocks, from a India poultry producer, were assessed over three consecutive production cycles on the same farm. Performance parameters included body weight, feed conversion ratio (FCR), mortality rate, cost economics, and intestinal histopathology.

Results showed a two-point improvement in FCR and a 1.47% reduction in mortality in the vaccinated flocks, leading to a cost benefit of INR 1.60 (0.019 US\$) per kg of body weight. Histopathological analysis also indicated good gut integrity, which correlated with improved performance.

Thus, coccidiosis vaccination offers a promising alternative to anticoccidials, addressing both drug resistance and bird welfare concerns.

Poster 49

Evaluation of The Development of Resistance Following Prolonged Use of Synthetic Anticoccidials in a High Challenge Floor Pen Model

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Abstract

Prolonged use of a single chemical coccidiostat has been associated with the development of resistance in coccidia strains. However, the nature and timeline of resistance development has not been well characterized for all anticoccidials. A previous series of studies using an undefined challenge indicated a gradual decrease in efficacy over time among tested treatments.

The purpose of this series of studies was to assess the development of resistance to four synthetic anticoccidials when used over four successive broiler grow-out cycles. Additionally, two of the anticoccidials were used at higher inclusion levels to increase the likelihood of resistance development. In contrast to the previous study series, a challenge inoculum was collected and used to supplement the natural challenge in the litter during each study. Resistance development was assessed based on feed conversion ratios (FCR), average body weights, and mortality by feeding phase. Coccidiosis lesions were evaluated at 16, 22, and 26 days. The results of this series are pending.

Poster 50

Fortifying Immunity: How Dried Egg Product Enhances Resistance in *Eimeria*-Challenged Turkey Poults

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Abstract

Eimeria-induced coccidiosis remains a critical challenge in poultry production, compromising gut health, immunity, and overall growth. Analyzing blood chemistry after infection can reveal biological impacts of coccidiosis, aiding research for treatment and prevention. This study explores the therapeutic potential of dried egg product (DEP) in mitigating the biochemical and immune disruptions caused by *Eimeria* infection in turkey poults. 120 day of hatch turkey poults were allocated into three groups: non-challenged control, *Eimeria*-challenged control, and *Eimeria*-challenged with DEP supplementation (300 CBU Ct). Birds were orally challenged with *Eimeria* via oral gavage at 14 days of age, and samples were collected on day 19. Blood samples were collected for biochemical analysis with i-STAT cartridge CG8+. *Eimeria* infection led to significant metabolic and respiratory imbalances, as indicated by decreased pH and disturbances in HCO₃, tCO₂, and base excess. However, DEP supplementation partially restored these parameters, stabilizing electrolyte levels (iCa) and metabolic indicators (Glu, Hct, Hb). Furthermore, DEP-treated poults exhibited enhanced immune responses, with flow cytometry revealing increased expression of CD4+, CD28+, CD44+, and MHC II+, suggesting improved T-cell activation and antigen presentation. These findings highlight DEP's potential as a dietary intervention for reducing the physiological burden of *Eimeria* infection while enhancing immune resilience in turkey poults. DEP presents a promising alternative strategy for managing coccidiosis in commercial poultry production by improving biochemical homeostasis and immune function.

Poster 51

Resistance to Ionophores, did it change with Raised Without Antibiotics programs

Greg Mathis

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Abstract

Did the elimination of Ionophore (IONO) usage with RWA allow for a reduction in field levels of IONO anticoccidial resistance. Three anticoccidial sensitivity tests (AST) were conducted. Coccidia was isolated from farms that never used IONOs (Never), that continued to use IONOs (Cont.), and RWA farms (no IONO usage for 5-8 years. Treatments: No Additive, challenged (NMI) and nonchallenged (NMU); Chemical Class (CHEM): Zoalene, Amprolium, Nicarbazine, Robenidene, Coyden, Diclazuril, and Decoquinate; IONO Class: Monensin, Salinomycin, Narasin, and Lasalocid. Each challenge isolate contained *E. acervulina*, *E. maxima*, and *E. tenella*. Across all 3 ASTS, NMU grew similarly (avg. FCR 1.429). NMI across all three ASTS produce a similar moderate challenge with all species (avg. FCR 2.829 and LES 2.7). Both Chemical and IONO significantly controlled all coccidia challenges. For the Never AST, the avg. IONOs control (FCR 1.639 and LES 1.0) was significantly better than Chemical (FCR 2.077 and LES 1.3). For the Cont. AST, the avg. Chemical control (FCR 1.753 and LES 1.4) was significantly better than IONO (FCR 1.953 and LES 1.9). For the RAW, the avg. IONOs control (FCR 1.664 and LES 0.9) was significantly better than Chemical (FCR 1.992 and LES 1.3). Results showed that the never using IONOs coccidia isolate was very sensitive (low resistance); the continuous usage has led to moderate resistance; and RWA which “rested” the IONO may have resulted in less IONO resistant coccidia on those farms. This information will be useful as more poultry purchasers are able to source birds fed IONOs.

Poster: Diagnostics

Poster 52

A Comparative Assessment of Serological Diagnostic Methods in Poultry Health Monitoring

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Vaxxinova International B.V., Amman, Jordan

Abstract

Serological testing is a valuable tool for disease diagnosis and health monitoring in the poultry industry. However, results can differ based upon serological test used and/or sample types. This study systematically assesses three key aspects of serological diagnostics: (1) the performance of various commercial ELISA kits, (2) the correlation of ELISA kits specific for H9 and H5 versus hemagglutination inhibition (HA/HI) tests, and (3) the suitability of different sample types, blood strips, serum strips, and serum in diagnostic applications.

In the first analysis, four commercial AEV ELISA were compared. The results showed that two kits consistently delivered the most efficient and reliable detection of antibody titers. In the second trial ELISA and HA/HI tests specific for H5 and

H9 antibody detection were compared. While ELISA offered a practical, high-throughput option, HA/HI tests demonstrated superior consistency in sensitivity and specificity over time, particularly for low-titer samples.

The goal of the third trial was to compare the use of whole blood strips, serum strips, and serum via ELISA. Whole blood strips consistently produced higher titers compared to serum and serum strips, suggesting their potential as a cost-effective and reliable alternative for field diagnostics. Additionally, the correlation between serum and serum strip results in HA/HI tests was analyzed, revealing comparable accuracy but highlighting practical advantages of serum strips, more convenient in terms of shipping.

This comprehensive evaluation provides critical insights into the performance, reliability, and practical application of serological diagnostics in poultry health management.

Poster 53

Genotyping Survey of Infectious laryngotracheitis virus (ILTV) in Broiler Breeder Flocks

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Abstract

Infectious laryngotracheitis virus (ILTV) strains circulating in commercial poultry in the United States are classified into five main genotypes (GT): vaccine and vaccine-like viruses (GT II and IV), virulent vaccine revertant strains (GT III and V), and virulent non-vaccine related viruses (GT VI). In 2023 and 2024 a significant increase of GT VI ILTV cases in broiler breeder flocks was observed. A survey of broiler breeder flocks was conducted to determine whether these flocks are serving as a sub-clinical reservoir of GT VI viruses. Six companies were represented in the survey including 13 complexes, 48 farms located in nine states. A total of 239 trachea samples were received; 65 samples were from non-endemic regions and 174 samples were from endemic regions. Thirty-one percent of all farms were PCR positive for ILTV. One sample from each positive farm (n=15) was selected for genotyping. Fourteen of the 15 samples had sufficient viral genome to conduct genotyping. Twelve samples were identified as GT II/III, one as GT VI, and one sample showed a mix of GT II/III and GT VI. The twelve GT II/III samples originated from broiler breeders vaccinated with the TCO vaccine, hence the virus detected was the vaccine administered. The one sample identified as GT VI originated from a TCO vaccinated broiler breeder flock with clinical signs of ILT. Results from this survey indicated against the hypothesis that vaccinated broiler breeder flocks serve as a reservoir of GT VI viruses.

Poster 54

Metagenomics as a diagnostic game changer in a multidisciplinary context: the example of lymphoid neoplasms in captive Asian Houbara bustards

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Abstract

The Asian Houbara bustard (*Chlamydotis macqueenii*) is an endangered steppic bird, object of conservation breeding projects. Lymphoid neoplasms represent economically-important, viral-induced conditions in poultry. However, they have been rarely investigated in Asian Houbaras, despite their regular occurrence in captive birds. In order to clarify their etiology, 10 cases from 2022 were selected for characterization. Affected birds originated from a breeding center in UAE and ranged between 1 and 16 year of age. Clinical findings included progressive weight loss or sudden death. At necropsy, the majority of birds (6/10) appeared emaciated and exhibited hepato-splenomegaly (7/10). Multiple white nodules were scattered in liver (5/10), digestive tract (2/10), heart, spleen and bone marrow (1/10). Microscopically, round neoplastic cells with a lymphoblastic morphology were infiltrating the majority of the organs. Immunophenotyping of tumoral cells revealed CD3⁻CD268⁺ B cells (3/10), CD3⁺CD268⁻ T cells (1/10), and CD3⁻CD268⁻ cells (2/10), while it appeared inconclusive or was not attempted (4/10). Metagenomic analysis was conducted on 4 formalin-fixed and paraffin-embedded liver samples, including 3 neoplastic birds (CD3⁻CD268⁺, CD3⁺CD268⁻ and CD3⁻CD268⁻ neoplasia) and a healthy Houbara. Large amounts of reticuloendotheliosis virus (REV) were detected in the T-cell lymphoma, while no significant viral agents were present in the other samples. Subsequently, REV IHC on the T-cell lymphoma revealed abundant viral antigen, both intralesionally and in a variety of epithelial cells. Additional studies are needed to assess the role and prevalence of REV in captive Houbaras. The simultaneous occurrence of spontaneous neoplasms should also be considered.

Poster 55

Serum Biochemistry preliminary surveillance on broiler breeder by using MiniChem system

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Abstract

A preliminary study of serum biochemistry surveillance has been done in a clinically normal broiler breeder flock in different ages (5 weeks, 20 weeks, 30 weeks, 40 weeks and 50 weeks) from both sexes by using miniChem Bioguard Corporation, Taiwan. Blood were collected, separated for serum and sent to laboratory for analysis within 6 hours. Sample (serum) volume in 140ul, plus diluent 750ul were dispensed into the microfluidic disc. Avian & Reptile test kit (CH14-1) with 20 parameters or biomarkers was applied, each took 12 minutes per samples to analyze. Biomarkers and test results were: ALB 0-2 g/dL (Albumin), AST 230-380 U/L (Aspartate aminotransferase), TBA 30-45 umol/L (Total bile

acid), Ca 8-16mg/dL(Calcium), CK 1300-3800U/L(Creatine kinase), Cl- 95-120mmol/L(Chlorine), GLU 200-245mg/dL (Glucose), K+ 6-8mmol/L(Potassium), Na+ 136-160 mmol/L(Sodium), PHOS 2.5-8.5mg/dL(Inorganic phosphorus), TP 3-7g/dl (Total protein), UA 0.5-17 mg/dL(Urea), ALT 3-10 U/L(Alanine aminotransferase), BUN <2.5-20 mg/dL(Blood urea nitrogen), LDH 260-1300U/L(Lactate dehydrogenase), TCH 95-165 mg/dL (Total cholesterol), *GLOB 1-4.2g/dL(calculated globulin), *A/G 0.4-0.68 (calculated Albumin/Globulin ratio), *Na/K 14-27(calculated sodium/potassium ratio), *AST/ALT 24-110 (calculated Aspartate aminotransferase/ Alanine aminotransferase ratio). It was concluded baseline or reference range could be established following this observation or more surveillance.

Poster: Enteric Health

Poster 57

An overview of a novel combination of postbiotic and phytogetic to support mitigating colibacillosis in poultry

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Abstract

The study summarizes the benefits of a novel combination (Biostrong™ C-Protect, Bio-CP) of *Saccharomyces cerevisiae* fermentation-derived postbiotic (SCFP) and saponin-based ingredient derived from *Quillaja saponaria*, to support reduction in the severity of APEC challenge in poultry. In Experiment 1, Bio-CP was evaluated in broilers (120 birds/treatment) intratracheally (i/t) challenged with APEC at 28d. For experiment 2, pullets fed with or without Bio-CP (48 birds/treatment) from 0d were challenged (i/t) with APEC at 9 weeks to evaluate the benefits one week post challenge. In a third experiment, layers at 79 weeks were transitioned to a ration with or without Bio-CP (28 birds/treatment) for 56 days with an APEC challenge (i/t) on 28d of the experiment. Data were analyzed with treatments as main effect, and pens/birds as random effect, at a significance of $P < 0.05$. The results from Experiment 1 showed that Bio-CP reduced ($P < 0.05$) perihepatitis lesions and APEC load in lungs compared to challenged control, resulting in 26% reduction in APEC related mortality. In experiment 2, pullets fed with Bio-CP had lower ($P < 0.05$) air sacculitis lesions and improved body weight gain (9.1%) compared to challenged control. In experiment 3, feeding Bio-CP supported reduction of APEC load in lungs of challenged birds and helped improving hen day egg production and average egg mass in layers. Overall, the results from these trials showed that the novel combination of SCFP and Quillaja saponin-based ingredient could support mitigating the severity of APEC challenge and help promoting performance in poultry.

