Welcome Reception & Poster Session

5:45 - 6:45pm Tuesday, 29th July, 2025
Room Gallerias
Moderators Suzanne McComb

Each person will be provided with a drink (both alcoholic or non-alcoholic) ticket. Light snacks will also be available for consumption.

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

Elena Marzo[1], Sandra Castanyer[1], Enrique Carceller[2], Martina Dardi[2], Evelyn Guirado[1]
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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.

Eman Gadu[1,2], Karen Krueger[1], Amro Hashish[1,3], Mostafa Shelkamy[1,4], Maria Chaves[1], Yuko Sato[1], Mohamed El-Gazzar[1]
[1]Iowa State University, Ames, USA. [2]Mansoura University, Mansoura, Egypt. [3]Animal Health Research Institute, Cairo, Egypt. [4]Suez Canal University, Ismailia, Egypt

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**

Elena Marzo[1], Evelyn Guirado[1], Ester Taberner[1], Sandra Castanyer[1], Idoia Teixidor[1], Martina Dardi[2], Sonia Feu[1]
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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 ($10^{5.8}CCID_{50}$/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**

Evan VanBeusekom[1], Miranda Painter[2]
[1]University of Minnesota, St. Paul, USA. [2]Iowa State University, Ames, USA

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

Evelyn Guirado[1], Sandra Castanyer[1], Enrique Carceller[2], Martina Dardi[2], Elena Marzo[1]
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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

Evelyn Guirado[1], Sandra Castanyer[1], Martina Dardi[2], Elena Marzo[1]
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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®

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

Mohamed Selim, Muhammad Luqman, Ravi Gupta, Kada Parfait, Tamer Sharafeldin, Sunil Mor
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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

Evelyn Guirado[1], Juan Luis Criado[2], Martina Dardi[2], Elena Marzo[1]
[1]Hipra Scientific S.L.U., Amer, Spain. [2]Laboratorios Hipra S.A., Amer, Spain

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 ($10^{5.4}CCID_{50}$/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 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**

Francisco Rojo[1], Ricardo Franco[2], Lilia Castellanos[3], David Dueñas[2], Arturo Orea[4], Luiz Sesti[5]
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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.**

Abdulkarim Shwani, David Suarez
USDA-ARS-USNPRC, Athens, USA

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)**

Abdoulaye Soumboundou
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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 assed 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**

Joaquin Caceres
Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, USA

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**

Zyne Baybay[1], Andrew Montecillo[2], Airish Pantua[1], Milagros Mananggit[3], Rene Jr Romo[4], Esmeralda San Pedro[4], Homer Pantua[1], Christina Leyson[5]

[1]BioAssets Corporation, Santo Tomas, Batangas, Philippines. [2]University of the Philippines Los Baños, Los Baños, Laguna, Philippines. [3]Department of Agriculture Regional Field Office III, Pampanga, Philippines. [4]Philippine College of Poultry Practitioners, Quezon City, Philippines. [5]Department of Science and Technology, Bicutan, Philippines

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

Mahmoud Samir[1], Mohamed Tarek[1], Moaaz Selim[2], Tamer El Shaarawy[3,2], Ayman El-Deeb[4,5,2]
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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

Marina Gaimard[1], Stephanie Lesceu[2], Chloe Redal[2], Jean-Emmanuel Drus[2], Catherine Lefebvre[2], Marianne Zorbas[2], Philippe Pourquier[2], Rafael Forero[1]
[1]IDvet INC, Hampton, USA. [2]Innovative Diagnostics, Grabels, France

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).

Mariana A Pinheiro[1], Thais M. Lima[2], Rocio Gerez[1], Diego E. Ventura[1], James T. Krehling[1], Miria F. Criado[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University,, Auburn, USA. [2]Department of Cellular and Molecular Biology, School of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirão Preto, Brazil

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 Log_{10}/ml in DF-1, 8.3 Log_{10}/ml in duck, 8.0 Log_{10}/ml in QM5, and 7.8 Log_{10}/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**

Florent Lavigne[1,2], STEPHANIE CASTAGNOS[1], Elizabeth Moreau[1], Clementine Caudron[1], Thierry Langlade[1], Benoit Mousset[1], Christophe Cazaban[1], Jean-Luc Guerin[2], Sylvain Comte[1]
[1]Ceva Animal Health, Libourne, France. [2]Veterinary University of Toulouse, Toulouse, France

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. Enzootic 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 23 **Antimicrobial Resistance profiles of emergent *Enterococcus cecorum* causing systemic disease in chickens**

Roxana Sanchez-Ingunza[1], Lifang Yan[2], Martha Pulido-Landinez[2]
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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 Lohmman 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**

Caleb Skow, Melha Mellata
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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 respective 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 stains, 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 i*solates 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. Additionaly, 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 subespecie *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 $2.0x10^7$ 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 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 iin 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 menigintis 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.**

Shayne Ramsubeik
University of the West Indies, St. Augustine, Trinidad and Tobago

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 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

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 HCO_3, tCO_2, 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
Southern Poultry Feed & Research, Athens, USA

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 52 A Comparative Assessment of Serological Diagnostic Methods in Poultry Health Monitoring

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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

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 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 weigth gain, feed conversion ratio, enhance villi heigth 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 determinate the effect of a blend probiotics and enzymes on histomorphic measures and growth perfomance. 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 was challenged with *Clostridium spp.* (5×10^8 CFU/ ml) + *Eimeria spp.* (5×10^4 ooquists) 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 convertion 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 compare 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 57 An overview of a novel combination of postbiotic and phytogenic 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-

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 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 SMCFA-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 ($1x10^9$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 $1x10^8$/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. The16S 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 poults measuring infection rate, lesion scores, and performance in a CRD. In S1, 2 out of 8 poults/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 poults were directly inoculated with 10^5 (L) or $2x10^5$ (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 poults 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 $1×10^8$ 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 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

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 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 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 CRIPSR 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

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 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

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 slidesplates 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 eldeberry 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-a, IFN-y, 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 EID_{50} 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 97 **Detecting Multiple Avian Reovirus Genotypes from Field Samples using a Novel S1 Segment PCR and Oxford Nanopore Sequencing**

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Callison
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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 (σC) 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 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 CD8α+ T cells; significantly higher than HVT alone. In addition, CVI-LTR and CVI-LTR + HVT, but not other treatments, increased the proportion of γδT CD8αα cells, which have been related to cytotoxic ability. A decrease in CD8β and activated CD8β 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.

Camilo Andres Medina Santos[1], FABIAN QUINTERO[2], JUAN CARLOS CARDENAS[2], LUCAS COLVERO[3]

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**

Ana Claudia Zamora[1], Roshan Paudel[1], Caley Ellington[1], April Skipper[1], Daniel Maekawa[2], Maurice Raccoursier[1], Maricarmen García[1]

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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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Brazil

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

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

Brian Jordan, Po-Hsin Yu, Kalen Cookson, Daniel Bautista
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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 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

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 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, ΔvIL4, 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, ΔvIL4-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.

173 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

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 dechorionation, 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.

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 armathum, D.wrightii, Trachyspermumammi, Azadirachta indica, Bambusabambos (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 poults. 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 poults in commercial settings.

Poster 134 USDA's National Animal Health Monitoring System Upcoming Study: Poultry 2025 Small Enterprise Study

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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 Poults

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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 poults. 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 poults. An initial trial was conducted in young poults 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.