Poster 58

Blend of Probiotics and exogenous enzymes as Alternatives to Antibiotics for the Prevention and Control of Necrotic Enteritis in Chickens

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Abstract

Background: Probiotics and enzymes can substitute antibiotics growth promoters (AGPs) from broiler diets. Also, both improve body weight gain, feed conversion ratio, enhance villi height and decrease crypt depth in chickens. **Methods:** A total of 720 male chicks (one-day-old, weighted 45.82 ± 0.25 g) were selected to determine the effect of a blend of probiotics and enzymes on histomorphologic measures and growth performance. Chicks were randomly allocated to four treatment with six replicates and thirty birds per replicate. The dietary treatments were as follows: Low dose of probiotics and enzymes (T1, basal feed + Probiotics + enzymes 0.5 g/kg) and Medium dose of probiotics and enzymes (T2, basal feed + Probiotics + enzymes 0.75 g/kg), antibiotic group (T3, basal feed + bacitracin 0.25 g/kg) and Control group (T4, basal feed). All broilers were challenged with *Clostridium spp.* (5×10^8 CFU/ml) + *Eimeria spp.* (5×10^4 oocysts) at 14d, 15d and 16d of the study. **Results:** Chickens supplemented with bacitracin (T3) or probiotics + enzymes (T1-T2) enhanced ($p < 0.05$) the final body weight, feed conversion ratio and average daily gain in overall phase compared with control group (T4). However, There was not significant statistical difference between bacitracin group (T3) and probiotics + enzymes groups (T1-T2). Moreover, supplementing with Probiotics and enzymes (T1-T2) or bacitracin (T3) enhanced the VH/CD ratio after challenge compared with control group but there was not significant statistical difference between groups. **Conclusion:** The supplementation of probiotics + enzymes improve the growth performance and could replace the use of bacitracin like growth promoter.

Poster 59

Comparative metagenomic analysis of jejunal and cecal microbiota in broilers with subclinical enteric infection

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Abstract

Subclinical enteric infections can disrupt the composition and functionality of gut microbiota, impacting poultry health and performance. This study examined the metagenomic profiles of jejunal and cecal microbiota in broilers exposed to subclinical enteric challenges. Two groups were included: an unchallenged negative control and a challenged positive control group. Subclinical infection was induced in the challenged group using a tenfold dose of a live coccidia vaccine on

day 14, followed by oral administration of *Clostridium perfringens* (10^8 CFU) on day 18. Jejunal and cecal contents were collected from nine birds per group on day 21 for shotgun metagenomic sequencing. Raw sequence reads were trimmed and mapped to the most recent core nucleotide BLAST database, with taxonomic classification performed using Kraken2 and species-level normalization conducted via Bracken. Alpha diversity analyses revealed no significant differences in microbial richness or evenness within the jejunum. However, Shannon, Simpson, and Fisher indices indicated significantly reduced microbial diversity in the ceca of challenged birds, despite no observable differences in beta diversity in either intestinal segment. Taxonomic profiling demonstrated a marked increase in *Lactobacillus* species within the jejunum and an overrepresentation of pathogenic *Shigella* species in the ceca of challenged birds. Concurrently, there was a notable depletion of key commensal taxa including *Blautia hansenii* and *Lachnoclostridium* sp. YL32. Microbial co-occurrence network analysis further highlighted disrupted community interactions and shifts in key taxa, indicating destabilized microbial ecosystems in the challenged group. These analyses provide insights into the dynamics of jejunal and cecal microbiota during subclinical enteric challenges.

Poster 60

Comparative study of the Jejunal microbiome composition in conventional and raised without antibiotics feeding systems in broiler chickens.

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Abstract

Introduction

Excessive antimicrobial use in broiler chicken feed has led to antimicrobial resistance. With growing consumer demand, the Canadian broiler industry is moving toward raised without antibiotics (RWA) programs. However, with the reduction of antimicrobial use, diseases such as necrotic enteritis may become more prevalent. In order to study the impact of RWA versus conventional broiler raising systems, more information is required on the microbiome of broilers raised under both systems. Therefore, the objective of this study was to compare the jejunal microbiome in both systems.

Materials and methods

Six commercial broiler operations with both conventional (n=6 barns) and RWA (n=6 barns) systems were selected. At 25 days of age, broilers (n=8/barn) were euthanized and jejunal contents were collected for 16S rRNA gene amplicon sequencing. Serum samples from birds (n=10/barn) of 33-37 days of age were analyzed for infectious bursal disease virus (IBDV) and chicken infectious anemia virus (CAV) using ELISA. Production data (barn size, feed program, vaccinations, treatments, mortality, condemnations, density) were recorded.

Results

Relative abundance of jejunal microbiota from both systems was presented at the phylum, family, and genus levels. The most abundant phylum was Firmicutes followed by Pseudomonadota in both systems. All barns were negative for CAV, while 5 of 6 farms tested positive for IBDV. The mortality was higher in RWA systems, along with total condemnations and stocking density.

Conclusions

The jejunal microbiome in both systems was dominated by Firmicutes, followed by Pseudomonadota.

Poster 61

Comparing the effectiveness of two short and medium chain fatty acid-based products in Cocci-Challenged broilers

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Abstract

A 57-day trial was conducted under commercial conditions using 1,800 Ross 708/YPM chicks to evaluate the effect of short- and medium-chain fatty acid-glyceride (SMCFAG) blends on broiler performance during a cocci challenge. Chicks were sourced from a single breeder flock and allocated across 12 replicate pens per treatment (50 chicks/pen) into three groups: a negative control (NC) without SMCFAG supplementation, and two treatments (SMCFAG 1 and SMCFAG 2) where SMCFAG blends were included at 2.0, 1.5, 1.0, and 0.5 lbs/ton during starter (day 1-14), grower (day 15-30), finisher (day 31-44), and withdrawal (day 45-57) phases, respectively. SMCFAG 1 contained glycerides of propionic, butyric, caprylic, capric, and citric acids, while SMCFAG 2 included glycerides of butyric, caprylic, capric, lauric, and citric acids. All birds were challenged on day 15 with a 20x dose of Cocci-Vac B52. Performance data, including body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR), were analyzed via ANOVA and Student's t-tests.

On day 35, SMCFAG 2 significantly improved BWG ($p = 0.0005$) and FCR ($p = 0.001$) compared to NC and SMCFAG 1. Similar trends were observed on day 57 (BWG: $p = 0.0067$; FCR: $p = 0.0387$). Improvements in this trial were likely due to SMCFAG 2-specific lauric acid glycerides or glyceride ratios in SMCFAG 2, which may have modulated immune responses and mitigated gut dysbiosis post-challenge. Further research is needed to explore the mode of action of SMCFAG glycerides during vaccine challenges.

Poster 62

Early Gut Colonization: Probiotic Spray on Incubating Eggs and the Impact on Microbiome Development in Chickens

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Abstract

Understanding the effects of early gut colonization by spraying probiotics on incubating eggs is important, as it is a noninvasive and industry feasible method of introducing probiotics to the embryo and chicks at hatch. The objective of this study was to understand spray application of probiotics during incubation, on microbiome development of specific pathogen free (SPF) leghorn chickens.

SPF eggs were grouped as 1-9 (n=50). Groups 1-5 were sprayed with bacterial broths (1×10^9 CFU/ml) containing either *Enterococcus faecalis*, *Bifidobacterium pullorum* sp *gallinarum*, *Lactobacillus plantarum*, *Ligilactobacillus salivarius*, *Pediococcus acidilactici* at 15 and 17 days of embryonation (DOE). Group 6 was sprayed with probiotic mixture at both 15 and 17 DOE. Group 7 was given a probiotic mixture of 1×10^8 /bird at D1 post-hatch (PH) and group 8 was given the probiotic mixture during the first week PH. Group 9 was incubated and raised separately without administering any probiotics. Jejunal contents were collected at 2-, 10-, 20- and 30-D PH. The 16S rRNA amplicon sequences were obtained using nanopore and analyzed using EPI2ME.

The microbial composition of birds that received probiotics was different compared to negative control group. The groups treated with probiotics had a high relative abundance of genus *Lactobacillus* at day 10 of age compared to the negative control group. This study showed early establishment of gut microbiota with the pre hatch application of probiotics. Further analysis of microbiome data is underway.

Spraying probiotics on incubating chicken eggs is feasible technique to promote colonization of probiotics in the intestine of the embryo.

Poster 63

Effects of early postbiotic supplementation on intestinal chemokine expression and their receptors during subclinical necrotic enteritis in broilers

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Abstract

Necrotic enteritis, caused by *Clostridium perfringens*, disrupts the intestinal epithelium and compromises its function and defenses. This study assessed the effects of in ovo and post-hatch administration of a *Saccharomyces cerevisiae*-

based postbiotic on broiler intestinal immune response during an NE challenge. Embryonic day (d) 18 Ross 708 fertile eggs were injected with 0.2 mL of either water or postbiotic. Hatchlings were divided into four groups: 1) NC (in ovo water, not challenged); 2) PIW (postbiotic in ovo and drinking water, not challenged); 3) NC+ (NC + challenge); and 4) PIW+ (PIW + challenge). NE was induced via oral gavage with 3,000 *Eimeria maxima* sporulated oocysts on d14, followed by two doses of approximately 1×10^8 CFU/mL/bird of *C. perfringens* on d19 and d20. On both d14 and d21, six birds/group were sampled to assess mRNA abundance in jejunal tissues. On d14, mRNA abundance of only CCL20 was significantly greater ($P=0.021$) in PIW birds compared to NC but not different ($P=0.788$) on d21. CCL5 abundance was significantly greater ($P=0.017$) on day 21 in the PIW+ birds in comparison with those in NC. No other significant interaction effects were observed at these time points. These results suggest differential modulation of chemokines, namely CCL20 and CCL5, in broilers under NE challenge. The CCL20/CCR6 axis plays a critical role in recruiting immune cells, while CCR5/CCL5 contributes to gut inflammatory responses. These preliminary findings highlight the potential of *S. cerevisiae*-based postbiotics in mitigating NE-associated intestinal inflammation and immune dysregulation in broilers.

Poster 64

Efficacy of an attenuated coccidiosis vaccine to reduce the excretion and colonization of *Salmonella Infantis* in chickens infected with *Eimeria spp.*

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Abstract

Salmonella Infantis, commonly found in poultry farms, poses a risk to the food chain and potential human infection. There is no commercial vaccine against *S. Infantis* for broilers, and control relies on biosecurity measures. *Eimeria spp.* infestation can facilitate the infection of certain *Salmonella* serovars, however little is known about its effect on *S. Infantis*. This study aimed to evaluate whether *Eimeria spp.* infestation promotes *S. Infantis* infection and if an attenuated coccidiosis vaccine can reduce this risk.

Three experimental groups of 26 SPF chicks each were included. Group A was vaccinated with EVANT[®], an attenuated coccidiosis vaccine for broilers, and challenged orally with a combination of *Eimeria acervulina*, *E. maxima* and *E. tenella* at day 17 and *S. Infantis* at day 20. Group B was non-vaccinated and challenged as previously indicated. Group C was non-vaccinated and challenged only with *S. Infantis*. *Salmonella* excretion and colonization were evaluated through cloacal swabs on days 23, 25, 27, 30 and 34 and samples of liver, spleen and caeca at study days 27 and 34, respectively.

Results showed that previous infestation with *Eimeria spp.* increased the excretion/colonization of *S. Infantis* (B vs C). The vaccinated group with the coccidiosis vaccine (A) reduced this synergic effect compared to non-vaccinated group (B) and no differences were observed with the group challenged only with *S. Infantis* (C).

This study demonstrates that *Eimeria spp.* can promote *S. Infantis* excretion/colonization. Moreover, immunization with an attenuated coccidiosis vaccine could have a positive impact to reduce this risk.

Poster 65

Impact of postbiotics and phytogenics on turkeys challenged with *Histomonas meleagridis*

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Abstract

The objective of the present studies evaluated a combination of postbiotics and phytogenics (CPP) fed at 1.6 lb/ton on histomonas challenged poult measuring infection rate, lesion scores, and performance in a CRD. In S1, 2 out of 8 poult/cage were intracloacally challenged with 10⁵ *H. meleagridis* cells/mL at d13. Two groups were evaluated: control (C) and CPP, with 10 replicates/treatment and 10 (pre-challenge)/8 (post-challenge) birds/cage from 0-33 days. In S2, all poult were directly inoculated with 10⁵ (L) or 2x10⁵ (H) histomonads/bird at d10. Four groups were evaluated: L + no CPP, H + no CPP, L + CPP, H + CPP with 6 replicates/treatment and 6 birds/cage from 0-30 days. Birds were fed corn and soybean meal based basal diets. Data were analyzed independently using dietary treatments as main factors. S1 CPP contact birds exhibited a significantly lower horizontal transmission rate and incidence of cecal lesion scores than C (p<0.05). S1 CPP seeders exhibited numerically lower mortality as compared to C (-20%). Additionally, S1 CPP's body weight was significantly heavier throughout the trial. In S2, both liver and cecal lesion scores were reduced numerically in birds fed CPP, with lower reported incidences of severity compared to their respective non-CPP counterparts. In S2, post-challenge BW trended heavier and FI was significantly higher (p<0.05) in CPP birds versus non-CPP. Based on these trial results, dietary supplementation of CPP was supportive in minimizing clinical signs and production performance impact on poult challenged with histomonas.

Poster 66

Sulfated polysaccharides positively influence the cecal microbiota of broilers during a necrotic enteritis challenge

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Abstract

Necrotic enteritis (NE), caused by *Clostridium perfringens* (CP), is a major enteric poultry disease resulting in severe economic losses to the industry. A 42-day (d) NE model study investigated the effects of a sulfated polysaccharide additive on the cecal microbiota of broiler chickens. Day-old Ross 708 chicks (n=450) were allocated to one of three groups: 1) negative control (**NC**; corn/soybean diet), 2) NC + Avilamycin/Amprolium (**PC**), and 3) NC + 0.1% Algoguard supplementation (**AGS**). On d 14 and d 19, all birds were orally inoculated with 2,000 sporulated oocysts of *Eimeria maxima* and 1x10⁸ CFU of CP, respectively. On d 14 and d 21, cecal mucosal scrapings were collected, microbial DNA was extracted and sequenced via targeting the V3-V4 regions of the 16S rRNA gene and analyzed using QIIME2. PICRUST2 pipeline was used for functional prediction analysis. Alpha diversity indices (Shannon and Simpson) were significantly

greater in the ceca of PC birds compared to NC on d 21 but not on d 14. PICRUST2 results showed significantly greater expression of riboflavin metabolism-related genes in AGS birds compared to other groups on d 14. On d 21, AGS and PC groups showed significantly greater abundance of functional genes involved in sphingolipid metabolism and biosynthesis of Ansamycins compared to NC. This suggests that AGS promoted the proliferation of cecal microbes involved in biosynthetic processes, maintenance of cell membrane integrity, and immune signaling, thus providing better defense against CP. Therefore, supplementation of sulfated polysaccharides could promote growth of beneficial cecal microbiota.

Poster 67

Two products based on short- and medium-chain fatty acids have distinct effects on performance and biomarkers of *Enterococcus*-challenged broilers

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Abstract

An experiment was conducted to evaluate the effects of two short- and medium-chain fatty acid-glycerides (SMCFAg) mixtures on broilers naturally infected with bacteria causing skeletal infections and locomotor issues. Three groups were set up, each with 12 replicate pens and 40 male Cobb500 broilers per pen. The control group received no SMCFAg, while the other two groups were supplemented with SMCFAg products at decreasing levels (2.0, 1.5, 1.0 lbs/ton) across three dietary phases (days 1-14, 14-28, and 28-42).

The [C3-C10] group was fed glycerides of propionic (C3), butyric (C4), caprylic (C8), capric (C10), and citric acid. The [C4-C12] group received a similar blend but with lauric glycerides (C12) replacing C3. Performance metrics were recorded, and bacteria from femoral heads and caecal contents and caecal IgA levels were analyzed on day 42. Statistical analysis (ANOVA, t-test) compared the control and treatments.

Bacterial cultures were isolated in 86% of femoral head samples, with *Enterococcus* spp. identified in 80%. SMCFa-fed birds showed better performance, with higher body weight ($P < 0.004$) and lower feed conversion ratio (FCR; $P < 0.001$). The [C4-C12] group achieved the highest weight and lowest FCR, while the [C3-C10] group showed lower mortality (5.62% vs. 8.95%; $P = 0.010$) and a trend toward reduced APEC ($P = 0.075$). IgA levels were higher in the [C4-C12] group ($P = 0.027$).

In conclusion, both products improved broiler performance via distinct gut microbial and immune mechanisms, warranting further investigation of their effect on extra-intestinal translocation of enteric bacteria.

Poster 68

Use of activated diatoms, pronutrients and phenolic antimicrobial molecules for preventing intestinal challenges in broiler chickens

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Abstract

A trial was conducted for 42 days to evaluate the efficacy of activated diatoms, pronutrients and phenolic antimicrobial molecules to prevent enteric diseases. 400 broilers were distributed into 4 treatments groups with 5 replicate pens each. Treatments were a standard basal diet (SBD) as a Negative Control (NC); a SBD challenged with soybean 3% (SBDC) as a Positive Control (PC); a SBDC with activated diatoms, pronutrients and phenolic antimicrobial molecules at 0.5 Kg/t (SBDC+); a SBDC with Halquinol at 100 g/t (SBDC-). The inclusion of soybean 3% in the diet was due to it contains ANFs and cause intestinal dysbiosis.

Intestinal retention time was higher in SBDC+ ($P<0.05$) group of 20 minutes and 24 minutes on average compared to PC and SBDC- groups, respectively. SBDC+ group showed a slightly increase in the digestibility of protein (+ 2%) ($P<0.05$) compared to PC. Body weight (BW), body weight gain (BWG) and feed conversion (FCR) were significantly better in SBDC+ group than PC and SBDC- ($P<0.05$). Mortality was significantly lower in SBDC+ group (0%) ($P<0.0001$) compared to the others. Litter humidity was significantly higher in SBDC+ group ($P<0.05$) than PC and SBDC- and dirty cloaca was lower in SBDC+ than the others ($P<0.0001$). Levels *E. coli* were lower ($P<0.0001$) in SBDC+ and levels of *Lactobacillus* were higher ($P<0.0001$) in SBDC+.

In conclusion, the mentioned active ingredient is effective to prevent gut challenges in broilers improving performance and reducing mortality and pathogen counts.

Poster: Infectious Bronchitis Virus

Poster 69

A heterologous vaccination approach against Var2 (GI-23) infectious bronchitis virus.

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Abstract

Infectious bronchitis virus (IBV) causes an economically important upper respiratory disease in poultry that is extremely difficult to control because multiple antigenic variants of the virus with little cross-reactivity exist. There are numerous IBV types circulating around the world with most being relatively geographically restricted. However, a limited number of IBV types have been identified in multiple countries and have the characteristic of becoming widespread. One such IBV type is Variant 2 (Var2) which is a GI-23 lineage virus. Var2 was first identified in Israel, then in Iran, Egypt, Turkey,

Europe, Brazil and recently in Mexico. Mexican Var2 IBV sequences are similar to Brazilian virus sequences. Because Var2 is close to the US boarder and a homologous Var2 vaccine is not approved for use in the US, we wanted to determine if a heterologous vaccination approach using IBron, a GA08 type vaccine and IMass (Ceva Animal Health) could provide cross-protection against challenge with Var2. SPF birds were vaccinated at one day of age with both vaccines simultaneously. At 23 days of age, the birds were challenged with a pathogenic Var2 virus isolated from broilers in Brazil in 2022. At 5 days post challenge we did not observe clinical signs or ciliostasis in the vaccinated and challenged birds, contrary to positive controls. In addition, they had significantly reduced histological lesions in the trachea and challenge virus replication in the trachea, kidney and airsacs compared to nonvaccinated and challenged controls, indicating that the birds were satisfactorily protected against challenge with Var2.

Poster 70

Characterization of microRNA candidates at the primary site of infectious bronchitis virus infection

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Abstract

Infectious bronchitis virus (IBV) is the causative agent of infectious bronchitis (IB), a primarily respiratory disease affecting chickens, with the ability to disseminate to the gastrointestinal, renal, lymphoid, and reproductive systems. Tracheal epithelial cells are the primary target of IBV, and these cells play a vital role in the effective induction of the antiviral response and eventual clearance of IBV. The host immune system is regulated by a number of different molecular players, including micro-ribonucleic acids (microRNAs), which are small, conserved, non-coding RNA molecules that regulate gene expression of complementary messenger RNA (mRNA) sequences, resulting in gene silencing through translational repression or target degradation. We aimed to characterize and compare the microRNA expression profiles in chicken tracheal epithelial cells (cTECs) in vitro and the trachea in vivo upon IBV Delmarva/1639 (DMV/1639) or IBV Massachusetts 41 (Mass41) infections using small RNA-sequencing (RNA-seq). We found that the profile of differentially expressed (DE) microRNAs is largely dependent on the IBV strain and time point of sample collection. Furthermore, we predicted host microRNA-IBV viral RNA interactions. We identified the candidate microRNAs, such as gga-miR-155, ggamiR-1388a, gga-miR-7/7b and gga-miR-21-5p. Characterizing the interaction between IBV and the host cells at the level of microRNA regulation provides further insight into the regulatory mechanisms involved in viral infection and host defense in chickens following IBV infection.

Poster 71

De novo transcriptome assembly enhances detection of differentially expressed genes in avian coronavirus-infected chicken tissues

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Abstract

Reference transcriptomes represent incomplete collections of transcripts, as each tissue contains a distinct yet partially overlapping transcriptome. To address this, RNA-seq libraries from the kidney, ovary, and oviduct tissues of chickens infected with infectious bronchitis virus (IBV) strains Delmarva (DMV)/1639 or Massachusetts (Mass), as well as uninfected controls, were used for de novo transcriptome assembly. Samples were pooled per tissue, resulting in 12 assemblies. Transcripts were predicted using TransDecoder, annotated with Trinotate, and aligned against the *Gallus gallus* reference transcriptome (Ensembl). Redundant novel transcripts were discarded, and reference and novel transcripts were merged to create hybrid transcriptomes.

These hybrid transcriptomes were subjected to differential expression (DE) analysis and compared with the reference transcriptome. On average, ~11,000 novel transcripts were identified per assembly. The hybrid transcriptomes revealed approximately 30% more DE transcripts than the reference transcriptome alone. Virus-infected tissues consistently showed a slightly higher number of DE transcripts compared to controls, with tissue- and strain-specific differences observed. Notably, hundreds of genes identified as DE in the hybrid transcriptomes were absent from the reference-based analysis, with the oviduct showing the highest number of novel DE genes. These genes included Zinc finger proteins, RNA-binding proteins, polymerases, transmembrane proteins, kinases, transcription factors, chromatin-associated proteins, helicases, and cellular receptors.

De novo assembly of novel transcripts significantly enhanced the informativeness of DE analysis in IBV-infected chicken tissues. To validate these findings, qPCR with appropriate primers will be used, offering insights into the host response to IBV infection.

Poster 72

Detection and Control of Field Infectious Bronchitis Viruses in the Mexican Poultry Industry in a 10-year timeline

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Abstract

Infectious Bronchitis is caused by a gammacoronavirus which is highly contagious and is distributed worldwide causing serious economic burden both for broilers and layers. The high capability of this RNA virus to mutate and recombine has caused the emergence of many genotypes that do not strongly cross-protect between each other, as well as the frequent generation of antigenic variants that make their control very difficult by using single vaccines. Also, the use of

homologous vaccines for controlling these variants may induce the emergence of novel variants. The concept of Protectotype, briefly, the strategic use of two antigenically different vaccine viruses for controlling variants has proven successful in many areas of the world. By using Sanger sequencing, which detects the dominant sequence strain in samples, for a time span of 10 years in Mexico, during which we managed to record the prevalence of Arkansas-like detections which apparently was displaced by emergent Var 2-like strains. By applying the Protectotype combination of two strains; a Massachusetts that spontaneously agglutinates chicken red blood cells, and another belonging to the 793-B serotype, we were able to demonstrate that Arkansas-like strains that were detected previously were successfully controlled. Var 2-like strains proved to be more detectable than Arkansas-like strains and were controlled by means of a stricter vaccination application supervision. Using a haplotype mapping system, we were also able to demonstrate that the 793-B strain used for vaccination varied very little during that 10-year period, whereas Var 2-like strains varied extensively during a 1.5 year period.

Poster 73

Does Infectious Bronchitis Virus G1 23 Prevent Commercial Pullets From Reaching Sexual Maturity? A Case Report.

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Abstract

Infectious bronchitis virus is a highly contagious, worldwide distributed virus that has a high capability of mutating and recombining, causing the emergence of different genotypes that most vaccines fail to induce satisfactory immunity against. Certain strains have been detected with no previous history of viral circulation in any given country, which forces the local poultry industry to review or update vaccination programs or promote the enforcement of stricter supervision of vaccine application. Var 2 IBV strains were first described during the early 2000's in the Middle East and have since spread to several parts of the world. These strains have been detected in the Mexican poultry industry since the second half of 2022 in broilers. Recently, Var 2-like detections in layers have increased in flocks that had not been vaccinated in lay. In Western Mexico, in a layer pullet flock, following a respiratory case around 18 weeks of age, the service personnel noticed that it was low in body weight, and showed sexual immaturity. Samples were taken and a Var 2-like IB virus was detected, as well as high serological IBV titers. The flock was revisited at 33 weeks of age, and the birds showed a delay of follicular development, or an absence of sexual maturity. At that age, it was estimated that more than 20% of the flock's population was affected. The IB vaccination program consisted of conventional vaccines and no vaccination in-lay. This case calls for more research on the effect of certain IB viruses on physiological parameters.

Poster 74

Evaluation of Seroconversion and Safety for Various Vaccination Programs Against Infectious Bronchitis

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Abstract

Infectious Bronchitis Virus (IBV) is a highly contagious pathogen that leads to significant losses due to respiratory and urogenital issues. In areas where more than two different field strains or serotypes of IBV circulate, various vaccination programs are often used. In broilers, two or three different vaccine strains can be administered in the hatchery. To assess the immune response and the severity of post-vaccination reactions after the administration of two or three live vaccine strains at the same time, six groups of 40 day-old broilers were vaccinated via spray with different vaccine combinations. The vaccines' antigens used in this study included the serotypes Massachusetts, BR-I, and Var2. Birds were kept in isolated rooms and monitored daily. Seven and eleven days post-vaccination, both the vaccinated and control groups were evaluated for clinical signs. Trachea was also collected for evaluation of ciliary movement and histopathology analysis. At 42 days post-vaccination, blood samples were taken from 20 birds per group to assess the level of antibodies using three commercial Elisa kits. Results showed clear differences in clinical scores of post-vaccination reaction, ciliary movement scores, and visible lesions among the groups. However, there was no correlation between the number of vaccine strains used at the same time and the severity of post-vaccination clinical signs or lesions. Instead, the severity of symptoms and lesions was related to specific vaccines included in the study. Additionally, no significant differences in antibody levels were found between groups receiving two or three vaccine strains simultaneously.

Poster 75

The effect of TLR3 and MDA5 gene knockout on Infectious Bronchitis Virus replication in DF-1 cells

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Abstract

First observed in 1930, infectious bronchitis virus (IBV) remains a significant challenge for the poultry industry. It is well established that IBV typically does not replicate in immortal cell lines, with the exception of certain strains, such as the Beaudette strain.

Toll-like receptor 3 (TLR3) and melanoma differentiation-associated protein 5 (MDA5) are pattern recognition receptors (PRRs) in vertebrate hosts that detect pathogen-associated molecular patterns (PAMPs). Both PRRs are crucial for sensing viral double-stranded RNA (dsRNA), which can be generated as a dsRNA genome or as a replicative

intermediate. TLR3 detects dsRNA in endosomes, while MDA5 detects it in the cytoplasm. IBV replication depends on the production of intermediate dsRNA.

In this study, we evaluate whether DF-1 cells are susceptible and permissive to IBV replication using the Beaudette and Arkansas IBV strains. Furthermore, we assess whether knocking out TLR3, MDA5, or both genes in DF-1 cells allows or enhances the growth of these IBV strains. Additionally, we characterize changes in mRNA expression of genes associated with innate immune responses to further elucidate the impact of IBV infection in these cell lines.

Poster 76

Use of a vaccination protocol including live and inactivated IBV vaccines against a heterologous challenge with a Brazilian IBV variant

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Abstract

An effective protection against infectious bronchitis virus (IBV) is essential in broiler breeders and layers to ensure good egg production and quality. Designing a field vaccination protocol in the presence of an increasing number of IBV variants worldwide can be a challenge. In this study, we evaluated the use of a protocol against IBV in a flock of broiler breeders, including multiple applications of a live IBV vaccine containing a Mass strain (*Nobilis® IB Ma5*) and a single application of an inactivated vaccine containing a combination of a Mass strain and a variant strain, D274 (*Nobilis® RT IBmulti G+ND*). The live vaccines were applied in 3 different ages during the rearing period, prior to the inactivated vaccine, as primers, and in 4 different ages, during laying, following the manufacturer's recommendations. Sera was collected at the ages of 23, 37 and 56 weeks, for virus neutralization. The challenge virus was the Brazilian variant strain BR1 (GI-11). Dilutions were prepared including the sera and the challenge strain and inoculated in SPF eggs between 9 and 11 days of incubation. Living embryos were removed 6-7 days post challenge and evaluated for the presence of typical IBV lesions. All sera dilutions above 1:40 were able to prevent those lesions in the chicken embryos and thus were considered protective against the heterologous IBV challenge.

Poster: Infectious Bursal Disease

Poster 77

Biological behavior of an immune complex vaccine against Infectious Bursal Disease applied in commercial layers in Mexico

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Abstract

In certain areas of México commercial layer farms face various poultry disease challenges, particularly in regions with high prevalence of Avian Influenza, velogenic Newcastle Disease, Avian Infectious Bronchitis and Infectious Bursal Disease (IBD). This report focused on biological evaluation (molecular detection of vaccine antigen, histopathology, serology) of an immune complex (ICX) vaccine against IBD in the state of Jalisco in México. The ICX vaccine evaluated in this study utilizes, as its antigen, the IBD virus (IBDV) intermediate plus strain SIZA26 complexed in vitro with specific humoral antibodies against it. The data evaluation spanned four years, from 2021 to 2024, and involved multiple serological, histopathological, and molecular assessments to monitor the ICX vaccine take and efficacy in replacing field strains throughout the period. As their only vaccine against IBD, day old hens were vaccinated (SQ) at day 1 at different hatcheries. A total of 9,791 serum samples were collected for IBD Elisa serology, 2,760 bursas were examined for histopathology (European Pharmacopoeia Bursa Lesion Score), and 149 bursas were tested by real-time qRT-PCR from birds aged 28 to 70 days across different commercial layer flocks. Results indicate lower bursal lesions and lower an uniform antibody titers. In some companies, IBD variant strains variant strains were quite prevalent just before the use of the immune complex vaccine and after 2-3 broiler cycles with the ICX vaccine, the ICX vaccine antigen was by far the most detected strain (i.e., displacement of field IBDV).

Poster 78

Comparative Study of Two Live IBDV Vaccines; A Live vaccine and an Immune Complex IBD Vaccine in Commercial Broilers in the Philippines

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Abstract

Infectious Bursal Disease (IBD), also known as Gumboro Disease is an acute, highly contagious viral infection of young chickens caused by the IBD virus (IBDV). Live IBD vaccines are the main tool to actively immunize broilers. MB-1, a live attenuated hatchery vaccine was recently introduced into the Philippines. In this study, two groups of 40,800 commercial broilers were vaccinated by SC injection in the hatchery with MB-1 and ICX. Twenty (20) Blood samples were collected from both treatment groups at 1d, 14d, 21d and 28d of age. They were submitted for both IBDV ELISA Test and Newcastle Disease Virus (NDV) ELISA Test. At 14, 21, and 28 days of age, six (6) Bursa samples from each group were

collected and sent for real time PCR analysis. IBD ELISA Mean titers at 21d were similar in both groups. At Day 28, MB-1 vaccinated birds had significantly higher titers (3982) and lower CV (CV 97%) than ICX group (591, CV 395%). NDV ELISA mean titer results shows no significant difference between groups. MB-1 (MB strain) and ICX (Winterfield 2512) were detected from Day 28 samples by PCR. MB-1 vaccinated birds demonstrated superior broiler performance (Sur: 95.58%, BW: 1.67 kg, FCR: 1.515 kg) compared to ICX vaccinated birds (Sur: 95.48%, BW: 1.65 kg, FCR: 1.523). In conclusion the MB-1 group demonstrated significantly higher, earlier and more uniform IBD titers. These correlates to superior protection against IBD challenge with no negative effect on NDV ELISA titers and better broiler performance compared to ICX group.

Poster 79

Comparing the immunosuppressive effects of classical and variant infectious bursal disease viruses in broiler chickens

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Abstract

Variant infectious bursal disease virus (varIBDV) causes immunosuppression in chickens resulting in secondary infections. The most predominant varIBDV in Canadian broiler chicken industry is varIBDV SK09. The objectives of this study were to compare virulence of varIBDV SK09 and classical IBDV at the cellular level and to compare immunosuppressive abilities in broiler chickens. Broiler chickens were obtained and were grouped as 1-4 (n=55). Group 1 was challenged orally with vIBDV SK09 at 7 days post-hatch (DPH), group 2 with non-immunosuppressive intermediate IBDV strain D-78, group 3 with intermediate IBDV strain ST-12 and group 4 received saline and was kept as a negative control. B cells, T cell subsets, monocytes and macrophages of the bursa of Fabricius (BF) were analyzed by flow cytometry following IBDV infection at 5-, 14- and 28-days post IBDV infection (n=15/group). Additionally, groups of birds were challenged with IBDV as above at 7 DPH, and with avian pathogenic *Escherichia coli* at 20 DPH via the subcutaneous route, to study the immunosuppressive effects of IBDV strains, using clinical scoring, mortality, bacterial load and histopathology of the BF. Pre-exposure of broilers with varIBDV SK09 caused immunosuppression with significantly higher mortality and disease severity in broilers challenged with a virulent strain of *E. coli*. Further, severe bursal damage was observed on histopathological examination of BF in the varIBDV SK09 infected group in comparison with the control group. Findings of this study compared the impact of classical and varIBDV SK09 in broiler chickens.

Poster 81

Generation and evaluation of a recombinant Newcastle disease virus expressing the VP2 protein of a novel infectious bursal disease virus variant

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Abstract

Infectious bursal disease (IBD), caused by the infectious bursal disease virus (IBDV), severely impairs the chicken immune system, leading to substantial global economic losses. The emergence of a novel IBDV variant (nVarIBDV) poses significant challenges, resulting in severe bursal atrophy, immunosuppression, and the ability to evade neutralization by conventional vaccines. In this study, we utilized a genotype VII attenuated thermostable Newcastle Disease Virus (NDV) vector, rNDV-VII, to develop a novel IBDV vaccine that can protect young chickens against the nVarIBDV challenge while being suitable for storage and transport at ambient temperature. The recombinant virus, rNDV-VII-VP2, expressing the nVarIBDV VP2 protein, was generated using reverse genetics technology. Biological assessments revealed that the recombinant virus exhibited low pathogenicity and maintained similar thermostability, growth kinetics, and virus titers compared to its parental virus. Subsequently, the immunogenicity and protective efficacy of the rNDV-VII-VP2 vaccine were evaluated in specific pathogen-free (SPF) chickens. The results demonstrated that rNDV-VII-VP2 effectively elicited high titers of anti-IBDV antibodies and anti-NDV antibodies, caused no damage to immune organs or detectable virus shedding, and provided 100% protection against the nVarIBDV challenge. These findings suggest that rNDV-VII-VP2 is a promising bivalent vaccine candidate with the potential to effectively prevent infections caused by nVarIBDV and genotype VII NDV.

Poster 82

Pathogenicity assessment of “subclinical” infectious bursal disease virus strains

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Abstract

Infectious bursal disease virus (IBDV) is widespread in chickens with major losses due to clinical disease including mortality in older birds and severe immunosuppression in chicks. It is a bi-segmented RNA virus whose pathogenicity ranges from acute fatal disease to a subclinical condition. Current virus nomenclature describes 9 genogroups based on the A segment of RNA. Most of the field strains either belong to the A2 (typically US “variants”, which are also detected in an increasing number of other countries), or to the A3 (very virulent isolates which are more and more replaced by “reassortant” strains). The disease is characterized by a reduced clinical manifestation which often leads to misdiagnosis and under reporting. In this study we examined some representatives of these strains for their ability to induce pathology, including immunosuppression.

To this aim, two A2, and three A3 strains were tested in 4-to-5-week-old commercial broilers or SPF chickens, respectively.

Pathogenicity was assessed at several time points post-inoculation. Bursa of Fabricius as well as extra-bursal tissue samples were collected and processed by histopathology analysis (including immunohistochemistry and B cell staining in the SPF study); virus load was assessed as well using RT-qPCR.

Altogether, all the tested strains showed clear evidence of residual pathogenicity, including lymphoid organs damage, with limited recovery. These findings stress the importance of improved awareness of these subclinical strains, including a proper genetic characterization, and of appropriate control since they can induce immunosuppression which results in production losses in the field.

Poster 83

The Application of Subunit Vaccine against Infectious Bursal Disease in Chickens with Maternal Antibody

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Abstract

The infectious bursa disease (IBD) is a highly contagious disease in young chickens. The major neutralization epitopes of IBD virus is located on the viral structure protein VP2. The objective of this study is to investigate the efficiency of a subunit vaccine containing VP2 protein in chickens with maternal antibody. The subunit vaccines were expressed by *E. coli* and confirmed by Western blotting. Total 20 chickens with maternal antibody were grouped to 4 groups, negative control (NC), challenge control (CC), killed vaccine group (KV) which vaccinated with commercial killed vaccine, and subunit vaccine group (SV) which vaccinated with subunit vaccine. Chickens received vaccinations twice at 10 and 18 days of age and challenged with IBDV at 32 days of age. All chickens were terminated at 5 days after challenge and bursal gross and histopathological lesion scores were given to each chicken. The gross and histopathological lesion scores were (0, 0) for NC; (4, 4) for CC; (1, 0.6) for KV and (0, 0.4) for SV. The protections in KV and SV were 60% and 100%, respectively. The results indicated that the subunit vaccine containing VP2 protein is able to provide protection for chickens with maternal antibody against IBD.

Poster: Mycoplasma

Poster 85

CRISPR spacers in *Mycoplasma gallisepticum* live vaccines isolated from chickens and turkeys

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Abstract

The poultry industry regularly uses commercially available live attenuated vaccines, like F-strain, to control *Mycoplasma gallisepticum* infections. CRISPR-Cas systems, an adaptive immune system that defends against phages and mobile genetic elements, have been developed by certain bacteria. Such records are found through CRISPR spacers analysis, but little research has been done on them. In this study, we investigated the diversity of CRISPR spacers in twelve F-strain isolates from chickens and turkeys across the USA. The full genome libraries of isolates were generated using Illumina technology. The CRISPR spacer sequences were aligned and phylogenetic tree was constructed. The average number of spacers in the F strain isolates is 55.5, with a range of 32 to 141 spacers. Only 14.1 percent (85 spacers) were found in more than one genome out of the 604 spacers identified and repeated spacers were observed among multiple isolates. Phylogenetic analysis of the spacers did not distinctly differentiate F-strain from turkey and neither the older nor newer isolates showed discernible changes. Even though isolation were separated by 16 years, two F strains from the same state had identical spacers. We have generated an extensive list of 'memory' DNA acquired by MG vaccine isolates. There may be identical spacers in F strain vaccine isolates from different locations and at different time points. Also, spacers acquired may remain the same over a long period of time in a particular location. The acquisition and persistence of CRISPR spacers will probably be better understood as more genomes become available.

Poster 86

Effect of Field Use of Live Vaccine for the Control of *Mycoplasma Synoviae* in Broiler Breeders in Chile

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Abstract

Introduction

Mycoplasma synoviae (MS) is an infectious agent that causes considerable economic losses in the poultry industry. Among the tools for its control, biosecurity, antibiotic treatments and vaccines are used to try to reduce or prevent the disease. The objective of this work was to document the first Chilean experience in the control of MS using vaccines with the MS-H strain under field conditions.

Materials and Methodology

A total of 1,125,000 birds were subjected to a continuous vaccination with the MS-H strain (Vaxsafe MS). Before vaccination, tracheal swab samples were sampled for the detection by RT PCR of field MS strains. The vaccination was carried out 7 weeks old. A total of 12,360 tracheal swab samples collected at various ages for monitoring of vaccine and field strains using a DIVA PCR method. Meanwhile, the use of antibiotics and the detection of clinical cases associated with MS problems during the period were recorded.

Results

Vaccinated birds showed positivity only for the strain MS-H (until 57 weeks old). After 3 continuous years of vaccination, the detection of field MS decreased in prevalence from a positivity of 40% to 0%. Additionally, there was no use of antibiotics to control MS. Clinical signs, such as respiratory, lameness or eggshell abnormalities have not been observed in birds vaccinated with MS-H.

Conclusions

Under the conditions of this study, it was observed that, thanks to the continuous use of vaccination with the MS-H strain, it is possible to control MS infections in broiler breeding birds.

Poster 87

Genetic Characterization and Antibiotic Resistance of *Mycoplasma synoviae* S-56 and S-76 Strains from Recent Outbreaks in Northeast Georgia

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Abstract

Mycoplasma synoviae (MS) is a major poultry pathogen, that although usually results in subclinical infection, may also cause respiratory disease, infectious synovitis, and eggshell abnormalities, leading to significant economic losses. MS spreads both horizontally and vertically through transovarian transmission. While long-term control strategies like vaccination and eradication exist, antibiotics provide short-term relief from clinical effects as well as reducing the shed of MS and reducing horizontal and vertical transmission. Previous studies have linked genetic mutations and single nucleotide polymorphisms to macrolide and tetracycline resistance in MS isolates in the US. This research focused on comparing the “S-56” and “S-76” MS strains, the two predominant genotypes in Northeast Georgia over the last decade, to identify mutations associated with antibiotic resistance. *In vitro* antibiotic resistance tests were conducted on isolates to determine the minimum inhibitory concentrations (MICs) for tylosin and tetracycline. Genome libraries for each isolate were generated using Illumina technology for comprehensive genetic analysis. Further investigations will focus on the significance of these identified mutations and explore the potential role of other genetic variants that may influence MS pathogenicity, including genes linked to transmissibility, colonization efficiency, and immune response. Understanding these genetic factors is crucial for developing more effective strategies to control MS infections in poultry populations.

Poster 89

Quantitative Assessment of *Mycoplasma gallisepticum* (MG) Detection Methods in Relation to Cleaning Effectiveness

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Abstract

Mycoplasma gallisepticum (MG) is a bacterial pathogen that primarily affects the respiratory system of poultry with turkeys being more severely affected. The monitoring and control of MG plays a crucial role in maintaining the economic sustainability of the commercial poultry industry. MG methods of transmission (both vertical and horizontal) and its effects on morbidity and mortality supports the advancement of our knowledge of this pathogen, making it a very important area of study. Advances in understanding the clinical presentation, epidemiology, and monitoring of MG have been key to improving management strategies for this pathogen. This study aims to review clinical manifestations, epidemiological trends, and comparisons of diagnostic testing methods (PCR and serology) as well as cleaning protocols and environmental sampling results across 2 infected flocks. The primary objective of this study is to evaluate the effectiveness of current cleaning protocols in eliminating MG and to identify optimal testing, cleaning, and disinfecting strategies for reducing the risk of MG infections on farm. Data will include environmental testing results before and after cleaning to assess whether existing protocols adequately eliminated the pathogen. Ultimately, this analysis will offer recommendations on the most effective combinations of diagnostic testing and cleaning practices for ongoing monitoring and mitigation of MG in commercial turkey production.

Poster: Newcastle Disease Virus

Poster 90

Analysis of gene expression in DF-1 cells infected with LaSota Newcastle Disease Virus using RNAscope

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Abstract

Newcastle disease (ND), caused by avian orthoavulavirus serotype 1, is a highly contagious infectious disease of poultry. Improving vaccines is important to controlling the disease and reducing economic losses. Understanding the avian immune response to vaccination with live ND virus (NDV) can identify immune genes linked to disease prevention, serving as biomarkers of immunity. The RNAscope in situ hybridization technique makes it possible to visualize specific RNA molecules in cells, helping in the precise identification of gene expression. In previous studies, we identified innate immune genes upregulated shortly after vaccination with the NDV LaSota strain. Among the identified genes, we selected six that were considered the most relevant as immunity biomarkers: USP41, OASL, IRF7, GBP1, and IFIT5. This study aimed to use RNAscope to confirm the results and demonstrate the expression of these genes in chicken embryo

fibroblasts (DF-1) cell culture infected with the NDV LaSota strain. Thus, DF-1 cells were cultured in Dulbecco's Eagle's modification medium, seeded on microscope slides/plates and infected with NDV LaSota. At regular time points after the inoculation, cells were fixed and the immune genes as well as NDV RNA were detected by RNAscope. The results will be presented and discussed. Establishing this method to investigate the immune response after infection with NDV is the first step for using it in in-vivo trials.

Poster 91

Comparison of broiler vaccination programs using two vector HVT vaccines versus one vector HVT and one inactivated given at the same time in Mexico

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Abstract

Vector recombinant HVT-Newcastle (rHVT-F) vaccines have been proven to be efficacy and reducing challenge virus excretion in field ND challenged broilers. In this study, two farms were compared. The first farm vaccinated chicks with rHVT-H5 and rHVT-F simultaneously at the hatchery. The second farm used the rHVT-F vaccine along with an Avian Influenza killed vaccine (KV) vaccine. At 9 days of age, both farms were given a live conventional vaccine Newcastle LaSota, and they also received another KV vaccine containing Newcastle and Avian Influenza H5 antigens. To assess the replication by Real-Time RT-PCR of the rHVT vaccines, spleen and feather samples were collected from each farm at 21 and 28 days of age. Both groups tested positive for both rHVT vaccines (90-100%), confirming that no interference had occurred. Serological tests showed no significant difference between programs ($P \leq 0.5\%$). However, the flock vaccinated with both recombinant vaccines showed better performance, productivity index, efficiency, and accumulated mortality rates (3.8% vs 5.5%). This study indicates that there is no interference when administering two vector HVT vaccines differing only in their gene inserts and that vaccinated broilers can effectively be immunized against both inserts leading to improved productivity.

Poster 93

Effect of *Echinacea purpurea* and elderberry on growth parameters and immune response on chickens vaccinated and challenged with Newcastle disease virus

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Abstract

Echinacea purpurea (EP) stimulates the immune system in two ways: by activating phagocytosis and fibroblasts. Likewise, elderberry contains flavonoids which may have immunomodulation, anti-inflammatory, antioxidant and antiviral effects. A total of 150 male chicks (one-day-old) were used to determine the effects of using EP + elderberry in vaccinated chickens and challenged with a viscerotropic velogenic Newcastle disease virus strain (vvNDV). Chicks were divided in three groups. Two groups were vaccinated against ND at 1 (La sota strain) and 10 days-old (inactivated vaccine). The groups were as follows: Treatment group (vaccinated/basal feed + EP + elderberry 0.5 g/kg), Control group (vaccinated/basal feed) and one unvaccinated group without treatment. At 30 days of age, all birds were challenged with vvNDV. Mortality, clinical signs, growth parameters and immune response were evaluated. All unvaccinated chickens died at 5 days after challenged, while, no mortality was observed in the treatment and control group. Chickens supplemented with EP + elderberry enhanced the final body weight, reduced feed conversion ratio ($p < 0.05$) compared with control group. In regard to immunomodulatory effect, serological assays (ELISA and hemagglutination-inhibition) showed higher titles of antibodies against ND in treatment group compared with control group. Also, EP + elderberry up-regulated the expression of genetic markers of, IL-1, IL6, TNF- α , IFN- γ , GPx and SOD compared with control group. In conclusion, the dietary supplementation of EP + elderberry enhanced the growth performance and improved immune response of ND vaccine in chickens challenged with vvNDV.

Poster 94

New Castle Disease Virus effects on the microbiota composition in the lower respiratory tract of young chickens

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Abstract

While some research on the respiratory microbiota has been done, it is nowhere as well researched as the intestinal microbiota. However, similar reasons for investigating respiratory microbiota are applicable: A better understanding might allow the selection of biomarkers for more or less susceptible chickens and maybe even allow the use of respiratory probiotics. Vaccinations against Newcastle Disease (ND) are among the most common in the poultry industry, but their effect on the respiratory microbiota has not yet been described. Therefore, this study aimed to

characterize the lung microbiota in chickens after ND vaccination. Forty-eight spf leghorn hatchlings were placed in BSL2 isolators. Birds were divided into unvaccinated control, V4, B1, and LaSota groups. Vaccinated groups received 10^7 EID₅₀ of the respective strain in 100 µl via ocular route, while control was mock vaccinated with PBS by the same route. Twenty-four hours post-vaccination the right lung of five birds per group was collected. Total DNA was extracted, and regions V4-V5 of 16s gene were amplified and sequenced. Bioinformatic analysis identified the relative abundance, alpha & beta diversity, and regulated metabolic pathways. The control and LaSota group were composed of Firmicutes, Proteobacteria, Actinobacteriota, Cyanobacteria, and Bacteroidota. Chicks from V4 group lacked Bacteroidota and Cyanobacteria, while the B1 ones lacked the former but contained the latter. Other results to be presented and discussed.

Poster: Reovirus

Poster 97

Detecting Multiple Avian Reovirus Genotypes from Field Samples using a Novel S1 Segment PCR and Oxford Nanopore Sequencing

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Abstract

Avian Reoviruses (ARVs) have a significant economic impact on the poultry industry; with pathogenic strains causing viral arthritis/tenosynovitis, stunting syndrome, and other respiratory and enteric diseases. ARVs contain a 10-segmented dsRNA genome classified into three size classes: large (L1, L2, L3), medium (M1, M2, M3), and small (S1, S2, S3, S4). The S1 segment encodes three proteins, including the minor capsid protein sigmaC (σ C) that is important for infection, eliciting neutralizing antibodies, and commonly used to genotype ARV isolates. Given that specific ARV genotypes are often associated with particular diseases, rapid identification and genotyping of ARV, particularly in field samples, are becoming increasingly more important. In this study, we sought to develop a modified S1 PCR combined with Oxford Nanopore barcoding approach to type multiple genotypes within the same field sample. To test this, known genotyped isolates and field samples were collected and tested using the modified S1 PCR approach. This method combined with current sequencing tools will enable faster identification of emerging genotypes from field samples for environmental treatment and vaccine development.

Poster 100

The effect of simulated vertical transmission of Avian Reovirus on chicken embryos

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Abstract

Avian reovirus (ARV) is a significant pathogen in poultry, causing viral arthritis, tenosynovitis, and immunosuppressive effects. Previous studies have demonstrated the horizontal and vertical transmission of ARV; however, its effect on embryonic development and infectivity based on the inoculation site, yolk, or albumen, has not been extensively studied. This study aimed to simulate vertical transmission and compare the infectivity levels between yolk and albumen inoculation. An ARV inoculum was serially diluted to titers between 10^{+2} to 10^{-2} EID₅₀/mL. The experiment involved 10 groups, with five replicates of specific pathogen-free eggs each. Before incubation, the first five groups were inoculated with the virus of the selected titers into the albumen, whereas the other five groups were inoculated with the same concentrations into the yolk. The eggs were incubated under standard conditions. By d5, a total of 36% of the inoculated eggs were nonviable (18% infertile and the other 18% dead). At the end of the experiment (d19), 56% (28 out of 50) of eggs survived: 12-albumen inoculated, and 18-yolk inoculated. The embryonated eggs were euthanized for the collection of yolk material, allantoic fluid, jejunum, and hock joints. Interestingly, the yolk sacs of inoculated eggs were found enlarged, and thick with black foci. RNA will be extracted from these samples and will be quantified for ARV through RT-qPCR for viral loads. The results will be presented and discussed. This study provides information about the infectivity level of ARV between the albumen or yolk route of inoculation before incubation.

Poster: Vaccinology

Poster 102

Comparison of vaccination strategies to protect long-lived birds against Salmonella Enteritidis and S. Infantis using commercially licensed products

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Abstract

The goal of this study was to compare how two inactivated SE vaccines protect against SE and cross-protect against S. Infantis (SI) and explore the additive effect of including a live ST. **Study Design:** SPF Leghorns were raised to 10 weeks and vaccinated intramuscularly as follows: T1) No vaccine, T2) SE/E. coli subunit vaccine, T3) SE-ND-IB whole cell vaccine and T4) SE-ND-IB + Live ST. At 17 weeks, 19 birds per treatment were challenged with SE or SI at 10^9 CFU/bird orally. At 18 weeks, ceca and liver/spleen were collected for Most Probable Number (MPN) enumeration and enrichment for prevalence if negative on MPN. **Results and Discussion:** Against the SE challenge, all treatments gave non-significant reductions in liver/spleen loads and % super shedders. The subunit vaccine did not reduce cecal loads or % super

shedders but the two whole-cell treatments did with the addition of Live ST having the lowest numbers. Against SI challenge, the subunit vaccine showed no reductions in any tissues. The whole cell bacterin significantly reduced ceca loads but the addition of Live ST resulted in the greatest reductions that were significant in liver/spleen loads, % positive and super shedder liver/spleens, cecal loads, and % super shedders. The combination of whole-cell bacterin and live ST providing a level of cross-protection to a Group C of human concern is noteworthy.

Poster 103

Effect of MDV-1 vaccines administered alone or with HVT on the development of the chicken embryo immune system

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Abstract

In previous studies, we have demonstrated that HVT hastens the immunocompetence of chickens when administered in ovo. HVT immunostimulant effect is stronger than that of known adjuvants such as poly I:C. Preliminary studies have also shown that in ovo administration of CVI988 had minor to no adjuvant effect, but it enhanced the transcription of various cytokines when administered together with HVT. In the present study, we have evaluated how in ovo administration of two MDV-1 vaccines (CVI988 and CVI-LTR), alone or in combination with HVT, affects the percentage of activated T cells and macrophages in one-day-old chickens. Our results confirm that HVT strongly activates T cells more efficiently than either CVI988 or CVI-LTR. However, administration of CVI-LTR together with HVT had the strongest immunostimulant effect on activation of CD4+ and CD8 α + T cells; significantly higher than HVT alone. In addition, CVI-LTR and CVI-LTR + HVT, but not other treatments, increased the proportion of $\gamma\delta$ T CD8 $\alpha\alpha$ cells, which have been related to cytotoxic ability. A decrease in CD8 β and activated CD8 β T cells was found in the group vaccinated with CVI-LTR but not in the one vaccinated with CVI-LTR + HVT. Our results show that administration of CVI-LTR with HVT significantly enhances the adjuvant effect of HVT and can render chickens more immunocompetent at hatch.

Poster 104

End-of-cycle mortality in Broilers between different vaccination protocols against IB and ND using a statistical analysis model in Colombia.

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Abstract

Avian infectious bronchitis (IB) is a disease that causes a severe socioeconomic impact on the global poultry industry; highly contagious, initially characterized by respiratory signs in broiler chickens and Colombia is no exception. The

purpose of this study was to compare the performance of the Nobilis® IB Ma5 vaccine against other vaccine plans with other Massachusetts strains. The study was carried out in Colombia, in 4 companies in three areas of the country (Antioquia, Boyacá and Cundinamarca) and evaluated the zootechnical information of 134 flocks of broilers (35 million birds), 64 flocks using Nobilis® IB Ma5 and Nobilis® ND C2 (15 million birds, Group 1) and 70 flocks (20 million birds) using another Massachusetts strain (Group 2). A univariate and bivariate analysis, a crude linear regression model and a linear regression model adjusting for confusion variables were carried out, contrasting the zootechnical variables of broiler chickens at the end of the cycle after being vaccinated. The overall mortality result was 5.4% in total, 4.9% in Group 1 and 5.8% in Group 2. A 0.8% higher survival was obtained in Group 1 with a statistically significant difference ($p<0.05$) and adjusting for confusion variables (conversion, age of slaughter and company) a 0.7% higher survival in Group 1 with a statistically significant difference ($p<0.05$). Considering the production data of Colombia, \$39.2 COP per kilo of chicken produced was recovered through survival after vaccination using Nobilis® IB Ma5 and Nobilis® ND C2.

Poster 105

Evaluation of Passive Transfer and Protective Efficacy of Maternal Antibodies Against a Novel *Salmonella* Vaccine Candidate

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Abstract

Non-typhoidal *Salmonella* are major foodborne pathogens, primarily linked to human infection through contaminated poultry products. While vaccines have effectively controlled certain serovars, their use has led to the emergence of untargeted serovars underscoring the need for novel vaccine candidates that can provide improved protection against multiple serovars. Another key challenge in *Salmonella* control is early-life exposure of chicks to *Salmonella*, whose underdeveloped immune system heavily depends on maternal antibodies for protection. Consequently, the ability to provide passive immunity through maternal antibodies is a critical parameter in assessing a vaccine candidate.

This study evaluated a novel vaccine candidate (InvG) conserved in *Salmonella*, for its ability to induce anti-InvG antibodies and to transfer them to offspring via egg yolk, to provide protection. To this end, hens laying fertile eggs at a steady state of production were vaccinated with purified recombinant InvG and their serum samples were analyzed biweekly using an enzyme-linked immunosorbent assay (ELISA) after each vaccination. Eggs were collected weekly for five weeks post-vaccination, divided into three groups and used to: (i) evaluate egg yolk IgG (IgY) against InvG, (ii) obtain day-old chicks to measure serum IgG and intestinal IgA levels against InvG, and (iii) obtain day-old chicks to challenge them with *Salmonella* Enteritidis or *Salmonella* Typhimurium. Results showed that InvG-vaccinated hens developed robust antibodies that were transferred to their offspring through egg yolk. The progeny of vaccinated hens exhibited higher serum IgG and intestinal IgA titers, accompanied by reduced *Salmonella* colonization in intestines and organs compared to control groups.

Poster 108

Identification and geographical location of variant strains of the Infectious Bronchitis virus in commercial birds in Colombia during 2024.

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Abstract

Avian infectious bronchitis is a disease that causes a severe socioeconomic impact on the global poultry industry, in Colombia IBV has been present since 1963. In 2003 strains were found: GI-1, GI-16, GI-20 and GVI-1. In 2020, strains compatible with GI-16 and GI-11 are identified. The aim of this was to identify circulating strains that can generate health problems associated with IBV in industrial poultry production in Colombia. The study was carried out in 21 flocks from 12 poultry companies. Samples were taken from 10 animals per flock, with cloacal and tracheal swabs, imprinting two FTA cards, one per organ, and sending them for PCR and IBV typing with the X-OVO laboratory. Six typeable samples compatible with GI-11 were obtained, all of which were found in broiler chickens. Three tracheal samples (Ct 18.78, 19.65, 25.60) that did not react to any conventional PCR protocol or any sequencing protocol. Four samples were positive for IBV, but with very high Ct at the cloacal level, which could not be typed. One sample was positive for the Massachusetts type strain at the tracheal level (cloacal: negative). Another was identified compatible with the Ma5 strain at the tracheal level. Six samples appeared negative. In this study with 67.6% of positive results for IBV in the sampling carried out and where the main strain found corresponds to GI-11 (40%) confirm the presence of variant strains in Colombia is in increase, suggesting the need for new sanitary strategies to face the challenges for the country's industry.

Poster 109

Infection Rate and Disease from Infectious Laryngotracheitis Virus (ILTV) Chicken Embryo Origin (CEO) Vaccine: Gel Drop in Hatchery vs. Drinking Water

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Abstract

Field experience has proven that ILTV CEO-vaccines administered via drinking water at 12–14 days of age (doa) grant appropriate protection against ILTV challenge in broilers. However, this practice is becoming less cost-effective due to the intrinsic logistics of CEO drinking water administration in the field. To reduce cost and facilitate administration, some broiler production sectors are administering CEO-vaccine in the hatchery cabinet via gel drop. Previous knowledge indicated that to minimize CEO-vaccine reactions and induce optimal flock immunity, vaccine infection-rate must be rapid (3- 7 days post-vaccination) and achieve a high viral genome load per bird. This study assessed the CEO infection rate and clinical signs induced when CEO-vaccine was administered at one (doa) via cabinet gel drop to non-vaccinated

broilers (CEOgd), to previously in-ovo recombinant (HVT-IBD-LT+CEOgd) vaccinated, and via drinking water (CEOdw) at 14 doa. Infection rate was defined as percentage of birds positive for ILTV genome load in choanal cleft swabs collected at 3-, 6-, and 14-days post-vaccination (dpv). Results showed significant severe clinical signs in the CEOgd broilers, leading to 21% humane euthanasia. CEOdw group had the highest initial infection rate (87.5%) at 3dpv, compared to 27% and 50% for the HVT-LT+CEOgd and CEOgd groups. At 14dpv, CEOdw and HVT-LT+CEOgd groups carried significantly lower viral genome load than CEOgd birds. Severity of clinical signs, inferior CEO initial infection rate, and slower viral clearance of the CEO-vaccine when administered alone at one doa indicate that this early age is not optimal for CEO immunization.

Poster 110

Innovative serological assays for poultry vector vaccines monitoring and DIVA testing of Newcastle, Infectious Laryngotracheitis and Gumboro diseases

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Abstract

Vaccination is an essential tool for poultry disease control. For many years, vaccines have been either live attenuated or inactivated, with innovation coming from the use of multivalent vaccines. Today, innovation in poultry vaccinology include immune-complex vaccines and vector vaccines. Vector vaccines are made from a vector microorganism of which the genome has been genetically modified to encode an immunogenic protein of the disease of interest. Vectors in poultry vaccines are commonly the Fowl Pox Virus (FPV) or the Herpes Virus of Turkey (HVT). One or more genes may be inserted to ensure stronger protection or to widen the spectrum of protection to more diseases. Benefits associated with this technology include bio-security, efficiency, ability to breakthrough passive immunity, and long-lasting immunity. In addition, vector vaccines may be used to as part of DIVA (Differentiation between Infected and Vaccinated Animals) strategies. Given that the conventional serological kits do not efficiently detect seroconversion to vector vaccines, IDvet has developed tools to monitor vaccination with vector vaccines for Newcastle disease (NDV), Infectious Laryngotracheitis (ILT) and Infectious Bursal Disease (IBD).

Poster 112

Monitoring of the humoral response induced after vaccination with a vectorized biological HVT-ND ILT in commercial farms in Colombia.

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Abstract

Newcastle and Avian Laryngotracheitis continue to be a concern for the Colombian poultry industry. The inclusion of vectorized vaccines is a very useful tool for the control of these diseases worldwide and Colombia is no stranger to this premise. To determine the serological behavior of ILT in Colombia, serological sampling was carried out (19 sera per batch) in 31 farms of 15 companies (23 batches of commercial layers, 8 broiler chickens) using the specific gI, gB and traditional laboratory ELISA kits. ID Vet[®] in birds vaccinated with Innovax[®] ND ILT to determine if the serological response at that time was associated with field challenges. Monitoring was carried out before applying any other biological to control ILT (4 to 18 weeks). A positive titer for gI was observed in 93.5% of the samples with the specific ID Vet[®] kit. Discriminating non-positive animals in commercial layers of n=23, a positivity for gI of 34.7% was found. In positive animals, when adjusting for confounding variables, n=17 was found, with 35.3% of positive animals without apparent clinical signs. For broilers, out of a total of n=6, 10% of positive animals were found without apparent clinical signs. In this study, it was evident that the presence of gB or titration with the indirect kit was related to clinical signs compatible with ILT, unlike what was seen with the inclusion of Innovax[®]-ND-ILT, where they can be controlled.

Poster 114

Serological monitoring as a tool to evidence ILT viral shedding reduction due to continuous vaccination with a rFP-gB vector vaccine in broilers

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Abstract

Infectious Laryngotracheitis virus (ILTV) was detected in Peru in mid of 2008, causing severe economic losses to the broiler and commercial layer industries. During the first months of ILT outbreaks no vaccine was available to prevent the disease, only specific biosecurity measures were implemented. That same year, a vector fowl pox vaccine carrying the g B gene of ILTV (rFP-gB) represented the first tool to effectively prevent and control the disease. Though the reduction in the incidence of ILT cases and presentation of clinical signs was evident, no tools to serologically monitor vaccinated flocks were available. Recently, commercial kits that detect the serologic response of vector ILT vaccines were made available. This study shows the results of a 1-

year serologic monitoring at slaughter age of 5 broiler farms in one production area (1.8M birds per cycle) with high ILT challenge. All flocks were vaccinated (SQ) at day-one with a rFP-gB vector vaccine. The IDVet commercial kit that detects specific antibodies against ILTV-gB was used. Our results showed that the kit detects the response to the vaccine and the field virus infection in the flocks. We observed a constant decrease in the GMT and maximum titers obtained after every production cycle. These findings show the ability to serologically monitor the control of ILT with the use of a rFP-gB vector vaccine in broilers. A constant decrease in the obtained titers is an indirect measure of ILTV shedding control. For statistical analysis and data visualization, Python coupled with Numpy/Scipy modules were used.

Poster 115

The effect of the addition of different antibiotics on the pH of a diluent for cell associated vaccines

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Abstract

Marek's disease has been around for a long time, but still has high economic impact on the poultry industry. Control is achieved by vaccinating the birds in the hatchery. With the advent of recombinant vaccines technology, Marek's vaccines have gained even greater importance. Most of the current recombinant vaccines use the HVT (Herpesvirus of Turkey) as a vector, and these vaccines are critical for the control of other diseases such as Gumboro, Newcastle, and Laryngotracheitis. This study aimed to evaluate the effect of the addition of antibiotics on the pH of the cell associated vaccine diluent. A neutral pH is important for viability of the Marek's vaccine. Three different gentamicin products were used, and the volume of each one added to the diluent bag was adjusted to be equivalent to the dosage of 0.20 mg/kg. One Ceftiofur product was added to correspond to 0.80 mg/kg. The pH was measured every 15 minutes for 1 hour. No changes were observed in the pH of the diluent when Ceftiofur was used in relation to the negative control (pH 7). In treatments with the addition of gentamicin, a drop in the pH of the diluents was observed, with average pH 5.98, with no changes in the different time points for the same product. We can conclude there was a reduction of the pH of the cell associated vaccine diluent when the gentamicin products were added. Further investigated to verify the impact on the viability of Marek's vaccine.

Poster 116

Utilizing Vaccine Takes for Newcastle Disease Virus (NDV) to Evaluate Different Vaccine Combinations

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Abstract

Assessing vaccine takes using real-time PCR is commonplace in the broiler sector of the poultry industry to evaluate vaccination efficiency. This is typically done for IBV but can also be performed for NDV. A real-time PCR assay targeting the matrix gene of NDV provides a positive/negative determination which is useful for measuring NDV vaccine take. Since field data are sparse, an initial project was undertaken in a commercial broiler hatchery that combined different manufacturers NDV/IBV vaccines with other IBV vaccines and vaccine takes were performed on chicks 5-7 days post-vaccination. Results showed that take for the NDV vaccine that includes a milder, cloned B1 NDV (C2) strain was very poor (4-17% positive), while the take for the NDV vaccine that includes a less attenuated B1 strain was very good (90% positive). This process was then expanded to other hatcheries and the same trends held true; vaccine takes for the milder C2 type NDV were consistently poor (<10%) while takes for less attenuated B1 type NDVs were consistently good (>80%). This was true across manufacturer and NDV vaccine combination, where stand-alone NDVs and NDV/IBV combination products behaved the same. Controlled laboratory studies are needed to determine if these poor takes result in poor protection from challenge, but these data highlight the differences in viruses used to develop these products and should be considered when building a vaccine program.

Poster: Virology

Poster 117

Genetic analysis of Infectious laryngotracheitis virus reveals virus origin and estimated spreading route

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Abstract

Infectious laryngotracheitis (ILT) is a highly contagious upper respiratory disease. It leads to significant economic losses due to increased mortality and reduced egg production. In early 2024, severe ILT outbreaks were reported in a densely populated poultry in a state in Brazil where the disease had never been diagnosed before and vaccination had never been implemented. Within a short period time, ILT cases were identified in broiler and broiler breeder farms, resulting in higher mortality rates and a decrease in fertile egg production. To trace the source of the virus or viruses responsible for these outbreaks, samples from six affected farms were analyzed. Two fragments of the ICP4 gene from the ILT virus (ILTV) were searched and compared with previously identified field and vaccine strains in the country. Sequencing results indicated that all the farms were infected with a virus of non-vaccine origin. Phylogenetic analysis revealed that

all farms were affected by the same virus, classified as Genotype VI. Furthermore, the ILTV samples from this study were compared with viruses previously found in egg-laying regions of Brazil. The viruses from the recent outbreaks were found to be identical, with one strain (VI-4) suggesting a possible spread route. This strain had been detected in 2020 in an egg-laying area located 800 kilometers away from where the current outbreak occurred. The phylogenetic analysis helped to identify the potential origin of the virus and its transmission route. Overall, the findings emphasize the importance of molecular characterization in distinguishing between vaccine-derived and wild strains.

Poster 118

Genetic evolution of wild waterfowl low-virulence Newcastle Disease virus in commercial chicken eggs with maternal antibodies

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Abstract

Newcastle Disease Virus (NDV) is the causative agent of Newcastle Disease (ND), affecting various wild and domestic bird species, including poultry. Low-virulence NDV (loNDV) strains, including those from vaccine strains, have been documented to spillover from vaccinated chicken to wild birds. Less is known about the risk that wild aquatic birds pose to poultry, and the molecular mechanisms underlying viral adaptation in vaccinated poultry remain poorly understood. Our previous work in specific pathogen-free chicken embryos demonstrated that wild bird loNDVs can adapt to chicken hosts while maintaining their lentogenic nature. Most mutations occurred in the HN gene, indicating better host recognition, with additional mutations in P and NP genes potentially enhancing viral replication. The present study done in commercial chicken eggs containing antibodies against NDV aimed to investigate the impact of maternal immunity on loNDV genetic adaptations. LoNDV isolates from wild waterfowl were passaged in 10-days-old commercial chicken embryos for 10 passages, with allantoic fluid harvested after 3 days of inoculation. Viral growth and antibody titers were tested through real-time PCR for viral load quantification, hemagglutination, hemagglutination inhibition, and ELISA assays at each passage. The virulence of the last passage of each isolate was compared by embryo mean death time. Whole genome sequencing of the first and tenth passages was performed using Illumina technology, with BWA alignment and iVar variant calling against the LaSota reference strain to identify passage-specific mutations. The results show that the presence of maternal antibodies did not interfere with virus replication. Results will be presented comprehensively.

Poster 119

Genome sequence analysis of novel Nephropathogenic/Respiratory isolates belongs to Mas/GI-1- Infectious Bronchitis virus from broiler/layer chickens.

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Abstract

The major goals of this study was to monitor genetic changes in the viral genomes of some recent field isolates of the AIBV from broiler chickens. To achieve these goals, we tested several pools of tissue specimens (trachea and kidneys) from some suspected AIBV outbreaks in broiler chickens by quantitative real-time PCR (q-RT-PCR). We selected two samples, one from the trachea (IBV-4) and one from the kidney (AIBV-6), that showed the lowest Ct values in the q-RT-PCR for the next-generation sequencing (NGS). The full-length genomes of these two isolates were deposited in the GenBank (Accession Numbers: PQ468962 and PQ468963). The viral genome size of AIBV-4 and AIBV-6 was 27,475 and 27,469 nucleotides in length. IBV-4 have typical IBV genome organization (5'UTR, ORF1a, ORF1b, S, 3a, 3b, E, M, 4b, 5a, 5b, N, and 3'UTR), while IBV-6 lack 5b. These two IBV isolates belong to genotype GI-1 based on the phylogenetic using the full-length, the S, and the N protein sequences. The S1/S2 cleavage sites show polybasic amino acid sequences (RR-F-RR) as direct evidence of virulence of these isolates in chickens. The recombination analysis shows multiple recombination events of these isolates with some natural and vaccine strains. The potential major parent for both IBV-4 and IBV-6 was IBV Beaudette, and the potential minor parent was the AIBV Arkansas DPI. Vigilant monitoring of the AIBV sequences of the currently circulating strains in chickens is highly encouraged to develop novel vaccines and diagnostic assays that match the field circulating strains.

Poster 120

Infectious Laryngotracheitis Virus (ILTV) Serological Monitoring in Commercial Broilers

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Abstract

Infectious Laryngotracheitis (ILT) is an acute upper respiratory disease of chickens caused by the alphaherpesvirus *Gallid alpha herpesvirus 1* (Ga HV-1). It is characterized by conjunctivitis, dyspnea, coughing, and hemorrhagic tracheitis. In high ILT virus (ILTV) challenge areas, the disease is controlled by vaccination. Commercial broilers in the USA can be vaccinated with live attenuated chick embryo origin (CEO) vaccines and/or recombinant viral vector vaccines expressing different glycoproteins (rHVT-LT gI/gD or gB, and rFPV-LT gB,). Currently, no serological tools are routinely used for ILT disease diagnosis or for vaccination monitoring in broilers. The goal of this work was to perform ILTV serological monitoring in commercial broilers raised in high ILTV challenge areas receiving different vaccination programs: CEO applied by spray at 14 days, or via drinking water at 7 days, or via gel droplet at day 1, rFPV-LT *in ovo*, a combination of rFPV-LT *in ovo* and CEO in gel droplet at 1 day, and non-vaccinated birds. Three indirect quantitative ELISA kits coated with recombinant glycoprotein B (gB), recombinant glycoprotein I (gI), and purified ILTV antigen were utilized. The

strategic utilization of those ELISA tests allows differentiating infected from vaccinated birds (DIVA). A total of 1006 pre-slaughter serum samples were collected from 82 flocks. Different seroconversion patterns to gB and gI were observed based on the vaccination programs. Interpretation of these results and their potential application to help control ILT in the field will be discussed in this presentation.

Poster 122

Measurement of Trachea Submucosal Thickness as an Indicator of Infiltration Induced by Infectious laryngotracheitis virus (ILTV) Infection.

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Abstract

Unlike other avian respiratory pathogens, such as Infectious bronchitis virus (IBV) and *Mycoplasma gallisepticum*, where tracheal mucosa thickness provides an assessment of the level of immune cell infiltration induced by the pathogen, for ILTV, a similar measurement is not possible. ILTV lytic replication in tracheal epithelial cells causes epithelial destruction and sloughing of the mucosa into the tracheal lumen. Based on the hypothesis that ILTV lytic replication has minimal impact on the submucosa, this study aims to establish a procedure to measure the trachea submucosa thickness to assess the level of cellular infiltration that reaches the trachea during ILTV lytic infection. To test this hypothesis, specific pathogen-free chickens were intratracheally inoculated with the virulent genotype VI strain 1874C5 and its derived recombinant strain, Δ vIL4, which lacks the viral cytokine IL4 gene. Transverse sections of the upper trachea were collected at one- and four-days post-infection (dpi), fixed in formalin, and stained with hematoxylin-eosin for histopathological examination. The submucosal thickness was measured as the distance between the tunica muscularis and the cartilage at six positions in each tracheal cross-section using GRYPHAX® 2.2.0.1234 at 200x magnification. At one dpi, no significant difference in the submucosal thickness was observed; however, by four dpi, Δ vIL4-infected chickens exhibited significantly higher submucosal thickness compared to chickens infected with the 1874C5 strain, highlighting distinct inflammatory responses. Immunohistochemistry staining of infiltrating mononuclear phagocytes followed by Masson's trichrome staining of the tunica muscularis layer is being conducted to better depict the level of inflammation in the submucosa after ILTV infection.

Poster 123

Pathological, epidemiological features, and statistical study of histopathological changes in chicken transmissible viral proventriculitis

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Abstract

Transmissible viral proventriculitis (TVP) is a chicken disease whose etiology is not fully understood. This study aims to describe histopathological, macroscopic, and epidemiological data associated with possible new etiological agents. The samples comprised 62 broiler farms, 4 laying hen flocks, and 3 broiler breeders. The disease was identified by proventriculus thickening, confirmed through histopathological examination as the most reliable diagnostic method for TVP. Prevalence, clinical signs, gross lesions, epidemiological features, and statistical analysis were calculated. Microscopic findings confirmed the disease, which was classified into three distinct statuses: TVP characterized by the presence of both lymphocytic infiltration and necrosis; lymphocytic proventriculitis (LP) identified by lymphocytic infiltration alone, without the presence of necrosis (WP) denoting cases devoid of both lymphocytic infiltration and necrosis in the proventriculus. These statuses occurred at 23.6 %, 52.8 %, and 23.6 % rates, respectively. The disease prevalence was 20.9 % in flocks aged 15 to 40 days, with a mortality rate from 0.1 % to 0.5 % upon discovery. TVP and LP are marked by intense lymphocytic proliferation and necrosis, hinting at the involvement of infectious agents. Conversely, the absence of these characteristics in WP points to non-infectious etiologies for proventriculitis. The distinct proventricular wall hypertrophy observed in TVP and LP, as opposed to WP, reinforces the interpretation that, only for the conditions of this study, infectious agents amplify existing conditions rather than serve as primary catalysts for the disease.

Keywords:

Transmissible viral proventriculitis Lymphocytic proventriculitis Without proventriculitis Histopathology Proventriculus

Poster 124

Phylogenetic analyses of chicken astroviruses diagnosed at the Poultry Research and Diagnostic Lab associated with digestive and hatchability issues.

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Abstract

Chicken astroviruses (CAstVs) were isolated from tissue samples of chickens suffering from digestive problems, stunting and “white chick disease”. Chicken astrovirus detected from intestinal samples were associated with runting, poor condition, poor feathering problems and diarrhea. CAstVs were also detected in progeny and unhatched eggs from

breeder flocks experiencing “White chick” condition. These viruses were associated with histopathological lesions including hepatocellular vacuolar degeneration, glycogen accumulation and heterophilic and lymphocytic interstitial nephritis. During viral isolation, chicken astroviruses induced severe congestion, hemorrhages, and edema of abdominal muscles in embryos. A conventional RT-PCR method targeting CAstV ORF-1b and ORF-2 that corresponds to the viral capsid protein was carried out to detect CAstVs was carried out. Nucleotide sequences were generated and analyzed by phylogenetic analysis using Neighbor-Joining method. The phylogenetic analysis separated the different astrovirus into two phylogenetic groups, according to the system proposed by Dr. V. Smyth (Avian Pathol. 41:2, 151-159, 2012). Astroviruses associated with “white chick syndrome clustered in a separate clade of group B. Enteric astroviruses clustered in different clades of groups A and B. According to this study, the capsid protein of astroviruses associated with hatchability issues is genetically different from those astroviruses associated with enteric problems.

Poster: Wealth of Knowledge

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Exploring factors on histomoniasis development in broiler breeder pullets

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Abstract

Previous studies found that early infection of histomoniasis increased morbidity and mortality, and E. coli co-infection and early infection time (D7) increased the disease progression. This study aimed to continue to explore and reconfirm the role of stressors and various factors on histomoniasis in broiler breeder pullets. 880 Ross 708 breeder pullets were randomly assigned to 11 treatments: non-challenged control (NC); challenged control (PC); infection at day 18 (Inf18); feeding at 75% (R75), 50% (R50) amount of feed during D14-28; 10 ppb aflatoxin(AFL); 5 ppm fumonisin, 8 ppm deoxynivalenol (Don + Fum); 24-hour delayed placement (Delay); 0.2% Eastern redcedar hydrosol (ERC); 0.2% Western redcedar hydrosol (WRC); cocci vaccination (Cocci); fenbendazole (Deworm). Birds were raised in battery cages for 4 weeks with a standard restricted breeder diet. On D7, all treatments, except Inf18 and NC, were intracloacally inoculated with 100,000 histomonads/bird (Inf18 was inoculated at D18). Body weights were collected on D0, 7, and 28. The birds were terminated on D28, and D18 treatment was terminated on D39 and scored for histomoniasis. Results were analyzed using one-way ANOVA, SAS, Tukey HSD for mean separation with a significance of $P \leq 0.05$. D+F, delay placement reduced BW at D7 ($P < 0.0001$). At D28, R75 and R50 had reduced BW ($P < 0.0001$). D18 infection showed reduced ceca scores (0.725; $P < 0.0001$) and liver scores (0; $P < 0.0001$) compared to D7 infection. This data suggests that broiler breeder pullets are more susceptible to histomoniasis at an early age.

Poster 127

Data mining to assess trends in airsacculitis condemnations and their correlations with the situation of Infectious Bronchitis in Brazil

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Abstract

The emergence of the GI-23 lineage of Infectious Bronchitis Virus (IBV) in 2021 significantly increased airsacculitis condemnations in Brazil's poultry industry, leading to substantial financial losses. This study employed a Mixed Methods approach, integrating quantitative analysis of condemnation data with qualitative interpretation to provide a comprehensive understanding of airsacculitis trends and associated factors. Seven official public reports released by the Ministry of Agriculture, Livestock, and Food Supply were used as the quantitative database. These data were analyzed semiannually (H1: January to June; H2: July to December) from 2021 to 2024, to identify temporal patterns and evaluate the effect of vaccination with a homologous IBV GI-23 vaccine introduced in 2023. Qualitative insights were derived through the contextual analysis of secondary sources and technical reports, exploring potential links between seasonal variations, vaccination programs, and condemnation rates. The study focused on Southern Brazil, which accounts for 65% of the country's poultry production and 78% of chicken meat exports. In the period evaluated, 130,964,846 carcasses were condemned due to airsacculitis. The data revealed a 258% increase in condemnations from 2021 H1 to H2, coinciding with the initial cases of IBV GI-23. On average, H2 condemnations were 70% higher than H1, likely linked to winter conditions. In 2023 H2, condemnations dropped by 24% compared to 2022, following the introduction of the homologous vaccine. 2024 H1 condemnations dropped by 44% compared to 2023 H1. These findings highlight a seasonal trend, with higher condemnations in H2, and underscore the vaccine's effectiveness in reducing airsacculitis-related losses.

Poster 129

Effect of type of shed on productive performance in brown egg-type pullets during the growth stage

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Abstract

The aim of this study was to evaluate the effect of the type of shed on the productive performance of the brown egg-type pullets during the growth stage. A total of 1600 birds were evaluated during the first 14 weeks of life, they were select with homogeneous initial weight and randomly distributed in two groups, 800 birds in each one respectively, the first group was raised in a heated shed, the second group was raised in a traditional shed, both with the same productive and sanitary management, there were 4 repetitions and 200 birds per repetition. To evaluate the productive performance of the birds, data was collected on initial weight (g.), final weight (g.) uniformity (%), total feed consumption (g.), feed conversion (g/g/live weight) and viability (%) of each type of shed. The data were tabulated in the

Excel program, the SAS statistical program was used to perform T-Student test, with a significance level of 5%. The normality of the data was obtained using the Kolmogorov-Smirnov test and the homogeneity of variances using the Levene test. In the results, significant differences were obtained ($p < 0.05$) on the final weight, total feed consumption, feed conversion, uniformity and viability; Brown egg-type pullets reared in the heated house presenting better productive results, compared to pullets reared in a traditional house.

Poster 130

Optimization of Microinjection Techniques for the Introduction of Wolbachia into Litter Beetles (*Alphitobius diaperinus*)

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Abstract

Litter beetles are economically significant insect pests in poultry production because they transmit pathogens, cause structural damage to poultry houses, and reduce feed efficiency due to consumption of too many beetles by the birds that can lead to indigestion. Pesticide use remains to be the primary method for controlling beetle population, but a biological alternative that reduces the risk of insecticide resistance is needed.

Sterile insect technique (SIT) is a control method which involves releasing sterilized male insects into the wild to suppress population size through failed reproduction. Wolbachia are gram negative bacteria which possess the ability to induce cytoplasmic incompatibility (CI) resulting in sterilization through an enzyme that causes lack of egg viability. Release of Wolbachia infected male insects has been used for SIT to control insect pests. We conducted experiments to optimize microinjection techniques to introduce Wolbachia into beetle eggs. These include assessments of dechoriation, use of oil, and incubation conditions to improve hatch rate and survivability of injected eggs. Wolbachia injections via cytoplasmic transfer and SPG buffer were evaluated. PCR analyses confirmed successful Wolbachia presence in injected eggs and newly hatched larvae. Our next step will be to conduct a large-scale injection of eggs with the aim of establishing a stable Wolbachia-infected beetle colony for use in SIT applications.

Poster 131

Preserving Animal Welfare and Disease Management through Ethnoveterinary Practices: Insights from Tribal communities of Jammu and Kashmir, India

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Abstract

At nearly 6 Lakh, the transhumant population of Jammu & Kashmir is more than individual population of as many as 55 countries in the world. The knowledge of ethno veterinary medicine and its significance has been identified by the tribal communities of India through a process of experience over hundreds of years. The study was carried out in hilly areas of Jammu to document various ethnoveterinary practices being used by Tribal Farmers and to find out if ethnoveterinary practices still are the first line of defense for control of infectious diseases. The data was collected by means of well-structured questionnaires. Around 23 major ailments commonly found in different categories of livestock/animals and their treatment with herbal, plant based and traditional practices were identified. Leaves (30%) were the most used followed by whole plant and seeds. The most common plant were *Trachyspermum ammi*, *Curcuma longa*, *Morus nigra*, *Aloe barbadensis*, *S. officinarum*, *Luecaena lucocephala*, *S. officinarum*, *Zanthoxylum armatum*, *D. wrightii*, *Trachyspermum ammi*, *Azadirachta indica*, *Bambusa bambos* (L.) Voss. Moreover, it was found that first line of defense was the use of local herbs. The traditional system of treatment is one of the most important prevailing systems in the area where modern veterinary health care facilities are still in developing stage due to hilly terrain and long distance. Due to high production cost and resistance developed as a result of excessive use of antimicrobial drugs. It is very important to promote sustainable, cost effective ethnoveterinary measures to control diseases in livestock.

Poster 133

The Effect of Poult Holding Temperature and Humidity on 7 Day Mortality

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Abstract

The correlation between the temperature and humidity in the environment of newly hatched poults and seven-day mortality rates was examined. Each box was divided into four equal quadrants. 60 poults total were placed equally into three quadrants. LogTags were then positioned in the empty fourth quadrant, recording temperature and humidity every five minutes. Six poults, two from each quadrant were selected for vent temperatures. These measurements were taken after servicing, the night preceding transport, the morning of, and after placement. After placement, mortality rate was the only variable monitored for the next seven days. Data was collected from six hatches over six weeks. An analysis was performed to evaluate the correlation between environmental conditions, both prior to and at placement, and seven-day mortality rates. The preliminary results indicated an increase in vent temperatures between 6:30AM and 8:30AM, coinciding with the poults being loaded onto the truck and after their arrival at the placement site. The data suggests a temperature increase of 1.2 degrees Fahrenheit during this critical time frame. Analysis showed no prominent correlation between environmental conditions and mortality. These findings underscore the importance of monitoring

temperature and humidity in the housing environment of newly hatched poult. This study emphasizes the potential for improved management practices that could reduce mortality rates in commercial turkey farming. The inability to draw statistically significant conclusions, highlight the need for further investigation into environmental management strategies that may enhance the welfare and survival of poult in commercial settings.

Poster 134

USDA's National Animal Health Monitoring System Upcoming Poultry Studies: Small Enterprise and Upland Gamebirds and Ducks

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Abstract

Introduction

The United States Department of Agriculture's National Animal Health Monitoring System (NAHMS) is planning two upcoming poultry studies: the Poultry 2025 Small Enterprise Study and the Poultry 2027 Upland Gamebirds and Duck Study. The small enterprise study will include U.S. poultry operations with 1,000 to 74,999 table egg layer inventory, 1,000 to 99,999 broilers sold or moved annually, and 1,000 to 29,999 meat turkeys sold or moved annually. The upland gamebirds and duck study is still in the needs-assessment phase. Data collection is planned for fall of 2025 for the small enterprise study and early 2027 for the upland gamebirds and duck study.

Procedures/materials and methods

Both studies are in collaboration with the USDA's National Agricultural Statistics Service (NASS). For both studies, operations will be selected to participate using the 2022 Census of Agriculture list frame. The surveys will be multi-modal, with options for participants to complete the survey by paper, web, or telephone call with a NASS enumerator. The small enterprise questionnaire consists of one 20-page survey.

Results

Results from the small enterprise survey will include information on inventory and general management, movement, visitors and workers, equipment and vehicles, animals, litter handling, health information sources, and disease and health management. The upland gamebird and duck survey results will include topics on health management and biosecurity.

Conclusions

These studies will provide valuable baseline animal health and management information for these poultry sectors. Input is still needed for the upland gamebirds and duck study.

Poster 135

Utilizing a Sucrose-Based Flavor Enhancer in Commercial Turkey Poult

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Abstract

Upper respiratory challenges in young turkeys are often associated with decreased water and feed consumption. To confound this problem, there are also limited options of supportive care products that relieve respiratory symptoms in poult. Products that are often used in late brood and throughout finish to relieve congestion may decrease water intake even further when used in young birds. Sweetener products are utilized in other species to increase palatability of products and to limit water intake reductions. This study hypothesized that adding in a sucrose-based flavor enhancer with menthol-based or oregano-based products may increase water palatability and limit drops in water consumption when administered to poult. An initial trial was conducted in young poult to confirm safety at half, full, double and quadruple recommended dosages of the flavor enhancer product. Water consumption, bird weight, and daily mortality were recorded for the duration of the 2 week trial. A follow up study will be completed combining the flavor enhancer at different dosing rates with recommended doses of either a menthol-based or an oregano-based product. Birds will be followed from 2-5 weeks of age with product application occurring weekly. Water consumption, bird weight, and daily mortality will be recorded for the duration of this study.

Poster 136

What can the Eggshell Tell Us?: Field Experiences Reveal Health and Production Implications of Eggshell Quality

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Abstract

A scientifically proven and non-invasive evaluation tool was developed to assess breeders egg quality by examining eggshell translucency. This innovative method subjectively grades eggs (scored as 1, 2 or 3) based on translucent spots, visible when light passes through the eggshell. Research shows that eggs with high translucency produce weaker eggshells due to malformation of the shell ultrastructure, higher incidence of contaminated eggs, reduced hatchability, lower chick quality, and higher chick mortality. These negative outcomes reveal the vitality of eggshells as protective barriers against bacterial penetration, and membranes for water and gaseous exchange to ensure optimal embryo development. The future holds exciting possibilities as we explore whether this translucency scoring system can indicate underlying health conditions in breeders that may impact egg physiology, embryo formation, and progeny quality later in life. In this presentation, the audience will experience real-life examples of how this tool can capture changes in breeder flocks' health status due to avian health challenges including mycotoxins, Enterococcus infection, avian metapneumovirus, and others. Our goal is to engage poultry veterinarians to adopt this tool in the field, leveraging eggshell translucency to enhance decision-making in poultry health management.